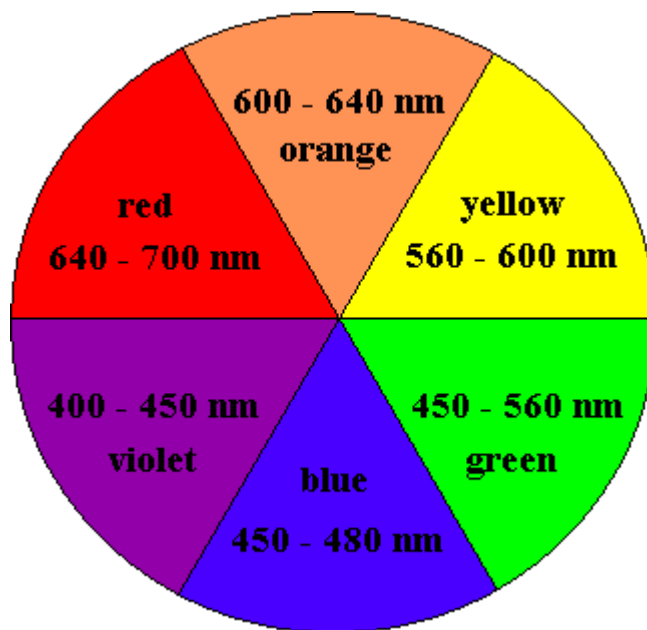


## A Beer's Law Experiment

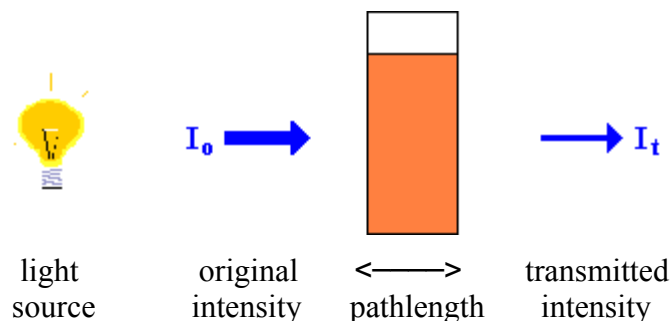
### Introduction

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.



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 is not absorbed, the intensity of the beam directed at the solution ( $I_0$ ) 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  $I_0$  indicates the percentage of incoming light absorbed by the solution. This is called the **percent transmittance**.

$$\%T = \left( \frac{I_t}{I_0} \right) \times 100 \quad (1)$$

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

$$A = -\log(\%T/100) \quad (2)$$

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  $\lambda_{\max}$ . In the first part of this experiment, you will determine  $\lambda_{\max}$  of **one** of the artificial food dyes by plotting absorbance versus wavelength. Figure I shows an example of this type of plot.

Once  $\lambda_{\max}$  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 ( $\epsilon$ ); the sensitivity of the absorbing species to the energy of  $\lambda_{\max}$ . 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:

$$A = \epsilon bc \quad (3)$$

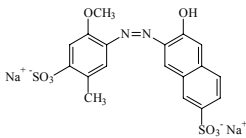
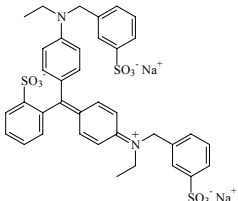
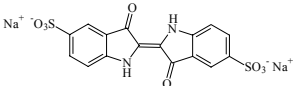
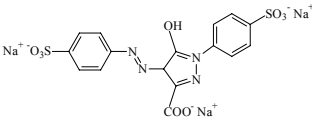
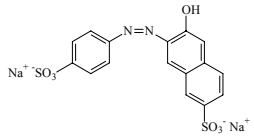
With this equation (or a calibration curve based on it), you can determine an unknown concentration or estimate what the absorbance of a certain solution will be as long as three of the four values in the equation are known.

In the second part of this experiment, you will determine the molar absorptivity of the dye chosen in the first part in aqueous solution. 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 third 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 5 being most common. The structures and **formulae** of the 5 are given below:

<b><u>Dye</u></b>	<b><u>Common name</u></b>		<b><u>Chemical Formula</u></b>	<b><u>Color</u></b>
<b>Red #40</b>	<b>Allura Red AC</b>		<b>Na<sub>2</sub>C<sub>18</sub>H<sub>14</sub>N<sub>2</sub>O<sub>8</sub>S<sub>2</sub></b>	<b>Orange-red</b>
<b>Blue #1</b>	<b>Brilliant Blue</b>		<b>Na<sub>2</sub>C<sub>37</sub>H<sub>34</sub>N<sub>2</sub>O<sub>9</sub>S<sub>3</sub></b>	<b>Bright blue</b>
<b>Blue #2</b>	<b>Indigotine</b>		<b>Na<sub>2</sub>C<sub>16</sub>H<sub>8</sub>N<sub>2</sub>O<sub>8</sub>S<sub>2</sub></b>	<b>Royal blue</b>
<b>Yellow #5</b>	<b>Tartrazine</b>		<b>Na<sub>3</sub>C<sub>16</sub>H<sub>9</sub>N<sub>4</sub>O<sub>9</sub>S<sub>2</sub></b>	<b>Lemon yellow</b>
<b>Yellow #6</b>	<b>Sunset Yellow</b>		<b>Na<sub>2</sub>C<sub>16</sub>H<sub>10</sub>N<sub>2</sub>O<sub>7</sub>S<sub>2</sub></b>	<b>Orange</b>

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 second part, you can determine the concentration of the dye from the graph.

Figure I: Absorbance versus wavelength plot of a food dye to determine  $\lambda_{\max}$ .

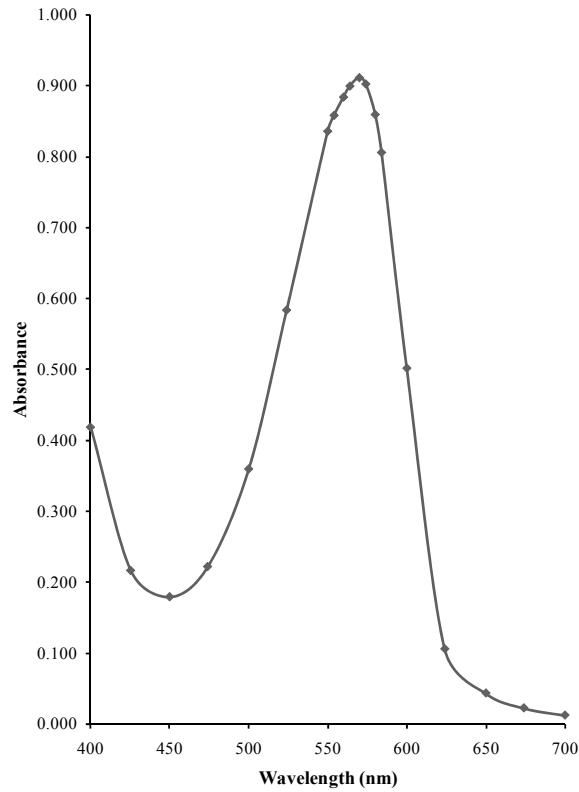
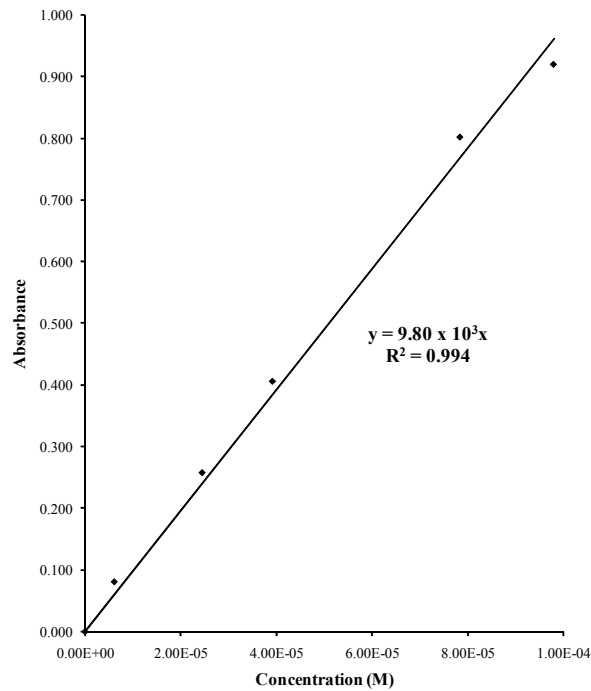


Figure II: Absorbance versus concentration plot of the same food dye in Figure I. The line goes through 0,0. The slope is  $\epsilon$  and  $R^2$  gives the fit (will be 1 if the fit is exact).

Calibration Curve of Food Dye at 572 nm



## Procedure

### Part I

1. Obtain a sample of red #40, blue #1, blue #2, yellow #5 **or** yellow #6 stock solution. Record the name, concentration and color of the solution you selected.
2. Fill a cuvette about half-full of the stock solution and another cuvette with a similar amount of distilled water (the **blank**).
3. Starting at a wavelength of 400 nm and working your way up to 700 nm, measure the absorbance of the stock solution at 25 nm intervals.

The wavelength is selected using the dial on the top of the instrument; to the right of the sample compartment. Set the Spectronic 20 to 0.0 %T (with nothing in the sample compartment) by adjusting the LEFT dial on the front of the instrument.

Place the **blank** cuvette into the compartment with the line on the cuvette aligned with the mark on the compartment (this keeps the pathlength constant). Adjust to 100.0 %T with the dial on the RIGHT. Some of the digital Spectronic 20's have a filter lever at the bottom. Make sure the lever is in the correct wavelength range.

Remove the **blank** and place the **sample** cuvette in the compartment and align as you did the blank. Record **absorbance (A)** by pushing the MODE button once.

Push the MODE button three more times to return to %T mode. Change the wavelength and **repeat the zero and 100.0 %T adjust**.

4. When you have readings for the entire spectrum, determine the wavelength with the **greatest** absorbance. Take readings at 5 nm intervals a little before and after this wavelength. For example, if the maximum absorbance was found at 450 nm, then to get a more accurate reading of  $\lambda_{\text{max}}$ , take absorbance readings at 440, 445, 455 and 460 nm. A graph of A versus  $\lambda$  (in **Data Treatment and Discussion**) will look like Figure I with a peak at the dye's  $\lambda_{\text{max}}$ .

### Part II

1. Determine (with calculations) how to make **at least** four dilutions of the original solution using 5-, 10-, 20- and 25-mL pipets and your two 100-mL and two 50-mL volumetric flasks that will give well spread out data points on the calibration curve (Figure II). You will have less error by pipetting only one time for each dilution.
2. Make the required solutions **as accurately as possible**.

3. Set the Spec 20 to the estimated  $\lambda_{\text{max}}$  determined in Part I and measure the absorbance of the four solutions **and** the original stock solution. Check the blank before each measurement.

### Part III 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 and II of the experiment. Ask the instructor for details. Record the color chosen.
2. Accurately (analytical balance) determine the mass 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 (from dispenser) 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 in 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 absorbance of the solution at the  $\lambda_{\text{max}}$  used in part II. Use 1:1 acetone/water as the blank.

### Questions

1. 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. Include the dye color in a proper title for the plot.

2. 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®.

### Data Treatment and Discussion

**Include** the following information, **with an appropriate discussion**. Show sample calculations.

Make a plot of A versus  $\lambda$ . Include **all** the data that you recorded for your dye. Make sure the plot is done properly: a **descriptive** title, axes labeled with units, data points clearly indicated, smooth line through points (you are not intending to demonstrate a mathematical relationship). Find  $\lambda_{\text{max}}$  to **3 significant figures** using a close-up plot.

Show **all** the calculations for the determination of the concentrations of the dilute solutions used to make the calibration curve. The concentrations are determined from:

$$M_{\text{new}} \frac{\text{mole}}{\text{L}} = \frac{M_{\text{old}} \frac{\text{mole}}{\text{L}} \times (\text{pipet volume}) \text{ L}}{(\text{volumetric flask volume}) \text{ L}}$$

Make a calibration curve by plotting A versus concentration. 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 points**. Determine the molar absorptivity,  $\epsilon$ , from the slope. Do not connect the points. Just 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. **Force the trendline to go through 0,0**.

Calculate the concentration of the solution in Part III using Beer's Law. Draw, by hand, the point (A, concentration) **on the plot** that corresponds to this value.

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.

### Conclusion

$\lambda_{\text{max}}$  **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** in your sample are to be given in the **first line** of the conclusion. Then address the following:

Does your solution obey Beer's Law? How do you know? If not, determine approximately which concentration range of the plot is not linear?

What is an advantage of colorimetric over titration determinations of concentrations?  
What is a disadvantage?