Name: _____

Chemistry 118 Laboratory
University of Massachusetts Boston

Beer's Law

LEARNING GOALS:

1. **Become familiar with the concept of concentration and molarity.**
2. **Become familiar with making dilutions and the calculations required for preparing diluted solutions.**
3. **Become familiar with the concepts of absorption of light and Beer's Law**
4. **Become familiar with graphing and determining the slope and intercept from a best fit linear regression using Excel.**
5. **Become familiar with using a Beer's law plot to find the concentration of a Commercial solution.**

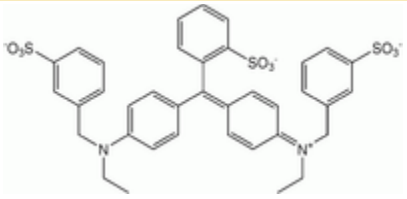
OBJECTIVE: To determine the concentration of food coloring dye in commercial juice using UV-VIS spectrometry.

INTRODUCTION:

All electromagnetic radiation consists of oscillating perpendicular electric and magnetic fields that travel through space at the same rate (e.g., the "speed of light", $c = 2.998 \times 10^8$ m/s in a vacuum). Any of the various kinds of electromagnetic radiation can be described in terms of frequency (ν) and wavelength (λ) by the relationship: $\nu\lambda = c$. The colors of transparent liquids are determined by the wavelengths of visible light that are absorbed and transmitted. Absorbance (the capacity of a substance to absorb radiation) and transmittance (the fraction of radiation at a specified wavelength that passes through a sample) are physical properties that all molecules have. For example, in this experiment we will use an aqueous solution of a commercial food coloring dye (blue, red and yellow).

Brilliant Blue FCF is a colorant for foods and other substances. It has the appearance of a reddish-blue powder. It is soluble in water, and solutions of this dye have a maximum absorption at 628 nm. Its CA Index Name is Benzenemethanaminium, N-ethyl-N-[4-[[4-[ethyl[(3-sulfophenyl)methyl]amino]phenyl](2-sulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-3-sulfo-, inner salt, disodium salt

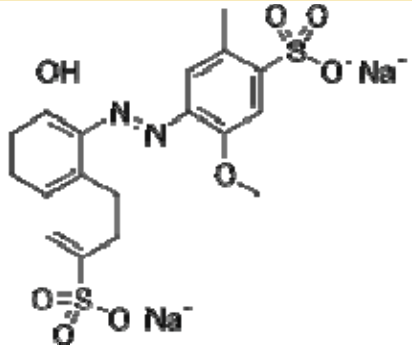
Brilliant Blue FCF



Molecular formula $C_{37}H_{34}N_2Na_2O_9S_3$

Allura Red AC is a red azo dye. It is used as a food dye. It has the appearance of a dark red powder. It usually comes as a sodium salt but can also be used as both calcium and potassium salts. It is soluble in water. In aqueous solution, its wavelegnth of maximum absorbance is 504 nm. Its melting point is at >300 degrees Celsius. Allura Red AC is one of many High Production Volume Chemicals. Red AC was originally manufactured from coal tar but is now mostly made from petroleum. Its CA Index Name is 2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt.

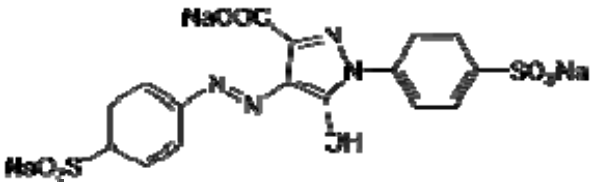
Allura Red AC



Molecular formula	$C_{18}H_{14}N_2Na_2O_8S_2$
Molar mass	496.42 g mol^{-1}
Appearance	Dark red powder
Melting point	>300 °C

Tartrazine (otherwise known as FD&C Yellow 5) is a synthetic lemon yellow azo dye used as a food coloring. It is soluble in water, and solutions of this dye have a maximum absorption at 427 nm. Tartrazine is a commonly used color all over the world, mainly for yellow, but can also be used with Brilliant Blue FCF (FD&C Blue 1, E133) or Green S (E142) to produce various green shades.

Tartrazine



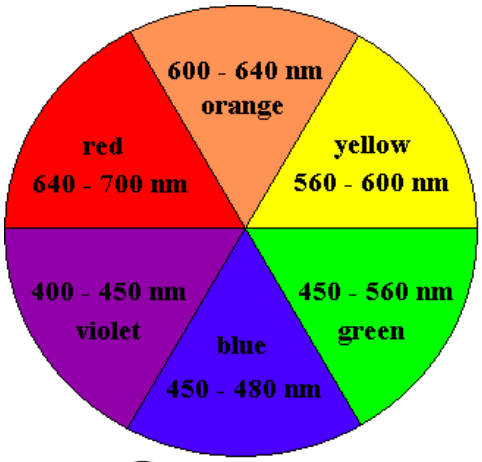
IUPAC name[hide]
Trisodium (4*E*)-5-oxo-1-(4-sulfonatophenyl)-4-[(4-sulfonatophenyl)hydrazono]-3-pyrazolecarboxylate

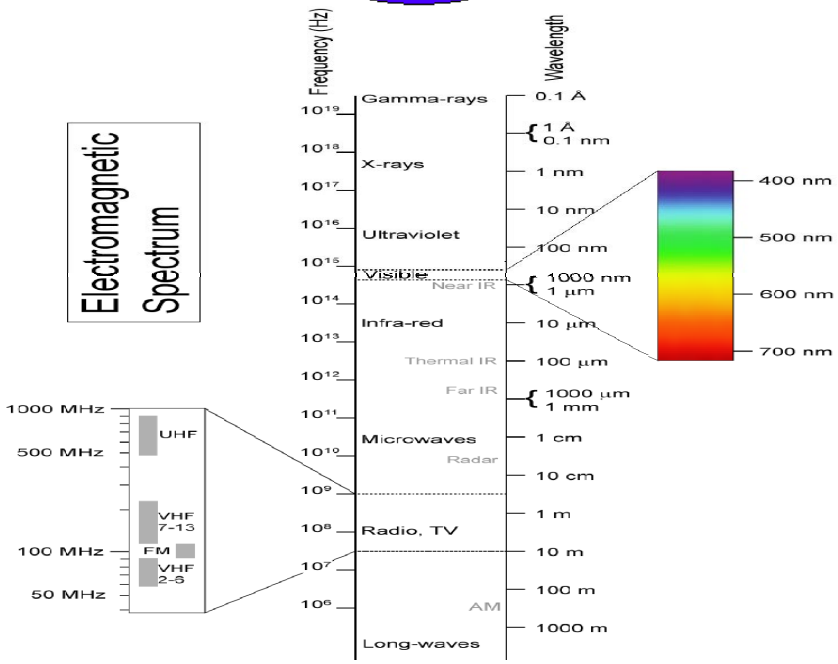
Properties

Molecular formula	$C_{16}H_9N_4Na_3O_9S_2$
Molar mass	534.3 g/mol

Absorption of light

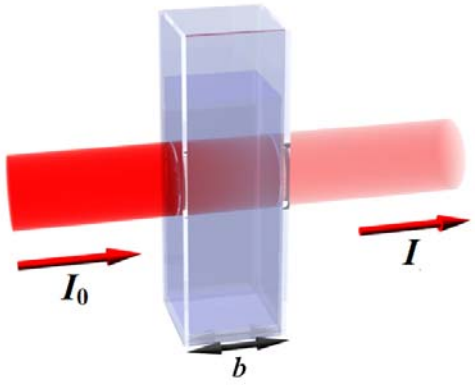
Absorption of light in the UV-visible region is due to electronic transitions between ground state and excited state valence electrons (the outermost ones involved in bonding). These dyes absorb visible light due to the network of conjugated double bonds in these molecules. Generally, the greater the number of conjugated double bonds in a molecule, the smaller the energy gap between the Highest Occupied Molecular Orbitals (where the outermost electrons hang out) and the Lowest Unoccupied Molecular Orbitals and thus the longer the wavelength of absorption. REMEMBER longer wavelengths correspond to smaller energetic transitions. The observed color of the dye is a consequence of the absorption of the complementary wavelengths (the ones opposite of it on the color wheel) by the molecule. For instance, an aqueous solution of Brilliant Blue FCF appears blue because orange light is being absorbed (600-650 nm). Aqueous solutions of Allura Red AC appear red because green light is being absorbed (480-560 nm). Aqueous solutions of Tartezine appear yellow because violet and blue light is being absorbed (400-450 nm). Notice Brilliant blue contains the most elaborate network of conjugate bonds and Tartezine contains the fewest number of conjugated double bonds.





As this suggests, a colored solution generally transmits visible light at many wavelengths, but the intensity of transmitted light is not always the same at each wavelength. For a given wavelength of visible light, if we compare the initial intensity of the visible light shining on the sample, I_o, to its intensity after it has passed through the sample, I, we can define the *transmittance* (T) as the ratio

(1) $T = I/I_o$



b is the *path length* - the distance the light travels through the sample (1.00 cm in this experiment)

The amount of light that is absorbed at a given wavelength is measured by the *absorbance* (A), defined as the negative of the logarithm of the transmittance:

(2) $A = -\log T = -\log(I/I_o) = \log(I_o/I) = \log(1/T)$

For a given solute, there is often one wavelength (or a small range of wavelengths) at which the absorbance is greatest (and therefore the transmittance the least). This wavelength is sometimes referred to as λ_{max}. For Bromophenol Blue, λ_{max} occurs near 590 nm (see figure below).

For a solution, the actual value of the absorbance, A, at any wavelength such as λ_{max} depends upon the following three factors:

ε, the *molar absorptivity*, a constant characteristic of the absorbing solute species (L/mol·cm); It is a unique constant for a given wavelength.
b, the *path length*, the distance the light travels in passing through the sample (cm); and,
c, the *concentration* in molarity (mol/L) of the absorbing solute species.

The relationship between these factors is called *Beer's Law*:

(3) $A = \varepsilon bc$, Absorbane is a unitless quantity.

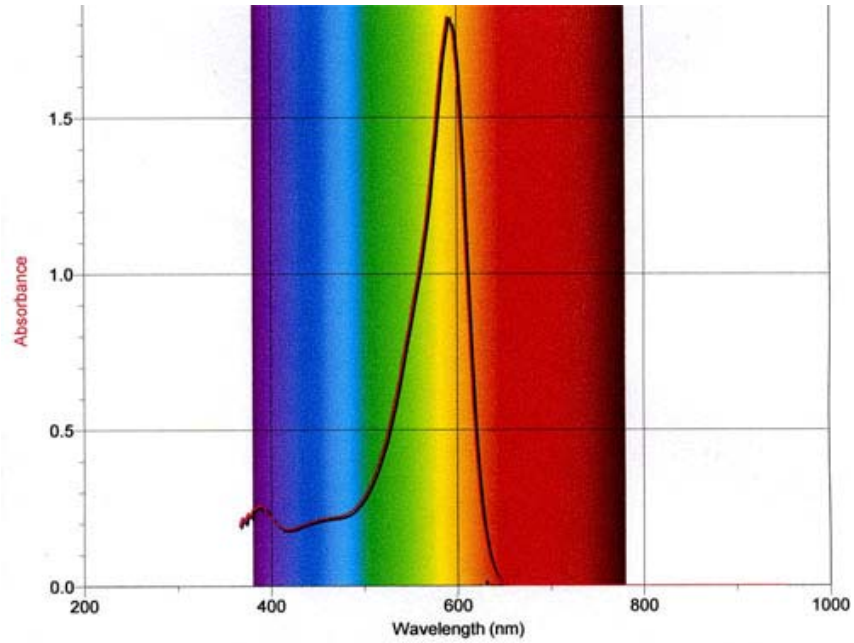
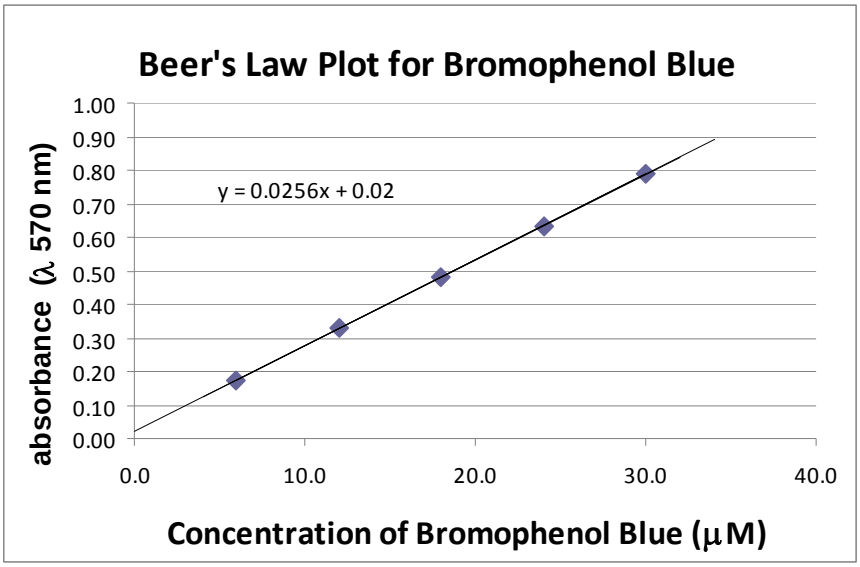


Figure 1: Bromophenol blue spectrum

You will use a spectrophotometer to measure the absorbances of dilute solutions of a dye. Because you will be using the same cuvette and spectrophotometer throughout the experiment, the path length, *b*, will remain constant. The molar absorptivity, ε, will also remain constant. Therefore, we can combine *b* and ε into a single experimental constant, *k*, and rewrite Beer's Law as

(4) $A = kc$, the units on k is L/mol.

Below is a typical Beer's Law Plot obtained in this experiment. The solute is a different dye, bromophenol blue, that is often used as an acid-base indicator.



Notice that the concentrations on the x-axis are in units of μM. These units are used so we do not have to use scientific notation on the axis, which often looks a bit cumbersome. The line between the points is the best fit line and the given equation represents the best fit line. The slope is 0.0250. Because the x-axis was plotted in μM, the eb (or k) actually equals $0.0250 \cdot 10^6$ = 25000 L/mol ($2.50 \cdot 10^4$ L/mol, with proper sig figs). We can use this plot to find the concentration of an unknown solution of Bromophenol Blue. The absorbance of the unknown solution is measured and the equation is used to find its concentration. For example if the absorbance of an unknown solution was found to be 0.445, its concentration is calculated as x = (y-b)/m or in this case (0.445-0.02)/0.025 = 17.7 μM.

MATERIALS:

Five 18 x 150-mm test tubes
5.00-mL Mohr pipet
Pasteur pipets
100-mL beaker or larger
Small beaker
Spectrophotometer
Cuvettes
$7.00 \cdot 10^{-6}$M Brilliant Blue in water
$2.00 \cdot 10^{-6}$M Tartazine in water
$2.00 \cdot 10^{-6}$M Allura Red AC in water
Distilled water
Various flavors of Gaterade

PROCEDURE:

Constructing a Beer's Law plot

1. Your instructor will assign you one of the three dyes. Turn on the spectrophotometer and set the wavelength appropriately to perform experiment on your dye. These instruments need time to "warm-up" before use.

2. Label the 18 x 150-mm test tubes 1 through 5. Fill test tube 1 approximately one-half to two-thirds full with your stock solution.

3. Draw up about 1.00 mL of stock solution into the Mohr pipet from test tube 1. With your finger on the top of the pipet to hold the liquid in, tip the pipet on its side and roll the liquid around to wet the inside of the pipet with stock solution. Drain this wash liquid into a 100-mL or larger beaker, which you will use as a waste container. However, since this experiment uses only food coloring at relatively low concentration, waste from this laboratory can be disposed of down the drain.

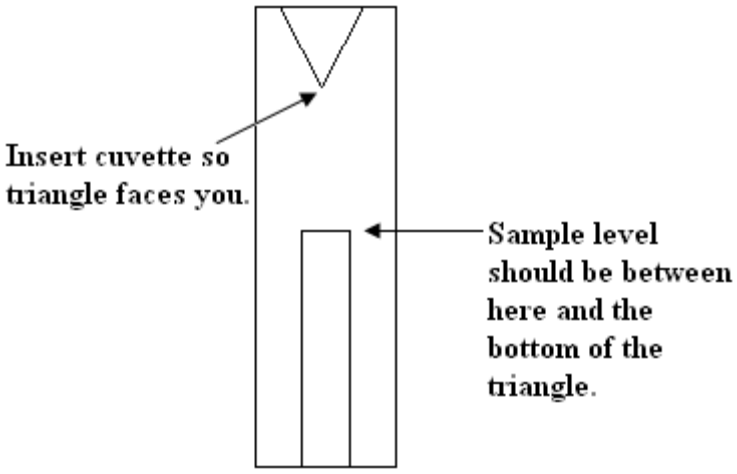
4. Using the 5.00-mL Mohr pipet, measure the amounts of stock solution indicated in the table below into test tubes 2 through 5.

5. Rinse the 5.00-mL Mohr pipet with distilled water. Measure the amounts of distilled water indicated in the table below into test tubes 2 through 5. Mix the solution in each test tube thoroughly by swirling it for about a minute. Calculate the concentration of the dye in each test tube and record in the last column of the table.

Test Tube	Volume stock Dye from Stock Solution (mL)	Volume water (mL)	Name and Concentration of Dye (M)
1	>5	0	
2	4.00	1.00	
3	3.00	2.00	
4	2.00	3.00	
5	1.00	4.00	

6. The solution in test tube 1 should be at least as high as the level in the other test tubes. If it is not, add additional stock solution.

7. Rinse out the cuvette with distilled water. Then fill the cuvette with distilled water so that the water level is between the bottom of the triangle and the top of the rectangle as shown in the figure below. Place the cuvette in the sample chamber on top of the instrument so that the triangle on the cuvette faces you. Close the lid of the spectrophotometer and zero the instrument as demonstrated by the instructor. Think about why you must close the lid before recording each absorbance. This sample is the blank sample. **The instrument settings from the blank must not be changed throughout the rest of the measurements. Think about why this is so.** Remove the cuvette.



8. You are now ready to measure the absorbances of your five samples. Add a small amount of the first sample (it is best practice to start with the most dilute solution) to be measured to the cuvette using a clean and dry Pasteur pipet. Swirl it around to wet the sides, and empty this wash solution into the waste beaker. Then, with the same Pasteur pipet, fill the cuvette to between the bottom of the triangle and the top of the rectangle. Close the lid and record the absorbance. Pour the solution from the cuvette back into its labeled test tube. Repeat the process for all five samples, and record your results to the proper number of significant digits in the following table:

Sample	Concentration of Dye (μM)	Absorbance
1		
2		
3		
4		
5		

Save all of your standard solutions until you are finished with the last part of this laboratory exercise.

9. Plot Absorbance vs. Molarity using Excel on one of the computers in lab**.**

10. Determine the slope and intercept of the best fit line through the points. What is your experimentally determined value of *k* in the Beer's Law equation $A = kc$?

    k = _____ μM^{-1}

    Is the intercept close to zero?

Do not change any instrument settings. Use the same cuvette for the next part of this experiment.

Determining the Concentration of the Dye in Commercial Juice Product

1. Obtain a sample of the commercial juice. It is critical that the color of the juice matches the color of dye you were assigned above. The commercial solution may be more concentrated than any of the solutions you used in making the Beer's Law plot. Therefore you will have to dilute the juice so that it gives an absorbance reading in the range of your Beer's Law plot.

   Rinse the 5.00-mL Mohr pipet with some of the unknown solution. Measure a portion of your unknown solution into a clean, dry 18 x 150-mm test tube. Record the volume of unknown solution used.

   Volume of unknown solution = _____

2. Now, add *measured* amounts of water to the sample, until it appears to be about as dilute as standard 3.

   Total volume of water added to make a successful solution =

3. Measure the absorbance of this solution.

4. Set up a new test solution with the same composition that yielded an acceptable absorbance above, using a single pipetting of the required amount of water. Measure the absorbance of this solution and record below. Determine its concentration by using the value of *k* from your Beer's Law plot.

   Volume of juice taken = _____

   Volume of H_2O added to make diluted solution = _____

   Absorbance of diluted solution = _____

   Concentration of dye in the diluted solution = _____

   Concentration of the dye in the original juice = _____

DATA SHEET:

Sample	Concentration of Dye (μM)	Absorbance
1		
2		
3		
4		
5		

Slope
Intercept

Abs = kc + b

Volume of unknown juice taken = _____

Volume of H_2O added to make diluted solution = _____

Absorbance of diluted solution = _____

Concentration of dye in the diluted solution = _____

Concentration of dye in the juice = _____

THE LAB REPORT

No abstract is due for this report. You need to submit your data sheet (pg 10), your Beer's Law plot and the answers the following questions. The data sheet is worth 30 points.

1. Submit a copy of the Beer's Law plot into your report. Be sure it has a title and the axes are labeled. (10 pts).

2. Why is it necessary to blank the spectrophotometer before making measurements? (5 pts)

3. One of the potential complications with performing spectroscopic experiments on commercial samples is that mixtures are complex and sometimes contain suspended solids. Commercial Gatorade appears cloudy due to some suspended solids. Explain how this might impact the results of this experiment. (5 pts)

4. Another potential complication is that many commercial samples are prepared from mixtures of dyes. Is the shade of the juice sample different than the shade of the standard dye solutions? Explain how this might impact the interpretation of results. (5 pts)

5. As mentioned above, this experiment replaced an analogous experiment that used 0.15 M $CoCl_2$ as the stock solution. Look up the Material Safety Data Sheets of $CoCl_2$ and compare its potential health hazards to the dye you used in today's experiment. Toxicity is always dose dependent. Discuss how a dilute solution of dye compares to a concentrated solution of cobalt chloride. Was the switch justified? (10 pts)