CHEM FAX!
P.O. Box 219 • Batavia, IL 60510
(800) 452-1261 • Fax (866) 452-1436
www.flinnsci.com • E-mail: flinn@flinnsci.com

FLINN SCIENTIFIC, INC
"Your Safer Source for Science Supplies"
Catalog No. AP7642
Publication No. 7642

Analysis of Food Dyes in Beverages

AP* Chemistry Big Idea 1, Investigation 1

An Advanced Inquiry Lab

Introduction

Assume an investigative role and design a valid procedure using spectroscopy and graphical analysis to determine the concentration of FD&C food dyes in sports drinks. The investigation will develop—and test—your skills in preparing accurate serial dilutions, understanding spectroscopic measurements, and extrapolating from graphical data.

Concepts

- Spectroscopy
- Absorbance vs. transmittance
- Wavelength
- Consumer science
- Beer's law
- Solution concentration

Background

The color of a solution is an important tool used by scientists to gain information about the composition of the solution. Color is a physical property that is useful for both qualitative and quantitative analysis. A *qualitative* method yields information about the nature or type of compound in a sample, whereas a *quantitative* method provides numerical data for the amount of a compound in a sample.

Spectroscopy is the study of the interaction of light and matter. A spectrophotometer is an instrument that uses electromagnetic radiation from a selected region of the electromagnetic spectrum, such as ultraviolet, visible or infrared light, to analyze the absorption or transmission of radiation by a sample. The basic function of a spectrophotometer is shown in Figure 1. The electromagnetic spectrum (see Figure 2) is the entire range of possible wavelengths or frequencies of electromagnetic radiation. In this investigation a visible spectrophotometer will be used—it scans the visible region of the electromagnetic spectrum, from 380 nm to 750 nm. Typical light sources for visible spectrophotometers include xenon and tungsten lamps.

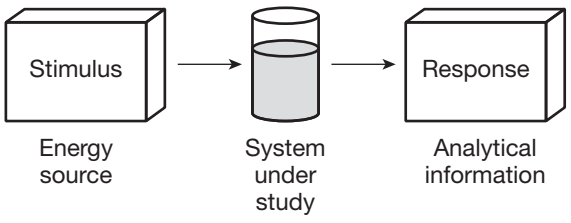


Figure 1.

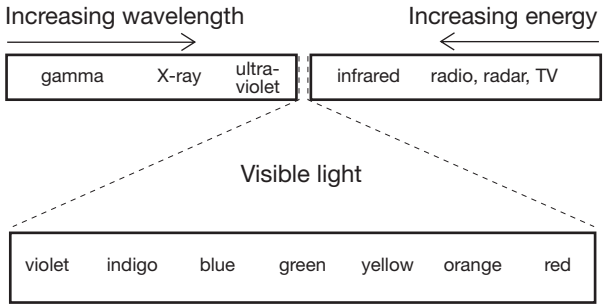


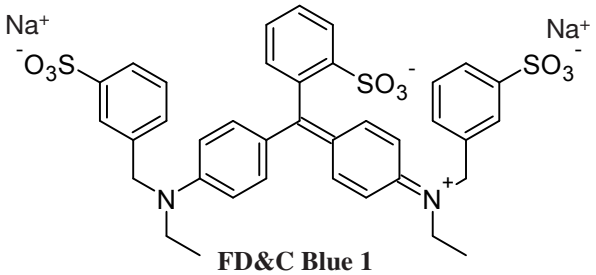
Figure 2.

Glass cuvettes or test tubes may be used as sample cells for visible spectrophotometers. More specialized spectrophotometers require quartz cells, which are "invisible" to and do not absorb ultraviolet radiation. In addition to the energy source used in spectrophotometers, a diffraction grating called a monochromator is also incorporated. The monochromator spreads the beam of light into the light's component wavelengths. The desired wavelength is then focused onto the sample cell to detect any absorption or emission of light by a substance in a sample.

Spectrophotometry is an analytical procedure that uses electromagnetic radiation to measure the concentration of a substance. The success of a spectrophotometric technique requires that the absorption of light by the substance being analyzed must be distinct or different from that of other chemical species in solution. How do scientists select the desired wavelength for spectrophotometry?

The absorption of visible light by a substance results from electron transitions, that is, the promotion of a ground state electron to a higher energy atomic or molecular orbital. Both light energy and electron energy levels are quantized, so that the specific wavelength of light absorbed by a substance depends on the energy difference between two electron energy levels. The optimum wavelength for spectrophotometric analysis of a substance is selected by measuring the *visible spectrum* of the substance, corresponding to a plot of absorbance (*A*) versus wavelength (λ, "lambda").

Just seven unique dyes are approved by the Food and Drug Administration for use in foods, drugs and cosmetics. These seven FD&C dyes give rise to the entire palette of artificial food colors. Three FD&C dyes, FD&C Blue 1, FD&C Red 40, and FD&C Yellow 5, are discussed in this advanced inquiry lab for the analysis of sports drinks and other beverages. The structure of FD&C Blue 1 is shown in Figure 3. Notice the extensive series of alternating single and double bonds (also called conjugated double bonds) in the center of the structure. This feature is characteristic of intensely colored organic dyes and pigments. Every double bond added to the system reduces the energy difference between the bonding and nonbonding molecular orbitals so that the resulting energy gap corresponds to visible light.



FD&C Blue 1
Figure 3.

A solution containing FD&C Blue 1 appears blue under normal white light—blue is the color of light *transmitted* by the solution. The colors or wavelengths of light that are *absorbed* by this solution are *complementary* to the transmitted color. A color wheel (see Figure 4) provides a useful tool for identifying the colors or wavelengths of light absorbed by a substance. The blue solution absorbs orange light and we would expect the visible spectrum of FD&C Blue 1 to contain a peak in the 600–640 nm region. The optimum wavelength for spectrophotometric analysis of a dye solution is generally determined from the *wavelength of maximum absorbance* (abbreviated λ_{max}, or "lambda max"). The value of lambda max for FD&C Blue 1 is 630 nm.

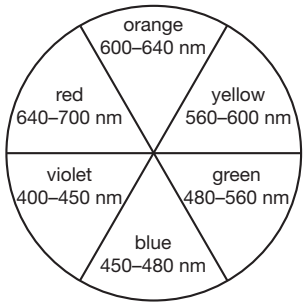


Figure 4.

The wavelength of light absorbed by a substance is characteristic of its molecular or electronic structure. The intensity of light absorbed depends on the amount of the substance in solution. Generally, the more concentrated the solution, the more intense the color will be, and the greater the intensity of light the solution absorbs. A digital spectrophotometer measures both the percent transmittance of light and the absorbance. When light is absorbed, the radiant power (*P*) of the light beam decreases. Transmittance (*T*) is the fraction of incident light (P/P_o) that passes through the sample (see Figure 5). The relationships between transmittance and percent transmittance (% *T*) and between transmittance and absorbance (*A*) are given in Equations 1 and 2, respectively.

$$\%\,T = T \times 100 = P/P_o \times 100\% \qquad \textit{Equation 1}$$

$$A = \text{absorbance} = -\log T \qquad \textit{Equation 2}$$

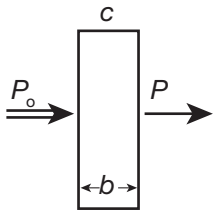


Figure 5.

The amount of light absorbed by a solution depends on its concentration (*c*) as well as the path length of the sample cell (*b*) through which the light must travel. See Equation 3, which is known as Beer's law. The constant *a* in the equation is a characteristic of a substance and is known as the molar absorptivity coefficient.

$$A = abc \qquad \textit{Equation 3}$$

Experiment Overview

The purpose of this advanced inquiry lab is to use spectroscopy and graphical analysis to determine the concentration of dye in a sports drink. The investigation begins with a an introductory activity for preparing a series of standard dilutions of an FD&C Blue 1 stock solution and measuring the percent transmittance of each. The results will be analyzed graphically to identify an optimum linear relationship among various functions (*T*, *% T*, log *T* and *A*) for a Beer's law calibration curve. The procedure provides a model for guided-inquiry analysis of the concentration of food dye(s) in sports drinks and other consumer beverages. Additional dyes, FD&C Yellow 5 and FD&C Red 40, are also available for optional extension or cooperative class studies.

Pre-Lab Questions

The visible absorption spectrum for FD&C Blue 1 is shown in Figure 6. The estimated concentration of the dye was 7.0 μM (7.0×10^{-6} M).

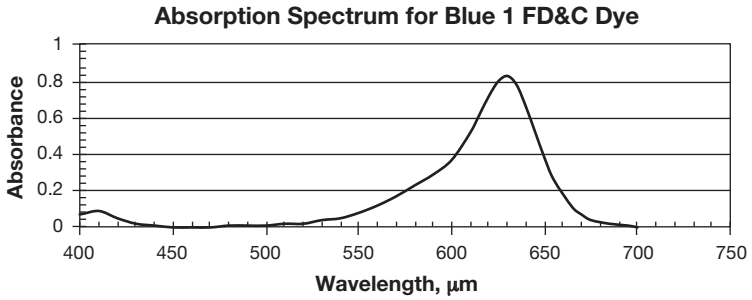


Figure 6.

1. What would be an optimum wavelength for measuring the absorbance versus concentration of a series of FD&C Blue 1 dye solutions? Explain your answer. Absorbance measurements are most accurate and sensitive in the range 0.2–1.0.
2. To construct a calibration curve, a series of known concentration standards is prepared. Using the estimated concentration of the FD&C Blue 1 stock solution, determine the concentration of each of the following dilutions. *Hint:* $M_1V_1 = M_2V_2$.

	Dye Stock Solution (A)	B	C	D	E	F	G	H
Concentration (Micromolar, μM)	7.0 μM							
Water (mL)	0	2	4	6	7	8	9	10
Stock Solution (mL)	10	8	6	4	3	2	1	0

3. Using the information provided in Question 1, predict the absorbance of each solution A–H at the optimum wavelength. Refer to Equation 3 in the *Background*. The value of *a* is 130,000 M^{-1} cm^{-1} and *b* = 1 cm.

Materials (for each lab group)

FD&C Blue 1 stock solution, 50-mL
Blue consumer sports drink, 10-mL
Water, distilled or deionized
Beakers, 50-mL, 2–3
Cuvets or test tubes, 13 × 100 mm, 3–8
Kimwipes or lens tissues
Pipet, serological, 10-mL
Pipet bulb or pipet filler
Spectrophotometer or colorimeter
Test tube rack

Safety Precautions

The FD&C dyes are slightly hazardous by eye and skin contact. The dyes have been stored with other, nonfood-grade chemicals and are not for consumption. Avoid contact with eyes, skin, and clothing. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Wash hands thoroughly with soap and water before leaving the laboratory. Please follow all laboratory safety guidelines.

Introductory Activity

1. Turn on the spectrophotometer and allow to warm up for 15–20 minutes.
2. Based on the maximum absorbance of the dye being tested, select the appropriate wavelength on the spectrophotometer.
3. Read the entire procedure. Construct an appropriate data table to record measurements and the results of calculations. *Note:* As part of a cooperative lab activity, your instructor may assign different groups to prepare and analyze different solutions, and to graph the results. Each group will need to transcribe data and analyze the results for all solutions in order to complete the guided-inquiry activity.
4. Obtain approximately 50 mL of stock solution containing FD&C Blue 1 dye.
5. Using a serological pipet for accurate volume measurements, dilute the stock solution as indicated in the following table to prepare 10 mL each of a series of standard solutions, B–H. Carefully mix each solution. *Hint:* To avoid contaminating the stock solution, first use the pipet to add the required amount of distilled water to each test tube. Rinse the pipet three times with the stock solution. Then measure and add the required amount of stock solution to each test tube.

Solution	**Stock (A)**	**B**	**C**	**D**	**E**	**F**	**G**	**Blank (H)**
Water (mL)	0	2	4	6	7	8	9	10
Stock Soln (mL)	10	8	6	4	3	2	1	0

6. Use the "blank" test tube (H) to set the 100% transmittance value for the spectrophotometer at the desired, optimum wavelength for this study.
7. Measure and record the percent transmittance (% *T*) of the stock solution and each standard solution (B–G) at the optimum wavelength. Remember to handle the test tubes only at the top and to polish the test tube with lens tissue. To avoid spills, do not place test tubes in the spectrophotometer if they are more than 75% full. Remove some solution if needed from each test tube before inserting it in the instrument.
8. Convert % *T* to transmittance (*T*) for each measurement, and calculate the appropriate values of both log *T* and –log *T*. Record all results.
9. Use Beer's law to calculate the precise concentration of FD&C Blue 1 in the stock solution. The molar absorptivity (*a*) of FD&C Blue 1 is 130,000 M^{-1} cm^{-1} at 630 nm and the path length (*b*) is 1 cm. Record the micromolar (μM) concentration (1 μM = 1×10^{-6} M) in your data table.
10. Prepare separate graphs of (a) % *T,* (b) *T,* (c) 1/*T,* (d) log *T,* and (e) –log *T* (on the *y*-axis) versus dye concentration (on the *x*-axis) for each solution. *Note:* Dye concentrations were calculated in the *Pre-Lab Questions* using the estimated concentration of the stock solution. Recalculate the concentrations of solutions B–G, if needed, based on your answer to Question 9. Use the dilution equation.

Guided-Inquiry Design and Procedure

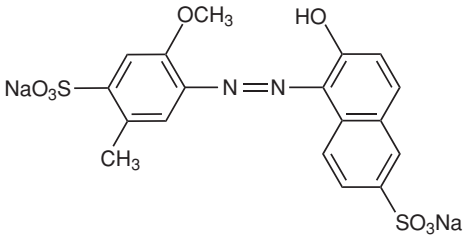
Concentration of FD&C Blue 1 in Beverages

1. Based on the graphs obtained in the *Introductory Activity,* identify the optimum linear relationship or *calibration curve* for quantitative analysis of the concentration of an "unknown" solution containing FD&C Blue 1 food dye.
2. Which graph would provide the most accurate means to determine the concentration of an "unknown" solution whose transmittance has been measured spectroscopically? Explain in terms of Beer's law and give an example of how the analysis would be carried out.
3. Consult the ingredients label for a blue-colored sports drink or other consumer beverage. Identify the dyes that are present and explain whether the beverage can be analyzed using the calibration curve described above.
4. Obtain the necessary spectroscopic data for the beverage containing FD&C Blue 1 food dye. Recall that absorbance measurements are most accurate in the range of A values from 0.2 to 1.0. Treat the beverage sample if needed to make sure the data is in the acceptable range.
5. Determine the concentration (micromolar, μM) of the dye in the beverage and calculate the amount (mass) of dye in milligrams per liter of the beverage. The molar mass of FD&C Blue 1 is 793 g/mole.

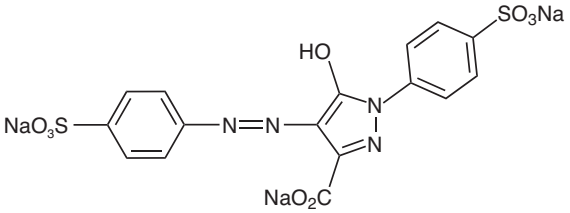
Opportunities for Inquiry

Absorbance Spectra and Analysis of Dyes

FD&C dyes are organic molecules with chemical structures containing multiple carbon rings with double bonds (see Figure 3). When molecules have a series of double bonds separated by single bonds, the bonding pattern is called conjugation. This pattern of bonding results in a reduced separation between the ground state and the excited state of the electrons. The energy difference corresponds to the energy of photons in the visible region. As the amount of conjugation increases, the energy of the absorbed photon decreases. FD&C Blue 1 dye should absorb light that is least energetic of the three, followed by Red 40 and Yellow 5 with higher absorbed energies. Measure the visible spectra of Red 40 and Yellow 5 and identify the wavelength of light that results in the maximum absorbance value, λ_{max}, for each dye. Plot the visible spectra of all the dyes on one graph to create an overlay. Compare the dyes and make observations. Prepare solutions and generate Beer's law plots to analyze the concentrations of these dyes in various beverages. The structures and molar masses of FD&C Red 40 and Yellow 5 are shown below in Figure 7.



FD&C Red 40
Molar mass = 496 g/mole



FD&C Yellow 5
Molar mass = 534 g/mole

Figure 7.

AP Chemistry Review Questions

Integrating Content, Inquiry and Reasoning

1. Calculate the value of *% T* for an absorbance value *A* = 1.5. Using this result, explain why absorbance measurements > 1 may not be accurate.

2. Spectrophotometric studies can be conducted on any colored compound. The transition metal group of the periodic table exhibits a wide array of different colored compounds. The complex ion tetraamminecopper(II) contains four ammonia molecules covalently bonded to a copper(II) ion. In aqueous solutions, Cu^{2+} ions will bond to four water molecules in a square planar geometry. The solution is a light blue color. The water molecules can be displaced by ammonia molecules, which form more stable complex bases than water. The appearance of the intense dark blue-violet color of the $[Cu(NH_3)_4]^{2+}$ ion is often used as a positive test to verify the presence of Cu^{2+} ions.

   a. Write a balanced chemical equation for the reaction of copper(II) sulfate and concentrated ammonia to produce tetraamminecopper(II) sulfate.

   b. $[Cu(NH_3)_4]^{2+}$ solutions exhibit a deep blue-violet color. How can you use spectrophotometry to confirm that this reaction has occurred and that the product formed is in fact tetraaminecopper(II) sulfate? Would you expect the wavelength of maximun absorbance (λ_{max}) for $Cu(NH_3)_4{}^{2+}$ to be greater than or less than λ_{max} for $Cu(H_2O)_6{}^{2+}$? Explain.

3. The electron transitions responsible for the colors of transition metal ions involve d → d transitions. Why are zinc ions colorless in aqueous solution?