Sustainable Spectroscopy:

Analyzing Sports Drinks with Beer's Law in a Green Chemistry Lab

Objectives

This experiment investigates the concentration of food dyes in sports beverages using spectrophotometric analysis, a fundamental technique in general chemistry. The goal is to understand the relationship between %transmittance and absorbance, apply Beer's law, and utilize green chemistry principles.

Green Chemistry Focus

This experiment minimizes environmental impact by

- employing safer chemicals like non-toxic food dyes and distilled water.
- reducing waste through small sample volumes.
- conserving energy by conducting experiments at room temperature.
- ensuring all materials used are biodegradable and environmentally friendly.

Tips

1. Several sports drinks contain FD&C Blue #1 food dye, such as Gatorade® Glacier Freeze and Powerade® Mixed Berry. Some sports drinks are formulated to be turbid; it is best to avoid using these “frosty” drinks because their turbidity will have an impact on the spectrophotometer readings.

2. A stock solution of Blue #1 food dye suitable for this investigation has a very low concentration. Start by dissolving 0.12 g of solid Blue #1 in 1.00 L of distilled water. Dilute this solution by a factor of 20. For example, add 50 mL of the solution to 950 mL of distilled water to make 1 L of Blue #1 stock solution for your students.

3. You can also use Blue #1 dye in a concentrated liquid form, such as can be found in grocery stores as part of egg dying kits. Prepare a stock solution by adding 6 drops of liquid Blue #1 dye concentrate to a liter of distilled water.

4. To determine the molar concentration of a stock solution of Blue #1, use a spectrophotometer to measure the absorbance of the solution at 630 nm. The absorbance reading should be between 0.7 and 1.0. You may have to dilute the stock solution to achieve an absorbance reading in this range. To calculate the molar concentration of the Blue #1 stock solution, divide the absorbance by 130,000 (the molar absorptivity of Blue #1 at 630 nm). For example, the molarity of a Blue #1 solution with an absorbance of 0.80 is

$$0.80/130{,}000\ M^{-1}cm^{-1} = 6.2 \times 10^{-6}\ M$$

5. Students can test for any FD&C food dye that is the sole coloring agent in a beverage. Follow a similar process to prepare a stock solution of any other of the seven FD&C food dyes. The literature values for the molar absorptivity of selected FD&C food dyes at a given wavelength are shown below:
   - Red 40: 2,900 $M^{-1}cm^{-1}$ at 503 nm
   - Yellow 5: 27,300 $M^{-1}cm^{-1}$ at 427 nm
   - Yellow 6: 22,200 $M^{-1}cm^{-1}$ at 482 nm
   - Red 3: 82,200 $M^{-1}cm^{-1}$ at 526 nm

Answers to Pre-Lab Analysis

1. **Understanding the Chemical Basis**
   - **Write the chemical formula for FD&C Blue #1 and discuss its role as a food dye.**

     The chemical formula for FD&C Blue #1 is $C_{37}H_{34}N_2Na_2O_9S_3$, also known as Brilliant Blue FCF. It is a synthetic dye used to impart blue color to food and beverages.

   - **Explain how its sulfonate ($-SO_3^-$) groups affect its water solubility.**

     The sulfonate ($-SO_3^-$) groups in FD&C Blue #1 make it dissolve easily in water because they have a negative charge, which helps them attract and interact with water molecules. Since water is a polar solvent, it can surround the charged sulfonate groups, keeping the dye molecules spread out and preventing them from clumping together. The sodium ions (Na^+) also help balance the charge, making the dye more stable in solution. This is why FD&C Blue #1 mixes well in sports drinks and other water-based foods.

   - **Using your knowledge of light absorption and molecular structure, explain why FD&C Blue #1 appears blue.**

     FD&C Blue #1 appears blue because it absorbs light in the red-orange region of the visible spectrum (around 630–650 nm). This is due to the delocalized ring structure of the FD&C Blue #1 molecule, which allows the electrons in the ring to absorb light in the visible wavelength region of the electromagnetic spectrum. When white light passes through a solution containing the dye, the molecules absorb longer wavelengths of light, and the remaining light that is reflected or transmitted is what we see. Since the dye absorbs red-orange light, the opposite color on the color wheel—blue—is what reaches our eyes, making the solution appear blue.

2. **Preparation and Dilution**
   - **Describe how to prepare 10 mL of each dilution listed in Table 1 from a stock solution of Blue #1 of known molar concentration.**

     For each concentration listed in Table 1, measure the appropriate volume of stock solution using a pipet and dilute with distilled water to make a total volume of 10 mL.

   - **Calculate the molar concentration of each solution.**

Table 1	
Test tube	**[Blue #1][1] (M)**
1	5.85×10^{-6}
2	4.68×10^{-6}
3	3.51×10^{-6}
4	2.34×10^{-6}
5	1.75×10^{-6}
6	1.17×10^{-6}
7	5.85×10^{-7}
8	0.00

[1] Calculations based on the concentration of the Blue #1 stock solution = 5.85×10^{-6} M

3. **Spectrophotometric Analysis**
   - **Explore how a spectrophotometer works and why it is an effective tool for measuring dye concentration.**

     A spectrophotometer measures the amount of light that passes through a sample (transmittance) and the amount of light absorbed by the sample (absorbance). It projects light of specific wavelengths through the sample and detects the intensity of light reaching the detector. By comparing the intensity of light before and after passing through the sample, the spectrophotometer calculates the absorbance, which is directly related to the concentration of the absorbing species (in this case, FD&C Blue #1).

   - **Predict how the %transmittance will change with different concentrations of Blue #1 dye.**

     As the concentration of Blue #1 dye increases, the %transmittance will decrease because less light passes through the solution.

4. **Green Chemistry Considerations**
   - **Discuss the environmental benefits of using a spectrophotometric method compared to other chemical analysis techniques.**

     Non-Invasive Analysis: Spectrophotometry is a non-destructive technique that requires small sample volumes, thus minimizing waste.

     Safer Chemicals: The use of non-toxic, biodegradable food dyes reduces the environmental impact compared to traditional methods that might involve hazardous metal salts.

Energy Efficiency: Modern spectrophotometers are energy-efficient and can perform rapid measurements, reducing energy consumption.

- **Identify the steps in your procedure where you can minimize waste and reduce the use of hazardous substances.**

  Accurate Measurements: Use precise pipetting techniques to minimize excess reagent use.

  Reuse Materials: Where possible, reuse cuvettes and other equipment after proper cleaning.

  Proper Disposal: Ensure that all waste, including dye solutions and disposable materials, is disposed of according to environmental safety guidelines.

  Dilution Practices: Prepare only the necessary amount of each dilution to reduce leftover solutions.

  Energy Conservation: Turn off equipment when not in use to save energy.

Sample Data and Supporting Calculations

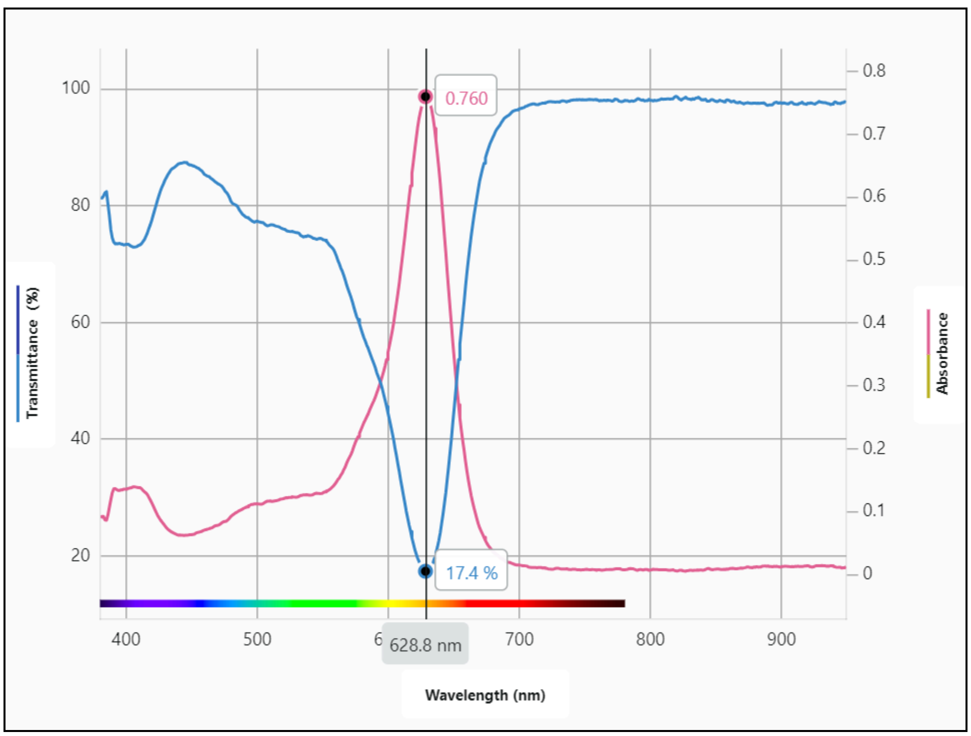

Figure 1 Overlapping transmittance and absorbance spectrum of FD&C Blue #1

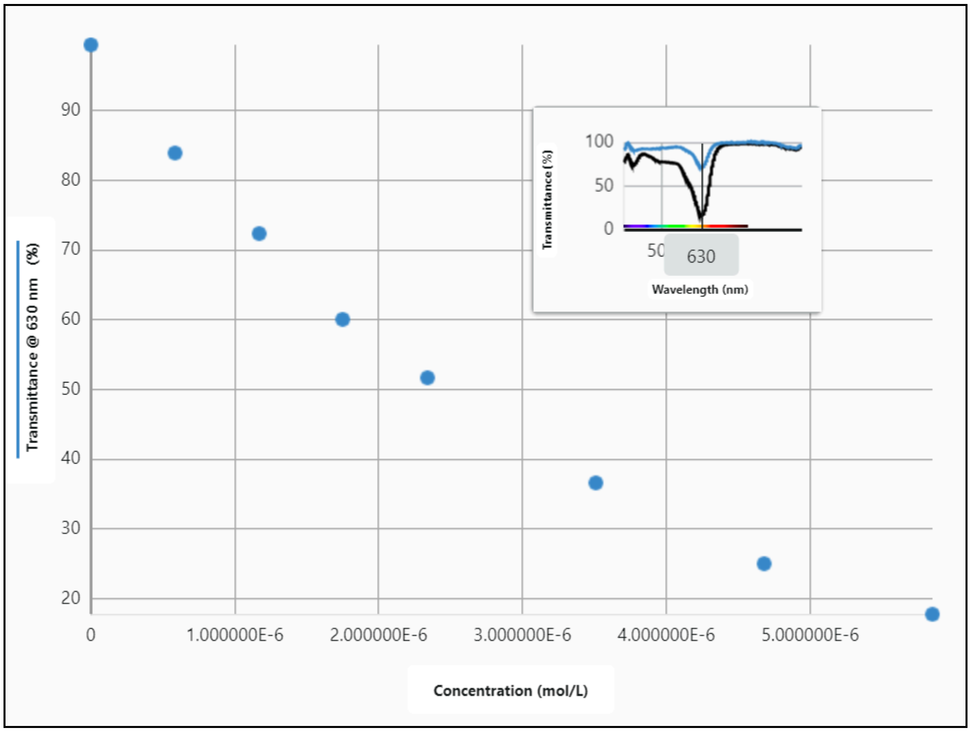

Figure 2 Transmittance vs. Concentration of FD&C Blue #1 dilutions

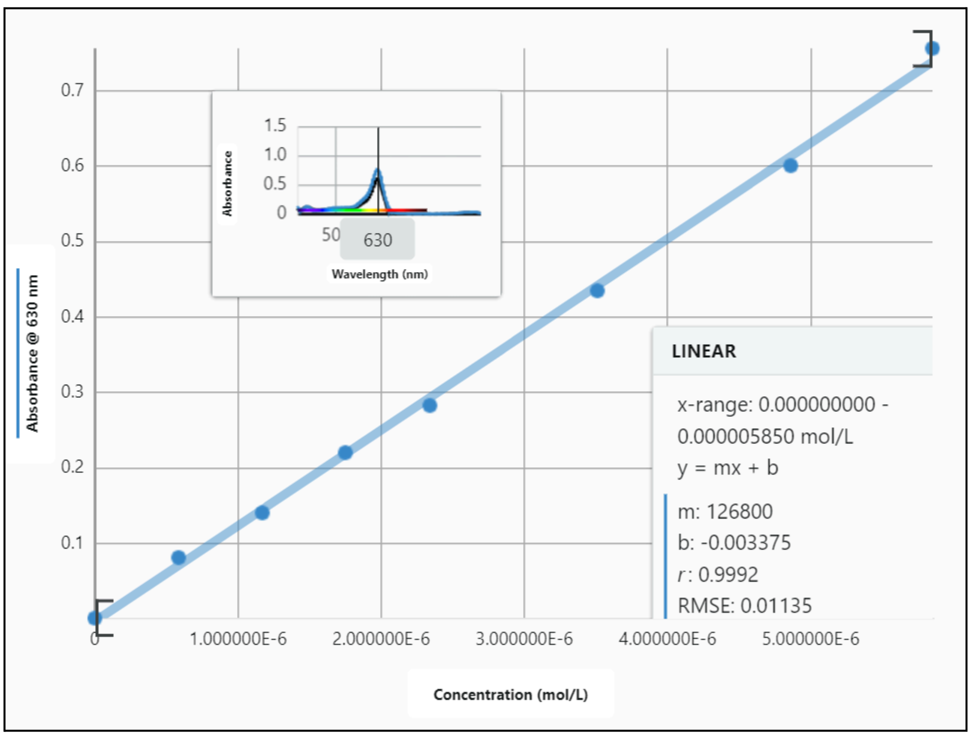

Figure 3 Absorbance vs. Concentration of FD&C Blue #1 dilutions

Answers to Post-Lab Analysis

1. **How does your Absorbance vs. Concentration graph demonstrate the proportionality predicted by Beer's law?**

   My Absorbance vs. Concentration graph shows a linear relationship, which matches what Beer's law predicts. As the concentration of FD&C Blue #1 increases, the absorbance also increases proportionally. This makes sense because, according to Beer's law ($A = \varepsilon c l$), absorbance depends directly on concentration when the path length (l) and molar absorptivity (ε) remain constant.

2. **Why is Absorbance vs. Concentration a linear relationship, while % Transmittance vs. Concentration is not?**

   My Absorbance vs. Concentration graph is a straight line, which shows that absorbance is directly proportional to concentration. This makes sense because of Beer's law ($A = \varepsilon c l$)—since molar absorptivity (ε) and path length (l) stay the same, the absorbance increases at a constant rate as concentration increases.

   On the other hand, my % Transmittance vs. Concentration graph is curved and not linear. This is because transmittance decreases exponentially as concentration increases. The relationship between transmittance and absorbance is logarithmic, meaning that transmittance drops quickly at low concentrations but changes more slowly at higher concentrations.

3. **Why do scientists prefer to use absorbance rather than transmittance to determine concentration?**

   Scientists prefer to use absorbance rather than transmittance to determine concentration because absorbance follows a linear relationship with concentration, as described by Beer's law ($A = \varepsilon c l$). This makes it easier to analyze data and accurately determine unknown concentrations.

   In contrast, transmittance is not linearly related to concentration because it follows an exponential decay as concentration increases. This means that small changes in concentration can cause large, non-linear changes in transmittance, making it harder to interpret.

4. **Report the concentration of Blue #1 food dye in the sports drink chosen by your group.**

   Two products were chosen for the sample results: Gatorade Glacier Freeze and Powerade Mixed Berry. Calculations to determine the molar concentration of Blue #1 for each follow:

   Gatorade Glacier Freeze

   absorbance @ 630 nm = 0.2084

   Calculating concentration of Blue #1 in Gatorade Glacier Freeze:
   $y = 1.27 \times 10^{5}x + 0.00356$
   $0.2084 = 1.27 \times 10^{5}[\text{Blue \#1}] + 0.00356$
   $[\text{Blue \#1}] = 1.64 \times 10^{-6}$ M

Powerade Mixed Berry
absorbance @ 630 nm = 0.3091

Calculating concentration of Blue #1 in Powerade Mixed Berry:
$y = 1.27 \times 10^5 x + 0.00356$
$0.3091 = 1.27 \times 10^5[\text{Blue \#1}] + 0.00356$
$[\text{Blue \#1}] = 2.43 \times 10^{-6}$ M

5. **Compare the concentration of FD&C Blue #1 you determined for the sports drink to the amount listed on the product label. Calculate the percent error using the equation:**

$$\%\ error = \left(\frac{|measured\ value - label\ value\ |}{label\ value}\right) \times\ 100$$

Is your measured concentration higher or lower than the labeled value? What potential sources of systematic or random error could have influenced your results?

Gatorade Glacier Freeze publishes their concentration of Blue #1 as 1 mg/L.

Experimental value was determined to be 1.64×10^{-6} M.

Convert this to mg/L: 1.64×10^{-6} M $\times$ 792.85 g/mol = 0.00130 g/L or 1.30 mg/L

(|1.30 mg/L – 1 mg/L| /1 mg/L) x 100 = 30% error

My measured concentration of 1.3 mg/L is higher than the labeled 1.0 mg/L. This could be due to calibration issues with the spectrophotometer, small pipetting errors, or fingerprint residue on the cuvette affecting light transmission. If the stock solution was slightly more concentrated than expected, it would also lead to higher values. Random fluctuations in instrument readings or slight temperature changes could contribute as well. Also, there could be variation in the bottles of Gatorade, their age, or batch consistency.

6. **If you were to drink a 16 ounce bottle of the sports drink tested by your group, how much food dye would you ingest? How many bottles of the sports drink could you consume before exceeding the FDA's Acceptable Daily Intake (ADI) for Blue #1, set at 12 mg per kg of body weight per day?**

Gatorade Glacier Freeze
16 fluid ounces = 473 mL
Blue #1 molecular formula: $C_{37}H_{34}N_2Na_2O_9S_3$
Formula mass = 792.85 g/mol
[Blue #1] from experimental results = 1.64×10^{-6}M
mol of Blue #1 in 16 ounces of Gatorade: 1.64×10^{-6} mol/L $\times$ 0.473 L = 7.76×10^{-7}mol
convert mol to mass: 7.76×10^{-7} mol $\times$ 792.85 g/mol = 0.000615 g or 0.615 mg of Blue #1

If I weigh 150 lbs, that is 68 kg. To consume 12 mg/kg, I would have to ingest
12 mg / 0.615 mg = 19.5 bottles per kg of body weight
68 kg × 19.5 bottles/kg = 1326 bottles of Gatorade Glacier Freeze

7. **Analyze how the experiment aligns with green chemistry principles, specifically focusing on waste minimization, the use of safer chemicals, and the reduction of hazardous substances. Discuss any practices that promote sustainability.**

   Waste Minimization: Using small volumes of dye solutions minimizes chemical waste. Reusing cuvettes and other lab equipment reduces waste.

   Use of Safer Chemicals: FD&C Blue #1 is a non-toxic dye and a safer alternative compared to many traditional chemicals used in spectroscopy, such as metal salts. Using distilled water as the solvent is safe and environmentally benign.

   Reduction of Hazardous Substances: Avoiding the use of toxic metal salts reduces the risk of hazardous waste and environmental contamination from waste disposal.

   Sustainability Practices: Using modern, energy-efficient spectrophotometers promotes sustainability by reducing energy consumption.

8. **Many colored solutions contain metals. Similar experiments used to investigate the relationship between %transmittance and absorbance use metal salts such as copper sulfate and nickel sulfate. Discuss the advantages and disadvantages of using food coloring to study transmittance and absorbance over solutions containing metal ions. Consider green chemistry principles and the results of your experiment.**

   Advantages of Food Coloring: Food dyes like FD&C Blue #1 are non-toxic and safer to handle compared to metal salts, which can be hazardous. Food dyes are biodegradable and have less environmental impact compared to metal salts, which can contaminate soil and water. Waste from food dye experiments can often be disposed of safely with minimal environmental regulations.

   Disadvantages of Food Coloring: The presence of other ingredients in food products can interfere with the measurements, making it challenging to isolate the effect of the dye. Some food dyes may be less stable than metal salts, potentially affecting the accuracy and reproducibility of measurements.

   Advantages of Metal Salts: Metal salts like copper sulfate and nickel sulfate are highly stable, providing consistent and reproducible absorbance measurements. Metal salts often have strong absorbance peaks, making them useful for studying the relationship between %transmittance and absorbance.

   Disadvantages of Metal Salts: Metal salts can be toxic and pose significant health risks, requiring careful handling and disposal. Disposal of metal salt solutions can lead to environmental contamination, requiring special waste management protocols.

Sustainable Spectroscopy:

Analyzing Sports Drinks with Beer's Law in a Green Chemistry Lab

Investigating the concentration of food dyes in sports beverages offers an excellent opportunity to explore green chemistry principles while learning about spectrophotometric analysis.

There are many different brands of beverages that fall under the general category of "sports drinks." Most of these beverages contain an FD&C food dye to color the beverage. There are a few options available to determine how much food dye is contained in a sports drink. A common, and very accurate, test method involves the use of an instrument called a spectrophotometer.

In brief, a spectrophotometer projects light through a small sample of a colored solution. The molecules in the solution allow some, but not all, of the wavelengths of light to pass through the sample and reach the spectrophotometer's detector. By carefully analyzing what happens to light as it passes through a sample of liquid, a great deal can be learned about some of the molecules in the liquid.

In this experiment, you will use a visible spectrophotometer, such as a SpectroVis Plus Spectrophotometer, to measure the concentration of FD&C Blue #1 food dye in sports drink. By adopting eco-friendly practices and using less hazardous materials, you will minimize environmental impact and promote sustainable scientific inquiry.

Pre-Lab Analysis

1. **Understanding the Chemical Basis**
   - Write the chemical formula for FD&C Blue #1 and discuss its role as a food dye.
   - Explain how its sulfonate ($-SO_3^-$) groups affect its water solubility.
   - Using your knowledge of light absorption and molecular structure, explain why FD&C Blue #1 appears blue.
2. **Preparation and Dilution**
   - Describe how to prepare 10 mL of each dilution listed in Table 1 from a stock solution of Blue #1 of known molar concentration.
   - Calculate the molar concentration of each solution.
3. **Spectrophotometric Analysis**
   - Explore how a spectrophotometer works and why it is an effective tool for measuring dye concentration.
   - Predict how the %transmittance will change with different concentrations of Blue #1 dye.
4. **Green Chemistry Considerations**
   - Discuss the environmental benefits of using a spectrophotometric method compared to other chemical analysis techniques.

- Identify the steps in your procedure where you can minimize waste and reduce the use of hazardous substances.

Materials

- ☐ computer, Chromebook™, or mobile device
- ☐ Vernier Spectral Analysis® app
- ☐ visible spectrophotometer, such as the Go Direct® SpectroVis® Plus (GDX-SVISPL)
- ☐ plastic cuvettes
- ☐ 10 mL graduated cylinders
- ☐ plastic Beral pipets or eyedropper
- ☐ test tubes
- ☐ test tube racks
- ☐ FD&C Blue #1 food dye stock solution
- ☐ distilled water
- ☐ sports drinks with Blue #1 food dye

Investigation

Prepare Dilutions

Using the information you collected in your pre-lab analysis, prepare dilutions from stock solution of Blue #1 of known molar concentration.

Table 1

Blue #1 solution	**Blue #1 stock solution (mL)**	**Distilled H_2O (mL)**	**[Blue #1] (M)**
1	10	0	
2	8	2	
3	6	4	
4	4	6	
5	3	7	
6	2	8	
7	1	9	
8	0	10	

Part 1: Exploring Transmittance and Absorbance Across the Spectrum

1. Launch Spectral Analysis and connect the Go Direct SpectroVis Plus Spectrophotometer. Select **Advanced Full Spectrum.**

2. Select **Transmittance** as the Sensor mode. Start data collection. Follow the instructions to calibrate your spectrometer.

3. Collect a transmittance spectrum of your most concentrated sample. Make sure to stop data collection.

4. Switch to Absorbance mode: Open the Spectrometer Settings, ⚙, and select **Absorbance**. Start data collection. Stop when you have measured the same sample's absorbance.

5. Tap along the graph to find the wavelength of maximum transmittance ($\lambda_{max,T}$) and the wavelength of maximum absorbance ($\lambda_{max,A}$).

6. Analyze the spectra:
   a. Why does the solution appear blue?
   b. Which wavelengths of light are absorbed versus transmitted?
   c. Compare the shapes of the absorbance and transmittance spectra. How do they relate?

7. Insert a copy of the graph into your lab report by capturing a screenshot or exporting an image (click File, 🗋, and choose Export). Be sure to save your data file for file for future analysis.

Part 2: Investigating the Effect of Concentration on Light Absorption

8. Begin a New Experiment in Spectral Analysis. This time, select **Transmittance** and then select **vs. Concentration**.

9. Calibrate the Spectrophotometer (if necessary) and confirm the selected wavelength aligns with your expectations from Part 1.

10. Measure %Transmittance:
    a. Start data collection.
    b. When the transmittance value stabilizes, click **Keep** to store the data point. Enter the concentration of the sample. Click **Done**.
    c. Replace the cuvette in the spectrophotometer with the cuvette for the next dilution and click **Keep** when the value stabilizes. Enter its concentration and click **Done**.
    d. Repeat for each of the dilutions.
    e. When you have data for all of the dilutions, stop data collection.

11. Examine the graph and observe its shape.

12. Insert a copy of the graph into your lab report by capturing a screenshot or exporting an image (click File, 🗋, and choose Export). Be sure to save your data file for file for future analysis.

13. Repeat for Absorbance vs. Concentration:
    a. Begin a new Experiment in Spectral Analysis. This time, select Absorbance and then select vs. Concentration.
    b. Follow the same process for Absorbance vs. Concentration as you did for Transmittance vs. Concentration.

14. Insert a copy of the graph into your lab report by capturing a screenshot or exporting an image (click File, , and choose Export). Be sure to save your data file for future analysis.

Part 3: Discovering the Relationship Between Transmittance and Absorbance

15. Compare your graphs.
    a. Examine how the shape of the %T vs. Concentration graph compares to the Absorbance vs. Concentration graph.
    b. Which one shows a more predictable trend?

16. After analyzing your graphs, consider how transmittance and absorbance are mathematically related. Scientists use the following equation to describe this relationship:

$$A = -log(T) = log\left(\frac{100}{\%T}\right)$$

    Consider how this equation relates to your data. How does this equation explain the trend you observed in your graphs?

17. Now that we have explored absorbance and transmittance, consider how absorbance is related to concentration. Scientists use Beer's law to describe this relationship:

$$A = \varepsilon c l$$

    A = Absorbance (unitless)

    ε = Molar absorptivity (L/mol·cm), a constant for each substance at a given wavelength

    c = Concentration (mol/L)

    l = Path length of the light through the solution (cm)

    Since path length and molar absorptivity remain constant, absorbance is directly proportional to concentration.

    How does this explain the trend in your Absorbance vs. Concentration graph?

Part 4: Determining the Concentration of an Unknown Sample

18. Obtain a sports drink containing FD&C Blue #1 food dye. Use Spectral Analysis to measure the absorbance of the sports drink sample.

19. Determine the sample's concentration using either of the following techniques:
    - Graphically: Locate the absorbance value on your Absorbance vs. Concentration graph and find the corresponding concentration.
    - Mathematically: Use the best-fit equation from your graph to calculate the concentration.

Post-Lab Analysis

1. How does your Absorbance vs. Concentration graph demonstrate the proportionality predicted by Beer's law?

2. Why is Absorbance vs. Concentration a linear relationship, while % Transmittance vs. Concentration is not?

3. Why do scientists prefer to use absorbance rather than transmittance to determine concentration?

4. Report the concentration of Blue #1 food dye in the sports drink chosen by your group.

5. Compare the concentration of FD&C Blue #1 you determined for the sports drink to the amount listed on the product label. Calculate the percent error using the equation:

$$\%\ error = \left(\frac{|measured\ value - label\ value|}{label\ value}\right) \times 100$$

   Is your measured concentration higher or lower than the value on the label? What potential sources of systematic or random error could have influenced your results?

6. If you were to drink a 16 ounce bottle of the sports drink tested by your group, how much food dye would you ingest? How many bottles of the sports drink could you consume before exceeding the FDA's Acceptable Daily Intake (ADI) for Blue #1, set at 12 mg per kg of body weight per day?

7. Analyze how the experiment aligns with green chemistry principles, specifically focusing on waste minimization, the use of safer chemicals, and the reduction of hazardous substances. Discuss any practices that promote sustainability.

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