

Beer-Lambert Law using Vernier/Ocean Optics Spectrometer (V-SPEC)

From *Vernier Spectrometer User's Guide*

INTRODUCTION

The primary objective of this experiment is to determine the concentration of an unknown solution. You will use a Vernier/Ocean Optics spectrometer (V-SPEC). This spectrometer measures in the visible and near IR range.

You will prepare five crystal violet solutions of known concentration. Each solution is transferred to a cuvette that is placed into the spectrometer. The amount of light that penetrates the solution and strikes the photo cell is used to compute the absorbance of each solution. When you graph absorbance vs. concentration for the standard solutions, a direct relationship should result. The direct relationship between absorbance and concentration is known as "Beer's Law".

You will determine the concentration of an unknown crystal violet solution by measuring its absorbance with the spectrometer. By locating the absorbance of the unknown on the vertical axis of the graph, the corresponding concentration can be found on the horizontal axis. The concentration of the unknown can also be found by using the slope of the Beer's Law curve.

PURPOSE

- Prepare and test the absorbance of five standard Crystal Violet solutions.
- Calculate a standard curve from the test results of the standard solutions.
- Test the absorbance of a Crystal Violet solution of unknown concentration.
- Calculate the molar concentration of the unknown solution.

MATERIALS

Vernier/Ocean Optics (Vis-NIR) spectrometer	Unknown Crystal Violet solution
1.0×10^{-5} M crystal Violet Solution	50 ml or 100 ml beakers
Plastic beral pipets or eyedroppers	
Dilute Crystal Violet solutions (0.75×10^{-5} , 0.50×10^{-5} M, 0.25×10^{-5} M and 0.00001×10^{-5})	

PROCEDURES

PART I SET UP THE SPECTROMETER

1. Connect the spectrometer to the computer using an USB cable. The cuvette holder /light source should be attached to the Spectrometer.
2. Connect the power hub to the spectrometer and to a power outlet.
3. Start the Logger Pro software.
4. If the ROY G BIV background does not appear, select the Connect Interface → Spectrometer → Scan for Spectrometer from the experiment menu.
5. To calibrate the Spectrometer, chose Calibrate → Spectrometer from Experiment menu. Follow the instructions in the dialog box to complete the calibration.

PART II MEASURE THE ABSORBANCE OF AN AQUOUS SAMPLE

1. Fill a cuvette about $\frac{3}{4}$ full of 1.0×10^{-5} M Crystal Violet solution. Place sample in the cuvette holder of the spectrometer and click “Collect”. Click on “stop” to end the data collection. Examine the graph and note the wavelength region of maximum absorbance.
2. If you want to save the spectrum data, chose “Store Latest Run” from the Experiment menu.

PART III CONDUCT A BEER’S LAW EXPERIMENT

1. With a cuvette of 1.0×10^{-5} M Crystal Violet in the Spectrometer click on the “Configure Spectrometer Data” Icon located on the right hand side of the toolbar.
2. Under the “Set Collection Mode” icon in the display click “Abs vs. Concentration”. The wavelength of the maximum absorbance will automatically selected. You may choose a different wavelength by unclicking the wavelength and clicking another wavelength. Click “Ok” when done to close the display.
3. Place your first standard in the cuvette holder and click “collect”. Then click “Keep”. Enter the concentration of the standard and click ‘Ok’ . Repeat this step for the remaining standard solutions, After the final standard, click “Stop”.
4. Click “linear fit” to see the function for the standard solutions.
5. Place an unknown sample of the Crystal Violet solution in the cuvette holder. Chose “Interpolation Calculator “ (not Interpolate) from the Analyze menu. A helper box will appear, displaying the absorbance and concentration of the unknown. Click “Ok”.
6. If you want to save the data. Click “Store Latest Run” from the Experiment menu.

DATA ANALYSIS

1. Prepare a data table listing the concentrations and the absorbencies of the standards and your unknown.
2. Calculate the linear regression (best-fit line) equation of absorbance vs. concentration for the five standard solutions. Print or draw a graph showing the data and linear-regression equation for the standard solutions.
3. Determine the concentration of the unknown. Explain how you made this determination.
4. Describe an alternate method for determining the molar concentration of your unknown sample using the standard data.