# Beer's Law: Determining the Concentration of a Solution

## Background

Color additives are used in foods for a variety of reasons. Sometimes they are used to compensate for the natural color loss of food during storage or exposure to light or air. Sometimes they are used to enhance natural colors because off-colored foods are often mistaken for being lower in quality—for example, perfectly good oranges that are naturally dull orange/brown are sprayed with Citrus Red No. 2 to make them more appealing. Color is also introduced to what are otherwise colorless products, such as strawberry frosting, key lime pie, and tandoori chicken!

In 1900, there were about 80 man-made food dyes available to consumers. Due to standards in improving food safety, nine color additives are approved, seven of which are Food, Drug, and Cosmetic certified (FD&C). Allura Red (FD&C Red No. 40) is one of these color additives, and currently the most consumed.

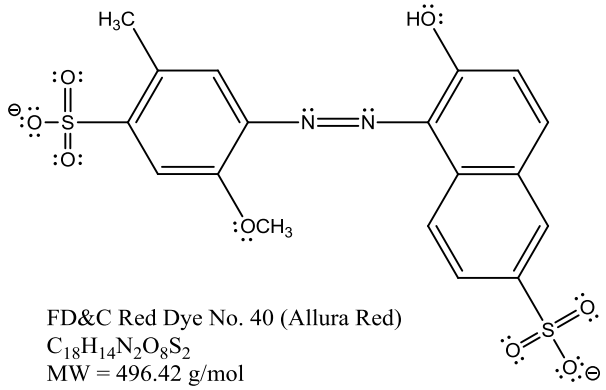


*Figure 1. Molecular Structure of Allura Red*

The safety of food dyes is rather controversial. Research has shown very little risk to humans in the consumption of approved dyes;[1] however, some consumers claim to have a sensitivity to artificial colors and flavorings and others believe the consumption of artificial colors is linked to hyperactivity or learning disabilities in children.[2] Without substantial evidence to support these claims, it is up to the public to use caution and good judgement in consuming color additives, as for any substance.

This experiment will give you the opportunity to quantify the amount of a color additive in several common products, such as Kool-Aid, Powerade, Gatorade, NyQuil, and sodas. How much food coloring is in these products? You'll find out!

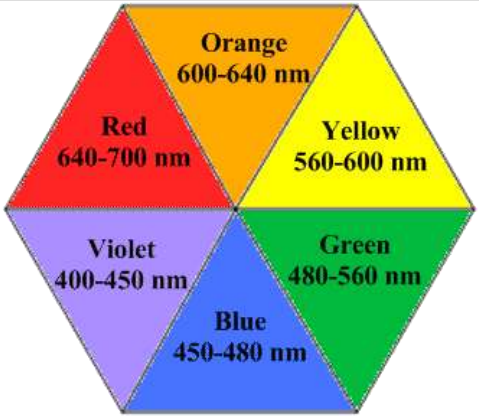


*Figure 2. Color wheel with approximate wavelengths of light corresponding to each color.*

## Principles of Colorimetry

The color of Allura Red solution is... red! Generally, the observed color is complementary to the color of light absorbed. In Figure 2, red is complementary to green. Thus, Allura Red absorbs primarily wavelengths in the 480-560 nm range. Wavelengths of 640-700 nm are not absorbed but transmitted, thus resulting in our perception of a red solution.

[1] FDA: How Safe are Color Additives? http://www.fda.gov/downloads/ForConsumers/ConsumerUpdates/ucm048960.pdf

[2] Beil, L. The Color of Controversy: Link between food dyes, childhood hyperactivity gets renewed attention. . Science News. August 27, 2011; 180 (5): 22. Available from: http://www.sciencenews.org/view/feature/id/333204/title/The_Color_of_Controversy_

The more Allura Red there is, the greater the absorption of green light. Thus, absorbance can be measured and the quantity of dye can be determined. The primary objective of this experiment is to determine the concentration of a common food dye, Allura Red, in various red-colored liquid products using Beer's Law and a technique called spectrophotometry.

***Here's how it works***

You will be using the colorimeter shown in Figure 3. There are four fixed LED (light emitting diode) sources of the following wavelengths: 430 nm, 470 nm, 565 nm, and 635 nm. Allura Red has the greatest absorption of light which is 500 nm. Therefore, the best wavelength of light provided by this colorimeter is 470 nm (blue light). (More expensive spectrophotometers have variable wavelengths, with a dial which can be set at any visible wavelength. You will use one of these in CHEM& 163!)

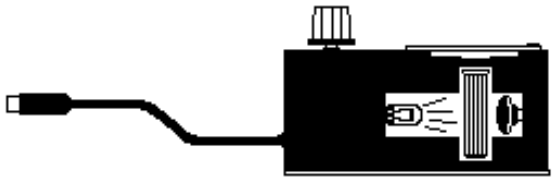

*Figure 3: Diagram of the colorimeter, showing the placement of the cuvette (sample) and the LED light source.*
*(Photo courtesy of Vernier Software & Technology).*

With 470 nm selected, the blue LED will pass through the solution and strike a photocell. A higher concentration of the colored solution absorbs more blue light and transmits less blue light than a solution of lower concentration. The computer-interfaced colorimeter monitors the light received by the photocell as either an *absorbance* or a *percent transmittance* value. (You will use absorbance.)

***How does the absorbance tell you the concentration?***

You will prepare a stock solution from which four solutions of known concentration (standard solutions) will be made. The absorbance of each will be measured. When a graph of absorbance vs. concentration is plotted for the standard solutions, a direct relationship should result, as shown in Figure 4. This is called a **calibration plot** since all concentrations are known. The linearity of this plot arises from the Beer-Lambert law (or Beer's law, for short) which states that the absorption of light by a substance is proportional to its concentration in solution:

$$A = \varepsilon \ell c$$

where A is the absorbance (unitless), ε is the molar absorptivity coefficient ($M^{-1}cm^{-1}$),
$\ell$ is the pathlength of the light through the cuvette (cm), and c is the concentration (M).
***Therefore, the concentration of Allura Red can be determined by measuring the absorption of light through the solution.***

The concentration of an *unknown* solution containing Allura Red is then determined by measuring its absorbance with the colorimeter that has been calibrated for Allura Red. By locating the absorbance of the unknown on the vertical axis of the graph, the corresponding concentration can be found on the horizontal axis (follow the arrows in Figure 4).

This is more accurately done using the equation of the line from a best fit (linear least-quares analysis) line:

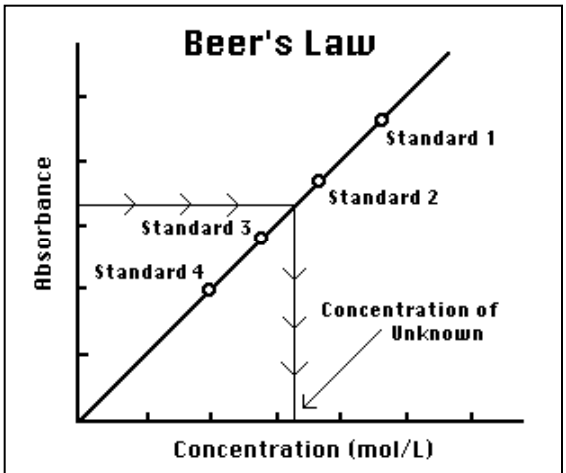


*Figure 4: Calibration plot to determine the concentration of an unknown by colorimetry. (Image courtesy of Vernier Software & Technology).*

The equation for Beer's law is a straight line with the general form of y = mx +b.

**Beer's Law:** $A = (\varepsilon \ell)c$

**with the general form** $y = (m)x$

where the slope, m, is equal to $\varepsilon\ell$. In this case, use the absorbance found for your unknown, along with the slope of your best fit line, to determine c, the concentration of the unknown solution.

*Think about it: Why should the y-intercept be zero?*

**Safety Precautions**


Allura Red is used in small amounts (< mg). Avoid eye and skin contact and avoid inhalation of dust. In case of eye contact, flush eyes with water for 15 minutes. For skin contact, wash with soap and water. Do not ingest.

# Procedure

Obtain and wear goggles! The food dye is considered safe in small quantities. However, take the same safety precautions as with other chemicals, in case it may cause skin/eye irritation.

**For steps 1-4, work in a group of 4 students at your station.**

1) Your prelab assignment was to calculate how much Allura Red you will need for 500.0 mL of a $1.90 \times 10^{-4}$ M Allura Red stock solution. This value should be entered in Table 1 as "calculated".

   Using weigh paper tared on a balance, carefully weigh to the nearest 0.001 grams the calculated amount of Allura Red required for the stock solution (it doesn't have to be exactly the amount you calculated). You will need a very small scoop of material. **Record the mass of Allura Red and weigh paper used.** After you transfer the Allura Red to your 500-mL volumetric flask, weigh the paper again to determine the mass of Allura Red delivered. This is called "weighing by difference".

2) **Make the stock solution**: To the 500-mL volumetric flask which now contains Allura Red, add enough water to fill the flask about 1/3 full. Swirl the flask to dissolve the contents. Continue to fill the flask with distilled water. As you get close to the mark, use a pipet to add water dropwise. Swirl the flask as you go. **DO NOT GO OVER THE MARK! (or you'll have to start over…)**

3) **Diluting the stock solution to make the standard solutions**: Obtain four 100-mL volumetric flasks and label them #1, #2, #3, #4. Rinse them out with tap water, then with distilled water. Pour 100-mL of stock solution into a clean, dry 150-mL beaker. *Think about it: Why does the beaker have to be dry? Why can the volumetric flasks be wet?* Using a graduated pipet with a 10-mL (Green) pipet pump, dispense 5-mL, 10-mL, 15-mL, and 20-mL of the stock solution into each of four flasks. Have each member in the group do one. Fill the flask half way with distilled water, swirl, then continue to fill to the mark. As you get

close to the mark, use a pipet to add water dropwise. **DO NOT GO OVER THE MARK! These are your four standard solutions of known molarity.**

4) **Calculations**: Determine the actual molarity of your stock solution and all four dilutions based on your mass of Allura Red and volumes of stock solution used. Record these in your data section. Include sample calculations, using significant figures and units.

**For the rest of the lab, work in pairs,**
**sharing your solutions with the neighbors at your bench.**

**5) Setting up the colorimeter:**

Plug in the colorimeter into Channel 1 of the Logger Pro Interface Box. Turn on the computer and open Logger Pro. File>Open>Chemistry with Vernier>Exp 11 Beer's Law (do not open 11B percents). The vertical axis is absorbance, and the horizontal axis is concentration (mol/L). (NOTE: If your axis reads % instead of mol/L, you have the wrong experiment opened!)

Your colorimeter comes with a bag of cuvettes. To have as few errors as possible, you will choose **one** cuvette to use the whole period.

*Think about it: Why does using the SAME cuvette for all samples reduce error?*

When using a cuvette, keep in mind:

- All cuvettes should be wiped clean and dry on the outside with a KimWipe.
- Handle cuvettes only by the top edge of the ribbed sides.
- All solutions should be free of bubbles.
- Always position the cuvette so the light goes through the clear windows (the direction of light is indicated by the white arrow on the colorimeter), not through the ribbed sides.

**6) Zeroing with a Blank (setting the absorbance of the solvent to zero)**:

This step is done once at the beginning of the data collection.

**Use distilled water as the blank. Do NOT zero with your samples.**

Prepare a *blank* by filling a cuvette 3/4 full with distilled water.
Holding the cuvette by the upper edges, place it in the cuvette slot of the colorimeter.
Set the wavelength on the colorimeter to 470 nm (blue) and press the CAL button. After it blinks, the absorbance should read 0 (or near 0).

**7) Measure absorbance of standard solutions to create a calibration plot:**

You are now ready to collect absorbance data for the four standard solutions. Click Collect. Your first data point will be the blank, since the absorbance is 0 in the absence of Allura Red. Click Keep, entering a concentration of 0. Empty the water from the cuvette. (Do **not** click Stop)

**When you rinse the cuvettes with water, the water dilutes the sample (concentration changes). Therefore, you must rinse the cuvettes with the sample (rather than water) so you can avoid changing the concentration of your sample. In this case, your samples are standard solutions #1, 2, 3, 4.**

**Rinse the cuvette twice <u>WITH Standard #1</u> and then fill it 3/4 full.** Wipe the outside with a KimWipe and place it in the colorimeter. After closing the lid, wait for the absorbance value displayed on the monitor to stabilize. Then click Keep, type the concentration in the edit box, and press the ENTER key. The data pair you just collected should now be plotted on the graph. NOTE: The concentration you enter should be the number you have calculated for your dilutions in step 4. You may need to adjust the column settings to view the correct number of digits in your data table. Make sure to also record the data in your data section. Don't rely on the computer!

Discard the cuvette contents into a beaker for waste (make sure you label it!). **Rinse the cuvette twice with Standard #2, and fill the cuvette 3/4 full.** Wipe the outside, place it in the colorimeter, and close the lid. When the absorbance value stabilizes, click Keep, type the concentration in the edit box, and press the ENTER key.

Repeat for Standards #3 and #4 for a total of five data points including (0,0). When you have finished with the last standard, click Stop.

Double check that you have all absorbance and concentration data pairs recorded in your data section in your notebook.

**8) Data Analysis**

Examine the graph of absorbance versus concentration. You may need to Autoscale the graph (Analyze>Autoscale). To see if the plot represents a direct relationship between these two variables, click the Linear Regression button, . A best-fit linear regression line will be shown for your four data points. This line should pass near or through the data points *and* the origin of the graph. The equation of the best fit line should appear.

**Record the equation and the correlation coefficient ($R^2$ value) in your data section. Make a sketch of the graph in your notebook. Print the graph, one for each student.**

**9) Determining the concentration of an unknown**

You will test the reliability of your calibration plot by analyzing an unknown sample provided by your instructor.

To determine the concentration of Allura Red in the unknown using your calibration plot, you need to make sure that its absorbance is in the range of your standard unknowns. If it is not, you cannot extrapolate because you do not know if the relationship will be linear outside of the range of absorbances you measured.

Depending on how much time has elapsed since zeroing the colorimeter, you may want to repeat it at this point. Use a distilled water blank and reset the colorimeter to read zero.

Obtain a sample of your unknown and measure the absorbance.
Is the absorbance WITHIN the range of the absorbance values of your standard solutions? It may read -0.061, or 2.36, or 3.5. Do not be surprised if it is too high or unreadable, your unknown may need to be diluted.

If the absorbance of your unknown is out of range, use volumetric glassware and graduated pipets to prepare a diluted unknown solution that has an absorbance in range of your calibration plot. (You may have to try this more than once. Tip: Look at your standard solutions!)

**Record how you made this dilution: record the volume used of the original unknown solution; record the final volume of the diluted solution.**

Measure and record the absorbance of the diluted solution. Using your calibration plot (the equation of the line, calculate the concentration of Allura Red in the diluted unknown solution.

Use the concentration of the diluted unknown solution (calculated using the calibration plot) and the volumes used to make the dilution to calculate the concentration of the original unknown solution. Use the following equation:

$$C_{original}V_{original} = C_{diluted}V_{diluted}$$

There are many forms of this equation. You may be more familiar with this version:

$$M_1V_1 = M_2V_2$$

Ask your instructor for the correct concentration of unknown solution, from which you will determine how accurate you were by calculating a % error.

$$\%\,\text{error} = \left|\frac{\text{experimental - true}}{\text{true}}\right| \times 100\%$$

**10) Determining the concentration of Allura Red in various products (Optional)**

Choose a product for your station to test. You may have brought your own, or you may use one of the samples provided. If each station chooses a different one, they can be compared at the end of class.
Which product did you choose? Record its identity in your data section.

Obtain a sample of your product and measure the absorbance. Is the absorbance WITHIN the range of the absorbance values on your calibration plot? If not, your product needs to be diluted. Repeat all steps to make a diluted solution as in step 9 above.

Class data may be pooled for the other products. Write your value on the white board, along with group member names.

**Waste**: Since this lab requires very small amounts of an approved food dye, it is acceptable to discard the contents of your waste beaker in the sink with plenty of water. Rinse all glassware used with water and return volumetric flasks to your instructor's cart.

- **DON'T FORGET TO REMOVE THE LAST SAMPLE FROM THE COLORIMETER!!!**
- **REMOVE ALL TAPE/LABELS FROM YOUR GLASSWARE.**
- **DO NOT LEAVE VOLUMETRIC FLASKS AT YOUR STATION!!!**

# Calculations – Complete in your lab notebook.

*Show all steps! Use significant figures and units.*

- Calculation for the molarity of the Allura Red stock solution that you prepared.
- Calculation for the molarity of Allura Red in Standard Solution #1.
- Calculate the molarity of **diluted unknown solution** from the calibration plot (equation of the line). Attach your graph.
- Calculation for the molarity of Allura Red in the **original unknown solution**.
- Calculate the % error for your **original unknown solution** compared to the value obtained from your instructor.
- Show these calculations to your instructor before you leave lab.

Name _____ Date_____

Lab Partner_____ Section _____

# REPORT SHEETS
# Beer's Law – Attach your graph!

Don't forget to label units and use an appropriate number of significant figures.

Table 1: Preparation of Stock Solution

| | Mass of Allura Red (g) | | Stock Solution Concentration (M) |
|---|---|---|---|
| | Calculated | Used | |
| | | | |

Table 2: Standard Solution Concentrations and Absorbances

| | Standard Solutions | Volume of Stock solution used (mL) | Concentration (M) | Absorbance |
|---|---|---|---|---|
| | Blank | 0.00 | Distilled water (solvent only) | |
| | 1 | 5.00 | | |
| | 2 | 10.00 | | |
| | 3 | 15.00 | | |
| | 4 | 20.00 | | |

Record the equation of the line:_____

Record the $R^2$ value (Correlation coefficient) = _____

Table 3: Solution Concentrations and Absorbances for given Unknown

| | |
|---|---|
| Absorbance of original unknown solution | |
| Volume of original unknown sample used for dilution | |
| Volume of final diluted unknown solution | |
| Absorbance of diluted unknown solution | |
| Calculated molarity of diluted unknown solution | |
| Calculated molarity of original unknown solution | |
| Instructor provided concentration of original unknown solution | |
| % error | |

**Don't forget: Attach a copy of your graph to the lab report sheets.**

# Postlab Questions:

1) Why should the y-intercept of any Beer's Law Plot equal zero? Consider the experimental set-up, and answer from a conceptual perspective.

2) Consider if the stock solution was made improperly in the procedure, such that the volumetric flask was filled to just above the line.

a) How would this affect the slope of graph? Would it increase, or decrease, or stay the same? Explain.

b) How would this affect the concentration determined for your unknown sample? Would the reported unknown concentration be incorrectly high, or incorrectly low, or stay the same? Explain.

3) The molar absorptivity coefficient, $\varepsilon$, is specific to Allura Red under the conditions of this experiment. Report the value **and** unit of $\varepsilon$ in your experiment using your graph. (The pathlength, $\ell$, is 1 cm across the cuvette. Assume that it is exactly 1 cm.)

4) Based on your % error, give two reasonable explanations for any deviation from the expected value. **Do not state human error (e.g. spilled sample), calculation error, rounding error, significant figures, etc. These are all errors that can be controlled and reduced or eliminated!**

# Pre-Lab Assignment:
# Beer's Law

*This experiment covers the concept of molarity, dilution, and Beer's Law. Show your work and use significant figures.*

**1)** What is Beer's law? Write down the equation and label the symbols (include units).

**2)** If the concentration of a solution is reduced by half, how will this affect the absorbance of a solution?

**3)** A **volumetric flask** will be used to make your stock solution and dilutions in this lab. Suppose you fill the flask with the desired substance and then accidentally add distilled water to just above the mark.

a) Will the concentration be higher or lower than desired? Explain.

***A volumetric flask***

b) Will you be able to read the new volume?

**4)** Step 1 in the procedure of this experiment requires you to make 500.00 mL of $1.90 \times 10^{-4}$ M Allura Red (MW 496.42 g/mol). How many grams of Allura Red would you need to weight out? Show your calculation below. <u>Copy your final answer to your data section.</u>

**5)** Suppose the molarity of Allura Red in your stock solution is $1.90 \times 10^{-4}$ M and you take 5.00 mL of this solution, place it into a 100.00 mL **volumetric flask** and fill it to the mark with distilled water. What is the concentration of the diluted solution (dilution)? Show your work below.