7/07 Name________________________________

Introduction to Beer's Law: Colorimetry of Food Dyes

Introduction:

A simple and accurate method used to determine the concentration of colored solutions is spectrophotometry. The use of a spectrometer and the principles of Beer's Law, allow one to plot a graph of Absorbance vs. concentration of various colored solutions and obtain calibration curves (also known as standard or working curves or Beer's Law Plots). It is simple matter to then use these curves and determine unknown concentrations of these solutions.

Beer's Law states that the **absorbance (A)** of a solution is dependent on three factors: (1) the **molar absorptivity (a)**, the value of which depends on the absorbing species and on the wavelength used, (2) the **path length (b)** of the solution through which the light must past, and (3) the **concentration (c)** of the solution.

$$A = abc$$

What is the absorbance? To define it, we must first need to define the transmittance (t) which is simply the ratio of the intensity of the light transmitted by a sample (I) to the intensity of the light incident on the sample (Io):

$$T = I/I_o \qquad \%T = I/I_o \times 100\%$$

If **<u>all</u>** the light is absorbed by the sample, %T = 0; if **<u>no</u>** light is absorbed the %T = 100%. The absorbance is then defined by the equation:

$A = \log 10(1/T) = \log_{10}(I_o/I)$

We can see, therefore, that when T = 0.10 (%T = 10%), A = 1.0, and that when T = 100 (%T = 100%) that A = 0.00. The most accurate results are when the absorbance readings are in the range of A = 0.10 to 1.0 (or %T = 80% to 10%).

This experiment makes use of a single colored species at a fixed wavelength and all of the cuvettes have the same path length; therefore, a and b are constant. Thus, according to Beer's Law, a plot of the absorbance versus concentration should be linear with a y-intercept of zero. Of course, small deviations from this are to be expected. That is why knowing the R or R2 value is helpful…it indicates how closely the substance adheres (follows) Beer's Law.

<u>Procedure:</u>

Part A: Obtaining a Spectral Curve

Consult your instructor to find out which colored stock solution you should use. Then fill one cuvette about two-thirds full with distilled water (the blank) and a second cuvette with the same amount of your colored solution.

Set the spectrometer to scan from 800 to 400 nm. Calibrate using the blank, then insert the cuvette containing the colored sample. Click on Collect to run the scan. Make a note of the peak(s) you see for you solution. Record the wavelengths for them.

7/07 Name________________________________

Part B: Beer's Law

Then use the spectral curve that you produced to determine the wavelength at which that solution has its maximum absorption (λ_{max}). Use a 10 mL graduated pipet to make up the following dilutions:

(a) 1 mL stock : 4 mL distilled H_2O
(b) 2 mL stock : 3 mL distilled H_2O
(c) 3 mL stock : 2 mL distilled H_2O
(d) 4 mL stock : 1 mL distilled H_2O

Mix the solutions thoroughly. Calculate the Fraction Stock Solution for each dilution as a decimal fraction (i.e. 1 mL stock : 4 mL distilled water gives 1 mL stock/5 mL total solution, so the fraction of stock solution is 0.20).

Set the wavelength of the spectrometer to λ_{max}. Then insert a cuvette three-fourths filled with distilled water into the cell compartment (this is the blank) and again calibrate the spectrometer.

Rinse the other cuvette with a small amount of the 0.20 stock solution. Pour this out into the sink, then transfer enough of the 0.20 stock solution to three-fourths fill the cuvette. Insert the cuvette into the sample compartment and click on Collect. When it shows an absorbance, click on Keep, then type in the concentration of your standard (0.20) and click on Okay. **DO NOT** click on Stop. Repeat with each of the other dilutions and the undiluted stock solution, **<u>then</u>** click on Stop. Save the graph. Now put some of the solution prepared for you by Mrs. Sibert, and record the absorbance reading.

Calculations and Questions:

1. Use your graph and the absorbance of the unknown to determine its concentration.

2. Use the Equation of the Line to calculate X, which is your concentration. Compare this value with that obtained from using the graph itself.

3. How well did your solution adhere to Beer's Law? Explain.

4. Look at the original spectral curve for your color. Explain the obvious mismatch between the wavelengths of the peak(s) and the color of your solution. Label the color of each peak and low points on the spectral curve.