Experiment 37A

EDIBLE COLORS: BEER’S LAW AND FOOD DYEST

MATERIALS: test tubes 13x100, 16x125; volumetric flasks: 500 mL, 50 mL(2), 25 mL(2); pipets: 10 mL(2), 5 mL; beakers: 100 mL(2); plastic buret funnel; glass stirring rod; plastic droppers; Spec 20 spectrometer; Genesis scanning spectrometer (optional); centrifuge; FD&C Yellow Dye #5; POWERade®.

PURPOSE: The purpose of this experiment is to prepare a series of solutions of a pure compound, FD&C Yellow Dye #5, and to use those solutions to create a calibration curve. With the calibration curve the students may analyze commercial beverage samples for dye content. This lab is designed to introduce students to absorption spectroscopy and Beer’s Law, as well as the preparation of standard solutions and generation of a standard curve.

LEARNING OBJECTIVES: By the end of this experiment, the student should be able to demonstrate these proficiencies:

1. Identify and use the following equipment: Spectronic 20 spectrometer, centrifuge and volumetric glassware.
2. Prepare solutions of known concentration by mass and by dilution methods.
3. Understand the relationship between the colors or wavelengths of a solution and the colors or wavelengths absorbed or transmitted by the solution.
4. Generate a calibration curve for absorption spectroscopy.
5. Create graphs and use them to determine the concentration of an unknown solute.

PRE-LAB: Complete the Pre-lab questions on p. E37A-7 before lab.

DISCUSSION: Color is an important property of foods that adds to our enjoyment of eating. Nature teaches us early on to expect certain colors in certain foods, and our future acceptance of foods is highly dependent on meeting these expectations. The novelty effect of adding green color to ketchup (or beer) is entertaining because we are so accustomed to the usual appearance of familiar foods. The joke is lost on people who find the adulterated foods completely unpalatable. The practice of adding coloring agents to foods has been around since antiquity. Until the last century, the additives were virtually all taken from natural materials. However, “natural” does not necessarily mean safe. For example, in the 1800s candy was almost always contaminated with one or more of the following: HgS for bright reds, CuSO₄ for strong blues, Cu(C₂H₃O₂)₂ for greens, and even Pb(C₂H₃O₂)₄ or PbCO₃ for whites. All natural, but all toxic. The use of toxic metal salts as colorants was banned in the US in 1906, but seven synthetic color additives were permitted; these were the first “certified” food colors. Now, the Food and Drug Administration (FDA), one of the country’s oldest consumer protection agencies, certifies only nine specific compounds for use in foods, drugs and cosmetics (FD&C) – one of them is “FD&C Yellow #5” the compound examined in this experiment. Other colors are obtained by using combinations of the certified agents.

FD&C Yellow #5, or simply Yellow 5, is chemically known as tartrazine, Na₃C₁₆H₁₇N₄O₇S₂. Tartrazine provides the lemon yellow color seen in many beverages such as POWERade®, Gatorade®, Kool-Aid®, and the like, and contributes to the color of numerous other foods and other consumer products. The structure of tartrazine is shown in the figure. Tartrazine is one of many synthetic “azo” dyes, named for the −N=N− group between the rings. It is relatively stable so products with this agent do not readily lose their color, and it is very strongly colored, so only a small amount is required. As suggested by data in the MSDS, it is quite safe at levels typically ingested.
In this lab we will determine the amount of tartrazine (Yellow 5) in samples of commercial drinks like POWERade®. Because the substance is strongly colored, and virtually no other colored species are present, quantification of Yellow 5 can easily be accomplished through absorption spectrophotometry. (If there were other colored species present the analysis could still be done this way, but it becomes a bit more complicated.) As described in detail in Appendix I (http://www.chemistry.usna.edu/manual/apdxI.pdf), solutions of a given color will absorb the wavelengths of light complementary to the observed color. Thus, we measure the absorbance of solutions of the yellow food dye tartrazine at 425 nm (blue-violet), not in the yellow region (near 590 nm). In order to quantitatively determine the unknown amount of tartrazine in a sample of POWERade®, we need to know the mathematical relationship between the measured absorbance at 425 nm and the actual amount of dye. We do that by first creating set of standards, a series of solutions of known concentration of tartrazine; this allows us to determine the relation between absorbance and concentration for this specific compound, at one specific wavelength. The general relation between absorbance and concentration is known as the Beer-Lambert law.

\[ A = \varepsilon c l \]

where \( A \) is the absorbance, \( c \) is the concentration (usually in moles/liter), and \( l \) is the pathlength, the length of solution through which the light passes. (For our cells, \( l = 1 \) cm.) By using our standards to create a calibration curve, a plot of measured absorbance vs. known concentration, we can determine the proportionality constant \( \varepsilon \) (known as the molar absorptivity). Once we know that, we can use the absorbance determined for our POWERade® sample and work backwards to determine the actual amount of Yellow 5 in the beverage. Calibration curves and absorption spectrophotometry are very common tools of analytical chemistry; you will utilize such analyses several times this year. For more details, see Appendix I.

One final point about the analysis of real-life samples – they can be complicated. Commercial beverages have many constituents, some of which might be insoluble. They may be there for nutritional reasons, or simply cosmetic ones. (Among other things, they might provide the cloudy or frosty appearance desired by the manufacturers.) Light scattering from small particulates in the sample can interfere with the analysis by making it appear that less light gets through the sample to the detector. In many cases, this particular problem can be solved by centrifugation. The rapid spinning of the sample pushes heavier particles to the bottom of the tube, leaving the top much clearer and more suitable to spectrophotometric analysis. The small bubbles evolving from highly carbonated beverages can have the same effect, but that is not really amenable through centrifugation. Degassing the solution by heating or other means may eliminate that problem.

REFERENCES
PROCEDURE:

The instructor will demonstrate the use of the Spec 20 spectrophotometer, centrifuge, and volumetric glassware. Take notes for future use.

Samples of POWERade® will be provided. If you wish to analyze a drink of your own choice you may do so, provided that:

a) the material contains **ONLY** FD&C Yellow #5 as coloring agent – check the label carefully; ask your Instructor for guidance if you are unsure. Some drinks which may qualify are Gatorade® (Lemon-lime), Mountain Dew®, and Kool-Aid®; these are available in the Mid Store or local grocery stores. Although these directions are optimized for POWERade®, the same scheme can be followed for most beverages.

b) the bulk of the material stays outside in the corridor. Food materials brought into the lab become chemical samples and should no longer be consumed.

EACH midshipman should make up at least two of the standard solutions in Part B.

Part A: Preparing the sample and stock solutions.

1. Obtain a large test and fill it about 80% full with the beverage sample. Wipe off the outside and place the test tube in a laboratory centrifuge. Obtain another sample from classmates, or a test tube filled with water to the same level, and place it in the centrifuge chamber opposite the original test tube. Make sure there is a balancing tube for every tube in the centrifuge, as explained by your Instructor. Close and latch the lid. Turn the timer knob left to the “hold” position. You will run the centrifuge for about 30 minutes, or until all your standards are prepared and you are ready for the sample. Record the identity of the sample, and the manufacturer’s suggested serving size (which must be listed on the label).

2. Use the analytical balance and a clean, dry 100 mL beaker to accurately weigh out (4 decimal places!) about 0.01 g of pure FD&C Yellow #5. Add about 50 mL of distilled water and use a clean glass stirring rod to stir until completely dissolved. Quantitatively transfer this sample through a clean small funnel to a clean 500 mL volumetric flask. (It is OK if the flask has water in it, as long as it is clean.) Rinse the beaker and stirring rod with distilled water several times, adding the rinsings through the funnel into the volumetric flask. (The idea of “quantitative transfer” is to move into the flask every molecule of Yellow 5 that you weighed out. The multiple rinsings help ensure that the transfer is complete.) After several rinsings of the beaker, remove the funnel and fill the flask to the mark with distilled water. (Add the last drops of water with a disposable pipet.) As demonstrated by your instructor, invert and/or swirl to mix well. This is solution #1, the “stock” solution from which you will make your other standards. You should already know an approximate value of the concentration of this stock solution, based on your Pre-Lab calculation. Now determine the ACTUAL concentration based on your mass data and the volume of the solution.

   approximate tartrazine concentration (from Pre-Lab) ___________________ mol/L

   ACTUAL tartrazine concentration (from data) ___________________ mol/L

Part B: Preparing the standard solutions by dilution of the stock solution

1. Pour about 5 mL of your stock solution into a clean 100 mL beaker, rinse the beaker and discard the rinse. Pour about 75 mL of your stock solution into the rinsed beaker. Rinse the 10 mL and 5 mL pipets with the stock solution and discard the rinses. (To rinse a pipet, draw some of the solution up into the pipet, manipulate the pipet so that the solution contacts all interior surfaces, and then discard the rinse.) Pour more stock solution into the beaker as necessary.

2. Clean your two 25 mL and two 50 mL volumetric flasks, including stoppers. Finish cleaning with a final rinse of distilled water. It is **NOT** necessary to dry the insides of these flasks; DO NOT stand them on end in an attempt to do so! Use a marker to label the 25 mL flasks #2 and #3, and label the two 50 mL flasks #4, and #5, respectively.

3. Using your rinsed pipets and your clean volumetric flasks, prepare your remaining standard solutions by dilution of
the stock solution (solution #1) according to the following table:

<table>
<thead>
<tr>
<th>SOLUTION #</th>
<th>PIPET</th>
<th>SOURCE</th>
<th>RECEIVER</th>
<th>CONCENTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>10 mL</td>
<td>stock soln.</td>
<td>25 mL volum. #2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10 mL</td>
<td>stock soln.</td>
<td>25 mL volum. #3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10 mL</td>
<td>stock soln.</td>
<td>50 mL volum. #4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5 mL</td>
<td>stock soln.</td>
<td>50 mL volum. #5</td>
<td></td>
</tr>
</tbody>
</table>

e.g., for Solution #2, use your rinsed 10 mL pipet to transfer 20 mL of the stock solution into the 25 mL volumetric flask labeled #2. (For this particular case you must use the pipet twice.) Fill the flask to the mark with distilled water. Invert and swirl to mix thoroughly. Perform the other dilutions in a similar manner. From the known concentration of the stock solution, determine the concentrations of your four other standard solutions. Show the calculation for solution #2 below. Have your instructor check your work for all four solutions before proceeding.

Instructor OK ________________

Part C: Preparing and using the spectrometer.

1. Use a marker to draw a small vertical line on the neck of the cuvette. This will serve as an alignment mark; when you put the cuvette in the spectrometer, always align this mark with the marker line at the front of the sample compartment. If you use the same cuvette for your blank and all of your samples you will avoid any problems caused by inconsistencies of the glass in different tubes.

2. Your instructor will demonstrate the use of the Spec 20 spectrophotometer. Set the wavelength to 425 nm. If your spectrometer is so equipped, make sure that the Filter lever is in the correct position. NOTHING should be in the sample chamber when setting the 0 %T. Use distilled water to set the 100 %T. After the initial settings you should not have to adjust any of the knobs again.

3. When the spectrometer is set up, measure and record the percent transmittance or absorbance of your standard solutions as follows. Pour a small amount of Solution #5 into the empty cuvette. Rinse and discard the rinse. Pour more of Solution #5 into the cuvette. (Fill only about 2” high!). Wipe off the outside of the cuvette with a tissue, and carefully place it in the sample chamber, paying attention to alignment as above. Record the %T or absorbance of the sample at 425 nm. Remove the cuvette, empty the contents, and repeat the process with Solution #4, then #3, then #2, and finally the stock Solution #1. Make sure to always rinse with the solution you are about to put into the cuvette.

4. When all of your standard solutions have been measured, go back to your beverage sample (remember that?) in the centrifuge. Turn off the centrifuge (return the timer knob to the vertical position) and WAIT until the rotor stops turning. Remove the sample tube, trying not to agitate it, and look carefully at the contents. At the bottom of the tube, and along one side, you will probably see a small amount of white solid. You want to avoid disturbing that material. Either gently decant (pour off) or use a plastic dropper to remove a small sample of clear liquid above the solid. Use this to rinse your cuvette. Remove another small sample, put it in the cuvette and measure and record the %T or absorbance of the beverage at 425 nm. (NOTE: If you chose to use your own sample, the absorbance of the beverage might not fall within the range of the standards. Consult your Instructor if this is the case.)

Clean-up:

1. All solutions can be safely poured down the drain. Empty your volumetric flasks and rinse them with distilled water. Lie them on their sides to drain; DO NOT leave them in an inverted position! It is not necessary that they be dry.

2. Rinse your pipets by drawing water into them and then expelling it. It will NOT work to simply hold them under the faucet!

3. Clean your remaining glassware and return it to your station. If there is any yellow color left in the glassware, it is NOT CLEAN! Pay special attention to any container that held the beverage; sugary residue becomes sticky, messy, and can be a health hazard.
Data Analysis:

Your Instructor may require you to work up the results of your experiment by hand or on your calculator for submission on the day of the lab, or may expect a more formal report and analysis with Excel. Follow the directions specific for your own section.

1. Calculate the molar concentrations of your standard solutions (Solution #1 through Solution #5).

2. Create an Excel spreadsheet to present your data. Have it convert the %T values of your standard solutions to Absorbance values, and make a Beer’s Law plot of Absorbance vs. Concentration. Plot the Trendline for the data; be sure to show both the trendline equation and $R^2$ value on the plot.

3. Use the trendline equation to determine the molar concentration of Yellow #5 in your beverage sample.

4. Knowing the concentration of Yellow #5 in the sample, use the manufacturers “serving size” (recorded from the bottle label) to calculate the mass of the dye consumed in one serving. Pay attention to units!

Questions for Consideration

1. Beer’s Law is given as $A = \varepsilon cl$. The pathlength $l$ of your cuvette is 1.0 cm. What is the value of $\varepsilon$, the molar absorptivity of your dye at the wavelength of measurement? (HINT: Use your Beer’s Law plot to determine this.)

2. You calculated the amount of tartrazine dye consumed in one serving. Compare this to the toxicity data for tartrazine from the MSDS. (You may have to use the LD$_{50}$ for mice, since data for humans may not be available. Assume a body mass of about 75 kg for the sake of comparison.)

3. All food additives have been subject to claims (some true, some false) of adverse reactions. Find some websites dealing with reactions to food additives and identify some common complaints about Yellow #5. Be aware that most websites are NOT authoritative sources of information, since they rarely provide data or references that might enable you to make your own objective determination of the validity of their claims!

4. The Discussion section pointed out that cloudiness from insoluble materials may create problems for the analysis, since they scatter light away from the path through the sample. You removed most such particles by centrifugation. If you had not done so, how would your analytical result be affected? Would you determine a higher concentration of tartrazine in the beverage, a lower one, or would it not change? Explain your answer.

5. There are only nine certified food colors; all other colors are obtained by combination. For example, the orange color of orange Kool-Aid is obtained by mixing FD&C Yellow #5 and FD&C Red #40, in about a 3.6:1 proportion. The absorption spectra of these dyes overlap somewhat, which complicates the analysis because absorbances are additive. If the $\varepsilon$ value for Red #40 at 425 nm is 0.289 as large as $\varepsilon$ for Yellow #5 at that wavelength, what are the concentrations of the two dyes when the absorbance of the beverage at 425 nm is 0.75?

6. A simplified structural diagram of FD&C Red #40 is shown below. In such diagrams, every unmarked vertex is a carbon atom attached to one or more hydrogen atoms (as many as necessary to ensure that the carbon has a total of 4 bonds). What is the chemical formula and molecular weight of Red #40? List some structural similarities it shares with Yellow #5.
IN-LAB SUBMISSION WORKSHEET
Experiment 37A

1. Show your work for the calculation of the concentration of Solution #2, which you prepared by dilution of the stock solution.

2. Complete the table using data for your own solutions. Identify the beverage you used and the serving size:

<table>
<thead>
<tr>
<th>SOLUTION #</th>
<th>CONCENTRATION (M)</th>
<th>PCT. TRANSMITTANCE</th>
<th>ABSORBANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution #1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solution #2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solution #3</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Solution #4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solution #5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown (beverage)</td>
<td>-----------</td>
<td>------------------</td>
<td>------------</td>
</tr>
</tbody>
</table>

3. Make a plot of Absorbance vs. Concentration on the grid below. Draw the best straight line that fits your data points. (This may or may not actually touch your data points.) Determine the slope and intercept of the line, and write the equation for the line.

\[ \text{Abs.} = \text{_____} \times \text{(Conc)} + \text{_____} \]

4. Use the equation to determine the concentration of the colorant in the beverage solution. Show your work.

\[ \text{dye conc.} = \text{__________} \]

5. Based on your results, what mass of Yellow #5 is consumed in one serving of the beverage? Show your work.
COMPLETE THESE QUESTIONS PRIOR TO ATTENDING LAB. THEY WILL BE COLLECTED BEFORE YOU CAN START THE EXPERIMENT.


2. Go to http://www.dartmouth.edu/~chemlab/info/resources/spectrum/spectrum.html
   a. Click on the absorbance choice to change the y-axis to absorbance. Click (and hold down) on the first purple marker on the left and drag the line such that your spectrum looks like:
   
   ![Spectrum 2a](image)

   i. What color is the background of the graph? To what does this color correspond?

   ii. Now change the display to transmittance by clicking the transmittance choice. Sketch the spectrum that results and explain what you see.

   b. Click on the spectrum and make it look like:

   ![Spectrum 2b](image)

   i. What color is the background of the graph? To what does this color correspond?

   ii. Now change the display to transmittance by clicking the transmittance choice. Sketch the spectrum that results and explain what you see.

3. Your stock solution will be made by dissolving approx. 0.01 g of tartrazine in water and using a volumetric flask to bring the total volume up to 500 mL. What will be the approximate molar concentration of tartrazine in the stock solution? Show the calculation and result here, and copy the result to Part A of the Procedure section on p. E37A-3.

4. Use the color wheel in Appendix I to predict the wavelength region(s) expected to be absorbed by the Yellow #5 dye solutions (circle your answer):
   red orange yellow green blue violet

5. Look up the MSDS for tartrazine. Explain the meaning of LD_{50}. What is the LD_{50} for oral ingestion of tartrazine in mice? Record the value here and in “Question for Consideration” #2 on p. E37A-5.