CHE1031 lab: Solution concentration and dilution[1]

Background:
The focus of this chemistry course is aqueous solution chemistry. **Solutions** are homogenous mixtures **solute**, the compound present in lesser amount, and **solvent**, the compound present in greater amounts. Molecular compounds **dissolve** in solvents, that is they become evenly distributed throughout the volume of solvent. Ionic compounds **dissociate**, or come apart into their ionic components, in aqueous solvents and then those ions become evenly distributed throughout the volume of solvent.

One expression of the concentration of solutions used by chemists, and other scientists, concentration is **molarity (M)**, or the moles of solute per liter of solution. So molarity is a measure of the amount of compound of interest (say salt) in the total volume of solution, most of which is solvent (for example, water). Because adding solute to solvent increases the volume of the overall solution, it's essential that molarity uses volume of solution rather than volume of solvent.

$$\text{molarity (M)} = \frac{\text{moles of solute}}{1\text{ L of solution}}$$

Since molarity has two units (moles/L) it is also a **conversion factor** that allows us to convert amount of compound of interest (moles of solute) to volume of a solution of that compound.

Dilution is the process of decreasing the concentration of a solution (its molarity) by adding more solvent. While the amount of solute isn't changed, its concentration in the solution is lowered by dilution. For example, adding more water to a beaker of salt water 1) lowers the concentration of salt in the salt water solution and 2) increases the volume of the salt water in the beaker. This simple equation can be used to figure out how make any dilution:

$$M_1L_1 = M_2L_2$$

where M = molarity (mol/L)
L = volume (L)
1 = initial (stock solution)
2 = final (dilute solution)

The more concentrated solution, called the **stock solution**, is usually thought of as solution 1 or the initial solution while solution being created by dilution is the final solution, or solution 2. When given three values, simple algebra can be used to solve for the fourth value.

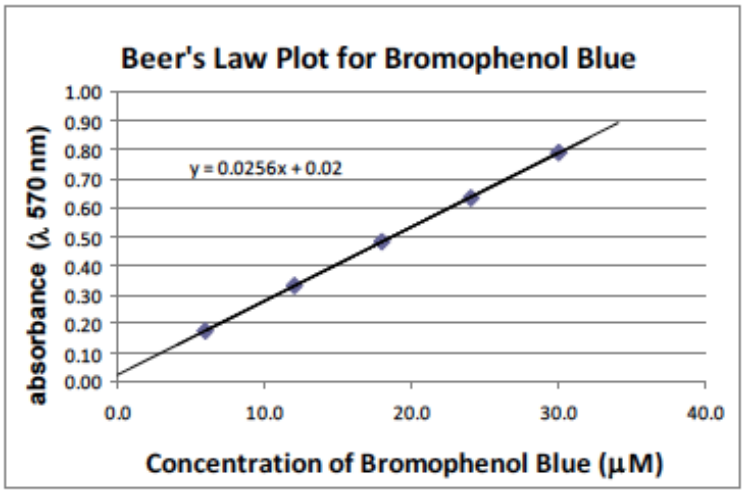
Absorption spectrophotometry is a technique that can be used to determine the concentration of a solution of a pure compound. Chemical compounds absorb light at a specific wavelength. The color of compounds is the wavelength of light that is not absorbed by the dye. The amount of light absorbed by a solution is directly proportional to the concentration of compound in that solution so absorption can be used to determine the concentration of the solution. The relationship between absorption of light is expressed by **Beer's law**:

[1] UMass Boston's Chemistry 118 Laboratory exercise on Beer's Law

A = εbc where A = absorbance at a wavelength (λ = nm)
ε = molar absorptivity for the solute (L/mol-cm)
b = path length in cuvette (cm)
c = concentration (M = mol/L)

If the dye and the cuvette's path length remain constant, we can combine them into a single constant (k = L/mol) and simplify Beer's Law: A = kc

The plot shown below demonstrates that the concentration of a dye – here Bromophenol Blue – is directly proportional to its absorbance at the wavelength of light at which is has maximal absorbance. We can plot a straight line and use **linear regression** to solve for the concentration (x) when we measure a solution's absorbance (y).



Again, algebra allows us to rearrange this equation to solve for concentration of the solute.

In this lab, you'll make a solution of a common and non-toxic dye in water, and then create a series of dilutions of that solution using the $M_1L_1 = M_2L_2$ equation. You'll then use visible wavelength spectroscopy to determine the accuracy of the concentrations of your solutions. The lab has three parts:

Part I: Creating a standard curve by diluting a stock solution
Part II: Reading absorbance and using linear regression
Part II: Determining concentration of dye in Gatorade

Learning objectives:

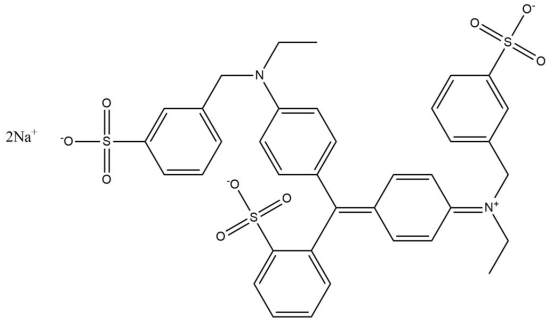
- Practical use of the concepts of concentration and molarity.
- Application of the concept of dilution
- Understanding of the concepts of absorption of light and Beer's Law.
- Use of linear regression to determine values from a standard curve.

Materials:

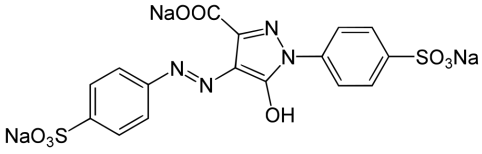
Chemicals	Equipment
Blue 1 (Brilliant Blue FCF)	Spec 20 spectrophotometer
Yellow 5 (tartrazine)	plastic cuvettes
Allura Red RC	test tubes, 18 x 150-mm
ddH2O	electronic balance, weigh paper, scoopulas
Gatorade, colored	pipettes and pipette rollers
	parafilm

Table I: Dyes and absorption characteristics

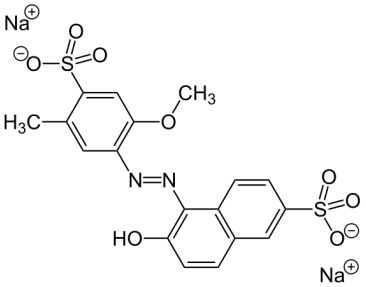
dye	Molecular formula	Maximum absorption (λ)	Molar absorptivity
Blue 1	C37H34N2O9S3Na2	628 nm	
Yellow 5	C16H9N4O9S2Na3	427 nm	
Allura Red RC	C18H14N2O8S2Na2	504 nm	



Blue 1



Yellow 5



Allura Red RC

Procedure:

Part I: Creating a standard curve by making serial dilutions of a stock solution

1. Be sure that the spectrophotometer is turned on and allowed to warm up.
2. Use a small beaker to get a sample of your assigned dye and record its identity and concentration.
3. Label a set of five test tubes 1 to 6.
4. Use a pipette to add the appropriate volumes of water to tubes 2 through 5.
5. Add your dye solution to test tube 1 until it is about 2/3 full.
6. Draw the dye solution from test tube 1 up into your pipet and dispense it back into test tube 1 in order to displace water and coat the pipet with concentrated dye solution.
7. Draw up 2.00 mL of dye solution from test tube 1 and dispense it into test tube 2 as completely as possible. Carefully vortex test tube 2.
8. Once mixed, draw up 2.00 mL from test tube 2 and dispense it into test tube 3. Vortex and then move 2 mL of solution from test tube 3 to test tube 4.
9. Continue until you reach the last test tube. Dispense and vortex. Tubes 2-5 should have 2 mL of liquid while tube 6 should have a full four mL of solution.

Table II: Serial dilution volumes

test tube	Previous dilution (mL)	ddH2O (mL)
1	> 5	0.00
2	2.00	2.00
3	2.00	2.00
4	2.00	2.00
5	2.00	2.00
6	2.00	2.00

Part II: Reading absorption and using linear regression

1. Check the wavelength setting of the spectrophotometer to be sure that it matches the maximal absorption wavelength of the dye you are using.
2. Pour at least 1 mL of each of your dilutions into a clean, dry cuvette.
3. Place the cuvettes into the sample rack in the spectrophotometer. The first sample, 1 or B, should be distilled water only, used as a blank. Then place your standard curve in the remaining cuvette holders.
3. Close the lid of the chamber and wait for the reading to stabilize.
4. Press the "auto zero" button to zero the blank.
5. Now press the up arrow to move the sample rack down and read the optical density (color and absorbance) of each of your sample. Record the OD490 values for the zeroed blank and each sample of the standard curve.

Part II calculations:

1. Use M1L1 = M2L2 to calculate the concentrations of dye in tubes 2 through 5.
2. Create an Excel table for your five tubes and the blank:
   Column 1 = concentration
   Column 2 = absorbance values
3. Use Excel to create a graph showing unconnected points (X,Y scatter). Your graph should look very similar to the one shown on the second page of this handout.
4. Under 'chart design', add 'trendline, linear' and choose to 'display equation on chart' and 'display R2 on chart'.
5. Rearrange the linear regression equation to solve for 'x', the concentration of dye.
6. Plug each absorbance value (y) into the equation and solve for concentration (x).
7. Calculate **percent error** for concentrations by comparing:
   (1) the concentration calculated using M1L1 = M2L2 (step 1); and
   (2) the concentration calculated using absorption (step 6).

Part III: Determining the concentration of dye in Gatorade

1. Use a small beaker to get a sample of Gatorade whose color matches the color of your dye.
2. Create a series 1:1 serial dilutions of the Gatorade using the volumes in Table II, treating the Gatorade as if it's the stock solution. Note that if the sample of Gatorade is "lighter" or less "intense" than the dye stock solution, you may not need to make as many dilutions. See the instructor if you need more help with that.
3. Read the absorption of your Gatorade dilutions using the spec set at the same wavelength used for the dye of the same color.

Part III calculations:

1. Use the linear regression equation from part II to calculate the concentration of dye in the Gatorade samples. Be sure to correct for dilution factors!
2. Calculate the mean and SD of your experimental Gatorade dye concentrations.