Experiment 3: CONCENTRATION
OF DYE IN GATORADE

Purpose: The concentration of the dye present in Gatorade is to be determined spectrophotometrically. A standard curve of the dye is prepared for this purpose. It is also used to provide us with the extinction coefficient of the dye.

Introduction: White light is composed of many different wavelengths of light combined together. A spectrophotometer is an instrument that shines a single wavelength of light of a known intensity into a solution and then measures the intensity of the light exiting the solution. If a solution contains any compound that absorbs light, the intensity of light exiting the solution will be less than what entered the solution. On the other hand, if none of the compounds in the solution absorb at that wavelength of light, then the intensity of the light exiting the solution will be the same as that entering.

The intensity of light that passes through an absorbing solution is dependent on the concentration of the absorbing compound in solution. In other words the more material in the solution absorbs the light, the less light will get through. This relationship is expressed by Beer’s Law (Equation 1).

\[ A = \varepsilon \ell c \quad \text{Equation 1} \]

“A” is the absorbance observed. "\( \varepsilon \)" is the extinction coefficient, also referred to as the absorptivity of the compound. It is characteristic of the substance that is absorbing light at a given wavelength. It represents how much light is absorbed at a particular wavelength. The units for this coefficient depend on the units used for the concentration and pathlength. Even for the same compound, the extinction coefficient varies with wavelength therefore it has no meaning without reference to the wavelength of the light. A green material might absorb a photon at 617 nm but may allow a photon at 448 nm to pass completely through it. The wavelength at which light is absorbed the most is called the \( \lambda_{\text{max}} \) (pronounced lambda-max).

“\( \ell \)” is the pathlength of the light through the solution. For most standard cuvets, the pathlength is 1.00 cm. “c” is the concentration of the solution, the units of which must be defined. Note that for a given substance at a particular wavelength, \( \varepsilon \) and \( \ell \) are both constants. If we combine them into one constant, the equation simplifies to show that \( A \) is directly proportional to the \( c \) of that substance (Equation 2).

\[ A = k c \quad \text{Equation 2} \]

If we were to prepare several solutions of various concentrations of the solute, measure the absorbance of each solution and then plot absorbance versus concentration, we should get a straight line with a y-intercept of zero and a slope of \( k \) (where \( k = \varepsilon \ell \)). With this graph (known as a standard graph or standard curve), we would be able to determine the concentration of the solute in any solution just by measuring its absorbance. Study Appendix 1, which has a discussion on standard curves.
Associated with absorbance, is the term, *transmittance*, $T$. It is the ratio of the intensity of light that emerges after passing through a material, $I$, to the input light intensity, $I_0$.

$$T = \frac{I}{I_0}$$  \hspace{1cm} \text{Equation 3}$$

If all of the light passes through a material (and none is absorbed), $A = 0$ and $T = 1$; in this situation, percent transmittance, $\%T = 100T = 100$. The relationship between absorbance and percent transmittance is expressed by Equation 4.

$$A = -\log T$$  \hspace{1cm} \text{Equation 4}$$

In older designs of the spectrophotometer, the $\%T$ scale is much easier to read because it is linear, as compared to the $A$ scale which is logarithmic. For this reason, we record $\%T$ and then calculate $A$. For newer designs, such as the instrument you will be using, the readings are digital and thus you will not have to deal with $\% T$. However, you should be aware of what transmittance stands for and how it is related to absorbance.

We had spoken of absorbance being specific for a given wavelength. How do we know which wavelength to use? It would make sense to take our absorbance measurements at the wavelength at which the solute is absorbing the strongest. This would be $\lambda_{\text{max}}$.

![Absorbance vs. Wavelength for Food Dyes](image)

Figure 3.1

To find $\lambda_{\text{max}}$, a plot of absorbance vs. wavelength for a given substance is collected. This plot is called an absorption spectrum. There are scanning spectrophotometers that can scan wavelengths very quickly to create an absorption spectrum. In your case, the spectrophotometers you will be using must be re-zeroed with water every time a new wavelength is
chosen. This is because water absorbs differently at each wavelength and therefore a different correction term must be subtracted out at each wavelength to compensate for it. After all, when we are measuring the absorbance of a solution, we do not want to be measuring the absorbance of the water as well. This makes collecting data for an absorption spectrum tedious, therefore you will not be asked to prepare it yourself, but it is provided in the graph shown above (See Figure 3.1). Use these spectra to select the appropriate \( \lambda_{\text{max}} \) for the color of dye assigned to you.

In this experiment you will be assigned a Gatorade of a particular color (red, yellow or blue) containing Red dye #40, Yellow dye #5 or Blue dye #1. You will first prepare a set of standard solutions (solutions of various but known concentrations) using the dye assigned to you and prepare a standard graph at the \( \lambda_{\text{max}} \) for that dye. You will use this standard graph to determine the concentration of the dye in a Gatorade sample. Study Appendix 1 which explains about trendlines and the \( R^2 \) Value.

Unfortunately, Beer’s Law only holds true at lower absorbance values. How low depends on the sensitivity of the spectrophotometer that you are using. The spectrophotometers that you will be using are sensitive to about an absorbance of 0.8. Above an absorbance of 0.8, the instrument becomes saturated and the readings no longer maintain a linear relationship. Keep this in mind as you prepare your standard solutions. The concentration of the Gatorade sample must also be such that the absorbance is below 0.8.

The original Gatorade samples will be too concentrated. To ensure that the absorbance reading of the Gatorade sample is within the usable range, you will need to prepare dilutions. When determining which dilution you should prepare, keep in mind that you should keep it simple such as a 1:2 or a 1:5 dilution. A 1:2 dilution indicates that the original solution has been diluted by adding enough solvent to double the volume. The diluted solution would therefore be \( \frac{1}{2} \) the concentration of the original. For a 1:5 dilution, you would add water to 1 mL of Gatorade to reach a total of 5 mL.

You might remember that volume is not additive. In other words, 1 mL of A added to 2 mL of B does not always add to 3 mL total, but in this experiment, for simplicity, we are going to treat volume as being additive. Thus, if we mix 2 mL of water to 6 mL of Gatorade, we assume the total volume to be 8 mL. To calculate the concentration of the diluted sample, use Equation 5. In our example, \( V_1 \) is 2 mL, and \( V_2 \) is 8 mL (the total volume, not 6 mL).

\[
C_1V_1 = C_2V_2
\]

Equation 5

\[
C_2 = C_1 \frac{V_1}{V_2} = C_1 \frac{2\text{ mL}}{8\text{ mL}} = C_1 \frac{1}{4}\text{ or } C_1 \frac{1}{4}
\]

In other words \( C_2 = \frac{1}{4} C_1 \). This is a 1:4 dilution.

You have probably come across a similar equation in your first semester of General Chemistry when you learned to do “dilution problems”: \( M_1V_1 = M_2V_2 \). When we use \( M \), we are referring to concentration in units of molarity (mole/L). The only difference here is we are
using C for concentration and not limited to units of molarity but could be in other units such as percent.

Food colors have been used for centuries because we have come to expect that our food will look a certain way. We expect that orange flavored drinks will be orange. They also help us identify the flavor of a food. For instance, lime and orange sherbets would be nearly indistinguishable based on appearance if not for the green and orange colors. In addition coloring agents add a festive appearance to foods—M&Ms candies if they were all colored gray, would certainly be less appealing. How many of you as kids ordered your snow cones based on color?

Historically coloring agents have also been added to foods to cover up inferior quality foods. At the beginning of the 20th century, when there was no regulation of color additives in this country, coloring agents were added to foods to mask spoiled foods, and some coloring agents marketed for inclusion in foods were poisonous. Since passage of the Federal Food, Drug, and Cosmetic (FD&C) Act of 1938, color additives in the U.S. have been the responsibility of the Food and Drug Administration (FDA). Dyes are still used to cover inferior products, but now the dyes must be approved (and the food must also meet basic quality inspections). At present, there are seven color additives certified for food use. Most are organic molecules similar to allura red (FD&C Red No. 40) that will be studied in this experiment. Its structure is shown in Fig. 3.2.

![Fig. 3.2. FD&C Red Dye No. 40 (Allura Red).](http://vm.cfsan.fda.gov/~dms/cos-221.html)
**Procedure:** *Work with one partner.*
You and your partner will be assigned a Gatorade of a particular color. Record the color in your notebook. Your standard curve is to be prepared for this particular color of dye.

**Preparation of a standard curve:**

1. Obtain a sample of stock dye solution of the color assigned to you and record the concentration from the bottle. Record the molar mass of the dye corresponding to color of your Gatorade.
   - Red No. 40  (molar mass 496.43 g/mol)
   - Yellow No. 5  (molar mass 534.35 g/mol)
   - Blue No. 1  (molar mass 791.84 g/mol)
2. From Figure 3.1, record the $\lambda_{max}$ for your dye.
3. Use two graduated cylinders and two dropper (one for the dye, and one for water) to prepare a series of dilutions that will be used to make a standard curve. Use the table below as an approximate guide for your dilutions. Record the exact volumes used, to the nearest $0.02\,\text{mL}$, in your lab notebook. You do not need to measure out exactly $9.00\,\text{mL}$ of original sample, but should record exactly what volume you used. Transfer each solution into a labeled, clean, and dry test tube (not a cuvet). To ensure that each tube is properly mixed, use a small piece of Parafilm to make a tight seal over the opening of each test tube and invert it multiple times. Ask your instructor to show you how to use Parafilm.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Volume of Original Sample</th>
<th>Volume of DI Water Added</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.00 mL</td>
<td>1.00 mL</td>
</tr>
<tr>
<td>2</td>
<td>8.00 mL</td>
<td>2.00 mL</td>
</tr>
<tr>
<td>3</td>
<td>6.00 mL</td>
<td>4.00 mL</td>
</tr>
<tr>
<td>4</td>
<td>4.00 mL</td>
<td>6.00 mL</td>
</tr>
<tr>
<td>5</td>
<td>2.00 mL</td>
<td>8.00 mL</td>
</tr>
<tr>
<td>6</td>
<td>1.00 mL</td>
<td>9.00 mL</td>
</tr>
<tr>
<td>7</td>
<td>0.00 mL</td>
<td>10.00 mL</td>
</tr>
</tbody>
</table>

4. Follow the instructions for the Unico 1100 spectrophotometers (Appendix 6), to measure the absorbance of each standard at the $\lambda_{max}$. When using the spectrophotometer, you will need to transfer each standard to a cuvet. To ensure that you do not spill sample inside the instrument, the cuvet should never be filled more than three-fourths full. Use Standard 7 as your blank to zero your spectrophotometer. Record the absorbance in your lab notebook. Remember to record the Instrument ID # in your lab notebook.

**Analysis of a Gatorade sample:**
*It is important that the absorbance of the Gatorade samples fall within the range covered by the calibration standards, and none has an absorbance above 0.8. Often unknowns are prepared at several dilutions, and then all are used to determine the concentration.*

*You will be measuring the absorbance of the original undiluted Gatorade and three diluted samples of the Gatorade. You will decide how to dilute the Gatorade. The objective is to have three diluted solutions that are in the “usable range.”*
5. First measure the absorbance of the undiluted Gatorade sample (Gatorade 1). Depending on how high the reading is, predict which three dilutions (Gatorade 2, Gatorade 3 and Gatorade 4) to prepare to ensure that they all fall into the proper range. For example, if the undiluted sample has an absorbance of 0.900 and make a 1:1 dilution (5 mL undiluted + 5 mL water) you would expect the absorbance to be 0.450, which would be in the usable range.

6. Carefully record how you prepared each dilution in your lab notebook as well as the absorbance of each of your four Gatorade samples.

**CALCULATIONS FOR THE STANDARD CURVE**
*To be completed before you leave the lab.*

The stock dye solution that is provided for you is 0.00100% by mass. You will first need to convert this value to molarity (similar to the calculation you did for Pre-Lab Question #5). Assume that the solution is dilute enough that its density is the same as that of water (1.00 g/mL). Show your calculations on the Calculations & Results Page (Item 1).

Calculate the concentrations of the standards using Equation 5 and complete the table on the Calculations & Results Page (Item 2).

Next, using Excel, and working individually, each of you should prepare a standard curve of absorbance versus concentration. (By convention, when we say “A vs. B” it means A is on the y-axis and B is on the x-axis.) Turning in any graph that is identical to another in the lab will be considered cheating.

**CONSIDERATION OF UNITS:** As previously mentioned, absorbance (y-axis) has no units. The x-axis has units of molarity (moles/L).

Refer to the check list in Experiment #1 as to what must be included on your graph.

**CALCULATIONS FOR THE CONCENTRATION OF THE GATORADE**
*To be completed before you leave the lab.*

Using the trendline from the standard curve, calculate the molarity of the diluted Gatorade samples (2, 3 & 4) from the absorbance values you measured. Show your calculations on a separate sheet of paper and enter your results on the Calculations and Results page.

Next calculate the concentration of the original Gatorade from the concentration of Gatorade samples 2, 3, and 4, by using Equation 5. (These values should be very similar to each other as you are working with dilutions of the same original Gatorade.)

**CALCULATIONS FOR THE EXTINCTION COEFFICIENT**
*This section may be completed at home if you run out of time.*
- Calculate $\varepsilon$ based on Beer’s Law and the $A$ and $c$ of one of your standards (Eqn 1). Choose wisely. Do not choose a standard not might be in error.
- Calculate $\varepsilon$ based on the trendline. Equation 2 shows us that the slope of the standard curve is $k = \varepsilon \ell$. Review the Introduction section as to what units are involved in this calculation. Complete the summary table at the bottom of the page.
Pre-Lab Assignment: Remember to answer these questions neatly in your lab notebook and turn in your carbon copies at the beginning of the lab. Always answer in full sentences and explain your answers.

1. a) Based on the absorption spectrum below, what is the $\lambda_{\text{max}}$ of the green dye?
b) Would having horizontal gridlines be helpful in determining the $\lambda_{\text{max}}$? Explain.
c) The graph below was created with Excel 2007 where the settings for the x-axis are as follows: Major Unit: 100 and Minor Unit: 50
   Examine the graph to see how these settings affect the appearance of the graph, then give the settings you might use to allow you to determine the $\lambda_{\text{max}}$ more precisely.

![Absorbance vs. Wavelength (nm) graph]

2. Explain the reason for preparing a standard curve in this experiment. How should the x-axis and the y-axis be labeled? (Note: The graph above is NOT a standard curve.)

3. Review the section on the previous page on units utilized in the standard curve.
   a) Remembering that slope is $\Delta y/\Delta x$, determine the units of the slope.
b) Next, consider the units of the y-intercept. What exactly is a “y-intercept”? It is the y-value when x equals zero. Determine the units of the y-intercept.

4. A diluted solution is prepared by mixing 4.00 mL of a $1.25 \times 10^{-4}$ M solution with 6.00 mL of water. What is the concentration of the diluted solution? Assume volume to be additive.

5. Malachite green is a toxic chemical (not a food dye!). It contains C, H, Cl and N. If a solution of malachite green dye is 0.00500% by mass, and malachite green has the formula $C_{23}H_{25}ClN_2$, what is the molarity of the solution? Assume the solution is dilute enough that its density is the same as that of water (1.00 g/mL). Show your calculation setup. (Hint: You can treat this as a unit conversion problem. Convert g solute/g solution to moles solute/Liter solution.)

(Pre-Lab continues on the next page.)
EXPERIMENT 3: CONCENTRATION OF DYE IN GATORADE

6. **Begin on a new page** on your lab notebook. Prepare the page as usual by filling in all the information at the top of the notebook page and stating the **Purpose** of the experiment. Neatly copy the data table shown below into your notebook.

Color of Gatorade = ____ Molar Mass of Dye = ____$\lambda_{\text{max}}$ for dye = ____
Instrument ID # ____

<table>
<thead>
<tr>
<th>Sample</th>
<th>Volume of stock solution</th>
<th>Volume of water added</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard 3</td>
<td></td>
<td></td>
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<tr>
<td>Standard 4</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Standard 5</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Standard 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard 7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gatorade 1</td>
<td>(undiluted)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Gatorade 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gatorade 3</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Gatorade 4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Post-Lab Questions:** *To be answered at home.*

*Calculations Question 3* can be done by hand. *Explanations should be carefully worded and TYPED.*

1. Compare the two extinction coefficients (from Beer’s Law and from the trendline). Which do you think is more accurate? Use complete sentences to explain your reasoning.

2. Locate and label the diluted Gatorade data points on your standard graph. Are there any data points that you think should not be used? Explain your reasoning for including or not including each of the three data points.

3. How is the precision of your three calculated concentrations of the original, undiluted Gatorade? Study Appendix 4 and calculate the standard deviation and relative standard deviation for the three concentrations. What does your relative standard deviation tell you about your precision? **SHOW YOUR CALCULATIONS,** step by step as shown. No credit will be given if the calculations are not written out.
Calculations & Results:  
Name: __________________________  
CHEM 124 Sec: ____  
Partner’s Name: __________________________

PREPARATION OF STANDARD CURVE

1. Calculate the concentration of the stock (original, undiluted) solution of dye in molarity.  
   Show your calculations.  
   Color of Dye: __________  
   Concentration of stock solution of dye in percent by mass: ______

2. Calculate the molarity of each standard solution and fill in the table. Show at least one sample calculation for the concentration.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Volume of Stock Soln (mL)</th>
<th>Volume of water added (mL)</th>
<th>Total Volume (mL)</th>
<th>Concentration of dilution (M)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard 2</td>
<td></td>
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<tr>
<td>Standard 3</td>
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<td>Standard 4</td>
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<td>Standard 5</td>
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<tr>
<td>Standard 6</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Standard 7</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

3. Use the table above to prepare a plot of absorbance versus concentration. Include a linear trendline, and display the trendline on the graph with the $R^2$ value. Have your instructor examine your graph before printing a copy of this graph, embedded in the spreadsheet. Remember that this graph should be an individual effort.

(continued on the next page)
Name:______________________________

CALCULATIONS FOR CONCENTRATION OF THE GATORADE

Show ALL calculations on a separate sheet of paper and then fill in the table below. See instructions on p. 34.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorbance</th>
<th>Concentration of diluted sample**</th>
<th>Concentration of original Gatorade***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted Gatorade 1</td>
<td></td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Gatorade Dilution 2*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gatorade Dilution 3*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gatorade Dilution 4*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

*Remember absorbance for these must be lower than 0.8.

**Calculate from absorbance and trendline.

***Calculate from concentration of dilute sample.

Briefly describe how these 3 dilutions were prepared.
(e.g. 4.00 mL Gatorade + 6.00 mL water)

Gatorade Dilution 2
Gatorade Dilution 3
Gatorade Dilution 4

CALCULATION FOR THE EXTINCTION COEFFICIENT:
This section may be completed at home if you are out of time.

Show calculations for ε based on Beer’s Law and one standard of your choice:

Show calculations for ε based on the trendline:

Summary:

<table>
<thead>
<tr>
<th>Color of dye =</th>
<th>Extinction coefficient (ε)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Based on Beer’s Law and one standard</td>
<td></td>
</tr>
<tr>
<td>Based on trendline</td>
<td></td>
</tr>
</tbody>
</table>

Type answers to Post Lab Questions.