SPECTROSCOPY

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INTRODUCTION

Spectroscopy is the study of the interaction of electromagnetic radiation with matter. All substances interact with electromagnetic radiation in a unique way. Our eyes act as fairly sensitive detectors of electromagnetic radiation that falls in the visible region of the electromagnetic spectrum. Electromagnetic radiation that contains all the wavelengths of visible light is known as white light. Objects appear to be a particular color because they selectively absorb some of the components of white light and reflect and/or transmit the others. The light that is not absorbed registers in the human eye as color. In spectroscopy, the detector is not your eye but rather an instrument that recognizes and measures electromagnetic radiation that is absorbed, transmitted, or emitted by a sample. Such an instrument is called a spectrometer.

A spectrometer sends a beam of light of a specific wavelength through a colored solution in a cuvette. The chemical compound responsible for the color absorbs light of certain wavelengths and transmits other wavelengths. The detector "sees" only the percentage of light that is not absorbed when it passes through the sample. The detector changes this energy into a measurable electrical current, which shows up on a scale that is calibrated in absorbance (A) units. The more concentrated the solution, the more light is absorbed.

For substances that produce colored solutions, there is a linear relationship between the concentration, c, of the substance in solution and the amount of light that is absorbed by the solution, A. Mathematically, A is related to c by the equation: $A = k \cdot c$, where k is a constant. A is unitless and c can have any units appropriate to concentration: %, moles of solute per L of solution, ppm, etc.

In this experiment, you will use a spectrometer. You will use food coloring to make standard solutions and prepare a calibration curve. You will then use the calibration curve to determine the concentration of an unknown solution. The use of a calibration curve to determine concentrations of unknown solutions is a common quantitative technique.

PROCEDURE

1. Double click the "Logger Pro" icon and allow the screen to open.

2. The spectrometer needs to be powered for about 5 minutes before using so do this step before preparing your solutions. Do not use the Go!Link with the spectrometer. Plug the spectrometer via provided USB cable to the computer USB port.

3. Arrange seven clean, dry, large test tubes (TT) in your TT rack. Number them 1 through 6. Label the first TT - BLANK.

4. Set up and load two 25 mL burets; one with deionized (DI) water and the other with your assigned stock dye solution (concentration: 100% dye).

5. Deliver the volumes (mL) of dye stock solution and DI water shown in the table below. Mix the contents of each tube well. The concentration (percent by volume) in each TT after the addition of water should be determined and entered in the table in your lab notebook.

TT	mL stock dye solution	mL DI H_2O	% v/v
BLANK	0	10.0	0
1	1.0	9.0	
2	2.0	8.0	
3	4.0	6.0	
4	6.0	4.0	
5	8.0	2.0	
6	10.0	0	100

6. Calibrate the Spectrometer; do not unplug the spectrometer during this experiment or you will have to start over.

   a. Return to the Logger Pro screen on the computer. Click: Experiment ; Calibrate ; Spectrometer:1.

   b. Allow the lamp to warm up for 90 seconds as displayed on the computer screen.

   c. Only touch the ridged faces of the cuvette, never touch the clear faces. Rinse and fill one cuvette (about ¾ full) with the blank. In this lab, the blank is deionized water. Rinse and fill (about ¾ full) a second cuvette with the most dilute of the solutions. **Gently blot** (do not scratch the sides of the cuvette) off any drips on the outside of the cuvette with a Kimwipe.

   d. Place the cuvette containing the blank in the spectrometer so that one of the clear sides is aligned with the white arrow at the top of the cuvette slot. Click: "Finish Calibration" ; OK.

7. Determine the Wavelength of Maximum Absorbance

   a. Place the cuvette containing the most dilute solution in the spectrometer.
   Click: Experiment ; Data Collection ; Full Spectrum ; Done. Click the rainbow icon labeled "Absorbance=…" in the upper left hand corner of the window. Change the "Wavelength Range" to 380-750nm. Close this box by clicking the "x" in the upper right hand corner of the window.

   b. Click the small green triangle in the toolbar labeled "Collect". After the line graph appears on the screen, click the small red square in the toolbar labeled "Stop".

   c. To automatically store the maximum wavelength go to the toolbar and select: Experiment ; Store Latest Run.

8. Generate the Calibration Curve.

    a. In the toolbar click: Experiment ; Data Collection ; choose Events with Entry in the Mode box. Highlight the word Event in the Column Name box and replace it with Concentration. Put percent in the Units box. Clear "Short Name". OK. You are ready to begin collecting data. Remove the cuvette from the spectrometer.

    b. Place the cuvette containing the blank back in the spectrometer. Click the begin data collection button (triangle) in the toolbar labeled "Collect". When the absorbance reading stabilizes, click the KEEP button located in the toolbar just to the right of the Red Stop Button (be careful that you **do not** accidentally click the stop data collection button (square)). Type in the concentration of the solution that is in the cuvette (do not include units). OK.

        Pour your samples back into the appropriate TT after you have measured each absorbance and discard them in the waste container only after you have acceptable data.

    c. Working in order of most dilute to most concentrated of the remaining standard solutions (not the unknown), rinse and then fill a cuvette with the solution that will be analyzed. Place the cuvette in the spectrometer. When the absorbance reading stabilizes, click the KEEP button (be careful that you **do not** accidentally click the stop data collection button (square)), and enter the concentration of the analyzed solution. Repeat until the absorbance of each solution has been determined.

    d. When the absorbance of all standard solutions (**not the unknown**) have been measured, click the stop data collection button (square) located in the toolbar.

    e. To determine the equation of the line for your calibration curve click: Analyze ; Linear Fit. A box should appear with the equation and a correlation.

        To receive full credit for this lab your calibration curve must be a good, straight-line graph, with a correlation coefficient of 99% or better (Corr: on the screen reads 0.9900 or greater). You should repeat the experiment until you get this proficiency. Work carefully. If you need to repeat the experiment, you still must be done with the write up and post-lab questions before the end of the lab period.

9. Rinse and fill a cuvette with your assigned unknown. Place the cuvette in the spectrometer. Record the absorbance in your lab report once the reading has stabilized.

10. Each lab partner's report must have a Logger Pro generated printout of the calibration curve attached to it. The printout must show the graph, the information needed to generate the equation (slope and intercept) for the line, and the correlation reading. To do this click: File ; Print. Uncheck the "Print Visible Spectrum on Wavelength Graphs" and change the "orientation" to landscape under properties. Be sure that the names of all lab partners are entered in the "Name" section and that the date box is checked.