***Lab 2. Spectroscopic Determination of Allura Red: How Much Dye is in my Gatorade?***

**Pre-Lab Directions – due at the start of lab (*read carefully to avoid loss of prelab points!*):**

1. **Read** this entire handout before starting. **Print** and **bring it to lab.** Refer to **‘The Lab Notebook’** as needed.
2. Complete the **Lab 2 Prelab Quiz** on Canvas (before its’ deadline)
3. Update the **Table of Contents**: Find the Table ofContents at the front of your notebook: **add** lab #, lab title, and the page # for the 1st page of this lab report.
4. Starting on a new page, complete the following **in your lab notebook** usingpermanent **INK**:
* **Header information:**  At the top of the report’s **1st page**, write the Lab #, Lab Title, date of the lab, Your full name, your section letter. You will add your lab partners names here during lab.
* Write a **Purpose statement;** *be sure to describe the* ***scientific goals of the lab,*** *not the ‘learning’ goals of the lab. Use complete sentences and an impersonal style here, and in the Method!*
* Write a **Method section**. **IMPORTANT:** You MUST ALSO look at the **post lab report** to write this section. Here are some questions that your section should provide the answer to:
* What data is collected in this lab and what equipment is used to collect the data?
* How is the data used in the Post lab report? (e.g., What calculations are done? What graphs are made? What equations are used?) Be sure to include a brief explanation the concepts behind each equation.
* After you have written the method…look back at the Purpose statement – does the method provide a description of exactly how the main lab goal accomplished? (it should!)
	+ Title the **Data and Observations** section and draw **ONE formatted data table** **large enough to allow for corrections** (multiple trials are not required) and ready to record the following:
	+ ‘Name’ for each solution: **standards** (#2, #3, #10, #15), unknown and Gatorade
	+ concentration of the ***stock*** solution used,
	+ volume used to make solution (standards, unknown and Gatorade)
	+ total volume made of each solution,
	+ molarity of each standard solution,
	+ measured absorbance at λmax for each sample tested (standards, unknown, Gatorade),
	+ value of λmax.
	+ **After the table, label and show the FOUR complete ‘Calculations’; one for the molarity (in mol/L) of each of the four standard solutions made in lab.** *How many sig figs do each pipet and the volumetric flask have?* R**ecord these molarity values in your table** (with correct sig. figs).

**Purpose**In this lab you will investigate the absorption of visible light by a colored compound, FD&C Red No. 40 (also known as Allura Red), a common artificial food dye. You will learn a new procedure, Visible Spectroscopy, for determining the concentration of a colored substance in solution. You will then use that procedure to accurately determine the concentration of an unknown solution of Red No. 40. **Extra credit points will be possible, depending on how accurately you and your team identify the concentration of the unknown solution.** Finally, you will use this procedure to determine the concentration of FD&C Red No. 40 in Gatorade.

**Figure 1**. FD&C Red Dye No. 40 (Allura Red). Allura red commonly comes as a disodium salt: C18H14N2O8S2Na2;

*molar mass = 496.42 g/mol*.

Background Information
The use of electromagnetic radiation to investigate chemical structure, behavior and concentration is a large and important field of chemistry. There are many important instrumental methods of chemical analysis that rely on the interaction between light and matter to probe chemical structure or to determine analyte concentration.. Nearly all regions of the electromagnetic spectrum have been used in chemical analyses of one sort or another. The lab you will do today uses the visible region of the spectrum and is used exclusively to analyze compounds that are colored in solution.

# Visible Spectroscopy

There are many ways to determine the concentration of a substance in solution. You used two methods in your Chemistry& 161 class (if you took it at GRC). In one experiment, you used titration to determine the concentration of calcium ions in an unknown sample. You also determined the density of alcohol/water solutions and used that property to determine the percent alcohol in an unknown sample. In that lab several solutions of known composition were prepared and their density was determined. A graph of density vs. percent alcohol for these “standard” solutions was prepared, and, using the equation of the ‘best fit’ line, the composition of the unknown was determined. That general procedure, using known standard solutions to determine the concentration of an unknown, is very similar to the procedure you will use today. The difference is that instead of measuring the density of the solution, you will be measuring the amount of light it absorbs. The amount of light absorbed by a solution is directly proportional to its concentration. This relationship is expressed mathematically by ***Beer’s law:***

***Beer’s law*: A = εbc (Equation 1)**

In Equation 1,

* **A** is ***absorbance***, the amount of light absorbed by the solution. Absorbance has no units.
* **ε** is the molar absorptivity (M-1cm-1). Molar absorptivity is a property of a substance that determines how much light it will absorb. Substances with higher molar absorptivity absorb more light, all other things being equal. For a given substance, molar absorptivity is a constant. [remember that M is the concentration unit, molarity, expressed as moles per liter]
* **b** is path length, or the “thickness” of the sample. Thicker samples absorb more light. This parameter is generally kept constant during an experiment.
* **c** is concentration, typically expressed in Molarity although any concentration units can be used as long as it cancels correctly with the units of **ε** .

Note that since **ε** and b are typically constant for a substance at a particular λ, Beer’s Law states that the Absorbance of a sample is directly proportional to its concentration. Thus we can rewrite Beer’s Law as:

**A = kc** where **k** is a constant equal to **ε**b. (Equation 2)



# Figure 2

# *Absorbance* is typically measured using a device called a spectrophotometer. A schematic diagram of a spectrophotometer is shown in this figure.

 Spectrophotometers have a detector which measures the intensity of light transmitted through the solution (It) as compared to the intensity of the incident light (I0). The ratio of It and Io can be used to indicate the percentage of incoming light absorbed by the solution. This is called the ***percent transmittance (%T).***

$$\%T= \left(\frac{I\_{t}}{I\_{o}}\right) x 100\% (Equation 3)$$

To apply Beer’s Law, we need a measure of the amount of light *absorbed*. Fortunately, these quantities are related. When more light is transmitted less is absorbed and visa versa. Mathematically:

$$A= -log \left(\frac{\%T}{100}\right) (Equation 4)$$

The higher the absorbance of light by a solution, the lower the percent transmittance. Modern spectrophotometers can display either Absorbance (A) or Transmittance(T), so in practice there is no need to use the equations above. *In this lab, all measurements will be made in the* ***Absorbance*** *mode.*

**Wavelength and Color**

A final consideration in this experiment is the relationship between the wavelength of the incident light and the amount of light absorbed. When colored solutions are irradiated with white light, they will selectively absorb light of some wavelengths, but not others. The remaining light, lacking the absorbed wavelengths, is transmitted and perceived by the eye (or by the spectrophotometer). A color wheel, shown below, illustrates the approximate ***complementary*** relationship between the wavelengths of light absorbed and the wavelengths transmitted. For example, in a blue substance, there would be a strong absorbance of the complementary (opposite it in the color wheel) color of light, orange. Substances that absorb blue light appear orange to the eye.



**Figure 3: Color wheel** with approximate **λ** values (nm) for each color light.

For a given substance, there is a wavelength**, λmax**, at which absorbance is highest and at which the solution is most sensitive to concentration changes. To find **λ**max for a specific substance, a plot of Absorbance vs. wavelength is collected using a spectrophotometer. This plot is called an ***absorption spectrum***. As an example, the absorption spectrum for a purple dye is shown in **Figure 4**, below. Note that maximum absorbance for the purple dye falls in the yellow region of the spectrum. Note the relative locations of purple and yellow on the color wheel in figure 2.

The spectrophotometers you will use in the lab can selectively emit light of any wavelength in the visible region of the spectrum. Which wavelength should you use for the Allura red solution? Can you make a prediction by looking at the color wheel?

Even though you can likely make a very good guess as to the **λ**max of Allura Red, we will use an instrument to measure its absorption spectrum and allow an accurate determination of the **λ**max.

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| **Figure 4.** **Absorption spectrum for a purple dye.** Analysis of this absorption spectrum indicates that the λmax for the purple dye is 572 nm. | **Figure 5**. **Calibration curve for a purple dye**. When A is plotted versus c, a straight line passing through the origin and with a slope of **b** is obtained. |

**What’s a Calibration Curve (or Standard Curve)?**

A spectrophotometer can measure the Absorbance of a solution, but it cannot automatically tell you the concentration of that solution. To relate the measured Absorbance to a concentration you must first prepare a set of standard solutions and measure the absorbance of each one. A ***standard curve***, also known as a ***calibration curve*** (fig. 5), is made by measuring and plotting the absorbance (**at λmax**) of several ‘standard’ solutions or solutions of known concentration. Recall that Beer’s Law (A = kc), shows that there is an expected linear relationship between Absorbance and concentration. Thus, when A is plotted versus c , a straight line passing through the origin and with a slope of k (k = **ε**b) should be obtained. The R2 value lets you know how well the line fits the data: R2 = 1 means that there is a perfect match between the data and the line. It should be noted that when solution concentrations are too high or too low that there are ***deviations from Beer’s law*** such that there is no longer a linear relationship between absorbance and concentration.

**Overview of this lab**

* Part A: Prepare a set of standard solutions of Allura Red with known concentration.
* Part B: Use one of the standard solutions to determine **λ**max. for Allura Red.
* Part C: Measure the absorbance of the standard solutions at **λ**max. and prepare a calibration curve.
* Part D: Measure the absorbance of an unknown and a sample of Gatorade.
* Use the calibration curve to determine the concentration of dye in the unknown and in the Gatorade.

###### Artificial Food Coloring Agents

Coloring agents have been used as food additives for centuries. They help us to identify foods visually. For instance, lime and orange sherbets would be nearly indistinguishable based on appearance if not for the green and orange colors. Coloring agents add a festive appearance to foods—M&Ms candies would taste the same if they were all colored gray, but would certainly be less appealing. Food additives are also added to foods because we have strong expectations about what colors should be associated with certain foods. All else being equal, would you buy an orange with a bright orange color, or one that is a mottled brown-green?

Coloring agents have been added to foods for less legitimate reasons as well. 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 inferior or spoiled foods, and some coloring agents marketed for inclusion in foods were indeed 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). A recent controversy in the news concerns the addition of a dye, canthaxanthin, to farm raised salmon. The dye gives the fish the deep red color consumers expect. After a lawsuit was filed here in Seattle by a consumer advocate group, local grocery chains were forced to label all fish containing the dye. Next time you are in the supermarket, stop by the fish counter and check it out!

The FDA divides coloring agents into two categories: certifiable and exempt from certification. The former are derived primarily from petroleum, while the latter includes agents derived largely from mineral, plant, or animal sources. Certified colors are further broken down in to water-soluble “***dyes***” and water-insoluble “***lakes***”, with most colors being available in both forms. At present, there are seven color additives certified for food use. One of these, allura red (FD&C Red No. 40), will be used in this experiment.

Red food dyes have a history of controversy. In 1960, additions to the FD&C Act of 1938 included the so-called ***Delaney amendment.*** This amendment prohibits the marketing of any coloring agent that has been found to cause cancer in humans or rats, regardless of the dose. For many years, FD&C Red No. 3 was the most important red dye used in foods. But, in 1938, a single study found that FD&C Red No. 3 could be associated with thyroid cancer in male rats. On the basis of that study, the FDA banned all uses of Red Lake No.3 and several uses of Red Dye No. 3. You may be old enough to remember that FD&C Red No.2 met a similar end several years ago, with the curious result that, for a time, there were no red M&Ms candies. As of today, Red Dye No. 3 remains certified for use in foods. However, in anticipation that the FDA may also ban Red Dye No. 3, food manufacturers have almost entirely abandoned this dye in favor of the relatively new (*and relatively untested*!!) FD&C Red No. 40.

More information can be found at: http://www.fda.gov/ForIndustry/ColorAdditives/default.htm

**PROCEDURE -** You will work as a **lab bench** to complete the following steps:

**Materials:**

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| * 1.894 x 10-4 M Stock solution of FD&C Red No. 40 (Allura Red)
* Gatorade Fruit Punch (FD&C red dye #40)
* Allura Red Unknown – **record** unknown # in your data table!
* 50.00 mL Volumetric flasks
* Various volumetric pipettes (TD; 0.00 mL precision)
 |  | - Pipette bulbs* Vernier spectrophotometer
* Cuvette
* *Kimwipes*
* D.I. Water
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 **CAUTION!** *An important factor that will influence the accuracy of your results in this lab is your ability to accurately pipette the Allura Red stock solution to make the standard solutions. You should test your pipeting skills before making the standard solutions from your stock solution.*

**Practice Using a Pipette (and clean it)**:

1. Each team member will prepare at least one of the four standard solutions. After determining which solution you will make, obtain the appropriate size pipette to use (e.g. 2.00 mL).
2. Weigh a 125 mL Erlenmeyer flask and then transfer the pipette volume (e.g. 2.00 mL) of DI water using the pipette and rubber bulb. Weigh the flask after transfer.
3. Assuming the density of water is 1.00 g/mL, use the mass of water transferred to determine the exact volume of liquid that was pipetted. Repeat this exercise until you can accurately (and precisely) reproduce volumes using the pipette. Note that this will also rinse and clean the pipette for use.

**Part A. Prep of Standard** Allura Red Solutions (from Stock solution)**, Unknown and Gatorade samples**

1. A stock solution of Allura Red will be available. **Record** the concentration of the Allura Red stock solution in your data table. Take about 60 mL of the stock solution in a clean, dry labeled beaker. Use this to prepare four standard solutions as described below.
2. Prepare the first of the standard solutions by quantitatively pipeting 2.00 mL of the stock solution into a *50.00 mL volumetric flask*. Dilute to the mark with DI water and mix well. Label the flask with your name and the number “2”. Use a disposable pipette or clean dropper to carefully add the final amount of DI water.
3. Repeat step 2 using 3.00, 10.00, and 15.00 mL samples to prepare the remaining standard solutions. The standard solutions will be used to generate the calibration curve and to determine λmax. For convenience, the standard solutions will be referred to here as solution 2, solution 3, solution 10, and solution 15.
4. Compare the calculated molarity values for the standard solutions (*your already calculated these in the prelab and recorded them in your table*) and ensure that all lab partners have the same values. These molarities will be entered into LoggerPro for the calibration curve in Part C.
5. Obtain an **unknown** solution of Allura Red from your instructor– **IMPORTANT-record** **the unknown number** in the data table. The unknowns are all too concentrated to be used as-is. Thus, *you will have to quantitatively dilute the sample before measuring its absorbance*. It is up to you to determine the appropriate dilution factor. Your goal is to prepare a diluted solution with an Absorbance that is *greater than that of the most dilute* standard solution and *less than that of the least dilute* standard solution. You don’t have the absorbance readings yet, so use the colors of the standard solutions as your guide. **Record** in the data table exactly how you diluted the sample (**volume used** and **final diluted volume**) and *remember to use this dilution factor when calculating the concentration of the original unknown.*
6. Obtain a sample of Gatorade from the lab cart. As with the unknown, the Gatorade will also need to be diluted in order to bring its Absorbance into the appropriate range. Dilute the sample as needed. **Record** in the data table how the sample was diluted (**volume used** and **final diluted volume**).

**Important Procedural Notes for the Spectrophotometer:**

* A Vernier spectrophotometer (figure 6) will be used to determine λmax. , create a calibration curve from the standard solutions and then measure the absorbance of the unknown and Gatorade samples.
* This instrument produces a complete absorption spectrum for your sample.
* Use the SAME cuvette for all measurement to avoid experimental errors.
* Avoid touching the two polished sides of the cuvette. Finger oils will interfere with the absorbance of light. Use Kimwipes to clean the outside of the glass.
* Both the DI water and the Allura Red samples in the cuvette should be kept free of bubbles, as these will scatter light and affect the measurements. If bubbles appear, try gently tapping the cuvette to dislodge them. If this doesn’t work, refill the cuvette with new sample.

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| Vernier Spectrometer | **Figure 6.** A Vernier spectrophotometer with a cuvette containing a Nickel (II) sulfate solution. |

**Part B. Determination of λmax with the Vernier Spectrophotometer**

The wavelength at which the absorbance is largest for a substance, is defined as λmax. Whatever the concentration, all samples of the *same substance* have the same value of λmax. The amount of light absorbed may vary with concentration, but the wavelength of the light absorbed remains the same. You will use *solution 10* to determine λmax for Allura Red, though any of the other standard solutions would also work.

1. Using a USB cable, connect a spectrometer to the FAR LEFT USB port on RED hub of the keyboard.
2. Start the Logger Pro program (under Vernier Software > Logger Pro); it will automatically launch the correct file, as long as you have the spectrometer connected **first**.
3. **Calibrate** the spectrometer (this sets absorbance value to zero when no solute is present)
	1. Prepare a *blank by* filling a clean cuvette ¾ full with DI water. Wipe the outside with a *Kimwipe*. Place the *blank* cuvette in the spectrometer, so the spectrometer light passes through the **smooth polished sides** of the cuvette.
	2. From the *Experiment menu* , select Calibrate ► Spectrometer. The calibration dialog box will display the message: “*Waiting ….. seconds for lamp to warm up*.” The minimum warm up time is 90 seconds, do not skip this. When the calibration is complete, click ‘Finish Calibration’ and then DONE.
4. **Determine λmax** of Allura Red and set up the data collection mode.
	1. Empty the blank cuvette, then rinse it twice with small amounts of the Allura Red solution **10**. Fill the cuvette **¾** full with solution 10, clean the outside with a Kimwipe, and place it in the spectrometer.
	2. Click. Click to complete the analysis. Examine the absorbance spectrum graph and note the wavelength region of maximum absorbance, **λmax**.
	3. From the *Experiment menu,* select *Store Latest Run* to saveyour absorbance spectrum.
	4. With solution 10 still in the spectrophotometer, click the **Configure Spectrometer Data Collection** icon, ,on the toolbar. A dialog box will appear: under *Set Collection Mode*, click **Abs *vs*. Concentration**. (The wavelength of maximum absorbance, **λmax**, will be automatically selected by the software. If you wish to select a new **λmax**, click on the graph or check the box next to the new **λmax** .) Click  to proceed.

## **Part C**. **Absorbance Measurements** of Allura Red standard solutions for the **Calibration Curve**

Run the samples in order from the *most dilute* to the *most concentrated*, if possible. (Why?) Run all samples, including the unknowns, in a relatively short time frame, before the instrument has time to drift off of the calibration.

1. Since it is already prepared, leave the cuvette containing solution 10 in the spectrometer. Click . When the absorbance reading stabilizes, click . Enter the previously calculated concentration of Solution 10 (in moles/L , this **must** be a numeric entry, e.g. ‘2.15E-6’) and click . **Record** the absorbance in the data table. Discard the cuvette contents and rinse well with DI water since solution 10 is more concentrated than your next solution.
2. Now, rinse the cuvette twice with solution 2, then fill it ¾ full. Wipe the cuvette and place it in the spectrometer. When the absorbance reading stabilizes, click . Enter the solution concentration (in moles/L) and click . **Record** the absorbance of solution in the data table. **Repeat this** for the remaining standard solutions.
3. **ONLY when you have finished testing all of the standard solutions should you click** . Check that the calibration curve is linear before discarding any solutions. *Are all of the points on the line or very close to it?* If any solutions are suspect, you will want to re-measure their absorbance and/or re-prepare them again.
4. Determine the best-fit line equation for the standard solutions, by clicking the “linear fit” button,, on the toolbar. In the box that appears, the slope (m) and y-intercept (b) values are given for the best-fit line, as well as the R2 (correlation) value. Note that for the slope, the notation “1.0E+004” means “1.0 x 104”. ***Write the equation*** *of the standard curve and the R2 value in your lab notebook.* (You will be using **MS Excel** to create a calibration curve in the Post Lab report. *Compare this equation to the one you create in Excel; they may not be identical due to sig fig differences, but they should be very similar*.

**Part D. Absorbance Measurements of Unknown** Allura Red solution and **Gatorade**.

1. Obtain an **unknown** Allura Red solution from your instructor– **IMPORTANT-record** **the unknown #** in the data table.
2. The unknowns are all too concentrated to be measured as-is. Thus, *you will have to quantitatively dilute the sample before measuring its absorbance*. It is up to you to determine the appropriate dilution factor. **Record** in the data table exactly how you diluted the sample (**volume used** and **final diluted volume**) and *remember this dilution factor when calculating the concentration of the original unknown.*
3. Rinse the cuvette twice with the *diluted* unknown solution and then fill it about ¾ full. Wipe the outside of the cuvette and place it into the spectrometer. **You don’t need to ‘click’ on anything!** Just **record** the **absorbance** at λmax that is displayed on the screen (lower right corner) for the diluted unknown solution.
4. Obtain a sample of Gatorade from the lab cart. As with the unknown, the Gatorade will also need to be diluted in order to bring its Absorbance into the appropriate range. Dilute the sample as needed. **Record** in the data table how the sample was diluted (**volume used** and **final diluted volume**). Measure and **record** its **absorbance** at λmax as you did in step 3 above for the unknown sample.

When measuring the absorbance of the *unknown it is essential that the Absorbance of the unknown be within the range of the standard solutions*. **If your absorbance reading for either sample is NOT between the reading for solution 2 and the reading for solution 15, you must prepare a different dilution of the sample and re-measure.**

1. Dispose of any of the remaining solutions in the waste container provided in the fume hood. Rinse all glassware thoroughly using DI water. Return all items to their proper locations. Wipe down the lab bench and wash your hands.
2. Check with your team members to ensure everyone has correctly recorded the **same** absorbance values for each solution, including the unknown and Gatorade sample. **Turn in the pages** from your lab notebook before leaving lab. **Don’t forget to include recorded observations and procedural notes.** Be sure to include the names of all lab bench team members in the header of the first page.

**POST LAB REPORT Instructions:**

Open the **Lab 2 Post Lab Report file** (on Canvas in the Lab Handout module) and **type** all of your responses into this MS Excel file, except as allowed in the directions provided. **Read the follow the directions carefully**!

One of the questions requires you to prepare and format a **calibration curve** using MS Excel and insert it into the post lab report form:

1. Create an **MS Excel** **x-y scatter** chart using the molarity and absorbance values for the four standard solutions. **IMPORTANT:** Remember that Excel needs the x,y data in **adjacent** columns to create a ‘chart’. The x-axis must be molarity and y-axis is absorbance; it should look similar to figure 5 in this handout.

 2. **Format** requirements for the calibration curve:

1. Use the trend line feature to display the linear best fit **line** for the 4 data points.
2. Display the MS Excel trendline **equation** and the **R2 value** on the graph (under Trendline Options)
3. Adjust the significant figures displayed on each axis, as needed
4. Add a descriptive chart title and axis titles, including units
5. Delete the ‘legend’
6. Once you have formatted the chart, copy and paste it into the appropriate box in the Post Lab Report. **Make it as large as possible** while still fitting inside the box provided.

**GRADING: (refer to the Lab 2\_Grading file on Canvas)**

1. **Lab 2 prelab quiz: Complete the quiz on Canvas** by the deadline.(5 pts – no late credit)
2. **Individual Lab Notebook work:**  Turn in the pages from your lab notebook containing the Purpose, Method, Data and Observations **before leaving lab**! Points will be deducted if the prelab work is not completed when the instructor checks it. (10 pts – no late credit)
3. **Post Lab Report (individual):** Type your answers into the Lab 2 Report template, then print and turn in on the assigned due date. Don’t forget to sign the declaration. (20 pts)
4. **Extra Credit Points:**  It is your responsibility to obtain the actual concentration for your unknown from your instructor before the Post Lab Report due date**. To receive these points**, you must not only achieve the accuracy below, but you must **also** show the correct calculation for both the unknowns’ molarity (based on your groups data) and the percent error in your post lab report **and** submit your report by the required due date.

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| **If your results have a percent error of:** | ± 2.5% | ±5 .0% | ± 7.5% | >10% |
|  **then your extra credit points will be:** |  3 pts | 2 pts | 1 pts | 0 pts |

*Acknowledgement: This lab is adapted from similar labs produced by my colleagues at GRC.*