VALENCIACOLLEGE

Experiment 10
Dye Concentration Using a UV-Vis Spectrophotometer

version 5

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In this experiment, you will determine the concentration of Allura Red Dye (FD&C Red No. 40) in a drink. By mixing a series of known concentration solutions of the dye and measuring absorbance ultraviolet-visible (UV-Vis) light for each, a calibration curve can be made to determine the unknown drink concentration.

Objectives

- Evaluate and apply the concept of solution concentration.
- Perform dilutions.
- Operate an ultraviolet-visible (UV-Vis) spectrophotometer.
- Express and interpret graphical data.

Learning Outcomes

- Understand and apply chemical quantities related to chemical compounds.
- Understand the relevance of quantum mechanics theory to the fundamental nature of light and spectroscopic data.
- Master essential laboratory techniques critical in the application of laboratory science study.

Definitions

- **Absorbance –** the amount of radiation absorbed by molecules in a sample, and therefore not transmitted through the sample, at a specific wavelength
- **Absorption spectrum –** a recording of electromagnetic radiation that is absorbed by a sample; for UV-Vis, units are typically absorbance versus wavelength in nm; the plural is spectra
- **Aliquot –** a small portion of sample solution
- **Calibration curve –** the graphic relationship of concentration to absorbance for a series of standard samples of known concentration that is used for determining the concentration of a substance in an unknown sample by comparison; it is often a straight line
- **Cuvette –** an optically transparent cell that holds the sample under study
- **Dilute –** to make a solution of lower concentration; add solvent
- **Frequency –** cycles per unit time; for example, wave crests per second in electromagnetic energy

- **Molar absorptivity –** a measure of how strongly a sample absorbs light at a given wavelength; it is a physical property of a compound
- **Organic molecule –** a molecule that contains carbon
- **Solute** – the component in lesser amount in a solution
- **Solvent** – the major component of a solution
- **Spectrophotometer –** an instrument for measuring the intensity of light transmitted, absorbed, or emitted from a sample
- **Standard solution –** a solution of precisely known concentration used to determine the concentrations of unknown solutions
- **Stock solution –** a solution of known concentration used to prepare more dilute solutions
- **UV-Vis –** an abbreviation for ultraviolet and visible
- **Wavelength –** the distance of one wave; for example, crest-to-crest length
- **Wavelength of maximum absorbance –** λ_{max} (read as lambda max), the highest point of absorbance in an absorbance band (peak); used to describe the UV-Vis activity of a sample

Techniques

The following techniques are used in the experimental procedure:

- Technique 1: Cleaning glassware
- Technique 6: Using a volumetric flask
- Technique 7: Using a graduated pipet
- Technique 11: Disposing chemical waste
- Technique 21a(Lake Nona) or Technique 21b(West): Using UV-Vis spectrophotometer

Introduction

Currently, there are eight synthetic food dyes approved for use in the United States by the Food and Drug Administration (FDA).[1] There has been much speculation that these artificial dyes are bad for health.[2] However, the evidence remains unclear; for example, the European Food Safety Authority and the FDA approve different dyes based upon their interpretations of the scientific research.[3] Some concern is justified given history. Since ancient times, colorants have been used to make food appear more attractive; such colorants included chalk to make bread appear more white, and lead(II) oxide and mercury(II) sulfide – toxic and poisonous heavy metal salts – to enhance the color of cayenne and curry powder and jams and candy. Allura Red AC (FD&C Red No. 40[a]) (Figure 1) is an organic molecule, a molecule that contains carbon, with chemical formula $C_{18}H_{14}N_2Na_2O_8S_2$ and molecular mass 496.42 g/mol.

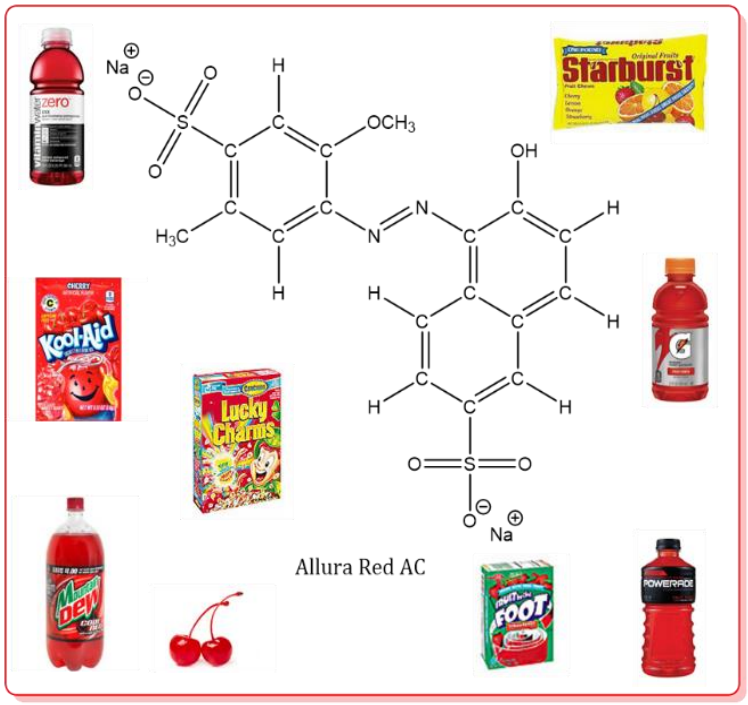

Figure 1. Chemical Structure of Allura Red AC, and Some Example Products.

In your scenario for this experiment, you have been contracted by a health organization, Don't Be Dyeing, that would like to begin research into the health effects of food dyes. They have asked you to determine the amount of Allura Red Dye in a common drink. From this amount, you can determine how many servings a consumer can drink without exceeding the FDA's acceptable daily intake (ADI) of 7.0 mg per kg of bodyweight per day.[4]

Unknown concentrations of solutions such as the amount of dye in a drink can be determined by spectroscopy, the measurement of how a substance interacts with electromagnetic radiation. Electromagnetic radiation is energy that travels through space with the characteristics of a wave. Light is specifically the visible region of the electromagnetic spectrum with wavelengths between about 400 nm and 700 nm, which is only a small portion of the entire spectrum. Our eyes detect different wavelengths of visible light as different colors. For example, light with a wavelength of 425 nm appears violet while light with a wavelength of 700 nm appears red. White light is a mixture of all the visible wavelengths.

[a] FD&C refers to the U. S. Federal Food, Drug, and Cosmetic Act, first passed in 1938.

When an object absorbs some wavelengths of white light and reflects others, it appears colored; the observed color is predominantly the colors reflected. So, blue food dye absorbs wavelengths around 600 nm (the orange wavelengths), reflecting the blue wavelengths back to the eye (Figure 2).

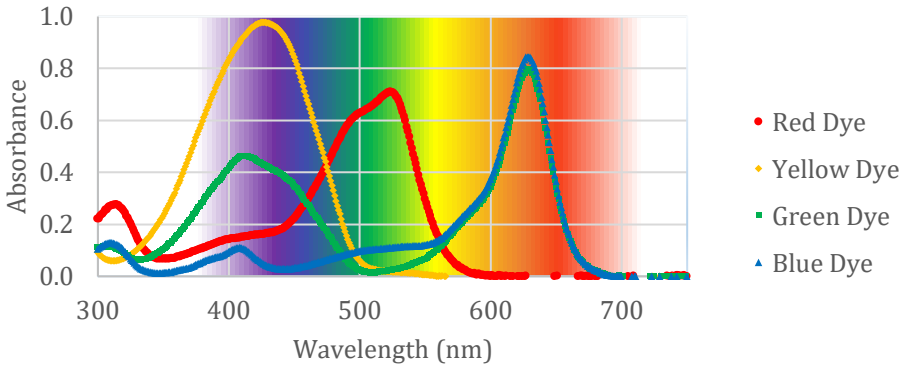

Figure 2. Ultraviolet-Visible Spectra of Food Dyes.

A color wheel can be useful for determining the color that a solution will appear based on the wavelengths absorbed (Figure 3).[5] If a solution absorbs wavelengths of one particular color, it will have the appearance of the color directly opposite it on the wheel. For example, FD&C Red No. 40 is known to absorb light around 504 nm, in the green range, therefore the solution will look red. FD&C Green No. 3 is known to absorb light around 628 nm, in the red range, therefore the solution will look green. However, mixtures of dyes can also be used. Green food dye is composed of FD&C Yellow No. 5 and FD&C Blue No. 1, and therefore absorbs wavelengths around 414 nm and 625 nm, transmitting blue and yellow together to make green.

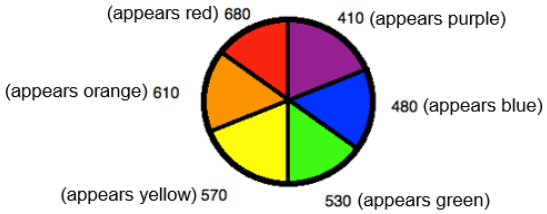

Figure 3. Color Wheel. Image used under a Creative Commons Attribution License (by 4.0) from OpenStax, Chemistry.[5]

The Beer-Lambert Law states that the absorbance of a substance is directly related to the concentration of the substance in solution:

$$A = \epsilon \cdot \ell \cdot c$$

where A is the measured absorbance, ℓ is the path length of light through the cell in centimeters, c is the molar concentration of the sample, and ϵ is the molar absorptivity or molar extinction coefficient of the sample in $cm^{-1} \cdot M^{-1}$.

The direct relationship of absorbance to concentration can be expressed by the graph of a line, and the equation of the line used to determine an unknown concentration from experimentally-determined absorbance. Let's examine how this experiment will work.

The substance in solution is placed into a cuvette, an optically transparent cell that holds the sample under study. The cuvette with its width (ℓ) is placed in the spectrophotometer along the path of the light beam (Figure 4). If a larger cuvette was used, the path length (ℓ) would increase, and more light would be absorbed. You will use one cuvette consistently to eliminate this variable.

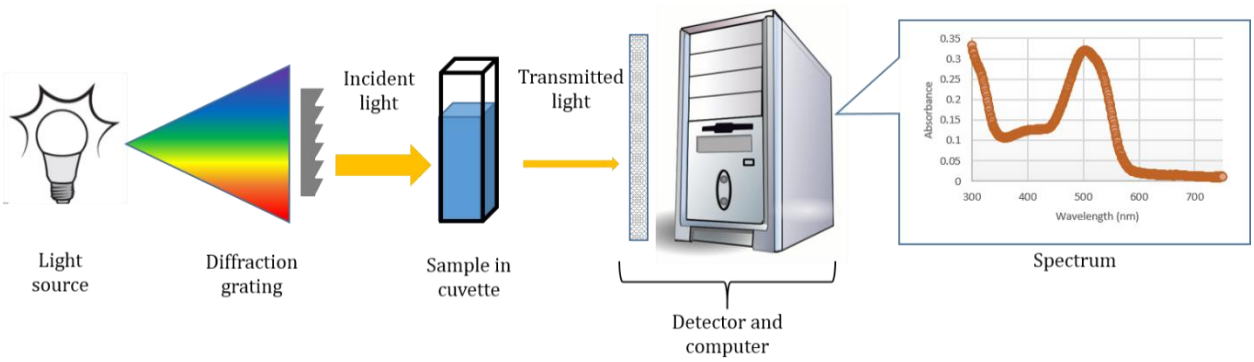

Figure 4. Simple Diagram of a Spectrophotometer.[67]

Incident light is the light that falls on a material. When light passes through the solution in a cuvette, the intensity of the incident light decreases due to absorption. The transmitted light is split into its different wavelengths by a diffraction grating and measured by the detector. If the intensity of the incident light is represented by I_o and the intensity of the transmitted light is represented by I, transmittance (T) is defined as the ratio:

$$T = \frac{I}{I_o}$$

Light absorption can also be represented as absorbance (A). Absorbance is the negative logarithm of the transmittance:

$$A = -log\left(\frac{I}{I_o}\right)$$

Molar absorptivity (ϵ) is a measure of how strongly the sample absorbs the light at a specific wavelength; it is a physical property of a compound. When working in concentration units of molarity, the unit of ϵ is $M^{-1} \cdot cm^{-1}$. So, absorbance is unitless. Examining the absorbance of Allura Red over the visible spectrum, you can see that the highest absorbance occurs at 504 nm (Figure 5). This wavelength is called the absorbance maximum or λ_{max}, and is read as lambda max.

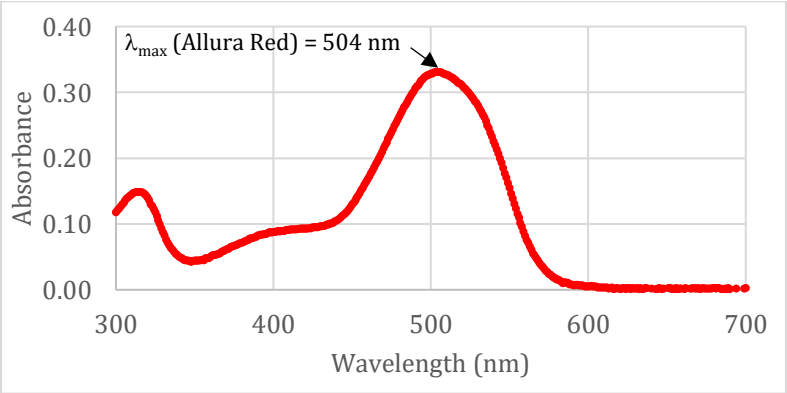

Figure 5. UV-Visible Spectrum of Allura Red AC.

On the spectrophotometer in the lab, you may be able to measure absorbance at only one wavelength at a time, so you will measure the absorbance at λ_{max}. The path length (typically 1 cm (ℓ)) and the molar absorptivity (Allura Red AC with its known ϵ) will be kept constant. By graphing the

absorbance versus concentration of several known solutions, a linear calibration curve can be made (Figure 6); an unknown concentration can be determined from the equation of the line. As the amount of solute increases (with increasing concentration c per the Beer-Lambert Law), more light will be absorbed.

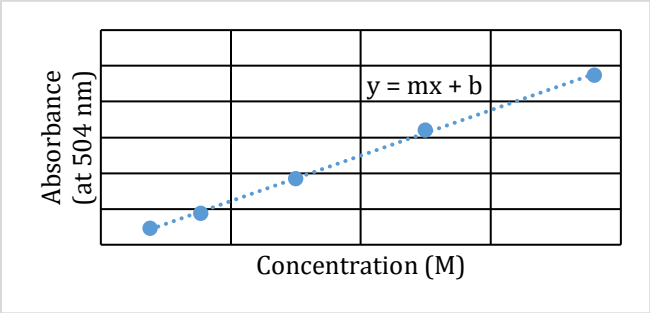

Figure 6. Beer-Lambert Graph Example.

In this experiment, you will use molarity to calculate concentrations:

$$Molarity\ (M) = \frac{moles\ of\ solute}{volume\ of\ solution\ (L)} = \frac{n}{V}$$

Solutions are stored often as concentrated stock solutions. You will require solutions less concentrated than the stock solution since the Beer-Lambert Law only works for small values of absorbance. To dilute a solution (make a solution of lower concentration) from the stock solution, a portion of the stock solution is measured into a new flask and more solvent is added. The following equation can be used to determine the aliquot, the volume of concentrated solution, needed to carry out the dilution:

$$M_1 \cdot V_1 = M_2 \cdot V_2$$

The moles of solute in the aliquot will be equal to the moles of the solute in the less concentrated or dilute solution. It is only the volume of solution that changes after dilution, hence molarity changes.

$$moles\ of\ solute\ in\ the\ aliquot = moles\ of\ solute\ in\ diluted\ solution$$

This process must be done quantitatively, which means the measurements of the quantities must be done accurately and precisely. For example, suppose an experiment requires 100.00 mL of 0.02500 M NaCl solution. The concentration of NaCl solution you have is 0.1000 M. What volume of the concentrated solution (0.1000 M) would be required for diluting to the required concentration and volume?

Upon rearranging the dilution equation:

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

$$V_1 = \frac{(0.02500\ M \times 100.00\ mL)}{0.1000\ M} = 25.00\ mL$$

To prepare the 0.0250 M solution, pipet a 25.00 mL aliquot of the 0.1000 M NaCl aqueous solution using a graduated pipet and transfer the solution into a 100.00 mL volumetric flask. Dilute with deionized water to the mark with mixing as in Technique 6: Solutions Using a Volumetric Flask.

The absorbance of a series of Allura Red Dye standard solutions with known concentrations will be measured. These data will be used to make a calibration curve (absorbance (A) at λ_{max} on the y-axis and concentration on the x-axis). By comparing the absorbance of the unknown drink to the known solutions, its concentration will be determined.

Experimental Procedure

List of Chemicals

- $\sim 7.0 \times 10^{-5}$ M Allura Red AC dye(FD&C Red 40)
- red-colored sports drink or juice containing Allura Red
- deionized (DI) water

Since these are food products, they are non-hazardous; however, since they are in the lab, they should never be consumed. Use Safety Data Sheet (SDSs) to learn about proper handling of these chemicals. (The SDSs are available in the laboratory or online.)

List of Equipment and Glassware

- 100-mL beaker
- 50-mL beaker
- watch glass
- 1-mL and 5-mL graduated pipets and bulb
- funnel
- transfer pipet
- five 10-mL volumetric flasks with stoppers
- UV-Vis spectrophotometer
- two cuvettes
- beaker for waste

Part A: Preparing Solutions

1. Pour about 30 mL of the Allura Red stock solution into a 100-mL beaker. Cover with a watch glass. Record its concentration, as written on the bottle, above Data Table 1.
2. Calculate how many mL of the Allura Red stock solution is needed to prepare 10.00 mL of each of the following standard solutions. Record these concentrations and volumes in Data Table 1:

Standard #	Concentration of Standard
1	3.50×10^{-6} M
2	1.40×10^{-5} M
3	2.10×10^{-5} M
4	2.80×10^{-5} M
5	3.50×10^{-5} M

3. Label the five 10-mL volumetric flasks with the standard number.
4. Condition the 1-mL graduated pipet and the 5-mL graduated pipet with the Allura Red stock solution.

5. Pipet the desired amount of Allura Red stock solution calculated for each standard into each volumetric flask. Use the 1-mL graduated pipet to deliver volumes of less than 0.900 mL. Use the 5-mL graduated pipet to deliver volumes between 1.00 – 5.00 mL.
6. Add DI water to fill each volumetric flask to the calibration mark. (If the volume goes over the mark, discard the solution, rinse the flask with DI water, and remake.) Cover with stopper and invert a several times to mix well.

Technique 6
Technique 7

Part B: Measuring UV-Vis Absorbances for the Calibration Curve

1. The instructions on calibration and use of the spectrophotometer will be found next to the instrument in the laboratory (Technique 21a (Lake Nona) or Technique 21b (West): Using a UV-Vis spectrophotometer).
2. Follow the directions to set the wavelength to 504 nm, the wavelength of maximum absorbance for Allura Red.
3. Fill the first cuvette to three-fourths its height with DI water for the blank solution. A blank solution is a solution that should contain everything (all reagents used) except the substance being analyzed. We will use water as the blank since the other species in the drink should not absorb in the visible range (e.g., sugar in water is colorless and clear). "Zero out" the spectrophotometer to give only the absorbance of the sample under study.
4. Fill the other cuvette to three-fourths height with the most dilute standard.
5. Measure and record the absorbance at 504 nm.
6. Then, use the next most concentrated standard to condition the cuvette. Fill it to three-fourths height, and measure and record the absorbance.
7. Repeat step 5 with all the standards.
8. If any of the absorbances are greater than 1.0, check with your instructor to prepare a more dilute sample.

Technique 21a
Technique 21b

Part C: Measuring the Absorbance of Allura Red in a Commercial Drink

1. Select a commercial drink from the options available. Record its name in Data Table 2 and pour about 20 mL of this drink into a 50-mL beaker.
2. Using a graduated pipet (don't forget to condition the pipet first), pipet 1.00 mL of the drink into a 10-mL volumetric flask.
3. Add DI water to fill the volumetric flask to the calibration mark.
4. Measure and record the absorbance at 504 nm on the same instrument used to measure the standards.
5. If the absorbance is greater than the absorbance of the most concentrated standard, check with your instructor to prepare a more dilute sample. Record, in Data Table 2, how this more dilute sample was prepared. Measure and record its absorbance at 504 nm. If no additional dilution was needed, write "Not applicable".

Part D: Create calibration curve and determine concentration of Allura Red in the Commercial Drink Selected

1. Use the data in Data Table 1 to prepare a calibration curve for Allura Red in Excel. You can follow the tutorial in Appendix 7 Using Excel to plot the graph.

a. Plot absorbance (on the "y" axis) versus concentration (on the "x" axis) in a scatter (XY) plot.
b. Using the Excel software, draw the "best fit line" through the data points; include the line equation and the R^2 value on the graph.
c. Add a title to graph.
d. Add titles to axes and units if applicable.

2. Use the line equation from the graph to calculate the concentration of Allura Red in the diluted sample of the drink. Record result in Data Table 2 and show calculation below the table.
3. Based on the way you prepared the diluted sample of the drink and its concentration, calculate the actual concentration of Allura Red dye in the commercial drink. Record result in Data Table 2 and show calculation below the table.

Clean up/Disposal

Technique 1
Technique 11

- Pour all waste into the waste container (Technique 11: Disposing Chemical Waste).
- Clean the glassware with soap and tap water, and discard in the sink. Rinse it twice with distilled water, dry the outside of the glassware, and replace in its original location (Technique 1: Cleaning Glassware).
- Place the graduated pipets tip up in the pipet canister.

References

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2. Potera, C. Diet and Nutrition: The Artificial Food Dye Blues. *Environ. Health Perspect.* **2010**, 118 (10), A428. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2957945/ (accessed June 24, 2017).
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Pre-lab

The pre-lab assignment must be completed before you come to the lab.

1. Calculate how many mL of an 8.0×10^{-3} M Allura Red stock solution will be needed to prepare 20.0 mL of the following solutions. Show your work for one example.

$$3.0 \times 10^{-3} \text{ M}$$

$$2.0 \times 10^{-3} \text{ M}$$

$$8.5 \times 10^{-4} \text{ M}$$

$$5.5 \times 10^{-4} \text{ M}$$

$$4.0 \times 10^{-4} \text{ M}$$

2. a) Graph the data set below and determine the equation of the line. Include your Excel graph and show your calculations. Make sure to add a title and axes labels and units to the graph. [See Appendix 7: Creating a Graph in Excel.]

Concentration (M)	Absorbance
2.2×10^{-6}	0.21
4.9×10^{-6}	0.43
6.2×10^{-6}	0.59
8.2×10^{-6}	0.79
1.0×10^{-5}	0.98

   b) Calculate the concentration of a solution with absorbance of 0.75.

3. Sudan 1 was a food dye approved by the FDA in 1918 but removed after six months for causing dermatitis;[3] now, it is listed as a carcinogen.[8] In solution, it has λ_{max} = 418 and 476 nm. What color does the Sudan 1 solution appear? Briefly explain.

4. Explain how to use a graduated pipet. Why was it chosen instead of a volumetric pipet in this experiment?

5. Print the data tables before coming to lab.

Post-lab

- Include Data Tables 1and 2 and calculations.
- Include your Excel graph.
- Include answers to the following questions:
  1. Based on your answer for the concentration of Allura Red dye in the drink, calculate the amount (in mg) of Allura Red dye that a consumer would obtain from an 8-ounce serving of the drink that you studied. The molar mass of Allura Red dye is 496.42 g/mol. In one U.S. fluid ounce, there are 29.6 mL.

  2. The FDA's acceptable daily intake for Allura Red dye is 7.0 mg/kg bodyweight per day. Calculate how many servings a 200-lb person may have per day (or if you would like to, calculate how many servings you may have per day). In 1.0 kg, there are 2.2 lbs.

  3. How many servings can a 5-year-old child, weighing 55-lb, drink in a day to remain below an acceptable daily intake of 7.0 mg/kg bodyweight?

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Experiment 10: Dye Concentration Using UV-Vis Data Tables

Experimental Data and Calculations

Name: _____ Date: _____

Lab Partner: _____ Section: _____

All measurements and calculated values must be reported with proper significant numbers and units. You can write any observations or description while doing the experiment.

Data Table 1. Preparation and Absorbance of Allura Red AC Standards.

Concentration of Allura Red AC Dye Stock Solution _____

Standard #	Volume of Stock Solution Used ( )	Concentration of Standards ( )	Absorbance
1			
2			
3			
4			
5			

Show your work for calculation of the concentration of standard 1:

Data Table 2. Preparation, Absorbance and Concentration of Allura Red in a Commercial Drink.

Drink Analyzed: _____

Dilution preparation: 1.00 mL of drink diluted with DI water to 10.00 mL	Absorbance:
Additional dilution preparation (if needed):	Absorbance:
Allura Red in diluted sample of drink	Concentration:
Allura Red in drink	Concentration:

Show your work for calculation of the concentration of Allura Red AC in the Commercial Drink:

Include graph and answers to the Post-Lab questions (page 11) with this Report.