Beer's Law: Determination of an Unknown Solution's Concentration

Minneapolis Community and Technical College

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Objective: To prepare a set of standard solutions for colorimetric analysis and use them to generate a calibration curve. The calibration curve will be used to determine the concentration of an unknown solution.

Prelab Questions: Read through this lab handout and answer the following questions before coming to lab. There will be a quiz at the beginning of lab over this handout and its contents.

1. Why not use green light to evaluate the green solutions used in today's experiment?
2. How many times is the colorimeter "calibrate" button pressed?
3. What precautions are taken when cleaning the cuvette?
4. What is a Beer's Law plot?
5. How many data points should there be on the Beer's Law plot?
6. What is the concentration of a solution created by mixing 3.0 mL of 0.25 M NaCl with 8.0 mL of distilled water.
7. Between colorimetry measurements, what precautions should be taken?
8. How do we know which way to put the cuvette in the colorimeter?
9. What is a "Standard?"

Recall the experiments with light absorption and food colorings. As you discovered, colored water solutions appear the way they do because of the light they *transmit*. For example, a blue solution transmits mostly blue light and that's what we see or detect.

In this week's experiment, we are going to utilize the absorption characteristics of a **green**, aqueous dye solution to determine its concentration. Because more concentrated solutions appear darker and dilute solutions appear lighter, you can use your eye to order or rank solutions of differing concentration if their differences are great enough. However, if the concentrations are similar, it can be difficult or impossible to compare them and see differences with the naked eye.

Instead of our eyes, we will be using an electronic device called a colorimeter (figure at right) to determine how light absorption is related sample concentration.

This device provides light at either **565 nm (Green)**, **635 nm (Red)**, **470 nm (Blue)** or **430 nm (violet)** wavelengths.

Light of the chosen wavelength passes through the sample and then strikes a detector which measures how much light gets through.

This information is sent to the computer where the Logger Pro data acquisition program determines the percent of the original incident light intensity that passed through the sample. This is known as percent transmission or simply **%T**.

In this experiment you will prepare and test six solutions known as "Standards." Each standard has a different concentration of green dye ranging from 0.00 M to 0.400 M. The first solution contains only distilled water and is referred to as the "blank". It is used to determine the 100% transmittance point (all light is passed) since there is no dye present. In subsequent trials, the concentration of the green dye is increased. Because these solutions absorb increasing amounts of light, less light reaches the light detector and a lower % transmittance is recorded.

After preparing the six standards and gathering the % T data, you will construct a graph of **absorbance vs. concentration** where absorbance is defined as

Absorbance = - Log(%T/100)

This graph, known as a Beer's law plot, is nearly a straight line for dilute solutions. You will use the trendline analysis of this straight line to determine the concentration of a green dye solution from its measured absorbance. *Part of your lab report grade will be determined by how close your measured unknown concentration is to the known value*.

Cuvette handling and sample volumes

Samples are transferred to a cuvette which is then placed in the colorimeter for measurement. The cuvette has ribbed sides and clear windows as in the figure at right.

Cuvette Tips:

- Keep cuvette's clear windows clean and free from fingerprints.
- Handle the cuvette by its ribbed surfaces only. Don't touch the clear windows.
- Clean the windows as needed with a damp tissue.
- Always rinse the cuvette with a small amount of the liquid to be measured. Discard the rinse solution.
- When working with multiple samples, start with the most dilute. That way, any dilute residue will not significantly affect the concentration of the next more concentrated sample.
- Use the same cuvette for all measurements.
- Fill the cuvettes with your samples 3/4 full.
- Tap the cuvette *gently* on the benchtop to eliminate bubbles that may be sticking to the inside surface of the clear windows.
- Use a lid on the cuvette to keep its contents from spilling.
- Always put the cuvette in the colorimeter the same way. This makes for more consistent measurements. Use the notch in the cuvette to reproducibly insert it in the colorimeter.
- When finished, rinse the cuvette with distilled water and leave it upside down on a paper towel to dry.

Experimental: Standard Preparation

1. Dispense approximately 30 mL of 0.400 M green dye stock solution in a clean/dry 100-mL beaker.
2. Dispense approximately 30 mL of distilled water in a clean/dry 100-mL beaker.
3. Rinse and fill two burettes: one for water and the other for aqueous green dye solution.
4. Obtain and label four clean, dry, screw top vials 2, 3, 4 & 5. Write the vial number on the lid of each vial.
5. Accurately dispense 2, 4, 6, and 8 mL of 0.40 M green dye solution into Vials 2 - 5.
   Record the actual volumes dispensed in the data table.
6. Accurately dispense 8, 6, 4, and 2 mL of distilled water into vials 2 - 5.
   Record the actual volumes dispensed in the data table.
7. Cover securely and shake each vial to mix the contents.
8. Keep the remaining 0.400 M stock dye in the 100-mL beaker to use as the sixth solution.
9. Calculate the concentrations of solutions 2 – 5 and record these values in the data table.

Experimental: Colorimeter Calibration:

1. Prepare a "blank" by filling a clean cuvette 3/4 full with distilled water.
2. Open the colorimeter door and place the water filled blank cuvette inside. Make sure the cuvette's smooth, transparent windows are facing the left and right of the colorimeter.
3. Note the position of the notch on the cuvette. Be sure to place the cuvette in the colorimeter the same way for all future measurements.
4. Close the colorimeter door.
5. Use the colorimeter's arrow keys to select the **635 nm (red)** light source.
6. Press the "CAL" button. When the red light on the colorimeter stops flashing, it is ready for use and the computer display should read close to 100% T.
7. If after calibration Logger Pro indicates something other than 100% (+/- 0.5%), alert the instructor who will then supply you with a new cuvette. Repeat the calibration procedure with the new cuvette.
8. Record the %T for the blank in your data table.
9. Use the same cuvette for all subsequent measurements.
10. Do not press calibrate again.
11. You must test all 6 standard solutions and your unknown solution before letting the next person use the colorimeter. They must recalibrate the colorimeter using their cuvette before performing their measurements.

Experimental: Standard Solution Analysis

1. When testing a series of solutions, always work from *low to high* concentration. This is because concentrated solutions leave residue that can significantly change the concentration of a more dilute samples.
2. Empty the water from the cuvette and rinse it *twice* with ~1mL amounts of the solution in vial #2 (the most dilute solution). Now fill the cuvette 3/4 full with the solution from vial #2.
3. Use the notch to properly orient the cuvette in the colorimeter.
4. Close the colorimeter door and wait for the %T value displayed on the monitor to stabilize. Record the %T value for vial #2 in your lab notebook.
5. Discard the contents of the cuvette in your waste beaker. Your waste beaker will be emptied into the sink at the end of the lab period.
6. Repeat the above procedure (Rinse…rinse… measure) for the remaining three vials and for the 0.400 M stock dye solution. The best results will be obtained if each measurement is performed consistently the same way.

Experimental: Unknown Analysis

1. Obtain an "Unknown" green dye solution from the lab instructor.
2. Record its number on your data sheet
3. Rinse the cuvette twice with the unknown solution and measure its %T as described above.

Experimental: Cleanup:

1. Pour all used or leftover green dye solutions down the drain.

2. Rinse your cuvette in distilled water and return it to your lab station. Position it upside down on a paper towel to dry.

3. Rinse the burets with distilled water and return them to your bench top. Position them upside down in the buret clamps for the next group of students to use.

4. Rinse all sample vials with distilled water and return them to the benchtop to dry. Leave upside down on a paper towel.

5. Examine the interior of the colorimeter for moisture. If it appears damp, alert the instructor.

6. Double check that you haven't left the cuvette in the colorimeter.

Prelab Questions: Answers

1. Green light is completely transmitted for a green solution. We learn nothing. Rather we use red light because it is absorbed to an extent that depends on how concentrated the green solution is.
2. The colorimeter is calibrated only once; for water also known as the blank. Therefore, the calibrate button is only pushed once in your experiment.
3. Handle the cuvette by the ribbed sides to avoid fingerprints on the clear windows. A clean, damp (distilled water) tissue is used to clean the cuvette's window surfaces.
4. A Beer's Law plot is essentially a calibration graph. Standard concentration is on the "X" axis and Absorbance is on the "Y" axis.
5. There will be 6 data points on your Beer's Law Plot. The unknown doesn't appear on the graph since we don't initially know its concentration.
6. 0.068 M
7. Rinse the cuvette twice with the next solution to be tested. Avoid fingerprints on the clear windows. Gently tap the cuvette on the benchtop to eliminate bubbles that may be sticking to the inside window surfaces.
8. A notch in the top of the cuvette is positioned the same way for each measurement insuring consistent insertion of the cuvette into the colorimeter.
9. A "Standard" is a solution of known concentration. We'll have six standard solutions that will be used to construct a Beer's Law plot which is then used to determine the concentration of an unknown solution.

C1151 Data Sheet
Beer's Law
Instructor Initials_____

Name_____
Date of Exp. _____
Lab Section _____

Your individual experimental report will be due at the beginning of class next week.

Data Table: (All entries must be in written in ink before you leave the lab).

Solution ID	Water mL	Stock 0.400 M Green	Concentration (M)	%T	Absorbance =-Log (%T/100)
1. Water Blank			0 M		
2					
3					
4					
5					
6			0.400 M		

Unknown #	Unknown %T	Unknown Absorbance	Unknown Concentration

Data Analysis:

1. Create an Absorbance (Y) vs. Concentration (X) graph using data from the six standard solutions above.

2. Perform a *linear* trendline analysis of your six data points.
   Display the equation and R^2 value on the graph with a minimum of 8 decimal places.

3. On the same graph, perform a *2nd order* polynomial trendline analysis of your six data points.
   Display the equation and R^2 value on the graph with a minimum of 8 decimal places.

4. Turn in your graph as part of this report.

5. In the space below, calculate the concentration of the unknown solution using both the linear and 2nd order polynomial trendline equations (Show all work for both. Use a separate page if necessary)

5. Choose the best unknown concentration from #4 above and report it with the correct number of significant figures in the unknown data table above.

Questions:

1. You now know the concentration of your unknown.
   How would you prepare new standard solutions to improve the accuracy of your unknown's concentration even more?

   _____

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2. Explain in your own words why we used red light to determine the concentration of a green solution.

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