EXP. 4 –DETERMINATION OF RED#40 AND BLUE#1 DYES IN KOOL-AID™

LABORATORY OBJECTIVES AND ASSESSMENTS

1. Understand the relationship between absorbance and concentration in Beer's Law.
   a. Discriminate between the need for single point or multiple point calibration.
   b. Calculate ε of a species at a specified wavelength.
   c. Using absorbance and ε, determine the concentration of an unknown dye in a solution.
   d. Relate the slope of a calibration curve to Beer's Law.
2. Understand the process of preparing standard solutions by serial dilution.
   a. Calculate the concentration of solutions made by dilution
   b. Express concentrations in molarity, %, or ppm.
   a. Use volumetric glassware properly.
   c. Design and describe a scheme for preparing a desired range of standards.
3. Understand the process of constructing a calibration curve.
   a. Correctly enter, inspect and plot data of concentration vs. response.
   b. Determine the least squares best-fit line to the data.

INTRODUCTION

Soft drinks are sold in a wide variety of colors: blue, red, yellow, purple or brown. These colors are usually the result of adding food colors, also called food dyes. While the other ingredients are usually similar among the soft drinks, they are not colored, so the addition of a food dye allows the product to have a trademark appearance. While some drinks have only one dye, most are a mixture of two or more so as to allow fine control of the color of the final product before bottling.

Food dyes absorb visible light intensely, so the amount added is very small. In this experiment we will determine the actual concentration of the red and blue dyes added to grape Kool-aid. To do this, it is first necessary to measure the pure dyes independently and from there construct **calibration curves** for each dye. Once the absorbance of the pure dyes is known, the measured absorbance of a mixture of these two can be used to determine the concentration of each dye in the mixture.

In the case of grape Kool-aid, the absorption of blue light by the red dye is negligible, as is the absorption of red light by the blue dye. So, in this case, we will be able to determine the concentration of red dye simply by measuring the absorbance at λ_{max} (red) and we can determine the concentration of blue dye simply by measuring the absorbance at λ_{max} (blue).

CONSTRUCTING A CALIBRATION CURVE.

For a discussion of Beer's Law and for the analysis of a mixture, refer to Harris chapters 18 and 19.

The absorption of light by matter is expressed by Beer's Law

$$A = \varepsilon bc \qquad (1)$$

where A is absorbance, ε , *epsilon,* is a constant of proportionality called the **molar absorptivity**, b is the path length through the solution, and c is the concentration of the absorbing species. It is important to realize that the absorbance of a species will be different at different wavelengths of light due to the different quantized energy states of the absorbing species. It follows that epsilon will a have unique value for each

wavelength. It is the case in most of our experiments that the path length of the cuvette will be 1.00 cm.

Last week we obtained ε (for bromine) by doing a single measurement of a solution of known concentration. In order to minimize (and quantify) the uncertainty in the measurement of ε, we must construct a standard calibration curve. Absorbance is measured for multiple solutions of different concentrations, and ε is then calculated from a plot of absorbance versus concentration, where the slope of the best-fit line (*m* in the linear equation, $y = mx + b$) is related to the ε in Beer's Law (Eq. 1).

$$A = (\varepsilon b)(\text{conc.}) + \text{intercept} \tag{2}$$

or substituting slope for εb (b is a 1.00cm.)

$$\text{slope} = \frac{(\mathbf{A} - \text{intercept})}{\text{conc.}} \tag{3}$$

Using the determined slope (at the specific λ_{max}) and the measured absorbance of the unknown solution one can determine the concentration of the absorbing species (in this case our red and blue dyes) in a sample of grape Kool-aid.

$$\text{conc.}_{\text{dye}} = \frac{(\mathbf{A} - \text{intercept})}{\text{slope}} \tag{4}$$

PREPARING STANDARD SOLUTIONS BY SERIAL DILUTION.

A serial dilution is a set of solutions created from a standard primary solution of a known concentration.

It is often necessary to prepare solutions of compounds that are very dilute (i.e., with very low concentration), especially if the measuring device is sensitive. This poses a problem in preparing a solution with precise concentration. For example, if you need a concentration of 0.1mg/L, you would have to weigh out 0.0001g and dilute to 1L. Due to the limitations of the balance the mass is not going to be very accurate or precise, and you would end up with 1L of solution when you only need 2-3 mL to fill a cuvette!

For example, in this experiment to prepare the 4 solutions (12.5 ppm, 5 ppm, 3.125 ppm and 1.25 ppm of the red and the blue dyes) for the calibration curves from a primary standard of 50 ppm dye, serial dilutions will be required. (Note: the 50 ppm Lab Stock Dye Solution will be supplied. Use the actual concentration on the label for calculations).

In every step of a dilution the amount of solute can be calculated using the relationship:

$$(C_{dilute})(V_{dilute}) = (C_{conc})(V_{conc}) \tag{5}$$

This process will work with any measure of concentration. In this lab the standards are prepared in concentration units of **parts per million, ppm**, which means grams of substance per million grams of total solution or mixture.

$$\text{ppm} = \left(\frac{\text{mass of solute}}{\text{mass of solution}} \right) \times 10^6 \tag{6}$$

We frequently equate 1 g of water with one ml of water, although this is only an approximation. Therefore 1 ppm corresponds to 1 $mg_{solute}/L_{solution}$ (=1 μg/mL).

We will want to prepare solutions of known concentrations of the dyes using the process of **serial dilution** for our calibration curves for the two dyes. Starting from a known Lab Stock solution four solutions of accurately known concentrations can be prepared following the process described below.

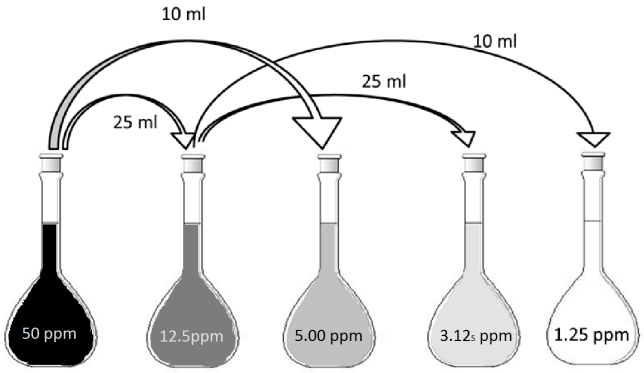

Figure 1. Schematic of the **Serial Dilution** process used for both dyes for this experiment.

To prepare:

12.5 ppm dye => pipet 25 ml of 50 ppm Lab Stock Dye Solution into 100-ml volumetric flask, swirl to mix, fill to the mark and invert to mix.

Using eq. 5 the concentration of the resulting solution is calculated as follows

$$C_{dilute} = \frac{(50.00\text{ppm})(25.00\text{mL})}{100.0\text{mL}} \quad (7)$$

$$C_{dilute} = 12.50 \text{ ppm} \quad (8)$$

5.00 ppm dye => pipet 10 ml of 50 ppm Lab Stock Dye Solution into 100-ml volumetric flask, swirl to mix, fill to the mark, and invert to mix.

3.125 ppm dye => pipet 25 ml of prepared ~12.5 ppm Dye Solution into 100-ml volumetric flask, swirl to mix, fill to the mark and invert to mix.

1.25 ppm dye => pipet 10 ml of prepared ~12.5 ppm Dye Solution into 100-ml volumetric flask, swirl to mix, fill to the mark and invert to mix.

EXPERIMENTAL PROCEDURE

WORK WITH A PARTNER

Stock solutions

An accurately known Lab Stock Standard solution containing ~50 ppm Red #40 and another containing ~50 ppm Blue #1 will be available. The bottles will be labeled with actual concentrations. **In a clean, dry and labeled container obtain an aliquot and record the Lab Stock Standard concentrations for each dye in your notebook**. With all solutions, be conservative; take only what you need, and NEVER pour anything back into a stock container.

Volumetric glassware must be used for all dilutions. Rinse glassware between different solutions with three rinses with the solution to be used to prevent from contamination from previous solutions.

Preparation of Calibration solutions:

Preparation of Red #40 Solutions.

- Prepare four Red #40 solutions for calibration containing 12.50, 5.000, 3.125 and 1.250 ppm in DI water.
- Prepare 100 mL of a 12.5 ppm (nominal concentration) solution as described above.

- Mix and pour into a clean, dry, and labeled beaker. Thoroughly rinse your 25 mL pipet (x3) with the 12.5 ppm solution.
- Pipet 25 mL of this 12.5 ppm solution into a rinsed 100 mL volumetric flask.
- Mix and dilute to the mark with DI water and mix thoroughly. This will give you 100 mL of a solution with the nominal concentration of 3.125 ppm.
- Pour this solution into a labeled beaker.

Continue the dilutions, using Table 1 as a guide, to prepare 100 mL of the remaining calibration standard solutions. Be sure to rinse the pipets and Vol. Flask each time a new solution is prepared.

Table 1. Calibration Solution Dilutions – Red#40
Stock Standard Red#40 concentration _____ppm
λ_{max} for Red#40 _____ nm

Starting Conc., ppm	Vol. pipetted, mL	Final Vol., mL	Final Conc., ppm	Absorbance
	25	100		
	10	100		
	25	100		
	10	100		

Preparation of the Blue #1 Solutions.

Prepare four Blue #1 standard calibration solutions containing 12.50, 5.000, 3.125 and 1.250 ppm in DI water using the same procedure as for the Red #40 solutions.

Table 2. Calibration Solution Dilutions – Blue#1
Stock Standard Blue#1 concentration _____ppm
λ_{max} for Blue#1 _____ nm

Starting Conc., ppm	Vol. pipetted, mL	Final Vol., mL	Final Conc., ppm	Absorbance
	25	100		
	10	100		
	25	100		
	10	100		

Absorbance Spectra and λ_{max} for red dye and blue dye:

- Open LoggerPro, connect the spectrophotometer and allow it to warm up. Obtain one cuvette. Calibrate the spectrophotometer with DI water. Use a clean and rinsed cuvette for each trial. Always fill the cuvette approximately ½ full.
- Rinse the cuvette by putting ~2-3 mL of the 12.5 ppm Red#40 dye solution into the cuvette. Pour into a waste container and repeat two more times.
- Now add enough dye solution to fill the cuvette ~½ way and place in spectrophotometer.
- To obtain the absorbance spectrum of the solution click the green "Collect" button. When the spectrum appears, click the red "Collect" button and save the file.

- Record the absorbance spectrum and determine λ_{max} using the "Examine" tool. λ_{max} is the wavelength at which there is maximum absorbance. If the absorbance at the λ_{max} is >1.0 then dilute the solution with DI water and remeasure the absorbance.
- Record the resulting absorbance at λ_{max} for the Red #40 dye. (This should be between 400nm and 700nm.)
- SAVE this file. Repeat the procedure to obtain and record the absorbance at λ_{max} for the Blue #1 dye.

Calibration Curves for red dye and blue dye.

- Select the rainbow icon to configure the spectrophotometer for the Absorbance vs. Concentration mode and select the appropriate radio button. To select the appropriate wavelength, unclick the default λ_{max}, and select the λ_{max} for the dye of interest, i.e., the wavelength of the maximum absorbance.
- Change the units to ppm and click OK.
- Calibrate the spectrophotometer at λ_{max} for the Red dye with the blank (DI water).
- With the blank still in the spectrophotometer click the green Collect button and then the KEEP button. Enter 0.00 for the concentration of the dye in the blank.
- Rinse and fill the cuvette with the most dilute Red #40 standard and insert into the spectrophotometer. Select the KEEP button and enter the <u>actual</u> concentration, in ppm, of the respective dye solution. Record the Absorbance in Table 1.
- Continue with the next most dilute Red #40 solution until the Absorbance of all calibration solutions have been recorded. **Save the file**.
- Change the wavelength to the λ_{max} recorded the Blue#1 dye and repeat for the Blue #1 standard solutions. Again SAVE your file.

Kool-Aid Unknowns

The Kool-aid sample is prepared according to the package directions and supplied in the laboratory.

Absorbance Spectrum of Grape Kool-Aid

- Rinse and fill the cuvette with the grape Kool-aid sample. Obtain and save the visible absorbance spectrum (A vs. λ) of the Kool-aid.

Absorbance of dyes in Grape Kool-Aid

- With the spectrophotometer set in the Abs. vs. concentration mode and the wavelength set to the **λ_{max} for the Red dye**, **record the absorbance**. If the Absorbance is above the absorbances from your calibrations dilute the Kool-aid 1:2 using volumetric glassware and remeasure.
- Repeat to obtain three discrete measurements of **Abs. of the Red dye** in the Kool-Aid.
- **Reset the wavelength to the λ_{max}** recorded for the Blue#1 dye.
- Using the same Kool-aid sample **obtain three discrete measurements of Abs.** of the blue dye in the Kool-Aid.

Calculation of Concentration (ppm) of dyes in Grape Kool-Aid

With the observed absorbances for the Kool-aid and the slopes of the respective calibration curves calculate the concentration with uncertainties in ppm of the Red#40 dye and the Blue#1 dye in the sample. Using Eq. 9 (Eq. 4 including the uncertainties) we have

$$\mathbf{conc._{dye}} = \frac{((\mathbf{A_{mean}} \pm sd) - (\mathbf{intercept} \pm \sigma_{\mathbf{y}}))}{\mathbf{m} \pm \sigma_{\mathbf{m}}} \qquad (9)$$

where A_{mean} is the **mean** of the three measured absorbances with uncertainty, **intercept** is labeled b(Y–intercept) (see the data box from LoggerPro) and is m = slope(**ε**). Be sure to record the respective uncertainties from the LoggerPro plot.

WHAT TO DO

To Be Turned in From Exp. 3-Bromination of Acetone:

Please hand in the following items in a packet STAPLED and in the following order:

a) Rate Equation

Write a summary of the Rate Law found by this experiment clearly showing;

a) value for average k (with units).
b) calculated rate law.
c) rounded rate law.
d) completed **Table 2. Results of Reactions at Room Temperature** from your notebook.

b) Answers to Questions

Compile on one to two pages from your lab notebook.
Always restate question and report answers to the questions in complete sentences.

c) Two Figures (Copy plot only, paste into Word Doc., add appropriate Title, add appropriate figure caption, print each figure on a full page, landscape, etc.).

a) Figure 1- A plot of Abs. vs. λ including λ_{max}. Use the figure caption for annotations.
b) Figure 2- A plot of all Abs. vs. time runs overlaid onto one plot. Use single letters to label each line in the plot and the figure caption should include the respective linear fit slopes.

Be Sure To Put Your Name on Every Page.

For Experiment 4

Before Lab: Read this experiment. Fill in the "Title bar" to include the experiment #, title, your name, partner's name, date, lab section, and TA's name. This information should be included on each page in your laboratory notebook for every experiment.
All prelab material should be **written in your laboratory notebook** and will checked by your CA prior to lab.
For this week's experiment your Prelab write-up should include, in addition to the completed title bars, the following sections.

NOTE: Tables in notebook should always be prepared larger than the samples in the lab write-up. Always leave plenty of room for data entry into the tables.

1. **Purpose** - After carefully reading the experiment, briefly state what you understand to be the purpose of this lab exercise.
2. **Table of Reagents** - Prepare a complete a Table of Reagents in your lab

notebook. Note that a table always has a number and a descriptive title. **ALL WASTES IN THIS EXPERIMENT CAN BE RINSED DOWN THE SINK WITH WATER.** The reagents include Red#40 and Blue#1.

3. Two tables for summarizing the dilution volumes, concentrations and resulting absorbances for the calibration dye solutions (see Tables 1 and 2).
4. A table, prepared for recording Absorbance for the Kool-Aid at both λ_{max}'s.

During lab: Carry out the procedure as written. Modify your procedure if you find it necessary, but be sure to record the modifications. You should record all observations and measurements in your notebook. Show sample calculations in your laboratory notebook. Be sure to obtain the data for all measurements in the experiment.

To be turned in: You will write a formal Results and Discussion for this lab. There is a template available on Moodle describing what should be in this report. You are collecting data in pairs BUT the written report is individual. You may work on the figures with your partner but make sure that when it comes to writing you are working alone. You will be turning in the report to Turnitin electronically as well as a paper copy (with copy pages Appendix).

RESULTS

Make sure that you introduce all of your tables and figures (not "graphs" or "plots") in the body of the Results section before you display them in your report. Text in the body of the Results section must lead the reader through the figures and tables. When referring to values in tables, indicate which numbers are measured data and which are calculated values. We expect that a good Results section will have more than one table. The content of these tables should include the final concentrations of your standard solutions, λ_{max} and the corresponding absorbance, slopes and uncertainties, and the absorbance of the sample and the calculated dye concentrations. Your first Figure should clearly plot the overlaid absorbance spectra for the two dyes individually. The second Figure should be the Absorbance spectrum (Abs vs. λ) for the Kool-aid. The last Figure(s) should clearly plot the two calibration curves and contain the line equation and R^2 value. Note: tables have titles and figures have captions. Figure captions should say what is contained in the figure and list any numerical values not easily discerned (such as the λ_{max}). The plots must be copied from LoggerPro and pasted into the formal report document. Above all else, your Results section should be a clear and linear narrative using the figures and tables to illustrate how you moved through the experiment and how the data builds on itself to reach the final answer.

DISCUSSION

A discussion is just that, discuss what your results say. Don't assume that just because your audience read the results they have drawn all the necessary conclusions. You need to discuss them. For example, you measured two different dyes but at the same concentrations. Were the slopes the same? Is this expected? Why might they be different? No really, what property of the molecules makes the slopes different? Is this OK? Primarily in this experiment you are trying to quantify the concentration of the dyes in the Kool-Aid but you don't have a known or "true" value for comparison. Therefore, you must convince your reader that you have done a good job in making your measurements. This can be achieved with a thorough description of the uncertainty in concentration and its sources. Use the propagation of error in your calculated concentration to identify the largest source of experimental uncertainty, and how the procedure could be modified to improve the uncertainty in the calculated concentration

of dye. Note: Citing "Student error" or "Human error" is not an acceptable part of any Discussion section.

APPENDIX

This section is used to include important items that don't necessarily fit in Results and Discussion or would be awkward to include. For this lab your Appendix should include your copy pages that show your calculations. Your propagation of uncertainty calculation must also be in your Appendix. This allows the reader to go look at the calculations if they want to further understand an item in a table or in the text. In the body of the Results or Discussion be sure reference the specific page in the Appendix where the calculation can be found.