A Beer’s Law Experiment
Prelab

1. What is the purpose of this experiment?

2. Using the absorbance versus wavelength curve given in Figure I, determine the approximate value of \( \lambda_{\text{max}} \) of the dye used to construct this curve. Then using this value of \( \lambda_{\text{max}} \) and the color wheel, determine the color of this dye.

3. Using the calibration curve and trend line equation given in Figure II, determine the concentration of a dye solution if its absorbance was measured to be 0.351. Then using this concentration and given that the volume of the dye solution was 100.0 mL, the molar mass of the dye is 466.56 g/mol, and the mass of Froot Loops® used to extract the dye was 2.5173 g, calculate the mass percent of the dye in the Froot Loops®.
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Introduction

There are many ways to determine concentrations of a substance in solution. So far, the only experiences you may have are acid-base titrations. There are other properties of a solution that change with concentration such as density, conductivity and color. Beer’s law relates color intensity and concentration. Using color can be much faster than doing a titration, especially when you have many samples containing different concentrations of the same substance. The tradeoff is the time required to make a calibration curve.

When colored solutions are irradiated with white light, they will selectively absorb light of some wavelengths, but not of others. This is because of the relationship between the electrons in a molecule (or atom) and its energy. Electrons in molecules and atoms are restricted in energy; occupying only certain fixed (for any given atom or molecule) energy levels. The electrons in molecules can jump up in energy levels if exactly the right amount of energy is supplied. This amount of energy can be provided by electromagnetic radiation. Visible light is one form of electromagnetic radiation. When this happens, the particular energy of light, which corresponds to a particular wavelength or color, is absorbed and disappears. The remaining light, lacking this color, shows the remaining mixture of colors as non-white light. A color wheel, shown below, illustrates the approximate complementary relationship between the wavelengths of light absorbed and the wavelengths transmitted. For example, a blue substance would absorb the complementary (opposite it in the color wheel) color of light, orange.

![Color Wheel Diagram]

When light is not absorbed, it is said to be transmitted through the solution. The wavelength or wavelengths that a substance absorbs can be determined by exposing the solution to monochromatic light of different wavelengths and recording the light transmitted.
If light of a particular wavelength in not absorbed, the intensity of the beam directed at the solution \((I_o)\) would match the intensity of the beam transmitted by the solution \((I_t)\). If some of the light is absorbed, the intensity of the beam transmitted through the solution will be less than that of the original intensity. The ratio of \(I_t\) and \(I_o\) indicates the percentage of incoming light absorbed by the solution. This is called the **percent transmittance**.

\[
\%T = \left(\frac{I_t}{I_o}\right) \times 100
\]  

(1)

A more useful quantity is the **absorbance** \((A)\) defined as

\[
A = -\log(\%T/100)
\]  

(2)

The higher the absorbance of light by a solution, the lower the percent transmittance. The wavelength at which absorbance is highest is the wavelength to which the solution is most sensitive to concentration changes. This wavelength is called \(\lambda_{\text{max}}\). \(\lambda_{\text{max}}\) is usually found by plotting absorbance versus wavelength for a variety of wavelengths. Figure I shows an example of this type of plot.

Once \(\lambda_{\text{max}}\) is determined, you can demonstrate how three variables influence the absorbance of a solution. They are the concentration \((c)\) of the solution, the pathlength \((b)\) of the light through the solution (also called the cell length) and the molar absorptivity \((\varepsilon)\); the sensitivity of the absorbing species to the energy of \(\lambda_{\text{max}}\). The pathlength, is usually fixed at 1.00 cm. The molar absorptivity depends on the substance, the solvent and \(\lambda\). The units for molar absorptivity are L/mole·cm for concentration in mole/L. **Beer’s law** is:

\[
A = \varepsilon bc
\]  

(3)

With this equation (or a calibration curve based on it), you can determine an unknown concentration or estimate what the absorbance of a certain solution will be as long as three of the four values in the equation are known.

In the first part of this experiment, you will determine the molar absorptivity of one of the dyes used as artificial food colors. You will vary the concentration of your solution and make a calibration plot of absorbance versus concentration. Beer’s law shows that absorbance is linearly
related to concentration. To determine the molar absorptivity, the slope of the best fit straight line is divided by the pathlength (usually 1). Figure II is an example of a calibration plot.

It should be noted that there are conditions where deviations from Beer’s law occur. This happens when concentrations are too high or because of lack of sensitivity of instrumentation.

In the second part of the experiment, you will determine the mass percent of your chosen dye in a consumable product sample, Froot Loops® cereal. Currently only 7 non-natural compounds are certified for food use in the US, with five being most commonly used. The structures and formulae of the five are given below:

<table>
<thead>
<tr>
<th>Dye</th>
<th>Common name</th>
<th>Chemical Formula</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red #40</td>
<td>Allura Red AC</td>
<td>Na₂C₁₈H₁₄N₂O₈S₂</td>
<td>Orange-red</td>
</tr>
<tr>
<td>Blue #1</td>
<td>Brilliant Blue</td>
<td>Na₂C₃₇H₃₄N₂O₉S₃</td>
<td>Bright blue</td>
</tr>
<tr>
<td>Blue #2</td>
<td>Indigotine</td>
<td>Na₂C₁₆H₈N₂O₈S₂</td>
<td>Royal blue</td>
</tr>
<tr>
<td>Yellow #5</td>
<td>Tartrazine</td>
<td>Na₃C₁₆H₉N₄O₉S₂</td>
<td>Lemon yellow</td>
</tr>
<tr>
<td>Yellow #6</td>
<td>Sunset Yellow</td>
<td>Na₂C₁₆H₁₀N₂O₇S₂</td>
<td>Orange</td>
</tr>
</tbody>
</table>

You will accurately weigh a sample containing a dye, extract the dye to make a solution and measure its absorbance. Using the calibration curve you obtained in the first part, you can determine the concentration of the dye from the graph.
Figure I: Absorbance versus wavelength plot of a food dye to determine $\lambda_{\text{max}}$.

Figure II: Absorbance versus concentration plot of the same food dye in Figure I. The line goes through 0,0. The slope is $\varepsilon$ and $R^2$ gives the fit (will be 1 if the fit is exact).
Procedure

Part I

1. Make the following dilutions to one of the dye solutions: (pipet volume/volumetric flask volume):
   10/100, 20/100, 20/50, 25/50

   Calculate the molarity (mole/L) of each diluted solution from the known molarity of the stock dye solution.

On the Netbook:

2. Double click LoggerPro.

Record Spectrum of Dye

3. Fill a cuvette with distilled water to make a blank. Dry the cuvette with a Kimwipe.

4. On the menu bar, select Experiment, Calibrate, Spectrometer:1. Wait for lamp to warm up.

5. Place blank in spectrometer with the line on the cuvette toward the arrow on the spectrometer. Click Finish Calibration. When it goes gray, click OK.

6. Put the stock dye solution in a different cuvette. Place it in the spectrometer the same way as the blank.

7. Click on the far right. When the spectrum appears, click .

Calibration Curve

8. Click to Configure Spectrometer to collect the calibration curve data.

   Under Collection Mode, select Absorbance vs Concentration. A $\lambda_{max}$ has already been selected (check mark on wavelength in list). Units of concentration should be mol/L. Single 10 nm band should be the option used. Click OK. A box will appear, asking whether you want to save data. Click Yes.

9. Rinse the cuvette with distilled water, then fill with distilled water. Place in spectrometer as before. Click . The absorbance should be very close to 0.000. Do not click the stop button until step 15.

10. Click Keep. A dialog box will appear asking you to enter the concentration. Enter 0, then click OK.
11. Rinse the cuvette 2x with the 10/100 solution, then fill. Place in spectrometer. Click keep after about 5 seconds. Enter the concentration (from step 1 calculations). A concentration of $1.00 \times 10^{-4}$ would be entered as $1.00e-4$. Click OK.

12. Repeat 11 with the 20/100, 20/50, 25/50 dilutions and the original solution. You will have 6 data points on the graph.

13. Click stop.

14. Make sure the calibration curve is selected by clicking on it. On the menu bar, click Analyze, then Linear Fit, or click . Look at the correlation value. A perfect fit is indicated with a value of 1. You should get 0.998 or better.

15. On the menu bar, click File, then Export As. Chose CSV (Excel, InspireData, etc). Save on your flash drive.

**Part II Extracting Food Dye from Froot Loops® Cereal**

1. Select the proper color Froot Loops® rings that contain the food dye that you used in Part I of the experiment. Ask the instructor for details. Record the color chosen.

2. Determine the mass (to 0.0001 g) of 10 rings in a tarred 50-mL beaker.

3. Grind the rings to a fine powder with a ceramic mortar and pestle.

4. Measure 25.0 mL of distilled water in a graduated cylinder and use the water to rinse the Froot Loops® powder into a 100-mL beaker. Rinse the mortar and pestle into the beaker with a little water from a wash bottle.

5. Using a hot plate, heat, with stirring, the Froot Loops® slurry until it just starts boiling. Remove from the hot plate and let the mixture cool to the touch.

6. Add 25.0 mL (graduated cylinder) of acetone to the cooled slurry.

7. Stir the slurry/acetone mixture on a cooled stirrer-hotplate until the solids settle easily and give a clear (not colorless) solution. This should be done for at least 5 minutes.

8. Letting the mixture settle, decant and filter the solution in the beaker directly into a 100-mL volumetric flask using fast, fluted 11.0 cm filter paper and a funnel. Keep as much of the solids in the beaker as possible to prevent the filter from clogging. Use a ring to support the funnel. Make sure the tip of the funnel is well into the neck of the volumetric flask so you do not lose any solution.

9. After nearly all the solution has drained into the flask, rinse the solids in the beaker once with about 10.0 mL of 1:1 acetone/water, let the mixture settle, then filter as before.
10. Fill the flask to the mark with 1:1 acetone/water.

11. Measure the spectrum (Part I, steps 5 – 9), then record the **absorbance** of the solution at the $\lambda_{\text{max}}$ used in part I. Use 1:1 acetone/water as the blank.

**Data Treatment**

Include the following information, **with some description of what you are doing.**

Show all the calculations for the determination of the concentrations of the diluted solutions used to make the calibration curve.

Plot the spectrum of the dye solution (A vs wavelength) in Excel. Do not show the data points – just a smooth curve through the points. Fix the wavelength range from 400 to 700 nm. Do not include the data page for this graph – there is too much data.

Plot the calibration curve (A versus concentration) in Excel. Include the four dilutions, the blank (an absorbance of 0.0 at 0.0 M concentration) and the original solution, for a total of 6 data points. Determine the molar absorptivity, $\varepsilon$, from the slope. Do not connect the points. Fit a linear trendline to the clearly indicated data points. Remove gridlines and adjust the font size for all your labels. Use the whole page. Include the equation of the line and the $R^2$ value on the plot. The $R^2$ value should be similar to what you saw in the laboratory.

Calculate the concentration of the solution in Part II using Beer’s Law.

Knowing the concentration and volume of the solution, the molar mass of the dye, and the mass of the Froot Loops sample, calculate the **mass percent** of the dye in the sample (as in the prelab).

**Conclusion**

Restate the $\lambda_{\text{max}}$ of the dye, the molar absorptivity of the dye (don’t forget the units), concentration of the dye (be sure to include which specific dye) in the Froot Loop extract (indicate the color of the Froot Loop used), and the **mass percent of dye** as a conclusion. Also address the following:

Does your solution obey Beer's Law? How do you know?