Lab: What Is the Relationship Between the Concentration of a Solution and the Amount of Transmitted Light Through the Solution?

Background:
In a colorimeter a beam of light is shined into a solution containing the sample, and the amount of light that comes out of the other side of the solution is detected and measured. By comparing the amount of light transmitted by the pure solvent to the amount transmitted when the sample is dissolved in it, a quantity called absorbance can be calculated. In this investigation, the relationship between absorbance and concentration of solution will be studied.

A stock solution of known concentration has been prepared by dissolving blue #1 dye (Brilliant Blue) in water. The blue #1 dye molecule is the only chemical species in solution. The absorbance of light by the solution will be measured using a colorimeter.

Purpose:
To explore the relationship between concentration of a solution and absorbance of light. To gain skills in using a colorimeter and Microsoft Excel.

Safety:
All waste can be disposed of down the drain and flushed with plenty of water.

Materials:

Colorimeter	Plastic pipet	Glass stirring rod
Vernier LabQuest	10 mL graduated cylinder	40 mL of stock solution
Plastic cuvette with lid	6 test tubes	10 mL of Gatorade
KimWipes	Test tube rack	

Procedure:

1. Setup and calibrate a colorimeter.
2. Using the colorimeter, collect and record absorbance data for each solution in Data Table 1.
3. Measure the absorbance of the Gatorade solution. Record this value in Data Table 2.
4. Wash all glassware and cuvettes. Return all equipment. Wash your hands with soap and water.
5. Determine the concentration in µM of each diluted solution in the data table. Remember, $M_1V_1 = M_2V_2$.
6. Login to **Google Classroom**, open Gatorade Data assignment.
   i. Using Google Sheets, create a calibration line by graphing Absorbance vs. Concentration.
   ii. Enter your name and lab partner(s) names.
   iii. Enter your data into each respective column.
   iv. Highlight Columns A and B.

v. Click the **Insert tab** from the top menu. Click **Chart types** tab. Choose the first Scatter plot (top left of the choices).

vi. Click **Customization** tab. Scroll to the very bottom.

   i. For "Trendline", select "Linear".

   ii. For "Label", select "Use equation".

   iii. Check the box that says "Show R^2".

vii. Click "Insert". Your graph with calibration curve will be placed into Google Sheets.

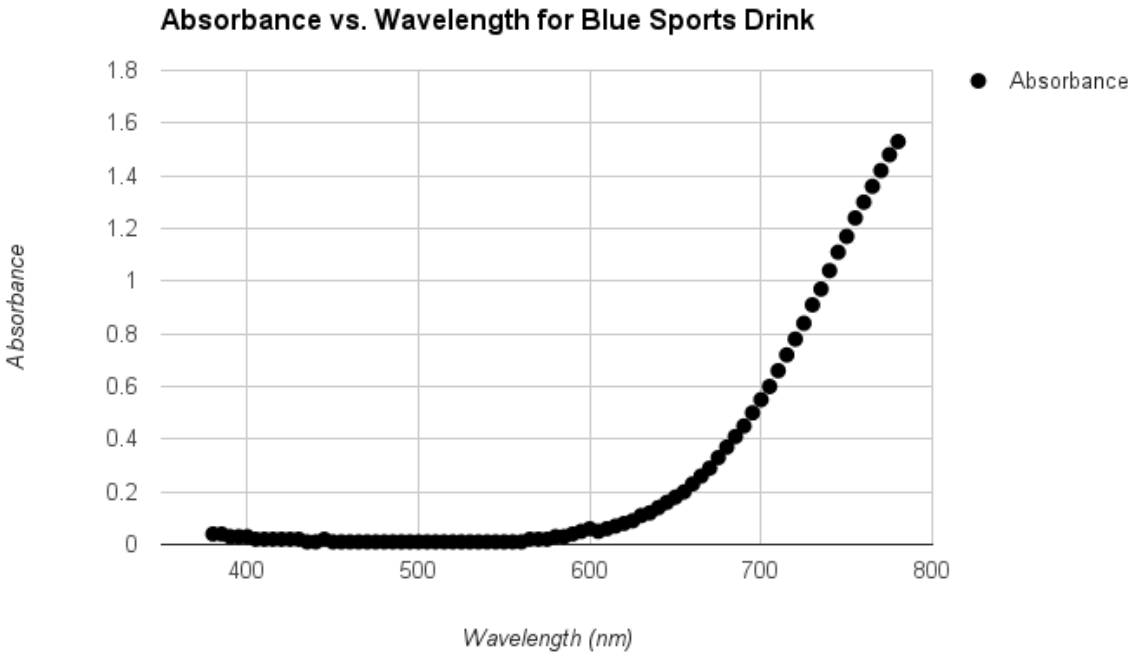
viii. Click on the graph, then click on the arrow in the top right corner that appears. Select "Advanced edit". Format the graph to include an appropriate title and label for x and y axes. Click "Update" to save changes.

ix. Record your calibration curve equation. Post-lab question #3.

x. Click "Turn-in" when complete.

Pre-lab:

1. A spectrophotometer was used to measure the absorbance of light by blue Gatorade at various wavelengths. The results are represented in the absorbance spectrum below.



   a. A student wants to test the effect of concentration of blue dye in Gatorade on the amount of light absorbed by the sports drink. What wavelength of light should the student choose? Justify your answer using the absorbance spectrum above.

   b. Why wouldn't the student want to use light the same color as the sports drink?

2. If 50.0 mL of a 3.20 µM NaCl solution is diluted to 200.0 mL, what is the new molarity, in M, of the resulting solution?

Data:

Table 1

Dilution Ratio mL stock/mL water	Absorbance
10 mL/0 mL	
8 mL/2 mL	
6 mL/4 mL	
4 mL/6 mL	
3 mL/7 mL	
2 mL/8 mL	
1 mL/9 mL	
0 mL/10 mL	

Table 2

Gatorade Sample Absorbance	

Post-lab and Data Analysis:

1. Determine the concentration of the diluted solutions. Show work for at least the 8mL/2mL dilution. You need not show work for the remaining dilutions. Record all answers in the table below.

Dilution Ratio mL stock/mL water	Molar Concentration (µM)
10 mL/0 mL	
8 mL/2 mL	
6 mL/4 mL	
4 mL/6 mL	
3 mL/7 mL	
2 mL/8 mL	
1 mL/9 mL	
0 mL/10 mL	

2. Analyze your calibration curve. Is the relationship between concentration and absorbance direct or inverse? Explain your reasoning.

3. What is the equation of your calibration line? Write the equation in y = mx + b format.

4. Using the calibration line equation and the absorbance value of Gatorade, determine the concentration in µM of blue #1 dye in the Gatorade solution.

5. Given, 1 μM = 1 x 10^{-6} M: express the concentration of blue dye #1 in the Gatorade solution in M (i.e. convert micromolar to molar).

6. Calculate the number of moles of blue dye #1 in a 500. mL bottle of blue Gatorade.

7. Determine the mass of blue #1 dye found in a 500. mL bottle of Gatorade. The molar mass of blue #1 dye is 793 g/mol.

8. Suppose a solution was too concentrated for an accurate reading with the colorimeter. The concentrated solution was diluted by placing 1.00 mL of the concentrated solution in 4.00 mL of water. The solution was then placed in the colorimeter and an absorbance was obtained and after a few calculations the molar concentration was calculated to be 3.5 x 10^{-6} M. What was the concentration of the original stock solution before dilution?

9. If a 0.10 M solution of a colored substance has a maximum absorbance at 500 nm and an absorbance of 0.26 at this wavelength, what will be the measured absorbance of a 0.20 M solution at 500 nm?

10. Using Beer's Law: A = abc (A = absorbance, a = molar absorptivity, b = path length, c = concentration), calculate the molar absorptivity of blue dye. Use a path length of 1 cm and the concentration and absorbance values for the original stock solution.