

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

Materials and Methods


Materials

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| - Stock solution of FD&C Red No. 40 (Allura Red) | - Various pipettes |
| - Gatorade Fruit Punch (FD&C red dye #40) | - Pipette bulbs |
| - Allura Red Unknown – record number in your lab notebook! | - Vernier spectrophotometer |
| - Five (5) 100.0 mL Volumetric flask | - Cuvettes |
| | - Kimwipes |
| | - D.I. Water |

I. Preparation of Standard Solutions of Allura Red from Stock Solution





1. Prepare the first of the six standard solutions by quantitatively pipetting 3.00 mL of the stock solution into a 100 mL volumetric flask. Dilute to the mark and mix well. Label the flask with your name and the number “3”.
2. Repeat this procedure using 5.00, 10.00, 15.00, 20.00 and 25.00 mL samples to prepare the remaining standard solutions. The standard solutions will be used to generate the calibration curve and to determine λ_{\max} . For convenience, later in the lab the standard solutions will be referred to as solution 3, solution 5, etc. Later, the exact concentration (in mol/L) of these stock solutions must be calculated.

II. Determination of λ_{\max} with a “Vernier Spectrophotometer”

1. Use a USB cable to connect a spectrometer to the computer.
2. Start the Logger Pro
3. **Calibrate the spectrometer.**
 - a. Prepare a *blank* by filling an empty cuvette $\frac{3}{4}$ full with distilled water. Place the blank cuvette in the spectrometer.
 - b. Select Calibrate ► Spectrometer from the Experiment menu. The calibration dialog box will display the message: “Waiting seconds for lamp to warm up.” The minimum warm up time is one minute. Follow the instructions in the dialog box to complete the calibration. Click .
4. **Determine the λ_{\max} Red No. 40 and set up the data collection mode.**
 - a. Empty the blank cuvette and rinse it twice with small amounts of the Red 40 solution from solution 10. Fill the cuvette $\frac{3}{4}$ full with solution 10 and place it in the spectrometer.
 - b. Click . A graph of the Red 40 solution absorption spectrum will be displayed. Note that one area of the graph contains a peak absorbance. Click to complete the analysis.
 - c. Save your graph of absorbance vs. wavelength by selecting Store Latest Run from the Experiment menu.
 - d. Print two copies of the graph—one for the white pages and one for the yellow pages in the results section of your lab report.
 - e. Click the **Configure Spectrometer Data Collection** icon, , on the toolbar. A dialog box will appear:
 - Select **Abs vs. Concentration** under Set Collection Mode. The wavelength of peak absorbance (λ_{\max}) will be automatically selected. If you wish to select a new wavelength, click on the graph or check the box next to the desired wavelength. Click to proceed.

III. Measurement of Absorbance for the Calibration Curve, Unknown and Gatorade

5. **Collect absorbance-concentration data for the six standard solutions.**
 - a. Leave the cuvette containing solution 10 in the spectrometer. Click . When the absorbance reading stabilizes, click . Enter the concentration of Solution 10 (in moles/L) and click . Record the absorbance of solution 10 in your lab notebook.

- b. Discard the cuvette contents. Using the solution 3, rinse and fill the cuvette $\frac{3}{4}$ full. Wipe the cuvette and place it in the spectrometer. When the absorbance reading stabilizes, click . Enter the concentration of Solution 3 (in moles/L) and click . Record the absorbance of solution 3 in your lab notebook.
 - c. Repeat Step 5b for the remaining standard red 40 solutions. When you have finished testing the standard solutions, click .
6. To determine the best-fit line equation for the Red 40 standard solutions, click the linear fit button, , on the toolbar. Write the equation of the standard curve in your lab notebook.
 - a. **Print** two copies of your absorbance vs. concentration graph, one copy for the white pages and the other for the yellow pages in the *results section* of your lab report.
 - b. Select **Save As** from the File menu and save your experiment file.
7. **Determine the concentration of an unknown red 40 solution.**
 - a. Obtain an unknown solution of Allura Red from the lab cart. Record the number of the unknown in your lab notebook and report the number to your instructor. When measuring the absorbance of the unknown it is essential that the Absorbance of the unknown be within the range of the standard solutions. In other words, the Absorbance of the unknown should be no lower than the most dilute standard, and no higher than the most concentrated standard.

The unknowns we have prepared for you 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 your lab notebook how you diluted your sample and remember this dilution factor when calculating the concentration of the unknown.
 - b. Rinse the cuvette twice with your diluted unknown solution and fill it about $\frac{3}{4}$ full. Wipe the outside of the cuvette and place it into the spectrometer. When the absorbance reading stabilizes, record the absorbance of the diluted unknown solution in your lab notebook.
 - c. Dispose of any of the remaining solutions as directed.
8. **Gatorade Analysis:** 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, then measure and record its absorbance at λ_{\max} .