

## CBL Laboratory to Determine the % by Mass of Yellow 5 and Blue 1 in Lemon-Lime Kool-Aid

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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. If you detect mistakes in the procedure please email me.*

### INTRODUCTION

Food products are artificially colored to ensure that they appear like they should taste and flavors are added so that they taste like they appear. Lemon-Lime Kool-Aid should taste like a mixture of lemons and limes and it should have a green color. To produce the green color, FD&C Yellow 5 and FD&C Blue 1 are added.

If you want to determine the % mass composition of colorants in each package of Lime Kool-Aid, you must perform a quantitative analysis. 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  $C$ =concentration. When the apparatus is designed to measure electromagnetic radiation in the visible range, the apparatus is known as a colorimeter and the technique is called colorimetry. It is possible to determine the concentrations of two absorbing species in the same solution if the absorbance of one species does not overlap the absorbance of the other. The maximum absorbance of FD&C Yellow 5 is 425nm and FD&C Blue 1 exhibits a maximum absorbance at 625nm. Since the Vernier's colorimeter has diodes that radiate at 430nm and 635nm, the colorimeter can be used in this analysis.

There are several experimental designs that might be applied to this type of analysis. [*See other procedures on the website for other approaches to the data treatment.*] In this lab you will prepare standard solutions that approximate the absorbances of a solution prepared from a package of the Kool-Aid, and apply Beer's Law. The dilutions are necessary because the colorimeter's effective range is between about 0.1-.6 AU (absorbance units) with better linearity at the lower end of the range. The calculation method is described below.

### OBJECTIVE

Using the analytical technique of colorimetry, the objective of the lab is to determine the % mass composition of FD&C Yellow 5 and FD&C Blue 1 in a package of Lemon-Lime Kool-Aid. [*To prepare a solution of Yellow 5 that the students can dilute to prepare a working standard, add 2.00 g of tartrazine (Aldrich Product Number 20195-2, Aldrich's website is <http://www.sigmaaldrich.com>) to a 500mL volumetric flask (or 600mL beaker or Erlenmeyer flask) and then dilute to the 500mL mark with water. The concentration is 4.00 g/L and the target is 0.200 g/L. By pipet, or by use of a graduated cylinder, transfer 25mL of this solution to a 500mL volumetric flask (or beaker or Erlenmeyer flask) and dilute to the 500mL mark with distilled water. This solution is 0.200 g/L. The students are to prepare a working standard solution of 0.00800 g/L from this solution. It should be noted that the tartrazine obtained from Aldrich (or any other source) is not 100% pure. Information from the manufacturer described the tartrazine used to develop this lab procedure to be 89% colorant. I do not know what technique was used to determine the % colorant. I have not used the purity in the calculations below. You should consider whether this factor should be included in the calculations. Modifying the equations to include the % purity might be an extension or extra credit problem for your students.*

*Following a similar procedure, prepare a solution of FD&C Blue 1 with a concentration of 0.00800 g/L that the students will dilute to produce a working standard solution of 0.00160 g/L. Add*

2.00g of FD&C Blue 1 (Aldrich Product Number 86114-6, Product Name-Erioglaucine ) to a 500mL volumetric flask and after dissolving, fill to the mark with distilled water. The concentration is 4.00g/L. Pipet 1mL of this solution to a 500mL volumetric flask and fill to the mark with distilled water. The concentration of this solution is 0.00800g/L.

### SAFETY

Wear goggles. It is advisable to wear latex gloves and a lab apron to protect your hands and clothes from stains.

### MATERIALS

- Colorimeter
- Cuvette
- TI 83Plus calculator
- CBL
- 100mL graduated cylinder
- Wash bottle
- Distilled (or deionized) water
- Pipet bulb
- 10mL serological pipet
- 1 dropper [or Beral pipet]
- Distilled water
- Working solutions of FD&C Yellow 5 and FD&C Blue 1
- Six 16mmX150mm test tubes [You can use inexpensive and reusable plastic cups available at any grocery store if you do not have enough test tubes.]
- 1 #6 rubber stopper
- One 50mL beaker. [You can use inexpensive and reusable plastic cups available at any grocery store if you do not have beakers.]
- One package of Lemon-Lime Kool-Aid
- Balance
- One 500mL beaker or flask
- One stirring rod

*[Volumetric flasks would produce more accurate results but the graduated cylinder will suffice. Consider discussing the issue of which glassware would produce the most accurate and precise results.*

*[If you are using 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 concentrations calculations. To conserve the laboratory time, you might consider having half the students perform the Yellow 5 analysis and half the students perform the Blue 1 analysis.]*

### **Preparation of the Standards of Known Concentration**

#### **FD&C Blue 1**

Your teacher will provide you with 25mL of a solution of FD&C Blue 1 at a concentration of 0.00800g/L. You will dilute this solution to prepare working standard with a concentration of 0.00160g/L.

1. Pipet 10mL of the FD&C Blue 1 provided by your teacher and add distilled water to the 50mL mark. Insert the stopper and agitate until you achieve a homogeneous solution. Label this solution  $WS_B$ . The concentration of this solution is  $C_{WSB}$ .

### **FD&C Yellow 5**

Your teacher will provide you with 10mL of a solution of FD&C Yellow 5 at a concentration of 0.200g/L. You will dilute this solution to prepare a working standard at a concentration 0.00800g/L.

2. Pipet 4mL of the 0.200g/L solution into the 100mL graduated cylinder and fill to the 100mL mark with water. Stopper the cylinder and agitate until a homogeneous solution is obtained. Label this solution  $WS_Y$ . The concentration of this solution is  $C_{WSY}$ .

### **Preparation of the Lemon-Lime Kool-Aid Solution**

You are to prepare a Lemon-Lime Kool-Aid solution at a concentration near the concentrations of the concentrations of the standards you prepared above.

3. Place a weighing paper or boat on the balance and record the mass.
4. Empty the package of Lemon-Lime Kool-Aid on to the weighing paper and record the mass.
5. Subtract the mass of step 3 from the mass of step 4 to obtain the mass,  $m_{KA}$ , of the Kool-Aid.
6. Quantitatively transfer the Kool-Aid to the 500mL beaker, dissolve the Kool-Aid in about 400mL of water and then fill to the 500mL mark with water. Label this solution KA1. The concentration of Yellow 5 in this solution is  $C_{KAY1}$  and the concentration of Blue 1 in this solution is  $C_{KAB1}$
7. Pipet 10mL of this solution to the 100mL graduated cylinder and fill to the 50mL mark with water. Stopper the cylinder and agitate until the mixture is homogeneous. Label this solution, KA2. The concentration of FD&C Blue 1 in this solution is  $C_{KAB2}$  and the concentration of FD&C Yellow 5 in this solution is  $C_{KAY2}$ .

### **Set Up of CBL for FD&C Yellow 5**

6. Plug the colorimeter into CH1 and connect the CBL to the calculator.
7. Turn on both the calculator and the CBL.
8. Press APPS on the calculator and start the ChemBio application.
9. Press ENTER when you see the "VERNIER SOFTWARE" screen.
10. Select #1, "SET UP PROBES".
11. Type 1 for "ENTER NUMBER OF PROBES:" and then press ENTER.
12. Select #4 for the colorimeter sensor.
13. Type 1 for the "ENTER CHANNEL NUMBER:" and then press ENTER
14. Use the arrows on the colorimeter to select the 430nm 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.]*
15. Rinse the cuvette and fill  $\frac{3}{4}$  full with distilled water. Insert cuvette into the colorimeter and press ENTER.
16. Press CAL on the colorimeter and wait for the red light to stop blinking. Press ENTER.
17. Select #2 for "COLLECT DATA".
18. Select #1 for "MONITOR INPUT". The absorbance of the water should be 0.001 or 0.002

### Absorbances of Working Standards and Lemon-Lime Kool-Aid at 430nm

19. Empty the cuvette and using the dropper, rinse several times with  $WS_Y$ . Fill the cuvette  $\frac{3}{4}$  full with  $WS_Y$ . Place the cuvette into the colorimeter and record the absorbance,  $A_{WSY}$ .
20. Empty the cuvette and using the dropper, rinse several times with KA2. Fill the cuvette  $\frac{3}{4}$  full with KA2. Place the cuvette into the colorimeter and record the absorbance,  $A_{KAY2}$ .
21. Empty the cuvette and using the dropper, rinse several times with  $WS_B$ . Fill the cuvette  $\frac{3}{4}$  full with  $WS_B$ . Place the cuvette into the colorimeter and record the absorbance. The absorbance should be 0.001-0.004. This step is to ensure that the FD&C Blue 1 does not absorb at 430nm.
22. Press the “+” key to stop the data collection.

### Absorbances of Working Standards and Lemon-Lime Kool-Aid at 635nm

22. Empty the cuvette and using the dropper, rinse several times with distilled water. Fill the cuvette  $\frac{3}{4}$  full with distilled water. Place the cuvette into the colorimeter.
23. Change the wavelength to 635nm and calibrate the colorimeter by pressing the CAL button.
24. Select #2 for “COLLECT DATA”.
25. Select #1 for “MONITOR INPUT”. The absorbance of the water should be 0.001 or 0.002
26. Empty the cuvette and using the dropper, rinse several times with  $WS_B$ . Fill the cuvette  $\frac{3}{4}$  full with  $WS_B$ . Place the cuvette into the colorimeter and record the absorbance,  $A_{WSB}$ .
27. Empty the cuvette and using the dropper, rinse several times with KA. Fill the cuvette  $\frac{3}{4}$  full with KA2. Place the cuvette into the colorimeter and record the absorbance,  $A_{KAB2}$ .
28. Empty the cuvette and using the dropper, rinse several times with  $WS_Y$ . Fill the cuvette  $\frac{3}{4}$  full with  $WS_Y$ . Place the cuvette into the colorimeter and record the absorbance. The absorbance should be 0.001-0.004. This step is to ensure that the FD&C Yellow 5 does not absorb at 635nm.
29. Press the “+” key to stop the data collection.

### CALCULATIONS

*[This data treatment assumes that if the standards and the sample are close in their concentrations, that any non-linearity in the absorbance vs. concentration curve will introduce insignificant error. There are of course other ways to treat the data. A traditional approach is to collect absorbance vs. concentration data for a series of working standards and use this data to either plot a graph or apply linear or non-linear statistical functions. The TI83Plus has both graphing and statistical capabilities. The course you take depends upon how much time you have.]*

#### Concentration of Yellow 5 in KA2

$$\text{If } A_1=abC_1 \text{ and } A_2=abC_2 \text{ and } ab \text{ is constant, then } \frac{A_1}{C_1} = \frac{A_2}{C_2} \text{ rearranging } C_2 = A_2 \cdot \frac{C_1}{A_1}$$

If,

$C_{KAY2}$  = the concentration of Yellow 5 in the Kool-Aid solution KA2.

$A_{KAY2}$  = the absorbance of the Kool-Aid solution KA2 at 430nm.

$C_{WSY}$  = the concentration of the Yellow 5 working standard,  $WS_Y$ .

$A_{WSY}$  = the absorbance of the Yellow 5 working standard,  $WS_Y$ .

then substituting these values into the equation above results in the equation below:

$$C_{KAY2} = A_{KAY2} \cdot \frac{C_{WSY}}{A_{WSY}}$$

### Concentration of Blue 1 in KA2

Based on the explanations above for Yellow 5, if:

$C_{KAB2}$  = the concentration of Blue 1 in the Kool-Aid solution KA2.

$A_{KAB2}$  = the absorbance of the Kool-Aid solution KA2 at 635nm.

$C_{WSB}$  = the concentration of the Blue 1 working standard,  $WS_B$ .

$A_{WSB}$  = the absorbance of the Blue 1 working standard,  $WS_B$ .

then substituting these values into the equation results in the equation below:

$$C_{KAB2} = A_{KAB2} \cdot \frac{C_{WSB}}{A_{WSB}}$$

### Concentration of Blue 1 and Yellow 5 in KA1

After determining the concentrations of Yellow 5 and Blue 1 in the diluted solution KA2, it is necessary to determine the concentrations of the colorants in the original solution KA1 which will allow us to determine the mass of the colorants in the package of Lemon-Lime Kool-Aid.

$C_{KAY1}$  = the concentration of Yellow 5 in Lemon-Lime Kool-Aid solution KA1

$C_{KAY2}$  = the concentration of Yellow 5 in the Kool-Aid solution KA2.

$C_{KAB1}$  = the concentration of Blue 1 in Lemon-Lime Kool-Aid solution KA1

$C_{KAB2}$  = the concentration of Blue 1 in the Kool-Aid solution KA2.

Since,

$$C_{KAY1} \cdot 10\text{mL} = C_{KAY2} \cdot 50\text{mL} \text{ therefore } C_{KAY1} = C_{KAY2} \cdot \frac{50\text{mL}}{10\text{mL}}$$

Since,

$$C_{KAB1} \cdot 10\text{mL} = C_{KAB2} \cdot 50\text{mL} \text{ therefore } C_{KAB1} = C_{KAB2} \cdot \frac{50\text{mL}}{10\text{mL}}$$

### Mass of Blue 1 and Yellow 5 in a Package of Lemon-Lime Kool-Aid

Since the mass of the colorants in KA1 is the mass of the colorants in the package of, we will determine the mass of the colorants in KA1.

$m_B$  = mass of Blue 1 in KA1 = mass of Blue 1 in the package of Lemon-Lime Kool-Aid

$m_Y$  = mass of Yellow 5 in KA1 = mass of Yellow 5 in the package of Lemon-Lime Kool-Aid

Since the concentrations we are using are in terms of grams per mL, then:

$$m_B = C_{KAB1} \cdot 500\text{mL}$$

$$m_Y = C_{KAY1} \cdot 500\text{mL}$$

### Percent Mass of Blue 1 and Yellow 5 in a Package of Lemon-Lime Kool-Aid

$$\% \text{ Blue 1} = \frac{m_B}{m_{KA}} \cdot 100 \qquad \% \text{ Yellow 5} = \frac{m_Y}{m_{KA}} \cdot 100$$

## 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 is the % by mass concentrations of Blue 1 and Yellow 5 in Lemon-Lime Kool-Aid?
2. How would you determine if your results are correct? *[Answers such as “compare results with other students”, or “contact the manufacturer of Kool-Aid”, or prepare an aqueous solution of Blue 1 and Yellow 5 same concentration as the experimental value and determine the absorbance of this “known” solution, are possible answers]*
3. If the Yellow 5 showed some absorbance at 635nm or the Blue 1 showed some absorbance at 430nm, would this mean you cannot use this procedure? *[Not necessarily, that is, you could measure the absorbances of the standard solutions of Yellow 5 at 635 and Blue 1 at 430 and then subtract these values from the sample absorbances.]*
4. Provide an explanation for the possibility that the true concentrations of the Yellow 5 or Blue 1 are lower than your experimental values. *[Accept reasonable explanations such as there are natural colorants used in the Lemon-Lime Kool-Aid in addition to the synthetic Yellow 5 and Blue 1.]*