EXPERIMENT: ***Quantification of Food Dyes in Drink Mix using Beer's Law***

Adapted from Sigmann,S.B.; Wheeler, D.E. *J. Chem. Educ.* **2004**, *81*, 1475-1478.

GOAL: To use UV/Vis spectrophotometry and Beer's Law to establish a calibration curve for a common food dye and, using the curve, quantifying the amount of the food dye in a powdered drink mix.

INTRODUCTION/THEORY/QUESTIONS TO BE ADDRESSED:

Food dyes are used in many foods and drinks available at local grocery stores. Two common dyes are Red #40/Allura Red AC($Na_2C_{18}H_{14}N_2O_8S_2$) and Yellow #5 /Tartrazine ($Na_3C_{16}H_9N_4O_9S_2$). Dye solutions absorb light according to Beer's Law which states that the amount of light a solution absorbs is directly proportional to its concentration.

$A = \varepsilon bc$, where A is the Absorbance, ε is the molar absorptivity, b is the path length of the cuvette, and c is the concentration of the sample in solution.

Although the amount of dye in commercial products is not publicized, it can be determined by using standard dyes. A calibration curve is generated by making five serial dilutions of a standard commercial dye and determining the absorbance of each solution. Then the concentration of each solution is plotted against its respective recorded Absorbance, and a linear regression on the line generates an equation of the line. The Absorbance of the sample solution is recorded and the absorbance is used in the calibration equation to solve for the concentration of the sample since ε and b are constants.

PROCEDURE:

Making Serial Dilutions of the Food Dye Standard

Each student will receive one of the food dyes as a stock solution from which to make serial dilutions.

Stock solutions:

0.0535 g yellow #5 diluted to volume with water in a 500 mL volumetric yielding 200 uM yellow #5

0.0496 g red #40 diluted to volume with water in a 500 mL volumetric yielding 200 uM red #40

First note the concentration of the stock solution. Then, using a measuring pipet, take 20 mL of the 400 µM stock solution and transfer to a 200 mL volumetric flask. Dilute to the volume of the flask with water. This will be solution A (20 µM). Take 50 mL of solution A and transfer to a 100 mL volumetric flask. Dilute to the volume of the flask with water. This will be solution B (10 µM). Take 50 mL of solution B and transfer to a 100 mL volumetric flask. Dilute to the volume of the flask with water. This will be solution C (5 µM). Take 50 mL of solution C and transfer to a 100 mL volumetric flask. Dilute to the volume of the flask with water. This will be solution D (2.5 µM). Take 50 mL of solution D and transfer to a 100 mL volumetric flask. Dilute to the volume of the flask with water. This will be solution E (1.3 µM). Label all solutions with their respective letters and **exact** concentrations.

Making a Calibration Curve for the Food Dye

Measure the absorbance of each dye solution three times each. When determining the concentration of Red #40, detect at its maximum Absorbance wavelength of 503nm. When determining the concentration of Yellow #5, detect at its maximum Absorbance wavelength of 427nm. Plot the calibration curve of dye concentration versus average Absorbance and determine the equation of the line.

Preparing the Drink Mix Solution

To prepare the drink mix to test, perform the following dilutions. For orange KoolAid, measure 1.0 g of the KoolAid drink mix into a weigh boat and transfer to a **1 L** volumetric flask. For pink lemondade KoolAid, measure 1.0 g of the KoolAid drink mix into a weigh boat and transfer to a **500 mL** volumetric flask. Dilute to the volume of the flask with water.

Measuring the Absorbance of the Drink Mix Solution

Place the drink mix solution in a cuvette and measure its absorbance three times at the maximum Absorbance wavelength of the food dye of interest.

Determining the Concentration of the Food Dye in the Drink Mix

Calculate the average absorbance of the drink mix solution and, using the equation of the line of the food dye calibration curve, calculate the concentration of the drink mix solution. Determine how much food dye is in 1 g of the Kool-aid drink and then, based on the mass of the entire packet, how much is in the Kool-aid packet. Alternatively, calculate how much dye is in a serving size.

DISCUSSION:

Discuss the importance of making accurate serial dilutions in order to determine the KoolAid dye concentrations. Why are serial dilutions better than making the standard solutions individually?

MATERIALS:

- Measuring glass pipets
- Pipet bulbs
- Pasteur pipets
- Weigh boats
- Drink mix
- Dye Standards
- Volumetric flasks
- DI water
- Spectrophotometer
- Balance