Lab: Concentration, Molarity, and Dilutions

Name _____

Purpose: To introduce the concepts of concentration, molarity, dilution and spectrophotometry (absorption spectra, standard curve, and Beers Law).

Introduction:

Color is an important factor in our choice of food. It can affect how we perceive flavor and how likely a product is to catch our eye. Food and drink manufacturers are aware of color's potential and often add dyes to their products to make them more appealing. Nitrates are added to meats to keep them bright red, sulfites are added to dried fruits to preserve their color, dyes are added to beverages to give them their characteristic look and indicate flavor. To a chemist, color can be a source of information.

In order for something to be colored, it must absorb (or sometimes scatter) some wavelengths of light more than others. How a chemical absorbs light depends on how that light interacts with its electrons; interaction with electrons is the central idea of chemistry. Qualitatively, color can tell us if a solution is acidic or basic, it can announce a chemical reaction or we can use it to detect the presence of a substance like iron in our water or starch in food. With the help of an instrument called a spectrophotometer, color can do even more. It can identify solutes and allow us to determine their concentration in solution.

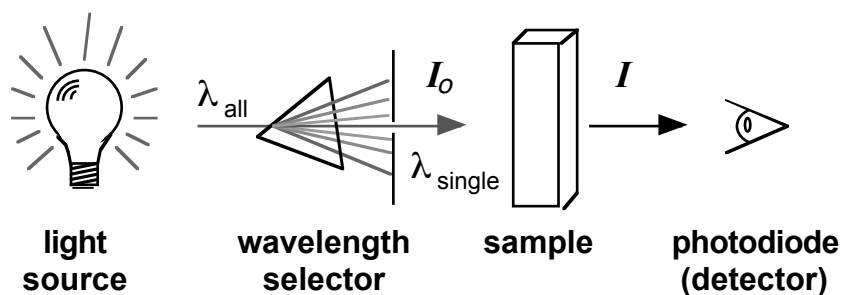***The following is a description of how the spectrophotometer works…***

Figure 1. Spectrophotometer.

A single wavelength of light (λ_{single}) generated by the **light source** is selected from all wavelengths generated (λ_{all}) by rotating a **wavelength selector**. The intensity of λ_{single} is denoted I_o. λ_{single} passes through the **sample** and is detected by the **photodiode**. If the sample absorbs any λ_{single}, then intensity of the light coming from the sample (I) will be different then the incident light intensity (I_o). The spectrophotometer can present data either as "transmittance" or "absorbance." Consider a situation where a sample absorbs the majority of the incident light (I_o); the amount of light transmitted (I) is close to zero. Under these conditions, we would say that due to the sample, very little light was "transmitted". We could also say that the sample "absorbed" most of the incident light.

We define the % transmittance (%T) as: $\%T = \dfrac{I}{I_0} \times 100$, where I and I_o are defined above.

We define the absorbance (*Abs*) as: $Abs = -\log\dfrac{I}{I_0} = 2 - \log\%T$

Question? So what wavelength do you set the spectrophotometer to when collecting the Beers Law plot data? The answer lies in the ***absorption spectrum***.

An absorption spectrum (or plot) is made by measuring, in the lab, the absorbance of a solution verses wavelength and then plotting this data in an xy scatter plot ("connect the dots") with wavelength being the x-axis (the independent variable) and absorbance being the y-axis (the dependent variable). As shown in the example to the right, this beta-carotene solution absorbs light in the 400-500 nm wavelength range.

Beers Law Plot
A Beers Law plot is made by measuring, in the lab, the absorbance of a solution verses its known concentration and then plotting this data in an xy scatter plot with concentration being the x-axis (the independent variable) and absorbance being the y-axis (the dependent variable).
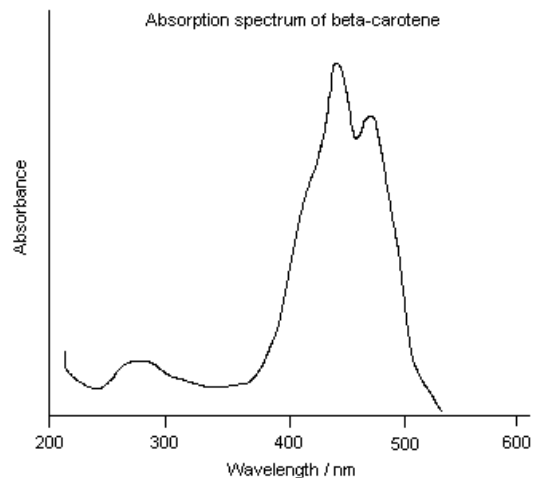
Lab Procedure (work in pairs):

Overview: You will use a spectrophotometer and a Beers Law plot (which you will create) to determine the amount of dye in a sample of sports drink. Most sports drinks are little more than salts, sugar and dye dissolved in water, making them ideal for this kind of analysis. By diluting a stock solution, you will make three solutions with known concentrations of dye, the absorbance of which you will measure and plot on a graph. You will also measure the absorbance of a sports drink. Finally, you will compare your standard solutions to the sports drink in Excel to determine the concentration of dye in the drink.

While working in the lab, you will use "volumetric glassware." Just like the balances can accurately measure mass, volumetric glassware can accurately measure volume. You will use two different pieces of volumetric glassware; a pipet (with pipet bulb) and a volumetric flask; the use of these will be demonstrated by the instructor.

Use of the volumetric flask:
1. Rinse the flask with several small portions of the desired solvent (in this case water).
2. In making a solution from a weighed solid it is best to dissolve the solid in small portions of solvent. Then transfer this concentrated solution to the volumetric flask and rinse the container several times with solvent, transferring the washings to the volumetric flask.
3. In making solutions from liquid or more concentrated solutions, pipet directly into the flask.
4. In either case (instructions 2 or 3) mix the solution well when about 3/4 of the solvent is added; it is okay to "shake" the flask if the solid material is not completely dissolved.
5. Then dilute to the mark. The bottom of the meniscus should just rest on the top of the mark. Be sure to look at the mark so that the back part is hidden by the front part. It is best to add the last few mL of solvent with a transfer pipet to avoid going over the mark.
6. Mix the solution thoroughly by holding the stopper (or parafilm) securely and invert the flask several times; ***SHAKING IS NOT RECOMMENDED***.

Step 1: Dilutions

1) Use a micro-pipette (P-1000) to transfer 1 mL (or 1,000 μL) of the concentration stock solution into a 100.00 mL volumetric flask. Fill the flask with DI water to the mark. Cover with a small piece of parafilm and invert several times. **This is solution 1.**

2) Use a 25 mL transfer pipette to transfer 25.00 mL of *solution 1* into a 50.00 mL volumetric flask. Cover with parafilm and mix as before. **This is solution 2.**

3) Use a 25 mL transfer pipette to transfer 25.00 mL of *solution 2* into a second 50.00 mL volumetric flask. Mix as before. **This is solution 3.**

Calculate the concentrations of each solution *in your lab notebook (show your work)*.

Step 2: Spectrophotometry Data collection

1) In order to gather meaningful data with a spectrophotometer, we must first measure the absorbance over a range of wavelengths to determine the wavelength that is most strongly absorbed by the dye. The "absorbance spectrum" will be displayed as a part of the pre-lab; sketch the absorption spectrum in your lab notebook, if not already done, and make sure to record the wavelength at the maximum absorbance (λ_{max} or "lambda max").

2) Make a table like the one below in your lab notebook and measure the absorbance at the λ_{max} for each of your samples, including the "drink." You will need to "blank" the spectrophotometer (once) before measuring your samples using water in the cuvette.

	Concentration	**Absorbance**
Solution 1		
Solution 2		
Solution 3		
Sports drink	To be determined…	

Step 3: Data analysis (to be done at a computer…one graph per pair…yes you can work together)

Now that you have all the data in the table above, you can determine the concentration of dye in your sports drink. To arrive at that answer, you must use Beers Law. Beers Law can be expressed in the equation below.

$$A = \varepsilon bc$$

Where *A* is absorbance and is dimensionless, *b* is path length or the distance light must travel through the sample in centimeters, *c* is the concentration in moles per liter (molarity, M), and *ε* is the molar absorptivity coefficient in units of cm^{-1}M^{-1}.

1) This analysis is a two-step process…first, determine the value of the molar absorptivity coefficient (*ε*). This is done by making a "Beers Law plot" in Excel of *A* (y-axis) vs. *c* (x-axis) for your known solutions (1, 2 and 3) ***DON'T INCLUDE THE DRINK ABSORBACNE***, then fit a trendline (linear) to those points; this is called a "standard curve" (although it is not a curve but a line). Select the option to display the equation for the trendline. The equation will be in the form y = mx + b, where m is the slope and b is the y-intercept (which should NOT be confused with the cell path length, *b*). **ENTER your trendline equation in your lab notebook.** (*Please note the the number of sig figs in the slope my only be one. If this is the case, "right-click" on the equation and format the text to "number"…1 decimal place.*)

The slope, m is equal to *ε* in the Beers Law equation.

2) Second…now that you know *ε*, you can use the absorbance of the drink and the Beers Law equation to solve for *c* in the sports drink; the path length (*b*) for the cuvettes you are using is 1 cm. **Show your work in your lab notebook.**

Once all data has been collected and the Beers Law Plot made, please turn in the graph and all carbon copies before leaving the lab.