Investigating Food Dyes in Sports Beverages

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 using 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 investigation, you will determine the concentration of an FD&C food dye in a sports beverage. You will use a Vernier SpectroVis Plus Spectrophotometer to conduct your tests.

PRE-LAB ACTIVITY

To prepare for the investigation, you will make a series of dilutions of FD&C Blue #1 food dye. You will be given distilled water and a stock solution of Blue #1 of known molar concentration, from which to make your dilute solutions. Prepare 10 mL of each dilution and calculate the molar concentration of each solution. Use Table 1 to record the information about the solutions you prepare. You will measure the % Transmittance of each solution in the Initial Investigation.

Table 1

Blue #1 solution	Blue #1 stock solution (mL)	Distilled H_2O (mL)	Concentration (M)	% Transmittance
1	10	0		
2	8	2		
3	6	4		
4	4	6		
5	3	7		
6	2	8		
7	1	9		
8	0	10		

INITIAL INVESTIGATION

1. Obtain and wear goggles.
2. Launch Spectral Analysis. Connect the Go Direct SpectroVis Plus Spectrophotometer to your Chromebook, computer, or mobile device.
3. Select % Transmittance and then select vs. Concentration.
4. Prepare a *blank* by filling a cuvette 3/4 full with distilled water. To correctly use cuvettes, remember:
   - Wipe the outside of each cuvette with a lint-free tissue.
   - Handle cuvettes only by the top edge of the ribbed sides.
   - Dislodge any bubbles by gently tapping the cuvette on a hard surface.
   - Always position the cuvette so the light passes through the clear sides.
5. Calibrate the spectrophotometer.
   a. Place the blank cuvette in the spectrophotometer.
   b. Select Finish Calibration. **Note**: If necessary, wait for the spectrophotometer to warm up before selecting Finish Calibration.
6. Select the optimal wavelength for the food dye (630 nm).
   a. Remove the blank cuvette from the spectrophotometer. Refill the cuvette with the stock solution of Blue #1 food dye.
   b. The live graph will update with the spectrum of the sample. Click or tap the desired wavelength or enter **630** as the Wavelength. Click or tap Done.
7. Collect data for the Blue #1 food dye solutions.
   a. Leave the cuvette of stock Blue #1 solution in the spectrophotometer.
   b. Start data collection.
   c. After the value displayed in the meter has stabilized, select Keep and enter the concentration in mol/L. Select Keep Point. The % Transmittance and concentration values have now been stored.
   d. Repeat the necessary steps to test the remaining Blue #1 dilute solutions.
   e. After you have finished testing all the food dye solutions, stop data collection.
   f. To save the file, click or tap File, 🗋, and choose Save.
   g. Record the % Transmittance readings in Table 1.

PLANNING FOR THE FINAL INVESTIGATION

For the Final Investigation, obtain a sports drink containing FD&C Blue #1 food dye. Discuss the results of the Initial Investigation, and based on the data, develop a plan that uses a spectrophotometer to determine the molar concentration of FD&C Blue #1 food dye in the sports drink. Prepare a procedure in sufficient detail that another group could follow it and achieve similar results. Consider the following issues during planning:

1. What is the relationship between % Transmittance and concentration of the Blue #1 food dye solutions tested in the Initial Investigation?

2. You measured transmittance of light through the Blue #1 solutions. There is another measurement you could have made, called *absorbance*, which relates to concentration in a linear fashion. How does % Transmittance relate to absorbance? To help you establish the relationship between % Transmittance and absorbance, complete Table 2. Next, prepare a graph of one of the columns as the y-values and the molar concentrations of the food dye as the x-values. Identify the graph that is the most linear *and* has a positive slope. **Note**: In Table 2, *T* represents transmittance, which is percent transmittance written in decimal form.

Table 2

Blue #1 solution	%*T*	*T*	1/*T*	log *T*	–log *T*
1					
2					
3					
4					
5					
6					
7					
8					

3. The Blue #1 food dye solutions tested in the Initial Investigation were pure in that the samples contained only the food dye and water. A sports drink contains a long list of ingredients. How does this difference play a role in determining how much food dye is contained in the beverage?

FINAL INVESTIGATION

As you carry out your approved plan, consider the following questions:

- Is there an optimum number of data-collection runs to achieve the best data?
- What is the best way to sample the sports drink to ensure that your sampling is the most reliable representation of the product?
- Is there a method of testing that provides more accurate data than the other methods?

ANALYZING RESULTS

When preparing your report, include

- A statement of the results: What was the concentration of Blue #1 food dye in the sports beverage chosen by your group?
- A description of the procedure that you used in the investigation, including any changes that were made to the method that was used during the Initial Investigation
- An analysis of the graphs and supporting calculations

Additional items to consider including in your report

- A comparison of your results with the results from other groups
- Recommended modifications to the procedure that would increase accuracy, save time, or ensure that liquids are handled more efficiently and safely

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INSTRUCTOR NOTES

1. In the Electronic Resources you will find multiple versions of each student experiment—one for each supported data-collection program (Spectral Analysis, Logger *Pro* 3, and LabQuest App). Deliver to your students the version that supports the software and hardware they will use. Sign in to your account at **vernier.com/account** to access the Electronic Resources. See Appendix A for more information. **Note**: The printed version of the book and the PDF of the entire book (found in the Electronic Resources) include only the Spectral Analysis version of this experiment (for Go Direct sensors).

2. 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.

3. 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 liter of distilled water. Dilute this solution by a factor of twenty. For example, add 50 mL of the solution to 950 mL of distilled water to make a liter of Blue #1 stock solution for your students.

4. 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.

5. 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

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

6. 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

7. The AP Chemistry kit for Lab 1 from Flinn Scientific (Flinn order code: AP7642) works well for this investigation.

8. **Important**: This investigation, as presented in the AP Chemistry lab manual published by the College Board, expects students to use a spectrophotometer to measure %T and absorbance. If you do not have a spectrophotometer, your students can collect Beer's law data for the samples of Blue #1 food dye and a sports drink with a Colorimeter, set at 635 nm. However, please understand that this changes a fundamental component of the investigation.

9. For additional information about the Vernier probeware used in this experiment, including tips and product specifications, visit **www.vernier.com/manuals** and download the appropriate user manual.

10. If you are using Go Direct sensors, see **www.vernier.com/start/go-direct** for information about how to connect your sensor.

CORRELATIONS

Primary Learning Objective

AP Learning Objective 1.15: The student can justify the selection of a particular type of spectroscopy to measure properties associated with vibrational or electronic motions of molecules.

Secondary Learning Objective

AP Learning Objective 1.16: The student can design and/or interpret the results of an experiment regarding the absorption of light to determine the concentration of an absorbing species in a solution.

NEXT GENERATION SCIENCE STANDARDS (NGSS)

Disciplinary Core Ideas	Crosscutting Concepts	Science and Engineering Practices
PS1.A Structure and Properties of Matter (HS-PS1)	Patterns Structure and function	Asking questions and defining problems Planning and carrying out investigations Analyzing and interpreting data Constructing explanations Engaging in argument from evidence

ESTIMATED TIME

The Initial Investigation is designed for a 45–60 minute lab period. The planning, execution, and analysis for the Final Investigation is likely to require at least one additional lab period. The amount of time necessary for the Final Investigation is dependent on the nature of the inquiry process followed by students. We encourage you to provide students with time to compare and discuss their experimental results with other lab groups.

MATERIALS

Make the following materials available for student use:

- SpectroVis Plus spectrophotometer
- data-collection program
- data-collection interface[1]
- plastic cuvettes
- 10 mL graduated cylinders
- plastic Beral pipets or eyedroppers
- test tubes
- test tube racks
- FD&C Blue #1 food dye stock solution
- distilled water
- sports drinks w/ Blue #1 food dye

[1]Required if using LabQuest App data-collection app, not required for Spectral Analysis or Logger *Pro*

PRE-LAB ACTIVITY

Blue #1 Solution	Blue #1 stock solution (mL)	Distilled H_2O (mL)	Concentration[2] (M)	% Transmittance
1	10	0	7.57×10^{-6}	13.42
2	8	2	6.06×10^{-6}	20.65
3	6	4	4.54×10^{-6}	29.86
4	4	6	3.03×10^{-6}	45.03
5	3	7	2.27×10^{-6}	55.04
6	2	8	1.51×10^{-6}	66.12
7	1	9	7.57×10^{-7}	80.98
8	0	10	0.00	99.36

[2] Calculations are based on the concentration of the Blue #1 stock solution = 7.57×10^{-6} M.

SAMPLE DATA AND SUPPORTING CALCULATIONS

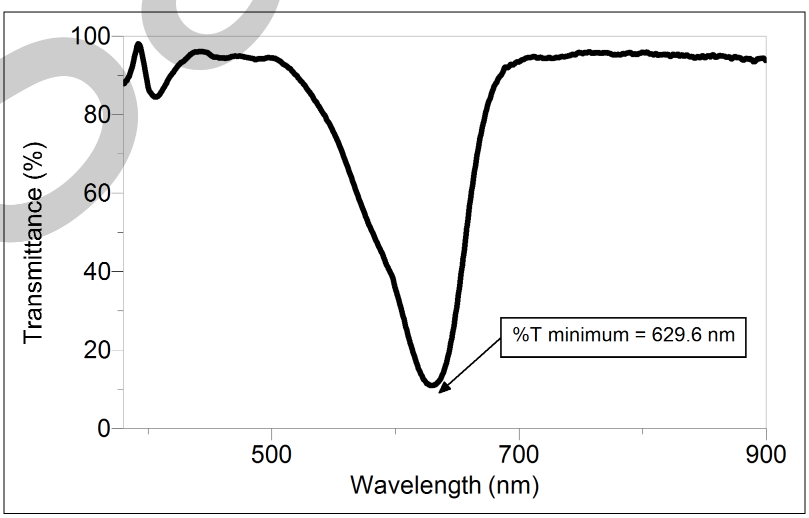
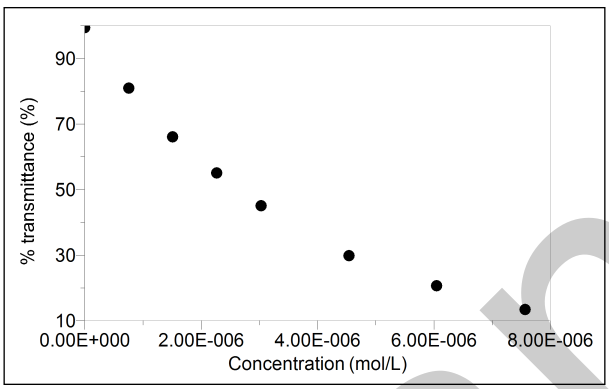


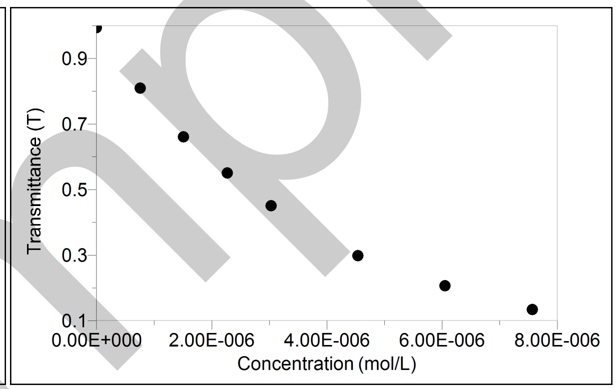
Figure 1 % Transmittance spectrum of FD&C Blue #1

The following data were taken with a SpectroVis Plus spectrophotometer set to 629.6 nm.

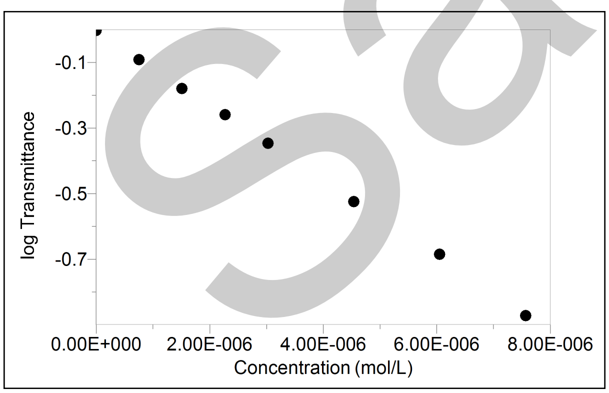
Test tube	[Blue #1] (M)	%T	T	log T	–log T
1	7.57×10^{-6}	13.42	0.134	–0.873	0.873
2	6.06×10^{-6}	20.65	0.207	–0.684	0.684
3	4.54×10^{-6}	29.86	0.299	–0.524	0.524
4	3.03×10^{-6}	45.03	0.450	–0.347	0.347
5	2.27×10^{-6}	55.04	0.550	–0.260	0.260
6	1.51×10^{-6}	66.12	0.661	–0.180	0.180
7	7.57×10^{-7}	80.98	0.810	–0.092	0.092
8	0.00	99.36	0.994	–0.003	0.003



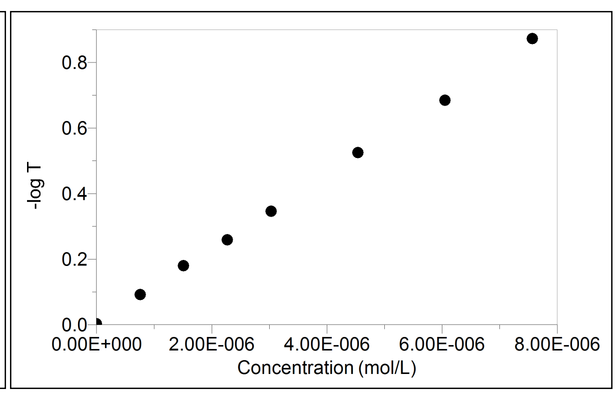
% Transmittance vs. *[Blue #1]*



Transmittance vs. *[Blue #1]*



log Transmittance vs. *[Blue #1]*



–log Transmittance vs. *[Blue #1]*

Figure 2   Samples graphs of transmittance data

Of the four graphs in Figure 2, –log Transmittance *vs*. [Blue #1] is the best choice to produce a linear fit equation with a positive slope. Students will find that the manipulation of the original

% Transmittance readings taken by the spectrophotometer in this graph is a means to achieve absorbance. Thus, this plot is also the established Beer's law graph of spectrophotometric data.

The linear fit equation for the data is

$$y = 1.14 \times 10^5 x + 0.00356$$

where y is absorbance and x is molar concentration.

Sample Calculations

Two products were chosen for the sample results: Gatorade Glacier Freeze and Powerade Mixed Berry.

1. The calculations to determine the molar concentration of Blue #1 for each food product are

   Gatorade Glacier Freeze

   % transmittance @ 629.6 nm = 61.89
   absorbance (–log T) = 0.2084
   calculating concentration of Blue #1 in Gatorade Glacier Freeze:
   $y = 1.14 \times 10^5 x + 0.00356$
   $0.2084 = 1.14 \times 10^5[\text{Blue \#1}] + 0.00356$
   $[\text{Blue \#1}] = 1.80 \times 10^{-6}$ M

   The concentration of Blue #1 in the Gatorade sample can be calculated by solving the Beer's law equation or by interpolating the linear fit equation on a graph.

   Powerade Mixed Berry

   % transmittance @ 629.6 nm = 49.08
   absorbance (–log T) = 0.3091
   calculating concentration of Blue #1 in Powerade Mixed Berry:
   $y = 1.14 \times 10^5 x + 0.00356$
   $0.3091 = 1.14 \times 10^5[\text{Blue \#1}] + 0.00356$
   $[\text{Blue \#1}] = 2.68 \times 10^{-6}$ M

   The concentration of Blue #1 in the Powerade sample can be calculated by solving the Beer's law equation or by interpolating the linear fit equation on a graph.

2. Students may extend their analysis of the data to calculate the mass or molar amount of a food dye found in a bottle of a sports drink. Perhaps a student group will ask themselves the question: If I drink a 16 ounce bottle of a sports drink, how much food dye am I also ingesting? Example calculations are shown below.

   Gatorade Glacier Freeze

   16 fluid ounces, or 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 = 1.80×10^{-6}M

   mol of Blue #1 in 16 ounces of Gatorade: 1.80×10^{-6} mol/L × 0.473 L = 8.51×10^{-7}mol

   convert mol to mass: 8.51×10^{-7} mol × 792.85 g/mol = 0.000675 g or 0.675 mg of Blue #1