<u>CBL Laboratory to Determine How Much Red Food Dye Must be Added to Produce the Target Color in Big Red</u>

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Revision 0

NOTE! In this document I have included explanatory information in italic type. You should omit the italic text to produce a procedure for your students. This procedure has been designed for teachers who have not purchased undiluted FD&C Red 40 (also known as Allura Red AC). If you wish to obtain Red 40 you can purchase it from Aldrich (Product Number 45884-8). Aldrich's website is http://www.sigmaaldrich.com.

If you find any mistakes in the procedure or calculations, please email me.

INTRODUCTION

Just as you follow a recipe to prepare a cake, companies follow recipes to produce the beverages you buy at the grocery. Concentrated syrups are sent to local bottling companies where water is added and, the beverage is injected into bottles and a cap added. Color is an important characteristic of a beverage and it is vital that the color intensity is consistent.

There are many dyes approved by the Food and Drug Administration for use by the food industry. FD&C Red 40 is a common component used in many food products including beverages such as Big Red.

Spectrophotometry *[for an explanation of spectrophotometry you can access standard analytical chemistry texts or go to* http://www.chem.ubc.ca/courseware/121/tutorials/exp3A/colorimetry *or* http://www.biology.ucsc.edu/classes/bio20L/content/basics/dyes.pdf *or* http://dl.clackamas.cc.or.us/ch105-05/colorime.htm*]* is a quantitative analytical technique based upon Beer's Law: A=abC, where A=absorbance, a=absorptivity, b=path length of the cuvette, and c=molar concentration. When the apparatus is designed to measure only electromagnetic radiation in the visible range, the apparatus is known as a colorimeter and the technique is called colorimetry.

OBJECTIVE

Assuming that the red food color available at many retail food stores is used to produce the red color in Big Red, you are to determine the volume of the red food color that must be incorporated into the concentrate to produce a 2L bottle of Big Red.

SAFETY

Wear goggles. Although the food color is not toxic it is advisable to wear latex gloves and a lab apron to protect your hands and clothes from stains.

MATERIALS

- colorimeter
- cuvette
- Kroger, GFS, or other food color that contains only FD&C Red 40.
- TI 83Plus calculator
- CBL
- 100mL graduated cylinder
- wash bottle
- pipet bulb
- 10mL serological pipet
- 1 dropper or Beral pipet *[I would advise obtaining graduated Beral pipets because they can be used to measure 1mL and as a general transfer device. Flinn Scientific has them available for about $21 for 500. Catalog number AP1516.]*

- 3X100mL beakers *[These can be replaced by inexpensive plastic containers]*
- rubber stopper
- distilled water

[100mL volumetric flasks would produce more accurate results but the graduated cylinder will suffice. If you have other calculators or the CBL2 or LabPro, you will have to make some modifications in the procedure.]

PROCEDURE

If you have the time, see if the students can devise their own experimental design. It is assumed that the students are already familiar with dilution and concentration calculations.

1. Read the procedure and then prepare a table to record your data.*[The table should have columns for recording the absorbance of the diluted Big Red and the diluted red food color.]*
2. Since Big Red is too concentrated to test directly, it must be diluted so that the absorbance is within the operating range of the colorimeter. Prepare diluted Big Red by using the 10mL serological pipet to transfer 8mL of the Big Red to the 100mL graduated cylinder. Add distilled water to the 100mL mark, stopper the graduated cylinder and agitate the contents to produce a homogeneous mixture. Transfer the diluted Big Red to a clean and dry 100mL beaker. Label the beaker BR_d. Rinse the graduated cylinder with distilled water.
3. In order to measure the absorbance of the red food dye, it is necessary to perform two dilutions. For the first dilution, using the 10mL serological pipet or a graduated Beral pipet, transfer exactly 1mL of the red food dye to the 100mL graduated cylinder. Add distilled water to the 100mL mark, stopper the graduated cylinder and agitate the contents to produce a homogeneous mixture. Transfer this solution to a clean and dry 100mL beaker and label the solution FD_{d1}. Rinse the graduated cylinder with distilled water.
4. For the second dilution, using the 10mL serological pipet or graduated Beral pipet, transfer exactly 1mL of FD_{d1} to the graduated cylinder. Add distilled water until the 100mL mark, stopper the graduated cylinder and agitate the contents to produce a homogeneous mixture. Transfer this solution to a clean and dry 100mL beaker and label the solution FD_{d2}. Rinse the graduated cylinder with distilled water.
5. Plug the colorimeter into CH1 and connect the CBL to the calculator.
6. Turn on both the calculator and the CBL.
7. Press APPS on the calculator and start the ChemBio application.
8. Press ENTER when you see the “VERNIER SOFTWARE” screen.
9. Select #1, “SET UP PROBES”.
10. Type 1 for “ENTER NUMBER OF PROBES:” and then press ENTER.
11. Select #4 for the colorimeter sensor.
12. Type 1 for the “ENTER CHANNEL NUMBER:” and then press ENTER
13. Use the arrows on the colorimeter to select the 470nm wavelength and press ENTER on the calculator. *[This set up assumes you have the second generation colorimeters. If you have the original colorimeters, you must modify the set up.]*
14. Using the wash bottle, rinse the cuvette and fill ¾ full with water. Insert cuvette into the colorimeter and press ENTER.
15. Press CAL on the colorimeter and wait for the red light to stop blinking. Press ENTER.
16. Select #2 for “COLLECT DATA”.
17. Select #1 for “MONITOR INPUT”. The absorbance of the water should be 0.000 or 0.001

18. Remove the cuvette from the colorimeter, and using the dropper, rinse the cuvette several times with FD_{d2}. Fill the cuvette about ¾ full with FD_{d2} and then put the cuvette into the colorimeter. Record the absorbance.
19. Empty the cuvette and, using the dropper, rinse the cuvette several times with the BR_d. Fill the cuvette about ¾ full with BR_d and then put the cuvette into the colorimeter. Record the absorbance. Empty and rinse the cuvette with distilled water.
20. Press the "+" key on the calculator to stop the data collection and then select #7 to quit the program. Turn off the CBL and calculator.

[Although the maximum absorbance for Red 40 is 502nm, performing the experiment at 470nm yields acceptable results. By trial- and- error I found that in the .08 to .3 absorbance range at 470nm the concentration vs. absorbance curve seems to be most linear. There are many products instead of or in addition to Big Red to investigate. If you select other products, either you or your students will have to determine the correct dilution to produce solutions that absorb in the target range. This is a good project for an AP Chem class. Other commercial products you might consider include: Faygo Redpop, Jones Cherry Soda, 7-UP Cherry, Big-K Red Cream Soda. Avoid products such as Gatorade that are turbid. You might discuss why turbid samples would not be appropriate. You also might remind the students that clear does not mean colorless.]

<u>CALCULATIONS</u>

[The calculations assume that there is a linear relationship between absorbance and concentration. This assumption is reasonable if the concentrations of the "known" and "unknown" are close. Depending upon the time available and the interest or prior knowledge of your students, you might consider discussing how to investigate the linearity of the relationship and how to address non-linear relationships. I should note that the term "concentration" below is a loose interpretation of the term.]

Assuming that the absorbance-concentration relationship is linear then the absorbance of one solution can be determined from the absorbance and concentration of another solution.

1. In this general Beer's Law equation, if $A_1 = abC_1$ and $A_2 = abC_2$ and ab is constant, then

$$\frac{A_1}{C_1} = \frac{A_2}{C_2} \quad \textit{rearranging} \quad C_2 = A_2 \bullet \frac{C_1}{A_1}$$

2. Calculation of the concentration of the food dye solution (FD_{d2}) used in this analysis, in terms of mL_u of undiluted food dye per mL_{d2} of the diluted solution, is shown below. Note that mL_u is the volume of the undiluted food dye used, mL_{d1} is the volume of dilution 1, and mL_{d2} is the final volume of the second dilution.

$$FD_{d2} = \frac{1.0mL_u}{100mL_{d1}} \bullet \frac{1mL_{d1}}{100mL_{d2}}$$

3. Calculation of the concentration of the diluted Big Red (BR_d).
Using the general relationship shown in 1 above and the concentration of the diluted food dye (FD_{d2}):

$$BR_d = A_{BRd} \bullet \frac{FD_{d2}}{A_{FDd2}}$$

Where: A_{BRd}=the absorbance of the diluted Big Red
A_{FDd2}=the absorbance of the second food dye dilution

4. The concentration of the food dye in the undiluted Big Red is calculated using the general equation for dilution:

Concentration before X Volume before = Concentration after X Volume after
(BR_u) (8mL) = (BR_d) (100mL)

Rearranging:

$$BR_u = \frac{BR_d \bullet 100mL}{8mL}$$

5. To determine the volume of red dye (V_{FD}) to add to the concentrate to produce a 2L bottle of Big Red use the equation below:

$$V_{FD} = BR_u \bullet 2000mL$$

[If you select a red food color manufacturer other than GFS or Kroger, or if you analyze a product other than Big Red, your dilution and final volumes may be different.]

RESULTS and CONCLUSIONS

[Included below are some suggestions as to thought provoking items for discussion. You must decide which are appropriate for your students or you might devise your own questions.]

1. What volume of red food dye must be added to the concentrate to produce a 2L bottle of Big Red?
2. How would you determine if your results are correct? *[Probably the best way is to prepare 2L of the solution and compare its absorbance with the absorbance of a sample of Big Red.]*
3. What assumptions are made in this experimental design? *[Examples: the concentration vs. absorbance relationship is linear; there are no substances in Big Red that absorbs at 470nm except Red 40.]*
4. If you dilute the volume of red food dye determined in #1 above and your absorbance values match the sample of Big Red, assuming the manufacturer uses the same food dye at the same concentration you are using, can you unequivocally conclude that this is the amount used by the manufacturer? Explain.
   [If there are species that absorb at 470nm, addition of the amount of dye determined in step #1 would lead to an erroneously high absorption.]
5. Why is concentrate sent to local bottlers for dilution and bottling rather than bottling at one national location and then shipping to the retail stores? It would seem that quality control would be easier and more reliable if the beverages were prepared at one location. *[Quality control would be improved if the production was centralized but the transportation costs would drive the price of the product prohibitively high.]*
6. If during step #3 of the procedure section you had, without noticing it, added 4mL of red food dye to the 600mL beaker instead of 3mL, would this make your calculated results for the amount of food dye to add in step #5 of the calculations, erroneously high, erroneously low, or would it have no effect? Explain*[Erroneously low. If you did not notice the error you would record 3mL. The absorbance of the Big Red would not change but the absorbance of the diluted food dye A_{FDd} in #3 of the calculations would be erroneously high which would make the calculated concentration of the Big Red, BR_d, erroneously low and this would make the value BR_u in #5 and V_{FD} in #6 also erroneously low.*
7. Which step (or steps) in the procedure, or experimental design, do you believe introduces the most error into the analysis? Justify your answer.
8. Explain in detail how you would change the procedure to produce a better result. You must defend your proposal.