Experiment 37

FV 10-17-05

COLORS TO DYE FOR: BEER'S LAW AND NATURAL DYES[1]

MATERIALS: volumetric flasks: 500 mL, 250 mL, 100 mL (3); pipets: 25 mL, 10 mL, 5 mL, 2 mL, 1 mL; beakers: 100 mL (3), 50 mL; vacuum filtration flask; plastic Buchner funnel; glass funnel; Spec 20 spectrometer; Genesis scanning spectrometer (optional); 0.1 M Na_2CO_3; frozen blueberries or skins from yellow onions; cotton balls or cotton cloth; Quercetin or blueberry dietary supplement capsules; alum and cream of tartar for mordanting solution.

PURPOSE: The purpose of this experiment is to prepare a natural dye and to quantify the amount of colorant used to dye a small sample of cotton material. 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, vacuum filtration and volumetric glassware.
2. Prepare solutions of known concentration by dilution.
3. Understand the relationship between the colors or wavelengths of a solution and the colors or wavelengths absorbed or transmitted by the solution.
3. Generate a calibration curve for absorption spectroscopy.
4. Create graphs and use them to determine the concentration of an unknown solution.

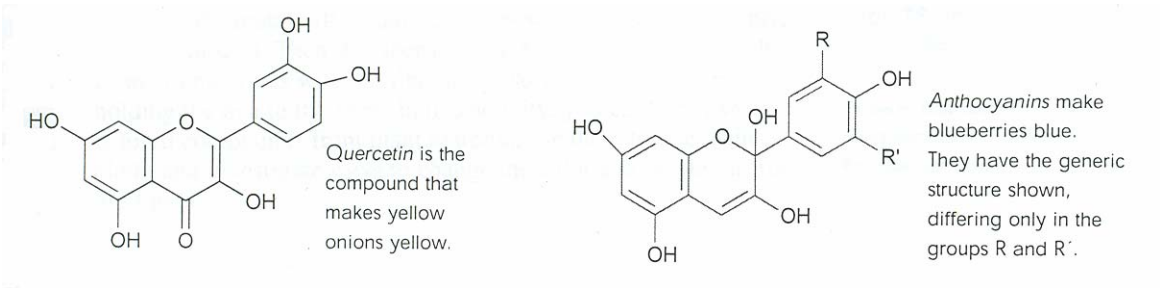
PRE-LAB: Complete the Pre-lab questions on p. E37-8 <u>before</u> lab.

DISCUSSION: Chemistry has always been a practical human endeavor, even before people understood what was going on in their crucibles and retorts. The practical arts of metallurgy, tanning, and dyeing flourished thousands of years ago. Most of these arts were developed by trial and error. The use of dyes can be traced to earliest history. The coloring properties of materials such as berries and bark were most likely discovered when clothing was accidentally stained by them. Dyes made from natural sources such as plants and minerals tend to produce colors that are not very bright and wash out easily. Some colors, such as blue and purple, were very difficult to produce. As a result, cloth dyed with these colors was so expensive that only royalty could afford to use them. Today, inexpensive synthetic dyes are available in nearly every color of the spectrum. Like the natural dyes, most of the colorants are large organic molecules with a significant amount of conjugation (alternating double bonds) in their structures. Small changes to the structure can change the wavelengths of light absorbed by the molecule, and hence change the color. With synthetic dyes, most kinds of fabrics can be dyed to a brilliant color, which can also remain quite color-fast (resistant to fading). Natural dyes are still used however, and even they can be made more permanent by use of a "mordant". In the mordanting process the fiber is treated with a solution of a metal salt (often an Al, Cr, Cu, Fe or Sn salt) before dyeing. Metal ions from the salt form strong bonds to the fiber and also to the dye, thereby holding the dye to the fiber. The mordanting mixture used here is a dilute solution of alum and cream of tartar, both available from the spice rack of a grocery store.

In this lab we will prepare a natural dye from the skin of yellow onions, or from blueberries. The structures of the two colorant molecules are very similar, but the colors they provide are very different. The active colorant in the onion skin is quercetin, $C_{15}H_{10}O_7$, which is classified as a flavonoid. Our "standard" material for this compound will be capsules of Jarrow Formula Quercetin-500®, analyzed to provide 500 mg of quercetin per capsule. The structure of quercetin is shown below. A significant amount of inactive material is also present in the capsule. The active colorants in blueberries are anthocyanins. This is a class of compounds with the general structure shown below, where the only differences among the compounds are the groups at positions labeled R and R'. For our purposes, we will assume that

[1] Adapted from *J. Chem. Educ.* **76**, 1688A (1999). Parts of lame title, structures and some discussion taken directly from that source.

we have only one such molecule, with H atoms in both of those positions, giving the molecular formula $C_{15}H_{12}O_6$. Our "standard" material for this compound will be capsules of Flavonoid Sciences Wild Blueberry IQ®, analyzed to provide 42.6 mg of anthocyanin per capsule. This supplement also contains other components that should not interfere with the analysis.



Since we are working with colored materials, absorption spectrophotometry provides a good way to identify and quantify the compounds involved. 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 onion dye quercetin at 382 nm (violet), not in the yellow region (near 580 nm). In this lab we will determine the concentration of a dye extracted from natural materials and used to color a small piece of cotton. We do that by creating a series of standard solutions of known concentration and utilizing the Beer-Lambert Law,

$$A = \varepsilon \cdot c \cdot \ell$$

to relate the Absorbance **A** to the concentration **c**. The molar absorptivity ε is a proportionality constant specific to the substance and wavelength of the measurement, and ℓ is the pathlength of light through the sample, 1 cm for the cells used here. See Appendix I for additional information.

PROCEDURE:

The instructor will demonstrate the use of the Spec 20 spectrometer, volumetric glassware and vacuum filtration set-up.

Midshipmen should work in pairs. Your instructor may assign you to work with a specific natural dye. Follow the directions pertinent to that material alone. Instructions for yellow onion skin dye begin below; those for blueberry dye begin on p. E37-4.

FOR YELLOW ONION SKIN DYE:

Part A: Preparing the stock solution.

1. In a 100 mL beaker, place 10 mL of 0.1 M Na_2CO_3 solution and 10 mL of water. Obtain a capsule of Quercetin-500® dietary supplement. Hold the capsule over the beaker while gently pulling apart the halves. (Twisting slightly may help to separate the pieces.) Pour the yellow powder into the beaker; try not to lose any of the material. Squeezing the opened capsule halves will help the material to flow out readily. Do not drop the empty capsule halves into the solution.

2. Stir or swirl the solution for a minute or two. There will be much undissolved material that sticks to the sides of the beaker. Use your squirt bottle of distilled water to periodically wash down the sides. Keep the total volume below 75 mL.

3. Carefully rinse a plastic vacuum Erlenmeyer flask with distilled water and drain. Place a piece of filter paper in the Buchner funnel and install that on the vacuum flask. (See Fig. 1 below.) Be sure that the flask is clamped to a ring stand. Connect the aspirator hose and turn on the water. Wet down the filter paper with distilled water by squirting a layer of water into the funnel.

4. Take your beaker with the contents of the capsule and swirl to suspend the material. With the suction on, pour the contents into the filter funnel. Swirl periodically to keep the solids suspended. When all of the liquid has been poured into the funnel, wash down the sides of the beaker with distilled water, swirl to suspend the solids, and pour into the filter funnel. Continue until all solids have been transferred to the filter funnel.

5. With the suction still on, wash the solids in the filter once or twice with distilled water. *Follow the next steps carefully to avoid drawing tap water into your sample!* With the water still flowing and suction still on, crimp the hose to the water aspirator and then disconnect the hose from the plastic funnel flask. Only now should you shut off the water and uncrimp the hose.

Fig. 1. Vacuum filtration assembly.

6. Quantitatively transfer the liquid filtrate from the filter flask into a clean 500 mL volumetric flask. (To quantitatively transfer, pour the material carefully from the filter flask into the volumetric. Rinse the inside of the filter flask with distilled water and pour the rinses into the volumetric. Repeat one or two more times.) Fill the flask to the mark with distilled water and swirl to mix. This is your stock solution. You should know the concentration from your calculation in the Pre-Lab. Enter your concentration here:

concentration of quercetin in stock solution _____

Part B: Preparing and measuring the standard solutions.

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 25 mL, 5 mL, 2 mL, and 1 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 250 mL and three 100 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 250 mL flask **#1**, and label the three 100 mL flasks **#2**, **#3**, and **#4**, respectively.

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

SOLUTION #	PIPET	SOURCE	RECEIVER	CONCENTRATION
1	25 mL	stock soln.	250 mL volum. #1	
2	5 mL	stock soln.	100 mL volum. #2	
3	2 mL	stock soln.	100 mL volum. #3	
4	1 mL.	stock soln.	100 mL volum. #4	

e.g., for Solution #1, use your rinsed 25 mL pipet to transfer 25 mL of the stock solution into the 250 mL volumetric flask labeled #1. 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 standard solutions. Show the calculation for solution #1 below. Have your instructor check your work for all four solutions before proceeding.

Instructor OK _____

4. Your instructor will demonstrate the use of the Spec 20 spectrometer. Set the wavelength to 382 nm. Pay careful attention to the use of the square plastic cuvettes and adapter. The side of the cuvette with a small inverted triangle (∇) should be placed on the OPEN side of the adapter. (See Fig. 2 below.) The white arrow of the adapter should face the white line on the sample chamber of the Spec 20. NOTHING should be in the sample chamber when setting the 0% T. Use distilled water to set the 100%T.

5. When the spectrometer is set up, measure the percent transmittance of your standard solutions as follows. Pour a small amount of Solution #4 into the cuvette. Rinse and discard the rinse. Pour more of Solution #4 into the cuvette. (Fill less than ¾ full!). Wipe off the outside of the cuvette with a tissue, carefully place in the adapter (paying attention to alignment as above) and insert the adapter into the sample compartment. Record the %T of the sample at 382 nm. Remove the cuvette, empty the contents, and repeat the process with Solution #3, then #2, then Solution #1.

Part C: Preparing the dye and determining colorant concentration.

(If directed by your instructor, based on the availability of samples, perform the following steps in separate beakers with two different types of onion skin.)

1. Tear the outer papery skin from a yellow onion into pieces no more than 1 inch square. Place a few pieces in a 100 mL beaker to cover its bottom with 1 or 2 layers of onion skin. Add 50 mL of water and bring the solution to a boil. Continue to boil for 5 minutes.

2. Use a plastic pipet to withdraw some of the colored solution (about 2 dropperfuls) and transfer it to a small clean DRY beaker. Allow it to cool, and then measure the absorbance of the solution at 382 nm.

3. Obtain a mordanted piece of cotton cloth or cotton ball and wet it with water. Using tongs, place it into the beaker so that it is completely submerged. Boil for 1-2 minutes. Use tongs to remove the dyed material and rinse it with water.

Clean-up:

1. All solutions can be safely poured down the drain. Place used onion skins in a collection bin, NOT the sink. 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. Using the pipet bulb and a beaker of distilled water, rinse your pipets with distilled water. It will NOT work to simply hold them under the faucet!

3. Clean your remaining glassware and return it to your station. Discard the used filter paper in the trash.

FOR <u>BLUEBERRY</u> DYE:

Part A: Preparing the stock solution.

1. In a 100 mL beaker, place 10 mL of 0.1 M Na_2CO_3 solution and 10 mL of water. Obtain a capsule of Flavonoid Sciences Wild Blueberry IQ® dietary supplement. Drop the capsule in the solution. Use the broad end of a stainless steel spatula to cut open the capsule (while beneath the surface of the solution), and then to squeeze the pierced capsule against the inside of the beaker. This will extrude most of the material.

2. Stir or swirl the solution for a minute or two, while continuing to squeeze and empty the capsule. There will be much undissolved material that sticks to the sides of the beaker.

3. Carefully rinse a plastic vacuum Erlenmeyer flask with distilled water and drain. Place a piece of filter paper in the Buchner funnel and install that on the vacuum flask. Be sure that the flask is clamped to a ring stand. (See Fig. 1 above.) Connect the aspirator hose and turn on the water. Wet down the filter paper with distilled water by squirting a layer of water into the funnel.

4. Take your beaker with the contents of the capsule and swirl to suspend the material. With the suction on, pour the contents into the filter funnel. Swirl periodically to keep the solids suspended. When all of the liquid has been poured into the funnel, wash down the sides of the beaker with distilled water (using as little water as possible), swirl to suspend the solids, and pour into the filter funnel. Continue until all solids have been transferred to the filter

funnel. The liquid main drain very slowly, but it will eventually pass through the filter. (Wax and other inert ingredients of the capsule clog up the filter pores.)

5. With the suction still on, wash the solids in the filter once or twice with distilled water, using as little water as possible. Allow to drain through. *Follow the next steps carefully to avoid drawing tap water into your sample!* With the water still flowing and suction still on, crimp the hose to the water aspirator and then disconnect the hose from the plastic funnel flask. Only now should you shut off the water and uncrimp the hose.

6. Quantitatvely transfer the liquid filtrate from the filter flask into a clean 100 mL volumetric flask. (To quantitatively transfer, pour the material carefully from the filter flask into the volumetric. Rinse the inside of the filter flask with distilled water and pour the rinses into the volumetric. Repeat one or two more times.) Fill the flask to the mark with distilled water and swirl to mix. This is your stock solution; label it Solution #1. You should know the concentration from your calculation in the Pre-Lab. Enter your concentration here:

concentration of anthocyanin in stock solution _____

Part B: Preparing and measuring the standard solutions.

1. Pour about 5 mL of your stock solution into a clean 100 mL beaker, rinse the beaker and discard the rinse. Pour about 50 mL of your stock solution into the rinsed beaker. Rinse the 25 mL, 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 three 100 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 three 100 mL flasks **#2**, **#3**, and **#4**, respectively.

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

SOLUTION #	PIPET	SOURCE	RECEIVER	CONCENTRATION
2	25 mL	stock soln.	100 mL volum. #2	
3	10 mL	stock soln.	100 mL volum. #3	
4	5 mL	stock soln.	100 mL volum. #4	

e.g., for Solution #2, use your rinsed 25 mL pipet to transfer 25 mL of the stock solution (which is already labeled Solution #1) into the 100 mL volumetric flask labeled #2. 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 standard solutions. Show the calculation for solution #2 below. Have your instructor check your work for all four solutions before proceeding.

Instructor OK _____

4. Your instructor will demonstrate the use of the Spec 20 spectrometer. Set the wavelength to 589 nm. Pay careful attention to the use of the square plastic cuvettes and adapter. The side of the cuvette with a small inverted triangle (∇) should be placed on the OPEN side of the adapter. (See Fig. 2 above.) The white arrow of the adapter should face the white line on the sample chamber of the Spec 20. NOTHING should be in the ample compartment when setting the 0 %T. Use distilled water to set the 100%T.

5. When the spectrometer is set up, measure the percent transmittance of your standard solutions as follows. Pour a small amount of Solution #4 into the cuvette. Rinse and discard the rinse. Pour more of Solution #4 into the cuvette. (Fill less than ¾ full!). Wipe off the outside of the cuvette with a tissue, carefully place in the adapter (paying attention to alignment as above) and insert the adapter into the sample compartment. Record the %T of the sample at 382 nm. Remove the cuvette, empty the contents, and repeat the process with Solution #3, then #2, then #1.

Part C: Preparing the dye and determining colorant concentration.

1. Pour 50 mL of distilled water into a clean 100 mL beaker. Place 3-4 blueberries in the beaker and mash them up with a stirrer. Bring the solution to a boil. Continue to boil for 5 minutes.

2. Use a plastic pipet to withdraw some of the colored solution (about 2 dropperfuls) and transfer it to a small DRY beaker. Allow it to cool, and then measure the absorbance of the solution at 589 nm.

3. Obtain a mordanted piece of cotton cloth or cotton ball and wet it with water. Using tongs, place it into the beaker so that it is completely submerged. Boil for 1-2 minutes. Use tongs to remove the dyed material and rinse it with water.

Clean-up:

1. All solutions can be safely poured down the drain. Place used blueberries in the trash, NOT the sink. 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. Using the pipet bulb and a beaker of distilled water, rinse your pipets with distilled water. It will NOT work to simply hold them under the faucet!

3. Clean your remaining glassware and return it to your station. Discard the used filter paper in the trash.

Data Analysis (both dyes):

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 #4).

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 the colorant in the solution you used to dye your cotton sample.

Questions for Consideration (both dyes):

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

2. Your standard solutions were made from a stock solution of "known" concentration. The concentration of the stock solution was based on the analysis provided with the nutritional supplement capsules. You probably saw evidence that suggests your stock solution may not have the concentration you believed. Explain. How would this affect your results?

3. What color was the stock solution for your sample? What was the wavelength setting used for your measurements, and to what color does that correspond? Explain the relation between these values for your dye molecule.

4. The "mordanting" mixture included "alum" and "cream of tartar", both obtained from a grocery store. What are the chemical formulas and chemical names of these materials?

5. Both of the nutritional supplements used as stock materials have numerous claims of health benefits. Look up the main component of the dye you used and describe the conditions it is reported to address, and the benefit it claims to provide.

Name _____ Section _____

Date _____

IN-LAB SUBMISSION WORKSHEET
Experiment 37

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. Circle the dye you used: yellow onion blueberry

SOLUTION #	CONCENTRATION (M)	PCT. TRANSMITTANCE	ABSORBANCE
Solution #1			
Solution #2			
Solution #3			
Solution #4			
Unknown (dye)	----------		

Show your calculation of the Absorbance for Solution #2.

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.

Abs. = _____ (Conc) + _____

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

dye conc. = _____

Name _____ Section _____

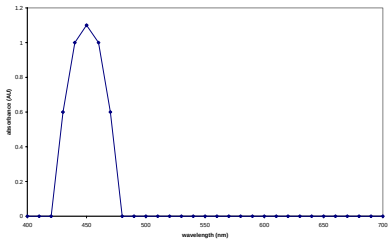
Date _____

PRE-LAB QUESTIONS
Experiment 37

Complete these questions prior to attending lab. They will be collected before you can start the experiment.

1. Read Appendix I in the lab manual (http://www.chemistry.usna.edu/manual/apdxI.pdf).

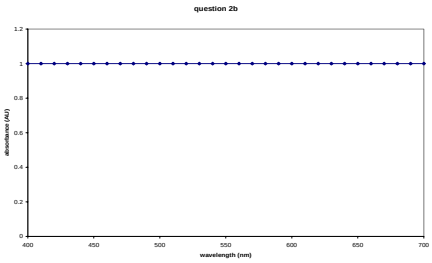
2. Go to http://www.dartmouth.edu/~chemlab/info/resources/spectrum/spectrum.html
   a. Click on the absorbance choice to change the y-axis to absorbance. Click (and hold down) on the first purple marker on the left and drag the line such that your spectrum looks like:



   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:



   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 standards for this experiment will come from commercial tablets of nutritional supplements. Calculate the molar concentration of the stock solutions from the data below, assuming all of the dye compound was transferred from the capsule to the solution. Record the concentrations here, and on pp. E37-3 and E37-5 of the Procedure.

Dyestuff	Standard Source	Compound	Formula	Assay of Capsule	Vol of Soln.	Conc. (M)
yellow onion	Quercetin-500®	quercetin	$C_{15}H_{10}O_7$	500 mg/cap	500 mL	
blueberry	Wild Blueberry IQ®	anthocyanine	$C_{15}H_{12}O_6$	42.6 mg/cap	100 mL	

4. Use the color wheel in Appendix I to predict the wavelength region(s) expected to be absorbed by the dye solutions (circle your answer):

yellow onion dye	red	orange	yellow	green	blue	violet
blueberry dye	red	orange	yellow	green	blue	violet