

Experiment 10

Beer's Law: Determining the Concentration of a Solution

Background

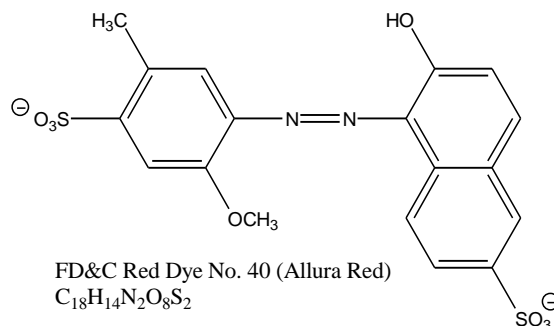
Color additives are used in foods for a variety of reasons. Sometimes they are used to compensate for the natural color loss of food during storage or exposure to light or air. Sometimes they are used to enhance natural colors because off-colored foods are often mistaken for being lower in quality—for 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 and key lime pie. A green key lime pie is more likely to be purchased by consumers than a beige one!¹

In 1900, there were about 80 man-made food dyes available to consumers. Due to standards in improving food safety, seven color additives were approved by the FDA (Food and Drug Administration) for use in foods.² Allura Red (FD&C Red No. 40) is one of the seven color additives certifiable for food use by the US Food and Drug Administration (FDA). (FD&C means Food, Drug and Cosmetic certified). Other dyes are permitted for drug and cosmetic use only (D&C) and external drug and cosmetic (external D&C).

The safety of food dyes is rather controversial. Research has shown very little risk to humans in the consumption of approved dyes but that doesn't guarantee there are no adverse effects in the population. 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. 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 a common product, Kool-Aid. Is it a lot of food coloring? You'll find out!



Principles of Colorimetry

The primary objective of this experiment is to determine the concentration of a common food-dye, Allura Red, in Kool-Aid using Beer's Law and a technique called spectrophotometry (colorimetry).

The Beer-Lambert Law states that the absorption of light by a substance is proportional to its concentration in solution:

$$A = \epsilon c l$$

where A is the absorbance (unitless), ϵ is the molar absorptivity coefficient ($M^{-1}cm^{-1}$), l is the pathlength of the light through the cuvette (cm), and c is the concentration (M).

¹ From the U.S. FDA/IFIC brochure, "Food Color Facts" (Jan 1993) <http://www.cfsan.fda.gov/~lrd/colorfac.html> accessed August 28, 2008.

²In addition to the seven, there are two additives that are restricted to specific uses.

The Allura Red solution used in this experiment has a red color. You will be using the colorimeter shown in Figure 1. In this device, blue light from the LED light source 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.)

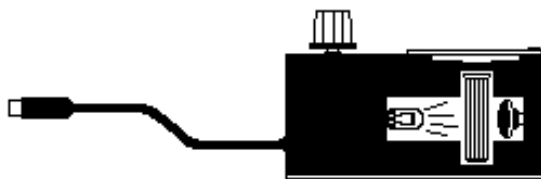


Figure 1: Diagram of the colorimeter, showing the placement of the cuvette and the LED light source.

You are to 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 2. This is called a calibration plot, since all concentrations are known.

The concentration of an *unknown* solution containing Allura Red is then determined by measuring its absorbance with the colorimeter. 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 2).

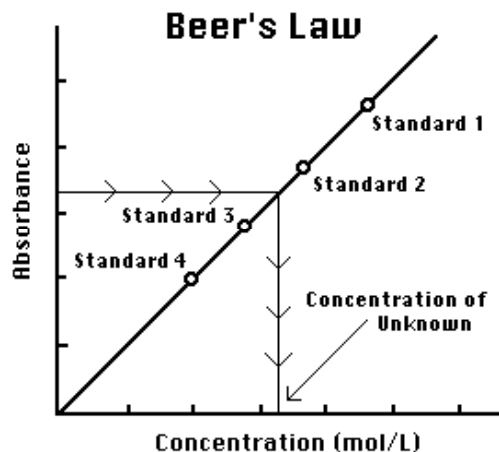


Figure 2: Calibration plot to determine the concentration of an unknown by colorimetry

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Experiment 10 Turn-in Sheets

Determining the Concentration of a Solution: Beer's Law

Procedure

Obtain and wear goggles!

- 1) Calculate the mass of Allura Red (MW=496.42 g/mol) required to prepare 500.0 ml of a 1.90×10^{-4} M Allura Red stock solution.

Approximate Mass of Allura Red needed?

- 2) Using weigh paper tared on a balance, carefully weigh to the nearest 0.001 grams the amount of Allura Red calculated (it doesn't have to be exactly the amount you calculated). You will need a very small scoop of material.
Record the actual mass of solid you use.

Actual Mass of Allura Red measured?

- 3) Make the stock solution: Quantitatively transfer all of the solid on the weigh paper to the 500-mL volumetric flask using distilled water. Add enough water to fill the flask about 1/3 full. Swirl the flask to dissolve the contents. VERY CAREFULLY add enough water so the bottom of the meniscus touches the etching for 500-mL. Swirl the flask as you go. **DO NOT GO OVER THE MARK!**

Ask your instructor if you are unsure where "the mark" is located.

Can you determine the concentration of the solution if you go over the mark?

- 4) Make the dilutions: For this part, pair up with another lab group. Obtain four 100-mL volumetric flasks and label them 5, 10, 15, 20. Rinse them out with tap water, then with distilled water. 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. **DO NOT GO OVER THE MARK!**
- 5) Calculations: Determine the molarity of your stock solution and all four dilutions. Show your work on the calculations page.

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- 6) Measuring the Absorbances: 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 Computers>Exp 11 Beer's Law (do not open 11B percents). The vertical axis has absorbance scaled from 0 to 0.6. The horizontal axis has concentration scaled from 0 to 0.5 mol/L.
(NOTE: If your axis reads % instead of mol/L, you have the wrong experiment opened!)
- 7) 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.

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).

Calibration:



This step is only done at the beginning of the period with distilled water. Do not calibrate 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 AUTO CAL button. After it blinks, the Absorbance should read 0 (or near 0).

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8) You are now ready to collect absorbance data for the four standard solutions. Click . Empty the water from the cuvette. Using solution 5, rinse the cuvette twice with the solution and then fill it 3/4 full. Wipe the outside with a tissue and place it in the colorimeter. After closing the lid, wait for the absorbance value displayed on the monitor to stabilize. Then click , 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 FROM YOUR DATA, NOT THE PRELAB VALUES!

9) Discard the cuvette contents into a beaker for waste. Rinse the cuvette twice with solution 10, 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 , type the concentration in the edit box, and press the ENTER key.

Repeat for solutions 15 and 20. solution for a total of four data points. When you have finished with the last solution, click .

10) In your Data and Calculations table, record the absorbance and concentration data pairs that are displayed in the Table window.

Data Analysis


11) 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 this. **Print the graph.**

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Determining the concentration of an unknown

- 12) To determine the concentration of an unknown by using the plot you obtained 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 don't know if the relationship will be linear outside of the range of absorbances you measured.

Your unknown is a sample(s) of Kool-aid, already prepared according to its label. Cherry-flavored Kool-aid contains Allura Red (Red No. 40). Obtain a small sample of the unknown in a clean DRY test tube. Rinse the cuvette twice with the unknown solution and fill it about 3/4 full. Wipe the outside of the cuvette, place it into the colorimeter, and close the lid. Read the absorbance value displayed in the Meter window.

(Important: The reading in Meter window is live, so it is not necessary to click  to read the absorbance value.) When the displayed absorbance value stabilizes, record its value in the Data and Calculations table.

Is the absorbance WITHIN the range of your previous absorbance values? If not, your Kool-aid needs to be diluted. Use your volumetric glassware and graduated pipets to create a dilution that works. Record how you made this dilution and the absorbance.

If time permits and at the discretion of your instructor, collect class data on the concentration of the original Kool-aid obtained.

DON'T FORGET TO REMOVE THE LAST SAMPLE FROM THE COLORIMETER!!!

Waste: 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.

Hint: You can use color to tell if your dilution absorbance will be in the range of your standard absorbances.

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Data and Calculations

Construct an organized data table with a column for the sample number (5, 10, 15, 20), the molarity of each, and the absorbance (which has no units). You may also wish to include a column for these samples: stock solution, original Kool-Aid, and diluted Kool-aid.

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

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Follow-up Questions

Complete these questions on your graph (for #2-5, use the back of your graph).

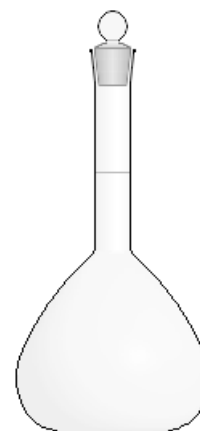
- 1) Show where the unknown falls on your graph by marking it with a pen. Determine the molarity of the diluted Kool-Aid by approximating this value from the graph and write it on your graph.
- 2) Another way to determine the concentration of your diluted Kool-Aid is to use the equation of the line from your data, $y=mx+b$. **Use this equation to solve the concentration of the unknown.** Show your work on the back of your graph.
- 3) Using the molarity you determined from #1 or 2 above of your diluted Kool-Aid, determine the molarity of your **ORIGINAL** Kool-Aid solution. Show all calculations on the back of your graph.
- 4) a) What does x and y represent in your graph, in terms of A , ϵ , ℓ , and c ? b) What does the slope of your best fit line, m , represent in these terms? (See the Beer-Lambert Law in the background section. Hint: The equation of a line is $y=mx+b$). c) The molar absorptivity coefficient, ϵ , is specific to Allura Red under the conditions of this experiment. Using your answer to #4b above, what is the quantity **and** unit of ϵ in your experiment? (The pathlength, ℓ , is 1 cm across the cuvette).
- 5) What type of errors (specific to this experiment) could you/did you encounter in this experiment? What experimental factors influence the accuracy and precision of the unknown concentration you report?

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Pre-Lab Assignment

This experiment covers the concept of molarity and concentration. See Chapter 6 in your textbook for guidance with the following questions/calculations. Show your work and use significant figures.

- 1) Search your house for a product that contains a color additive. List the product and the name of the color additive here.
- 2) Prepare a data table for the blank Turn-in sheet provided for this purpose. Follow the directions on which columns to make in your table. **This may be checked at the beginning of lab.**
- 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?
 - b) Will you be able to read the new volume? What will you do with this solution?



A volumetric flask

- 4) Step 1 in the procedure of this experiment requires you to make 500.00 mL of 1.90×10^{-4} M Allura Red (MW 496.42 g/mol). How many grams of Allura Red would you need to weight out? Write the answer below and also in the margin for Step 1 in the procedure. Show your work below.
- 5) Suppose the molarity of Allura Red in your stock solution is 1.90×10^{-4} M and you take 5.00 mL of this solution, place it into a 100-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.