

# Determination of the Allura Red Concentration in a Sports Drink or Other Commercial Product

## Goal and Overview

The spectral profile of Allura Red (red dye #40) will be measured using a Spec 20 spectrometer over visible wavelengths to determine the wavelength of maximum absorbance,  $\lambda_{\text{max}}$ . A Beer's Law plot constructed using absorbance data for a series of volumetric dilutions of a stock solution of Allura Red of known molarity will allow the quantitative relationship between absorbance and concentration to be determined, as well as the molar extinction coefficient,  $\epsilon$ , of Allura Red at  $\lambda_{\text{max}}$ . Absorbance data for a sports drink will be measured and, in conjunction with the proportionality found via the Beer's Law plot, the molarity of Allura Red in the product will be calculated.

## Objectives and Science Skills

Explain dilution and how it relates to moles of solute in a solution and how it changes the molarity of a solution.

Perform both standard and serial volumetric dilutions and calculate associated molarities. Understand and explain absorption spectroscopy and the mathematical relationships between percent transmittance (%T), absorbance (A), concentration (c), path length (b), and molar extinction coefficient ( $\epsilon$ ).

Apply linear fitting methods to find relationships between dependent and independent variables, such as %T (and A) and concentration; explain and apply Beer's Law.

Use absorption data to qualitatively and quantitatively analyze the concentration of Allura Red in solution.

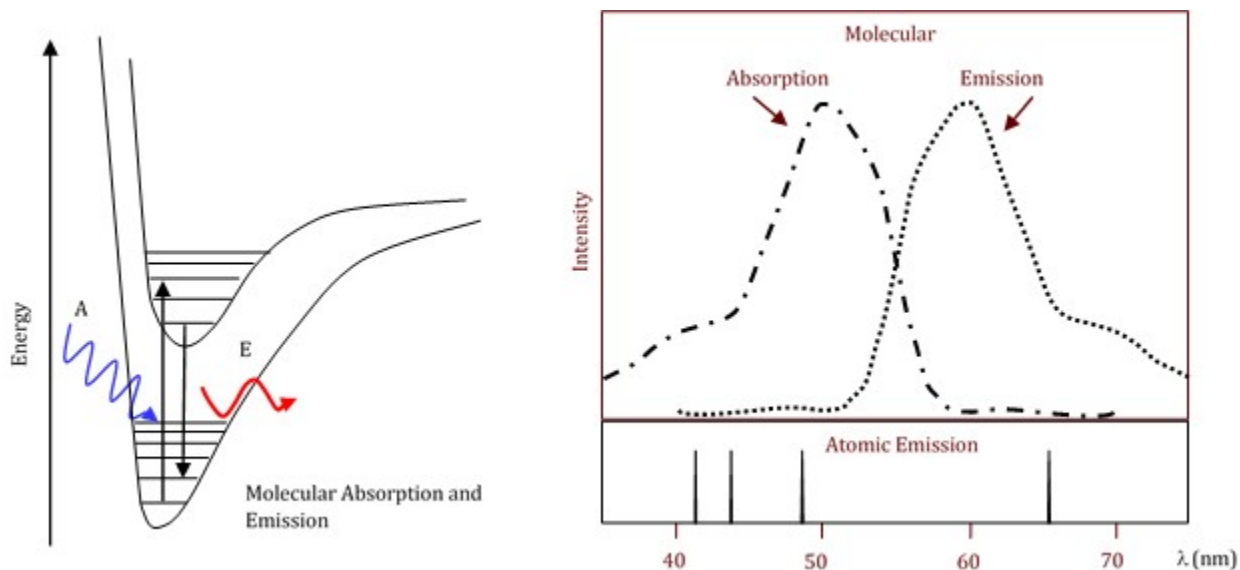
## Suggested Review and External Reading

Reference material and textbook sections on dilutions and on spectroscopy, supplemental course files

## Introduction

Spectroscopy, the study of the interaction of light with matter, can be used for both qualitative and quantitative analysis. This experiment uses the absorption ("absorbance", A) of visible light to measure the concentration of Allura Red molecules in solution.

The absorption of a photon of appropriate energy can cause a transition between allowed electronic and vibration levels in a molecule. The associated spectra are called band spectra because they appear as broad peaks (“bands”) vs. the lines observed in the atomic case.

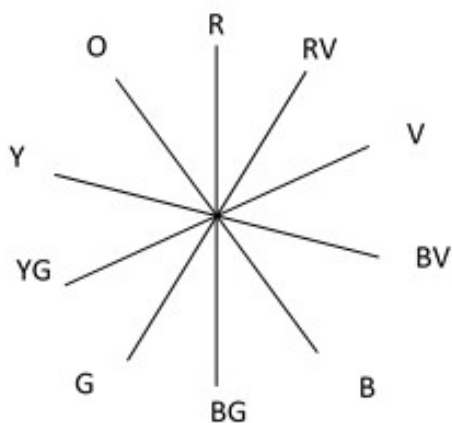


White light is a mixture of all visible wavelengths (400-700 nm). When white light shines on an object or solution that appears colored, a common reason for the perceived color is that molecules in the object or solution are absorbing some visible wavelengths while transmitting (or reflecting) the remainder; the mixture of the visible wavelengths not absorbed is observed.

Allowing for individual variation, many humans view the following wavelength ranges as the colors indicated:

Color	Wavelengths
Violet/indigo	400-450 nm
Blue	450-495 nm
Green	495-570 nm
Yellow	570-590 nm
Orange	590-620 nm
Red	620-700 nm

For an object to appear a color under white light, it must absorb the wavelengths of light corresponding to its complementary color. For example, green leaves absorb red and violet, reflecting green to our eyes.



Complementary colors are on opposite sides of the color wheel. When complementary colors are mixed, white light is the result.

When a color is observed (transmitted), its complementary color is absorbed.

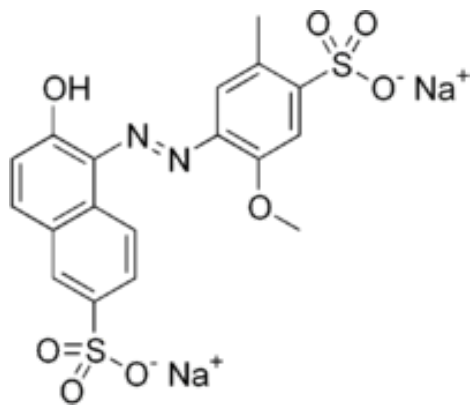
R = red, O = orange, Y = yellow, G = green, B = blue, V = violet

In terms of spectroscopic studies, few molecules undergo emission processes that can be measured, so absorption measurements are more common. In a typical experiment, a spectrometer is used to:

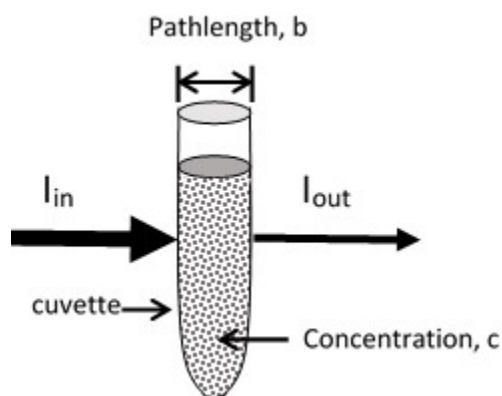
- scan over a range of wavelengths to identify which wavelength is most strongly absorbed by the molecule of interest ( $\lambda_{\max}$ );
- quantify the concentration of absorbing molecules in the sample by measuring how many photons are absorbed when light at  $\lambda_{\max}$  is passed through a sample.

Beer's Law describes the quantitative relationship between absorption (absorbance) and concentration. In this experiment, Allura Red is the absorbing species.

Allura Red has a molar mass of 496.42 g/mol. It usually comes as a sodium salt, which is soluble in water. In aqueous solutions, its  $\lambda_{\max}$  is about 504 nm. Allura Red was approved for use in food in 1971 after Allied Chemical Corporation subjected it to the most expensive and thorough testing program that had ever been given to a food dye.



## Absorption Spectroscopy and the Spec 20 Spectrometer



A solution of Allura Red in a cuvette is inserted into the sample holder of the spectrometer. Incident light of a known wavelength at an initial intensity  $I_{in}$  is directed through the solution. The emerging light has a reduced intensity,  $I_{out}$ .

The percent transmittance, %T, reflects the ratio of  $I_{out}$  to  $I_{in}$ , along with the distance the light travels through the sample (pathlength,  $b$ , in cm), the number of Allura Red molecules (concentration,  $c$ , in mol/L), and the identity of the absorbing species (molar extinction coefficient,  $\epsilon$ , in  $\frac{L}{mol \cdot cm}$ ;  $\epsilon$  is molecule- and wavelength-specific).

$$\%T = \left( \frac{I_{out}}{I_{in}} \right)^{-\epsilon bc} \times 100\%$$

You should read %T values from the Spec 20 because the scale is linear, allowing estimation between lines, and then convert to absorbance,  $A$ .  $A$  is the preferred unit because it changes linearly with concentration. Absorbance,  $A$ , is defined as the negative  $\log_{10}$  of  $T$ .

$$A = -\log_{10} \left( \frac{\%T}{100\%} \right) = \epsilon bc$$

Formulated by German mathematician and chemist August Beer in 1852, Beer's Law states that the absorbance of a dissolved substance is directly proportional to its concentration in a solution. By measuring changes in  $A$ , you can qualitatively infer concentration is changing; if you know  $\epsilon b$ , you can quantify the concentration of the absorbing molecules.

### Equipment List

25- and 50-mL volumetric flasks  
cuvettes (2) and large test tubes with rack

deionized water  
Allura Red (red dye #40)  
Spec 20 spectrometer  
assorted glassware, labeling tape and waterproof pen

## Procedure

You will:

Collect %T readings over a range of visible wavelengths, calculate A values, and plot A vs.  $\lambda$  (y vs. x) to create a spectral profile and to find  $\lambda_{\max}$ .

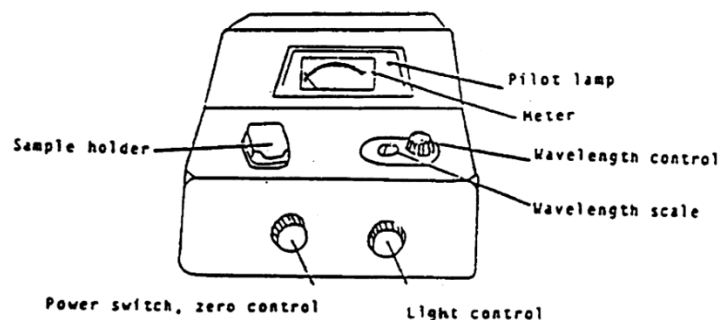
Take %T measurements at  $\lambda_{\max}$  using solutions of known concentration, calculate A values, and graph A vs. concentration (y vs. x) to create a Beer's Law plot. The best-fit line should be straight with a slope equal to  $\epsilon b$ ; the y-intercept should be 0. The slope divided by the pathlength (measured in cm) inside the cuvette is the experimentally-determined value for  $\epsilon$ .

Prepare dilutions of a commercial product, record their %T values, and calculate A. Using the slope from your Beer's Law plot, you will calculate the molarity of Allura Red in the diluted samples and adjust for the dilution ratio to find the original molarity in the commercial product such as a sports drink.

You must use the same Spec 20 for the entire experiment.  
Cuvettes are NOT test tubes. You only need TWO cuvettes for this experiment.

## How to Use the Spec 20

The power should be turned on and the spec should warm up for about 30 minutes before use.



**Two cuvettes** are needed: one for the blank and one for the sample.  
Do not fill cuvettes more than **2/3-3/4 full** (definitely not above the logo).  
Hold the cuvette **at the top**. Do not touch the middle (hand oil can interfere with absorption of light).  
If the outside of the cuvette is wet or dirty, wipe it gently with **kimwipes**.  
Put the cuvette in the holder with the **logo facing forward**.  
The sample holder flap must be **closed** when setting 0.0%T and when a cuvette is in it.  
Do **not drop or force** the cuvette into the holder; it should slide in easily.  
Do not break the cuvette and **do not pour liquid into the Spec 20**.

The measurements are made to one decimal place in % transmittance (%T), not absorption (A). %T is a linear scale so you can estimate between lines; A is on a log scale so you cannot read it accurately.

When making reading, the needle should line up with the mirror section behind where the reading is made so you know you are looking directly down at the needle to make accurate measurements.

## The Blank

Make sure the Spec 20 has been on for about 30 minutes.

**Set the wavelength** with the knob on the top of the Spec 20. Every time the wavelength is changed, you **MUST** blank.

With the sample holder empty and closed, use the **left knob** (zero control knob) to adjust the needle to **0.0 %T** (left knob, left side).

Put in cuvette containing the blank, close the sample holder flap, and use the **right knob** (light control knob) to adjust the needle to **100.0 %T** (right knob, right side).

Note: at very short wavelengths or very long wavelengths, sometimes you can't get the reading all the way to 100.0%. Usually, that's okay for your experiments.

The Spec 20 is now blanked.

Remember, the Spec 20 **MUST** be **re-blanked every time the wavelength is changed**.

## The Sample

Once the Spec 20 is blanked, you are ready to put the second cuvette containing the sample in (logo front, 2/3-3/4 full, lid closed).

**Record its %T** to one decimal place.

When finished with all measurements, the sample holder should be empty, the cuvettes should be thoroughly washed and put in the rack to dry, and the Spec 20 should be turned off.

Note: If the dial needle flickers or wanders after 5 seconds, let your TA or the stockroom staff know.

### Part 1. Spectral Profile of Allura Red to Find $\lambda_{\max}$

Note: if you are working with a partner, one person can do the spectral profile while the second person prepares the dilutions for part 2. You can switch roles halfway through if you'd like.

1. Take about 40 mL of the stock solution of Allura Red in a labeled beaker. This should be enough for parts 1 and 2.
2. Record the concentration of the stock solution to two significant figures.
3. Fill one cuvette about 2/3-3/4 full with the stock solution. For the blank, fill a second cuvette 2/3-3/4 with deionized water. **Cuvettes are NOT test tubes.**
4. Record %T to one decimal place over the entire visible range of wavelengths (400 - 700 nm). Do not forget to check 0.0 %T and to blank at each new wavelength.

From 400 to 480 nm, use 20-nm intervals; 480 to 540 nm, 10-nm intervals; and, from 540 to 700 nm, 20-nm intervals.

5. Calculate absorbance at each wavelength. If %T has three significant figures, A has three decimal places (# sig figs in %T = # decimal places in A).

$$A = -\log_{10} \left( \frac{\%T}{100\%} \right)$$

6. Determine the value of  $\lambda_{\max}$  by finding the wavelength with the lowest %T (highest A). Does your value of  $\lambda_{\max}$  make sense, given that you are looking at a red dye?
7. Set the Spec 20 to  $\lambda_{\max}$  for parts 2 and 3.
8. Create the spectral profile by graphing A vs.  $\lambda$ . This plot must be included in your results.

### Part 2. %T (and A) for Solutions with Known Concentrations of Allura Red

The Beer's law plot requires absorbance data for a range of concentrations that include the unknown concentrations you plan to measure (the sample). Four dilutions of the stock

solution of Allura Red will be made by serial dilution using volumetric glassware. Each of the four dilutions will be 1/2 the concentration of the one before it. The volume goes up by a factor of 2, so the concentration goes down to half of what it was.

$$M_1V_1 = M_2V_2 = n_{solute}$$

Making a solution by volumetric dilution follows the steps as the procedure outlined below. Change  $M_1$ ,  $V_1$ ,  $M_2$ , and  $V_2$  as needed.

You will make four serial dilutions of the stock solution.

You need only two 50.00-mL volumetric flasks (one will suffice) and one 25.00-mL volumetric pipet.

### Caution

Incorrect use of the pipet and pipetter can result in **serious injury**.

Please **do NOT force or jam the pipet** into the pipetter.

If you push too hard, the pipet can break and **cut your hand**.

Use **just enough force** to create a seal. The pipet does not “click” into place.

**Hold both** the pipet and pipetter at all times.

If you pull any solution into the pipetter, please give it to your TA so that it can be cleaned.

If you have trouble, **ask your TA for help**.

The Spec 20 may not be available while the spectral profile is performed. If this is the case, store your dilutions in large, labeled test tubes (NOT cuvettes).

1:2 dilution of the stock Allura Red solution

1. Fill your 50.00-mL volumetric flask ~1/4 full with deionized water.
2. Carefully pipet 25.00 mL of the stock solution into the flask.
3. Add deionized water until the bottom of the meniscus is close to the line around the flask's neck. Do not overfill the flask. Use a disposable pipet to add drops of deionized water until the bottom of the meniscus is at/on the line around the flask's neck.
4. Stopper the flask and, while holding the stopper in place, invert flask several times to thoroughly mix the solution. The concentration of this first dilution is 1/2 that of the stock solution (1:2 dilution of the stock).



5. Pour part of the 1:2 dilution into a large, labeled test tube (about halfway full) and make the second dilution (1:4 of the stock) from the remainder of the first dilution (1:2).

1:4 dilution of the stock Allura Red solution

6. Fill your 50.00-mL volumetric flask  $\sim 1/4$  full with deionized water.

7. Carefully pipet 25.00 mL of the 1:2 dilution into the flask.

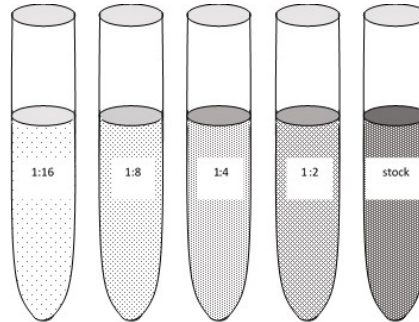
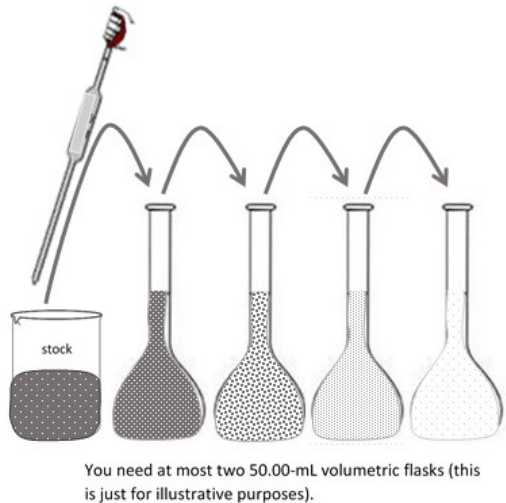
8. Add deionized water until the bottom of the meniscus is close to the line around the flask's neck. Do not overfill the flask. Use a disposable pipet to add drops of deionized water until the bottom of the meniscus is at/on the line around the flask's neck.

9. Stopper the flask and, while holding the stopper in place, invert flask several times to thoroughly mix the solution. The concentration of this first dilution is  $1/4$  that of the stock solution (1:4 dilution of the 1:2 dilution).

10. Pour part of the 1:4 dilution into a large, labeled test tube (about halfway full) and make the third dilution (1:8 of the stock) from the remainder of the second dilution (1:4).

11. For the 1:8 and 1:16 dilutions of the stock Allura Red solution, repeat the above procedure two more times.

Dilution	Dilution factor of stock solution	$V_1$ (mL of previous dilution)	$M_1$ ( $M$ of previous dilution)	$V_2$ (final mL of new dilution)	$M_2$ (final $M$ of new dilution)
0	none	----	$M_{\text{stock}}$	----	$M_{\text{stock}}$
1	1:2	25.00	$M_1 = M_{\text{stock}}$	50.00	$M_2 = 1/2(M_{\text{stock}})$
2	1:4	25.00	$M_1 = 1/2(M_{\text{stock}})$	50.00	$M_2 = 1/4(M_{\text{stock}})$
3	1:8	25.00	$M_1 = 1/4(M_{\text{stock}})$	50.00	$M_2 = 1/8(M_{\text{stock}})$
4	1:16	25.00	$M_1 = 1/8(M_{\text{stock}})$	50.00	$M_2 = 1/16(M_{\text{stock}})$



12. Measure the inner diameter of the cuvette in cm to two decimal places.

13. Check that the Spec 20 is set to  $\lambda_{\max}$ . Re-blank using deionized water.

14. Fill the sample cuvette 2/3-3/4 full with the 1:16 solution and record its %T value to one decimal place. Pour the solution into a large waste beaker and rinse the sample cuvette with deionized water.

15. Repeat step #13 with the 1:8, 1:4, 1:2, and stock solutions. You should have 6 data points (%T for  $M_{\text{dilution}}$ , including the blank at 0.0%T).

16. Calculate  $M_2$  and  $A$  for each solution ( $M_2$  values have same number of significant figures as  $M_{\text{stock}}$ ; a given  $A$  value's number of decimal places equals the number of significant figures in %T).

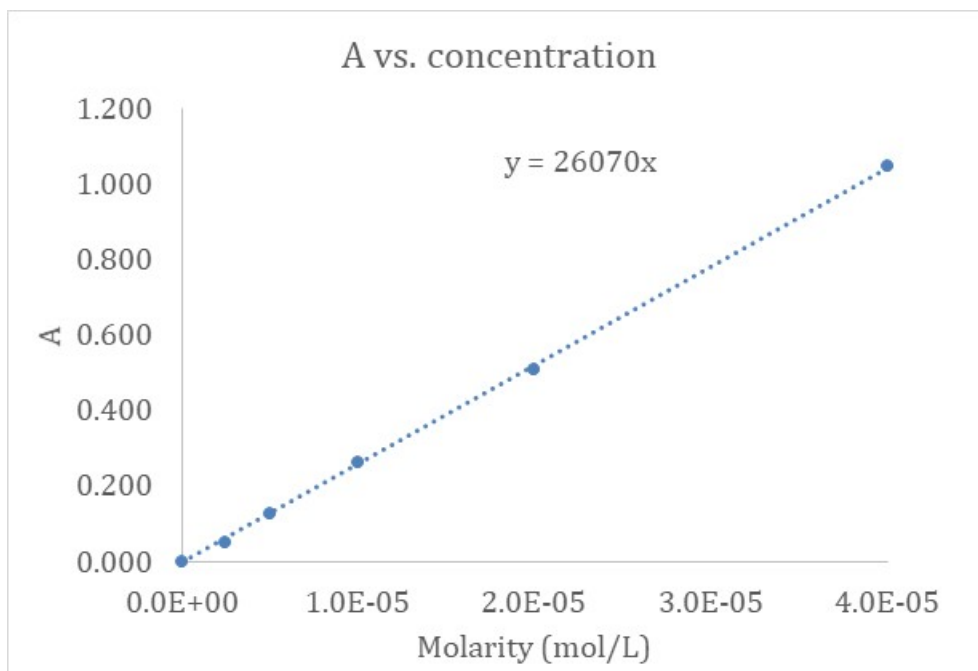
$$M_2 = \frac{M_1 V_1}{V_2}$$

$$A = -\log_{10} \left( \frac{\%T}{100\%} \right)$$

17. Plot absorbance,  $A$ , vs. concentration,  $M$ . See the supplemental instructions for using a program like Excel® or Google Sheets®.

18. Draw or otherwise determine the best-fit line for the data points. The line must go through (0,0);  $A$  for zero concentration of Allura Red should be zero.

An example plot is shown. Use your experimental data (your graph may not exactly match the one shown).



19. Determine the slope of the best-fit line to two significant figures (in the example, 26000). Use points on the line, not data points. Include units.

20. Calculate the molar extinction coefficient for Allura Red at  $\lambda_{\max}$  to two significant figures. Include units.

$$\text{slope} = \epsilon b \text{ so } \epsilon = \text{slope}/b$$

### Part 3. Determination of Allura Red Concentration in a Sports Drink (or Other Commercial Product)

The concentration of Allura Red in the drink is too high to read directly, so dilution is required.

1. Obtain about 7 mL of the drink in a small, labeled beaker. This is enough for three trials.
2. Make a 1-to-25 dilution using a 2.00-mL volumetric pipet and 50.00-mL volumetric flask.
3. Measure %T for the diluted sample.
4. Repeat two more times. Do not reuse one dilution.

- Calculate A for each sample.
- Calculate the concentration of Allura Red in each dilution to two significant figures using the rounded value of your slope from your Beer's Law plot.

$$A = \epsilon bc \text{ so } c = \frac{A}{\epsilon b}$$

- Take into account the dilution factor and report the concentrations of Allura Red in the original drink (undiluted) to two significant figures.
- Determine the average concentration and the standard deviation of the three values obtained from your three trials for both the diluted samples and the original drink.
- Calculate the average number of Allura Red molecules in one milliliter of sports drink.

$$\frac{\# \text{ Allura Red molecules}}{\text{mL drink}} = \frac{\# \text{ mol Allura Red}}{1 \text{ L}} \times \frac{6.022 \times 10^{23} \text{ molecules}}{1 \text{ mol}} \times \frac{1 \text{ L}}{1000 \text{ mL}}$$

Follow your TA's instructions for cleanup and waste disposal. Return any equipment and glassware borrowed clean.

## Results / Sample Calculations

Complete the online inlab or write a lab report as directed by your TA.

$\lambda_{\text{max}}$  and  $\epsilon$  of Allura Red (include spectral profile plot)

%T measurements for known concentrations (include Beer's Law plot)

%T of sports drink (dilutions and undiluted)

Average concentration and standard deviation of Allura Red in undiluted sports drink

# molecules Allura Red/mL drink

## Discussion Questions and Review Topics

What were the primary sources of error?

Does  $\lambda_{\text{max}}$  match what you expect for a molecule that appears red in solution?

How was Beer's law used?

What were the final results? Is absorption spectroscopy a useful technique for determining concentration? Why or why not? Can you think of any limitations to this technique?