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

Prelab Assignment

Before coming to lab:

- Complete the following sections of your report for this lab exercise *before* attending lab: Title, Introduction, Materials/Methods and Data Table(s). For the Materials/Methods section, simply summarize the overview of the procedure in your lab notebook and cut and paste copies of the procedure for parts I – III of the procedure onto both the white and yellow pages of your lab notebook. Ensure that the table of contents of your lab notebook is current.
- Read the lab thoroughly and answer the ***prelab questions*** that appear at the end of this lab exercise and be prepared to hand them in just before the start of lab.

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 or Red 40), 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 a solution of Red No. 40. Part of your grade will be based on the accuracy of your analysis. You will also use this procedure to determine the concentration of Red No. 40 in Gatorade.

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. To learn more about spectroscopy, and to prepare for this lab, read the following sections in your text:

"*Tools of the Chemistry Laboratory — Spectrophotometry in Chemical Analysis*", pp. 281–282

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 140 class. In one experiment you used a titration experiment to determine the concentration of calcium ions in an unknown sample. You also made measurements of solution density for various alcohol/water solutions and used that property to determine the percent alcohol in an unknown sample. As you may recall, in that lab you first prepared several solutions of known composition and measured their density. You then prepared a graph of density vs. percent alcohol for these "standard" solutions, and used the graph (or the equation of the line) to determine the composition of the unknown. 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 ($M^{-1}cm^{-1}$). 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.
- **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.

Note that since ε and b are typically constant, 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 (Equation 2)

Where **k** is a constant equal to εb.

Absorbance is typically measured using a device called a spectrophotometer. A schematic diagram of a spectrophotometer is shown below in Figure 1.

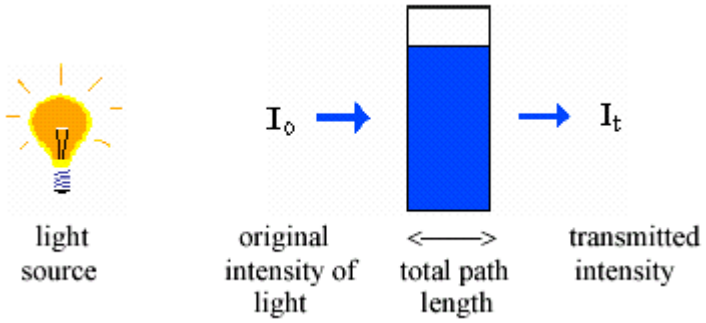

Figure 1. Schematic diagram of a spectrophotometer

Spectrophotometers have a detector which measures the intensity of light transmitted through the solution (I_t) as compared to the intensity of the incident light (I_0). The ratio of I_t and I_o can be used to indicate the percentage of incoming light absorbed by the solution. This is called the ***percent transmittance***.

$$\%T = \left(\frac{I_t}{I_o}\right) \times 100 \qquad \textbf{(Equation 3)}$$

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:

$$A = -\log(\%T/100) \qquad \textbf{(Equation 4)}$$

The higher the absorbance of light by a solution, the lower the percent transmittance. Modern spectrophotometers can display either Absorbance or Transmittance, so in practice there is no need to use the equations above. In your experiments you will make all of your measurements 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.

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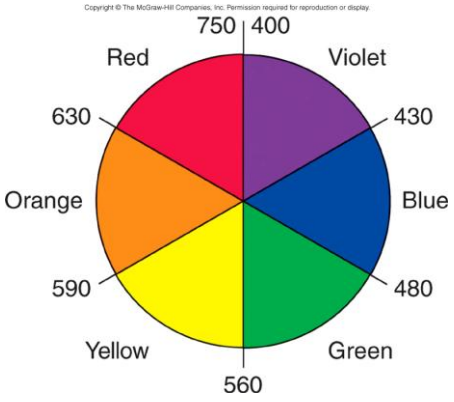


Figure 2. Color wheel with approximate wavelength values for different color light.

For a given substance, there is a wavelength**,** λ_{max}, at which absorbance is highest and at which the solution is most sensitive to concentration changes. To find λ_{max} a plot of Absorbance vs. wavelength for a given substance is collected. This plot is called an ***absorption spectrum***. As an example, the absorption spectrum for a purple dye is shown in Figure 3, below. Note that maximum absorbance for the purple dye falls in the yellow region of the spectrum.

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? 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 have an instrument available that will measure its absorption spectrum and allow an accurate determination of the $\lambda_{max.}$

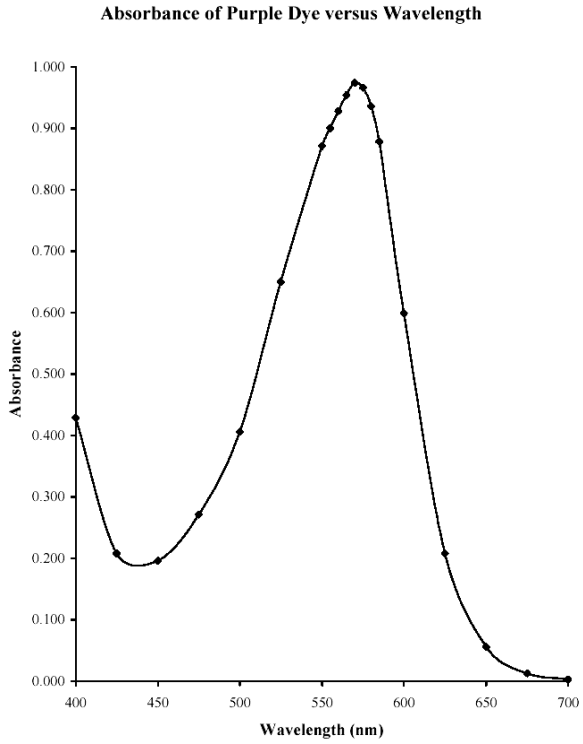


Figure 3. Absorption spectrum for a purple dye. Analysis of this absorption spectrum indicates that the λ_{max} for the purple dye is 572 nm

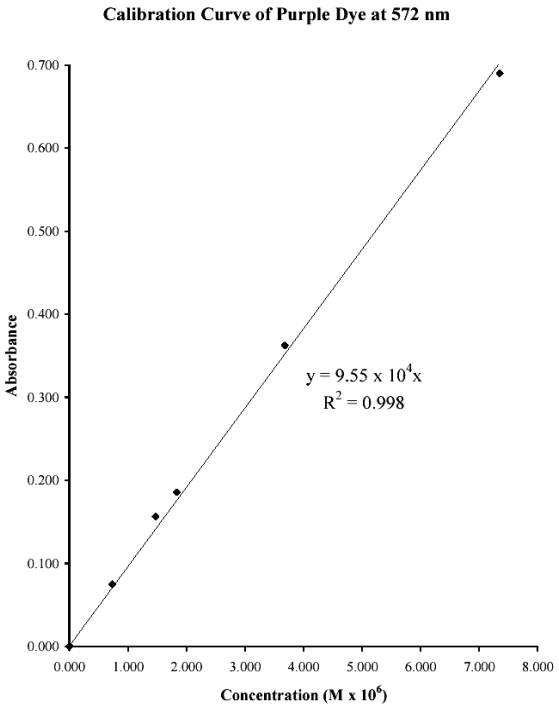


Figure 4. Calibration curve (standard curve) for a purple dye. When A is plotted versus C, a straight line passing through the origin and with a slope of (εb) is obtained. The R^2 value lets you know how well the line fits the data: $R^2 = 1$ means that there is a perfect match between the data and the line.

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 their absorbance. A ***standard curve***, also known as a ***calibration curve*** (fig. 4), is made by measuring and plotting the absorbance of several standard solutions—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 (fig. 4), a straight line passing through the origin and with a slope of k (k = εb) should be obtained. 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.

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. Its structure is shown in Fig. 1.

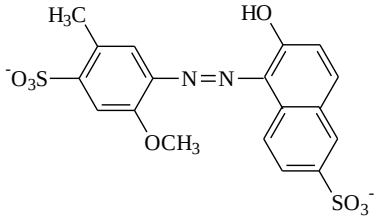


Figure 5. FD&C Red Dye No. 40 (Allura Red). Allura red commonly comes as a disodium salt: $C_{18}H_{14}N_2O_8S_2Na_2$; ***molar mass = 496.42 g/mol***.

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 about color additives in foods, drugs, and cosmetics can be found at http://vm.cfsan.fda.gov/~dms/cos-221.html.

Materials and Methods

Materials

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

You will work individually for this lab.

Overview of the procedure for this lab

- Prepare a set of six solutions of Allura Red with known concentration (standard solutions.)
- Use one of the standard solutions to determine $\lambda_{max.}$ for Allura Red.
- Set the spectrophotometer at $\lambda_{max.}$, measure the absorbance of the standard solutions and prepare a calibration curve.
- Measure the absorbance of an unknown (diluted) and of a sample of Gatorade (diluted).
- Use the equation of the calibration curve and the absorbance of each solution at λ_{max} to determine the concentration of the Red 40 dye in the unknown and in the Gatorade.

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

The lab technician will have available a stock solution of Allura Red. You will use this solution to prepare your standard solutions as described below. Make sure that you record the concentration of the stock solution of Allura Red in your lab notebook.

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 may wish to test your pipetting skills before making the standard solutions from your stock solution

Practice Using a Pipette

a. Get a pipette (e.g. a 5.00 mL pipette) and rubber bulb. Weigh a 125 mL Erlenmeyer flask and then transfer 5.00 mL of DI water using the pipette and rubber bulb. Weigh the flask after transfer.
b. 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.
c. Repeat this exercise until you can reproduce different volumes using the pipette

Procedure

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"

The wavelength at which a sample absorbs most strongly, i.e., at which the absorbance is largest, is defined as λ_{max}. Whatever the concentration, all samples of the *same substance* have the same value of λ_{max}. *The amount of light absorbed may vary, but the wavelength of the light absorbed remains the same.* In order to determine λ_{max} for FD&C Red No. 40, you will *use solution 10*, though any of the other standard solutions would also work.

You will use a Vernier spectrophotometer (figure 6) to determine λ_{max}. This instrument will produce a complete absorption spectrum for your sample—it works a like a ***scanning spectrophotometer***, a spectrophotometer that scans through the wavelengths of the visible spectrum and indicates the solution's absorbance at each wavelength (figure 7).

Figure 6. A Vernier spectrophotometer containing a cuvette containing a Nickel (II) sulfate solution.

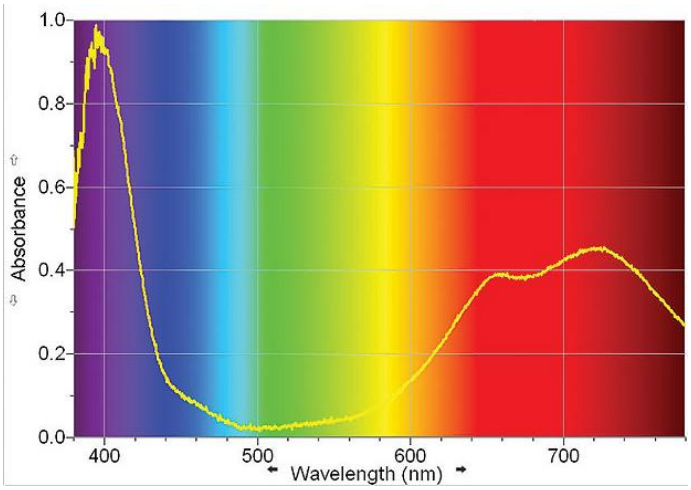


Figure 7. An absorption spectrum of a Nickel (II) sulfate solution. What is λ_{max}? Can you determine the color of the solution from this spectrum?

Procedure

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 ¾ 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 OK .

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 ¾ full with solution 10 and place it in the spectrometer.
    b. Click Collect. A graph of the Red 40 solution absorption spectrum will be displayed. Note that one area of the graph contains a peak absorbance. Click Stop 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 OK 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 Collect. When the absorbance reading stabilizes, click Keep. Enter the concentration of Solution 10 (in moles/L) and click OK. Record the absorbance of solution 10 in your lab notebook.
    b. Discard the cuvette contents. Using the solution 3, rinse and fill the cuvette ¾ full. Wipe the cuvette and place it in the spectrometer. When the absorbance reading stabilizes, click Keep. Enter the concentration of Solution 3 (in moles/L) and click OK. 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 Stop.

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 ¾ 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}.

Analysis of Results

1. Calculate the molarity of the standard solutions of Allura Red. Show sample calculations using correct units and significant figures.
2. Use *Excel* to prepare a calibration curve for your standard solutions. Fit a line to this plot, and use *Excel* to determine the equation of the best-fit line (i.e. the trendline) and the value for R-squared. Before printing, check that your name appears on the graph. If done correctly, your graph and the equation of the best fit line should be the same as the calibration curve you printed from step 6 of the procedure.
3. Use the equation of trendline and the absorbance of the Gatorade and your unknown to determine the concentration in **mol/L**, **g/L** and **mg/L** of FD&C Red No. 40 in the in the Gatorade and your unknown. *Don't forget to take into account the dilution of the samples!* Show all calculations clearly using units and significant figures. After you have calculated the concentration of your unknown, see the instructor to check the accuracy of your results. Remember, you will receive an additional grade based on the accuracy of your results! If you are unsatisfied with your results you may repeat the lab on your own time. However, any such make-ups must be *scheduled in advance* with the chemistry lab technician.

If your results have a percent error of...	**... then your grade will be:**		
	First Attempt	**Second Attempt**	**Third Attempt**
± 5.0%	100% (30 pts)	90% (27 pts)	80% (24pts)
± 7.5%	90%	80%	70%
± 10%	80%	70%	60%
± 15%	70%	60%	0%
± 20%	60%	0%	0%
> 20%	0%	0%	0%

5. Unfortunately, the mass of FD&C Red No. 40 in the Gatorade is unknown. Therefore, in order to evaluate the success/accuracy of your experiment, comment on the quality of the best-fit line in your calibration curve—that is, is the calibration curve linear? Does there appear to be a lot of scatter in the data? What is the R-Squared value? What is the meaning of the R-squared value? Does the y-intercept of the best-fit line have a value close to zero?
6. Let's look at why quantitative analysis is important. Because of its toxicity, the use of FD&C Red No. 2 as a food coloring agent is banned in the U.S., while the Allura Red (FD&C Red No. 40), although relatively untested, is used widely. An oral **LD50** is a concentration that represents the **l**ethal **d**ose in **50%** of subjects that ingest the compound being studied.
   a.) Assuming that the oral LD50 for FD&C Red No. 40 is 8 g dye per kg of body mass (see table below), how many liters of this soft drink used in this lab activity would a 60. kg person (132 lb.) have to drink before they would have even odds of ingesting a toxic dose of FD&C Red No. 40?
   b.) Now, compare this to the oral LD50 for FD&C Red No. 2, which is 0.8 mg dye per kg of body mass. If the soft drink manufacturer was using the same amount of FD&C Red No. 2 in their product as they did for FD&C Red No. 40, how many liters of this soft drink would the person, above, have to drink before they would have even odds of ingesting a toxic dose of FD&C Red No. 2?
   c.) Which quantity of soft drink seems like a more reasonable amount to drink in a day? Does it make sense to ban FD&C Red No. 2?

Toxicological Information (In Rats & Mice) for Allura Red source: http://www.jayindustriesonline.com/Allura_Red_msds.htm	
LD 50 Oral	6000-10000 mg/kg body weight
LD 50 intraperitoneal	3800-5000 mg/kg body weight
LD 50 skin	No adverse or pathological effect
LD 50 inhalation	Not Known
Eye Irritation	Not irritating to Eye
Dermal Irritation	Not irritating to skin.
Acceptable Daily Intake	0-2.5 mg/kg body weight

Acknowledgement: This lab is adapted from several similar labs produced by instructors at other colleges across the country.

Lab report Checklist:

Introduction

- Goal/purpose of the lab is stated clearly?
- Includes summary of background information?
  - Visible Spectroscopy
  - Beer's Law equation: **A = kC**
  - Wavelength, Color and Color Wheel
  - Calibration Curve
  - Artificial food colorings

Materials and Methods

- Summarizes the overview of the procedure?
- Has cut and pasted the procedures for parts I – III onto both the white and yellow pages?

Results

- Data *neatly* recorded in a ruled and easy to read table or tables that are numbered consecutively and each with an informative caption?
- Correct use of sig figs and units?
- Absorption spectrum printed in lab from step 4d is cut and pasted onto *both* the white and yellow pages?
- Standard curve printed in lab from step 6a is cut and pasted onto *both* the white and yellow pages?

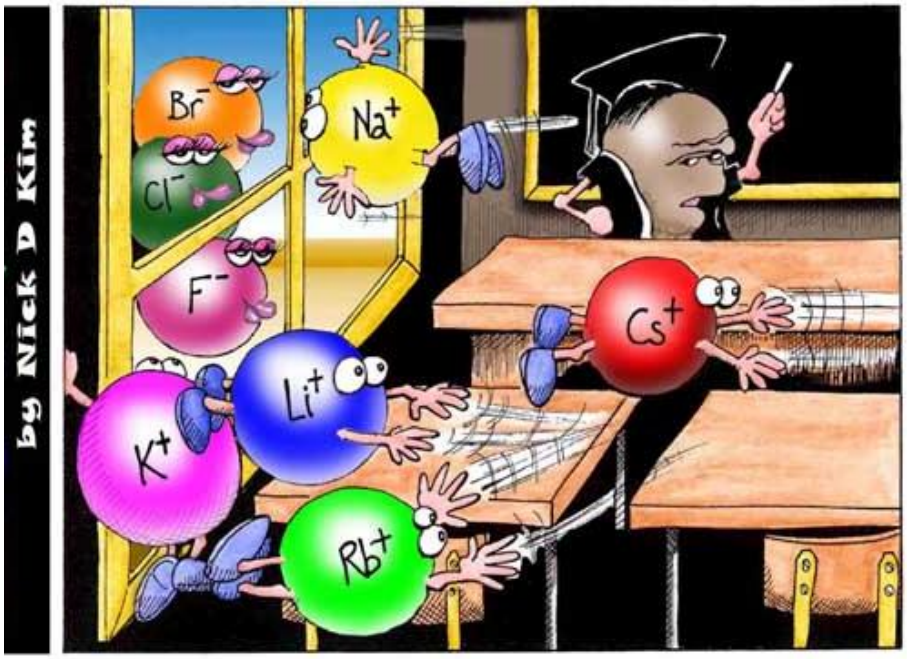
Analysis of Results

- Work is clearly labeled, neat and easy to follow?
- Sample calculation with units of the molarity of the standard solutions of Allura Red?
- Excel plot of calibration curve: A vs. [Allura Red]
  - Axes labeled fully with units and correct sig figs?
  - Has trend line with equation and R^2?
  - Name printed on graph?
  - Graph has proper title?
- **Unknown Analysis: Calculation of [Allura Red] in mol/l, g/L and mg/L**
  - Correct use of units and sig figs?
- Comments on the accuracy of the Gatorade analysis?Discusses...
  - R^2, its meaning?
  - Y-intercept (What should it be?)
- Calculates volume in liters of Gatorade a 60 kg person must consume before reaching LD50
  - Red No. 40 (Allura Red)
  - Red No. 2 (if used same conc. as red 40)
  - Does it make sense to ban Red No. 2?

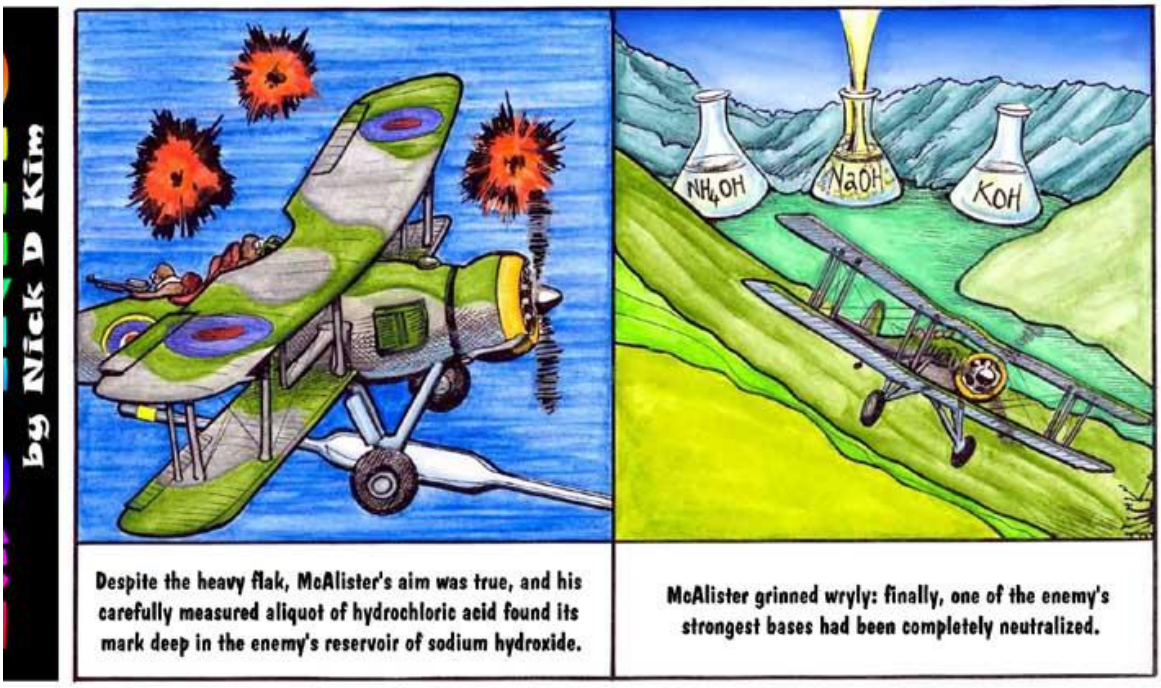
Conclusion

- Uses "*bullets"* to state concisely the major conclusions:
  - Equation of Calibration curve and R^2
  - [Red 40] in Unknown and Gatorade
  - LD50 volume of Gatorade using Red #2 and Red #40
  - Summarizes sources of error or uncertainty?

On the lighter side…



"Perhaps one of you gentlemen would mind telling me just what it is outside the window that you find so attractive...?"



Lab 2. Spectroscopic Determination of Allura Red Prelab Questions

Name _____

Lab Section_____ Team #_____

Instructions: Complete the following questions and hand in at the start of your lab period or when instructed by your instructor. Show your work with units and correct significant figures for all questions that involve a calculation. **Circle numerical answers.**

1. For each of the colored solutions below, consider the regions of the visible spectrum in which the compound should absorb light and then prepare a rough sketch of absorbance *vs.* wavelength for each solution. Label λ_{max} on each graph.

   a. Urine (i.e. a light yellow solution)

   b. Water colored a light red with food coloring

   c. Pure distilled water

2. A sample of a red dye and a sample of a purple dye both have the same concentration. Their Absorbance is measured using the same spectrophotometer. Will both samples give the same Absorbance reading? Why or why not?

3. Calculate the concentration of Red 40 in mol/L and mg/L for each of the six standard solutions in the table below that you will prepare in this lab by adding varying volumes of a 1.894×10^{-4} M standard solution of Red 40 to a 100.0 mL volumetric flask and then filling to the mark with DI water. *Show a sample calculation for solution 5*.

   Hints:
   i.) Once you've calculated the concentration of solution 5, the concentrations of solutions 10, 15, 20 and 25 are simple multiples of the concentration of solution 5.
   ii.) Recall from Chem 140: $V_{conc}M_{conc} = V_{dil}\ M_{dil}$
   ii.) You'll find the molar mass of Red 40 with figure 5 on page 5.

Solution Number	**Volume (mL) of 1.894×10^{-4} M Red 40**	**Concentration in Moles per Liter**	**Concentration in mg per Liter**
3	3.00		
5	5.00		
10	10.00		
15	15.00		
20	20.00		
25	25.00		

Sample Calculation for Solution 5: