Food Dyes and Beer's Law

What makes your drink blue?

Introduction

The color of light

White light, as we see it, is a mixture of all the colors of the spectrum. We are used to seeing raindrops scatter white light into its colors to form a rainbow, or seeing "rainbows" of light on a wall from sunlight that has been scattered by cut glass or a prism. If you perceive an object as being colored, as opposed to white, it is because colors other than the one you see are being absorbed by the object.

For chemical solutions, we can use an instrument called a spectrophotometer to pass light through the solution and measure which wavelengths are absorbed. You can predict what wavelengths will be absorbed at a simple level by taking the visible spectrum and wrapping it into a circle to make a spectroscopist's color wheel. With this wheel, the color that you see is the opposite of the color that is absorbed. If you know what wavelengths of the visible spectrum correspond to which color, you can predict where in the spectrum a chemical will absorb even before doing the experiment.

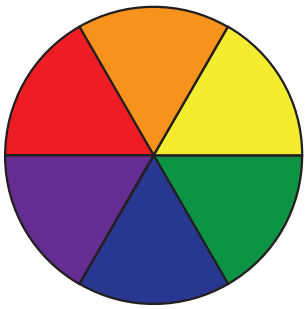


Figure 1. Color wheel

The wavelength of light is measured in nanometers: 1 nm is 1 x 10^{-9} meters. The visible spectrum in Figure 2 shows which wavelengths correspond to which color of light.

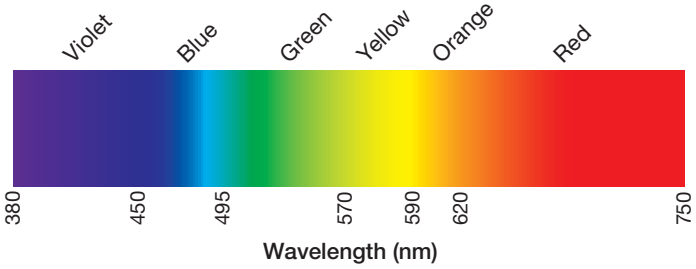


Figure 2. Visible spectrum

UV-Visible spectrophotometers

Measuring how much of which wavelengths of light are absorbed by a substance, and getting useful information about that substance from the results, is the scientific discipline of spectroscopy. The visible spectrum is one

part of the electromagnetic spectrum that we can access with equipment found in a typical chemistry laboratory. The basic principles of spectrum analysis can also be applied to other instrumentation that examine the ultraviolet, infrared, and radio frequency regions.

In a visible spectrophotometer, we shine a beam of light into a solution containing the sample, and detect how much of it comes out of the other side of the solution. By comparing the amount of light transmitted by the pure solvent to the amount transmitted when the sample is dissolved in it, we can calculate a quantity called the **absorbance**. Absorbance is directly proportional to concentration, so if you know the proportionality constant, you can use it to calculate the concentration of a substance in solution. Being able to answer the "how much?" question means that a visible spectrophotometer is a tool for doing *quantitative* analysis.

Knowing exactly which wavelengths of light are absorbed by a substance also gives us information that can be used to tell one substance from another or to determine whether a sample is a pure substance or a mixture. Being able to answer the "what is it?" question means that a visible spectrophotometer is also a tool for doing *qualitative* analysis.

Absorbance and Beer's Law

When colored solutions are irradiated with white light, the solution selectively absorbs incident light of some wavelengths. The wavelength of light where the absorbance is highest is used as the analytical wavelength. Once the analytical wavelength for a particular solution is determined, we can learn more about the solution through the relationship between absorbance (**A**) and three variables:

$$A = \varepsilon bc \qquad \text{Beer's Law}$$

The three variables are concentration of the solution (**c**), the pathlength of the light through the solution (**b**), and the sensitivity of the absorbing species to the energy of the analytical wavelength. When the concentration is expressed in molarity and the path length is measured in centimeters, the sensitivity factor is known as the molar absorptivity (**ε**) of the particular absorbing species.

Visible spectrophotometers are capable of displaying data in either of two scales:

- Percent transmittance (**%T**), which is a linear scale
- Absorbance (**A**), which is a logarithmic scale

The linear %T scale can be converted to absorbance where T is the percent transmittance expressed as a decimal (e.g., 22% = 0.22):

$$A = -\text{Log}_{10}\, T$$

The most important lesson to take home from this logarithmic relationship is the realization that when the absorbance is 1.0, only 10% of the light beam's full intensity is reaching the detector and when the absorbance is 2.0, only 1% of the light beam is reaching the detector. The accuracy and sensitivity of low cost instruments starts to suffer at absorbance values higher than 1.5.

Transmittance (or %T) itself is determined by the instrument by dividing the detector signal when measuring the sample (**I**) by the signal recorded for a "blank" solution ($\mathbf{I_0}$).

$$T = \frac{I}{I_0} \qquad \text{Transmittance}$$

When we work with cuvettes or test tubes where the path through the liquid is exactly 1 cm, the value of "b" in the equation for Beer's Law is simply 1, so it effectively drops out of the equation and simplifies it to A = εc. This means that:

- If you were to measure the absorbance of several solutions of known concentration, and plot the absorbance on the y-axis and concentration on the x-axis, the slope would be the molar absorptivity (ε) of the sample in solution.
- If you know the molar absorptivity, you can calculate the concentration (c) of a solution with ease by simply dividing the absorbance by ε (c = A/ε).

Purpose

In this experiment, you will make different kinds of measurement on various food dyes:

1. A scan of the visible spectrum recorded using a Thermo Scientific™ SPECTRONIC™ 200 Visible (Vis) Spectrophotometer* will show you which wavelengths are absorbed by each sample. You will identify a peak or peaks in the scan and record the wavelength of each peak. Officially, the wavelength at the top of the peak is

called the “wavelength of maximum absorbance”, which is abbreviated to λ_{max} (spoken as “lambda max”).

2. A single point measurement recorded at λ_{max} will be used to calculate the concentration of red, yellow, green, and blue food dyes in a solution. You will be able to determine which chemical dye was used in the solution samples and whether the dye is a single chemical food dye or a mixture of dyes.

3. Given a stock solution of known concentration, you will make a Beer’s Law plot by diluting the solution. You will then take a sports drink or soft drink and determine the molar concentration of the Blue No. 1 dye found in it. From this calculation and the molar mass of your dye, you will determine the mass of Blue No. 1 dye found in 591 mL of the solution – equivalent to a 20 fluid ounce bottle.

Experimental

Procedure

Making a measurement with the Thermo Scientific™ SPECTRONIC™ 200 Visible (Vis) Spectrophotometer*

1. Turn on the instrument and allow it to complete its startup sequence. Let the instrument warm up and stabilize for at least 30 minutes. Set up the experiment you want to perform in the spectrophotometer software. Obtain a square plastic cuvette or glass test tube to use in your experiments. If using a test tube cuvette, use a pen to place a mark near the top if the cuvette is not already marked with a white line. The mark allows you to ensure consistent placement into the instrument.

2. Add liquid to the cuvette until there is ~3 cm of liquid in the bottom (4 cm for test tubes). If plastic transfer pipettes are available, use one. The exact liquid level in the cuvette is not critical for good measurements as long as it is above 3 cm. Do not waste solution or risk spills by over-filling the cuvette.

3. Place the cuvette in the sample stage of the SPECTRONIC 200 Visible Spectrophotometer. If using a plastic cuvette, the clear sides should be on the right and left. If using a test tube cuvette, place it so that the mark faces to the right.

4. After the warm-up period, follow steps 2 and 3 using water or the appropriate “blank” solvent. Zero the instrument by pressing the autozero button.

5. For each subsequent measurement, empty and rinse your cuvette, shaking out as much of the rinse solvent as possible. When preparing samples, never return excess solution to the stock bottle. Pour all waste or excess into the appropriate waste receptacle. Follow steps 2 and 3 using your sample.

SPECTRONIC 200 Visible Spectrophotometer

*SPECTRONIC 200 Spectrophotometers are available on loan from Thermo Fisher Scientific™ at no cost. We will ship it to you, and you ship it back after one week. If you are interested in this program, please visit: www.thermofisher.com/spec200freetrial

Part 1. Scan the dyes

Prior to the lab, your instructor should have prepared dye solutions using the four packs of liquid food dyes from McCormick® Food Coloring containing red, yellow, blue and green dyes [1]. The actual concentration of the dye solutions is arbitrary, but they should be chosen to ensure the largest peak in each solution lies within the absorbance range of the spectrophotometer.

1. Run a scan of each dye solution from 400 nm to 700 nm.

2. Record the wavelength (λ_{max}) and absorbance at each peak in the spectrum. If the color is due to a mixture of dyes, two λ_{max} peaks will be present.

3. Enter this information in Data Table 1 in the Lab Report.

Data analysis: Determination of the dyes used in McCormick food coloring

Use the reference spectra in the Appendix to determine which chemical dye(s) are used to make each of the four colors from McCormick. Some of the colors are pure substances and some are mixtures of dyes. Enter your answers in Data Table 2 in the Lab Report.

Calculations: Molar concentration of dyes present in each solution

Use the Beer-Lambert Law equation (A = εbc), your measured absorbance values, and the molar absorptivity values in Table 1 below to calculate the molar concentration of each dye present in the four solutions tested. Write your answers in Data Table 2.

You will need to know the pathlength (b). If you have a standard square plastic cuvette the pathlength is 1 cm. If you are measuring in test-tube cuvettes or ordinary test tubes (without a pre-printed white line to help you to align them consistently) the pathlength will not be 1 cm. If this is the case, use a metric ruler to measure the pathlength of your cuvette and record it on the Lab Report.

Table 1

FD&C Dye	Molar Mass ($g \cdot mol^{-1}$)	ε ($L \cdot cm^{-1} \cdot mol^{-1}$)
Red 3 or Erythrosine (cherry red)	898	31,000
Red 40 or Allura Red AC (orange-red)	496	25,900
Yellow 5 or Tartrazine (lemon-yellow)	534	27,300
Yellow 6 Sunset Yellow (orange)	452	25,900
Green 3 Fast Green FCF (sea green)	809	43,000
Blue 1 Brilliant Blue FCF (bright blue)	793	130,000
Blue 2 Indigotine (royal blue; Indigo Carmine)	466	111,000

Part 2. Create a Beer's Law plot for Blue No. 1 dye

What is the relationship between the absorbance of a colored solution and its molar concentration? You will prepare a series of solutions of known concentration, measure their absorbance at λ_{max}, and plot the data.

Record the concentration of the stock solution: _____
(This will be given by the instructor.)

Dilutions: Take approximately 40 mL of the Blue No. 1 dye stock solution to your bench and prepare dilute solutions from it according to Table 2. These solutions will be your known concentrations of the dye. Calculate the molar concentrations of your solutions and enter them in Data Table 3 in the Lab Report. Report the concentrations as μM. Find the absorbance of your five solutions using the spectrophotometer and record in Data Table 3.

Table 2

Solution	Dilution Ratio (mL stock/mL water)
1 (stock solution)	10 mL/0 mL
2	8 mL/ 2 mL
3	6 mL/ 4 mL
4	4 mL/ 6 mL
5	2 mL/8 mL

Using your absorbance readings and the molar concentrations, construct a Beer's Law plot (plot the molar concentrations of your known solutions on the x-axis and the absorbance data on the y-axis). Use a spreadsheet program or a graphing calculator to plot your data and determine a best-fit line (trend line) to calculate the slope of your line. Record the slope of the line in the Lab Report.

Part 3. What's in that drink?

1. Obtain about 5 mL of the blue colored drink.
2. Measure the absorbance of the drink at λ_{max} for Blue Dye No. 1 and record it on the Lab Report.
3. Calculate the concentration of Blue No. 1 dye in the drink using the Beer's Law plot from Part 2.
4. Calculate the mass of dye present in a 20 oz (591 mL) bottle of the drink.
5. Record your calculations and answers in the Lab Report.

Disposal of chemicals:

All of the food dyes can be flushed down the sink with plenty of water.

Further reading/reference material

1. Sigman SB, Wheeler DE (2004) The quantitative determination of food dyes in powdered drink mixes. A high school or general science experiment. *J Chem Educ* 81: 1475–1478.

Lab Report

Food Dyes and Beer's Law

Name: _____

Date: _____

Section No. or Lab Period: _____

Part 1. Scan the dyes

Data Table 1

Color of Solution	λ_{max} (nm)	Absorbance
Red		
Yellow		
Green		
Blue		

Record the pathlength of your cuvette: _____ cm

Data Table 2

Color of Solution	Dye(s) contained in solution	Pure substance or mixture?	Conc. (mol/L)
Red			
Yellow			
Green			
Blue			

Questions

1. **What was the wavelength of light absorbed by the blue colored solution at its λ_{max}?**

2. **Using the information in the introduction, determine the color of light this corresponds to in the visible light spectrum.**

3. **How is the color of light absorbed by the colored solution related to its perceived color? Is there a connection between these two?**

4. **Show your concentration calculations for any two of the dyes listed in the table. Label the calculation with the name of the dye, box your answer, and write neatly!**

Part 2. Create a Beer's Law plot for Blue No. 1 dye

Data Table 3

Solution	Dilution Ratio (mL stock/ mL water)	Molar Conc. (μM)	Measured Absorbance
1 (stock solution)	10 mL/0 mL		
2	8 mL/2 mL		
3	6 mL/4 mL		
4	4 mL/6 mL		
5	2 mL/8 mL		

Plot of Absorbance vs. Concentration for Blue No. 1 dye (Beer's Law plot)

Staple your printed graphs to this report sheet and record the required data and answers in the spaces below:

1. **Record the slope of the best-fit line:** _____

Name _____

2. **Write the full equation (*y* = *mx* + *b* format) for the best fit line on the graph you just created using the slope.**

3. **The slope of the line is derived from the molar absorptivity (ε) of the dye and the path length (b) of the sample in the spectrophotometer. What is the path length of your cuvette?**

   Record the pathlength of your cuvette: _____ cm

4. **Use the slope of the line to determine the molar absorptivity (ε) of Blue No. 1 dye. Use the equation for Beer's Law to derive and include the units. Note that absorbance has no units. Show your calculation here:**

Remember:

- Staple hand-drawn or printed graphs to your lab report
- Staple the two sheets of the lab report together before you hand them in

Part 3. What's in that drink?

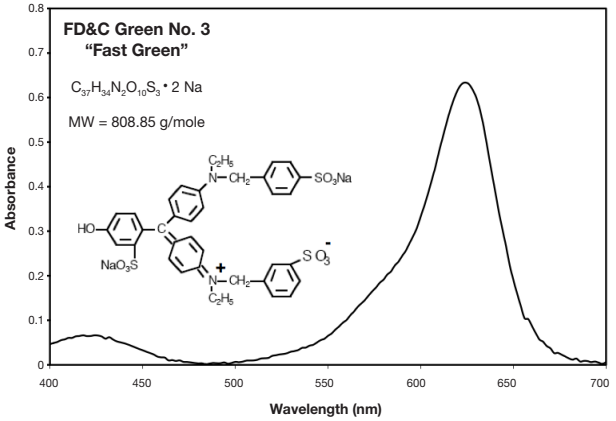
Absorbance of the blue drink: _____ at _____ nm

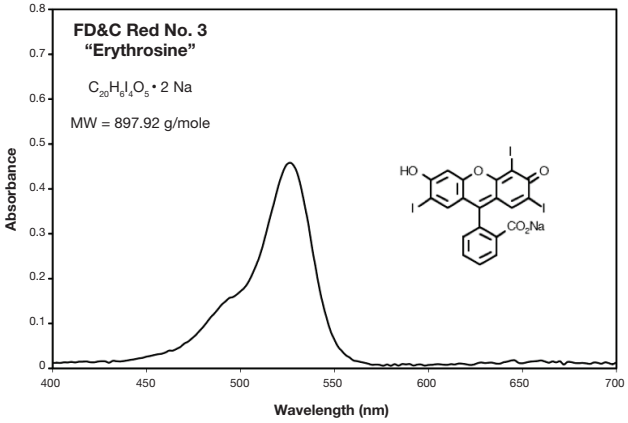
1. **Determine the molar concentration of the Blue Dye in the drink. Show all work.**

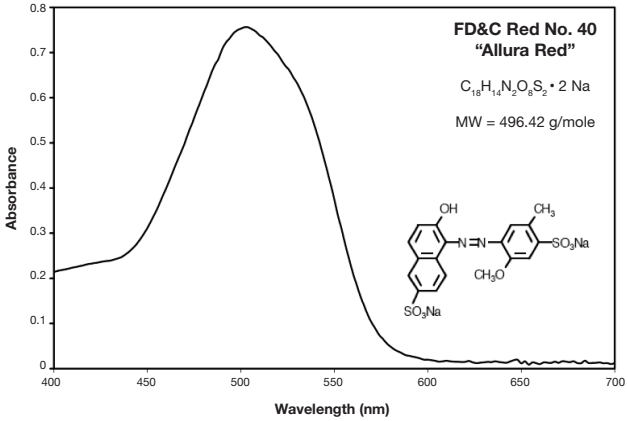
2. **Determine the mass of Blue #1 Dye found in 591 mL of the drink. Show all work.**

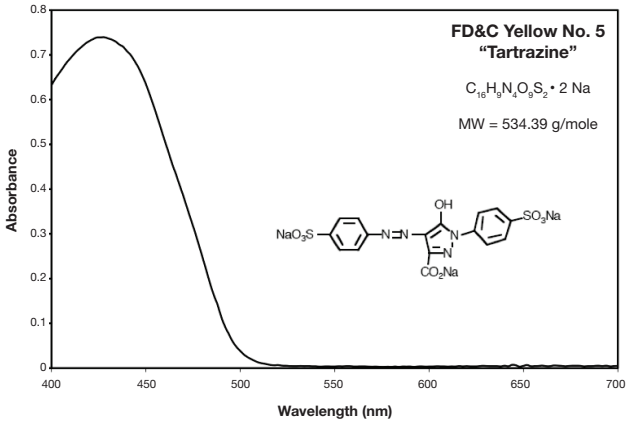
Appendix

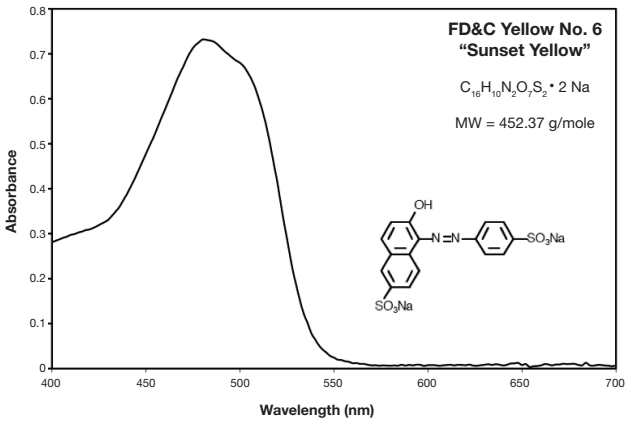
Reference spectra for FDA food dyes

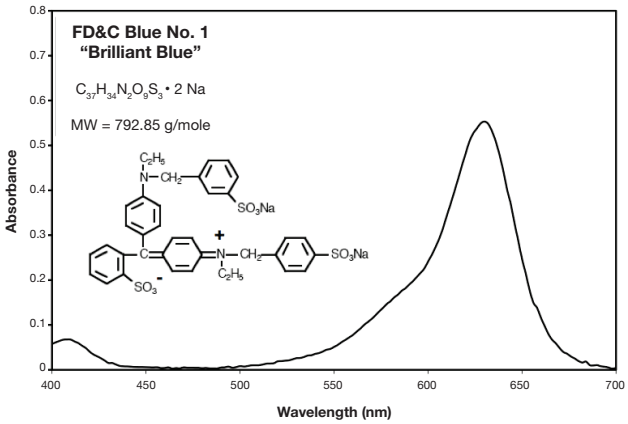


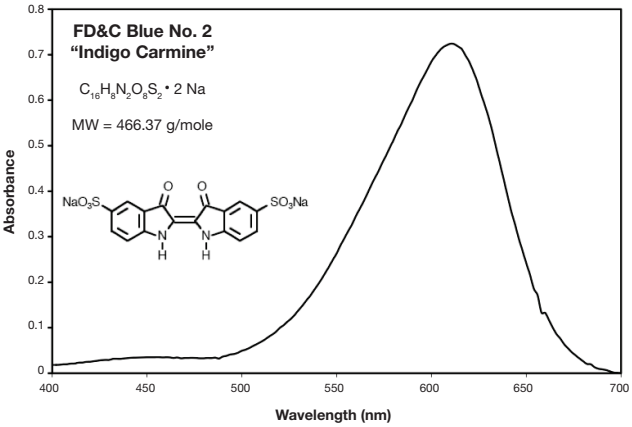












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