Crystal Violet Colorimetry:
a Beer's Law Investigation

INSTRUCTOR RESOURCES

The CCLI Initiative

Computers in Chemistry Laboratory Instruction

Learning Objectives

- 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.

Procedure Overview

- "ideal" simulated data is manually entered into the ***MicroLAB*** spreadsheet and a series of plots using different functions operating on the current readings are constructed in order to discover a linear relationship between the current and concentration variables.
- a series of standard crystal violet solutions are prepared and analyzed using the ***MicroLAB*** colorimeter. A Beer's Law plot of these concentrations is constructed using the relationship discovered with the simulated data.
- an unknown solution of crystal violet is analyzed in the colorimeter and the Beer's Law plot is used to determine its concentration.

Name _____ Section _____ Date _____

COLORIMETRY: A BEER'S LAW INVESTIGATION

Report Sheet

Dilution Table for Standards

	#1	#2	#3	#4	#5
Desired CV Molarity	2.00×10^{-6}	4.00×10^{-6}	6.00×10^{-6}	8.00×10^{-6}	10.0×10^{-6}
mL CV					
mL H2O					
Total mL	15.00	15.00	15.00	15.00	15.00

1. Record the number of your unknown_____ _____

____Record the absorbance of your unknown___ _____

____Record the concentration of your unknown _____

2. Show your calculations for the concentration of your unknown.

Name _____ Section _____ Date _____

COLORIMETRY: A BEER'S LAW INVESTIGATION

Report Sheet (Page 2)

3. As discussed by your instructor, Beer's Law is expressed by the equation $A = \epsilon bc$. In this equation, A is the absorbance of the solution, b is the cell path length (2.54 cm in this case), c is the molar concentration of the light-absorbing solute (CV), and ϵ is a proportionality constant called the molar absorptivity.

   a. In the space below, calculate the molar absorptivity for each of the five standard CV solutions and determine the average of the five values. Watch significant figures and be sure to include proper units. Note how well your values agree with one another. After all, it is supposed to be a constant!

   b. Compare the numerical value of your average molar absorptivity with the slope of the best straight line given on your Beer's Law plot. That slope represents the "best" estimate of ϵ from your experimental data.

Name _____ Section _____ Date _____

COLORIMETRY: A BEER'S LAW INVESTIGATION

Questions/Problems

1. When iron (III) chloride is added to a solution of salicylic acid a purple solution forms. The photocell current read with the colorimeter for a series of standard solutions is given below. Convert these values to absorbencies. (The blank current is 100.0.)

FeCl3	Transmittance	Absorbance
40.0×10^{-5} M	17.9	
32.0×10^{-5} M	25.0	
24.0×10^{-5} M	35.7	
16.0×10^{-5} M	50.2	
8.0×10^{-5} M	70.8	

2. What would be the effect of the following on the Beer's Law plot?

   a. At the higher concentrations used to construct the plot, some solute molecules react with one another to form a species that doesn't absorb visible light.

   b. A fingerprint is present on the solution cell where the light beam passes through.

   c. Some solute molecules react with one another to form a species that doesn't absorb visible light.

COLORIMETRY: A BEER'S LAW INVESTIGATION

Tips and Traps

1. Page 3 of the experiment has a large blank space where students are to make notes on the development of Beer's Law as provided by the instructor. One suggestion for development follows.

   A plot of Log(T) (y-axis) vs. concentration is linear, but the y intercept of the plot is not zero. A zero y intercept might be desired so that the measured function will be zero at zero concentration. For Log(number) to be zero, number must equal exactly one. To obtain a number exactly equal to one the easiest thing to do is divide the measured number by itself. At zero concentration the measured current is the blank current Io. Hence the solution to the intercept problem is to divide each current measurement by Io and then take the log of this ratio, i.e., Log(I/Io). **Have the students do this**. **NOW!** Use the **Add Formula** to calculate Log(current(I)/measured value of Io). For example, if Io was measured to be 370.23 microamps, then the function for Column C would be Log(I/370.23). "Click-drag" this formula to Column C.

   Also note that the slope of the line is negative, not positive. A positive slope might be desired so that the measured function will increase as concentration increases. To change the slope from negative to positive is easy. Simply multiply the function by -1. **Have the students do this NOW** by adding a new formula. "Click-drag" this formula to Column D of the spreadsheet to be -Log(I/Io) and to the **Y2** graph axis.

   The plot of Column D vs. concentration is now linear with a zero intercept and a positive slope. The function -Log(I/Io) is called the absorbance and Beer's Law has been established.

2. Since the concentration of the crystal violet solutions are so small, the concentration column should be formatted in Scientific Notation. This will allow proper printing of the data table from the Spreadsheet. To cause the spreadsheet to record the input in **scientific notation**, **right click** on that spreadsheet column and select **Column Properties**, and click on **Scientific Notation**.

3. Crystal violet stains, so appropriate care should be taken in its handling.

4. Be sure to caution the students to **PLACE THE CAP** before clicking **Add**.

5. Be sure the students mark the vial so it can be placed in the colorimeter chamber the same way each time.

COLORIMETRY: A BEER'S LAW INVESTIGATION

Suggested Answers for the Report Sheet

Dilution Table for Standards

	#1	#2	#3	#4	#5
Desired CV	**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	**3.00**	**6.00**	**9.00**	**12.00**	**15.00**
ml H2O	**12.00**	**9.00**	**6.00**	**3.00**	**0.00**
Total ml	**15.00**	**15.00**	**15.00**	**15.00**	**15.00**

1. Record the number of your unknown _____ _____

Record the absorbance of your unknown ___ _____

Record the concentration of your unknown _____

2. Show your calculations for the concentration of your unknown.

A = ϵ bc, Therefore, c = A/(ϵ b)
Students will use their own values of molar absorptivity "ϵ" and absorbance "A." The value of b is 2.54 cm.

3. As discussed by your instructor, Beer's Law is expressed by the equation A = ϵbc. In this equation, A is the absorbance of the solution, b is the cell path length (2.54 cm in this case), c is the molar concentration of the light-absorbing solute (CV), and ϵ is a proportionality constant called the molar absorptivity.

   a. In the space below, calculate the molar absorptivity for each of the five standard CV solutions and determine the average of the five values. Watch significant figures and be sure to include proper units. Note how well your values agree with one another. After all, is supposed to be a constant!

   ϵ = A/bc where b = 2.54 cm. Since "b" is a constant, and A is proportional to c, then ϵ should be a constant for each solution. The values will vary as a function of the experimental conditions.

   b. Compare the numerical value of your average molar absorptivity with the slope of the best straight line given on your Beer's Law plot. That slope represents the "best" estimate of ϵ from your experimental data.

____ **The average of the five molar absorptivity values should be very close to the slope of the Beer's Law plot.**

COLORIMETRY: A BEER'S LAW INVESTIGATION

Suggested Answers to Questions/Problems

1. When iron (III) chloride is added to a solution of salicylic acid a purple solution forms. The photocell current read with the colorimeter for a series of standard solutions is given below. Convert these values to absorbencies. (The blank current is 100.0.)

FeCl3	Transmittance	Absorbance
40.0 x 10-5 M	**17.9**	**0.747**
32.0 x 10-5 M	**25.0**	**0.602**
24.0 x 10-5 M	**35.7**	**0.447**
16.0 x 10-5 M	**50.2**	**0.299**
8.0 x 10-5 M	**70.8**	**0.150**

2. What would be the effect of the following on the Beer's Law plot?

   a. At the higher concentrations used to construct the plot, some solute molecules react with one another to form a species that doesn't absorb visible light.

      This factor produces non-linear plot behavior.

   b. A fingerprint is present on the solution cell where the light beam passes through.

      The Beer's Law plot line is displaced toward higher absorbance values.

   c. Some solute molecules react with one another to form a species that doesn't absorb visible light.

      This factor produces non-linear plot behavior.

COLORIMETRY: A BEER'S LAW INVESTIGATION

Sample Graphs

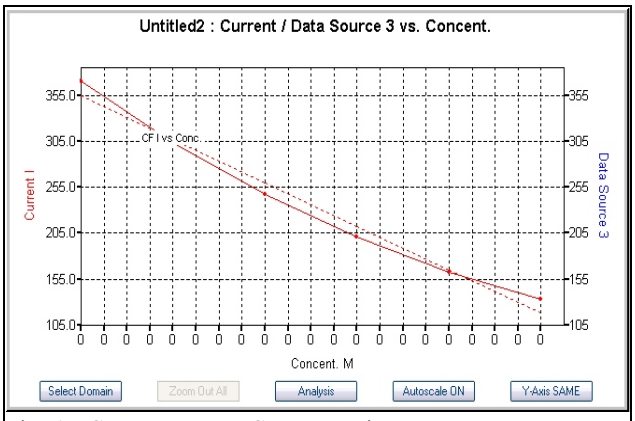

Fig. 1. Current versus Concentration.

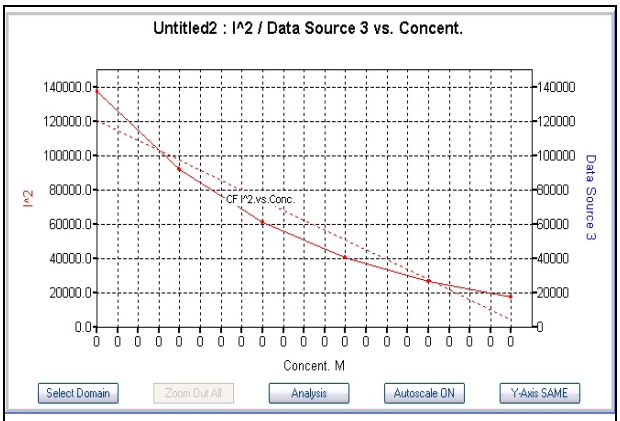

Fig. 2. Current squared versus Concentration.

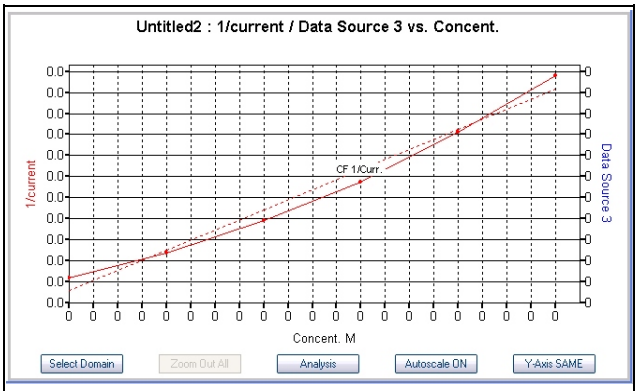

Fig. 3. 1/Current versus Concentration.

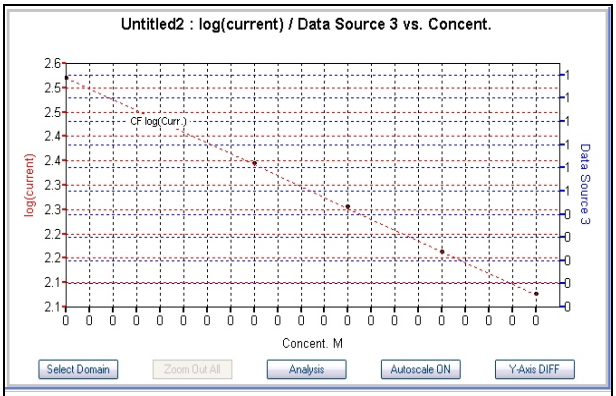

Fig. 4. Log(Current) versus Concentration

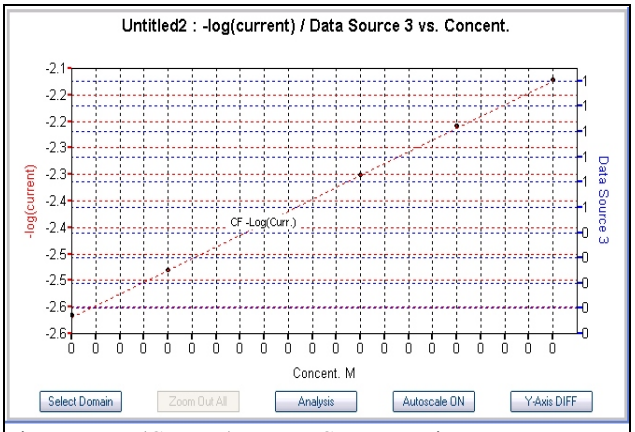

Fig. 5. -Log(Current) versus Concentration.

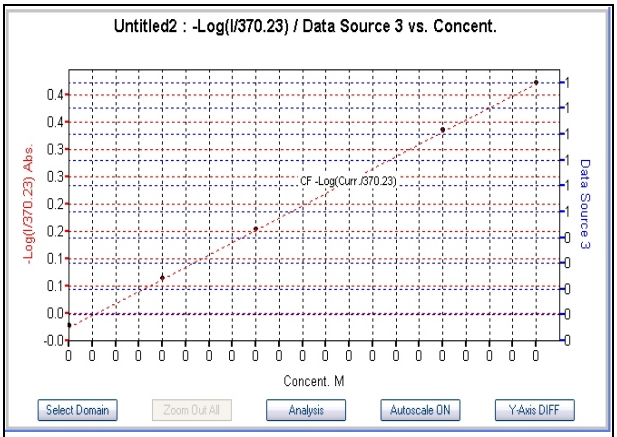

Fig. 6. -Log(Current/370.23) versus Concentration

COLORIMETRY: A BEER'S LAW INVESTIGATION

Laboratory Preparation (per student station)

Equipment
- ***MicroLAB*** colorimeter
- 6 colorimeter vials, 2.54 cm path length
- ring stand
- buret clamp
- buret with at least 15 ml capacity

Supplies
- KimWipes

Chemicals
Actual quantities needed are given below. A 50% excess is recommended.
- 1.00 x 10-5 M crystal violet solution (45.00 ml)

Safety and Disposal
- crystal violet may cause skin and eye irritation and the NaOH solutions are corrosive. Make sure students wear goggles at all times.
- all resulting solutions may be flushed down the drain with plenty of water.
- have students wash hands with soap and water before leaving the lab.