Separating Food Dyes

Objectives:

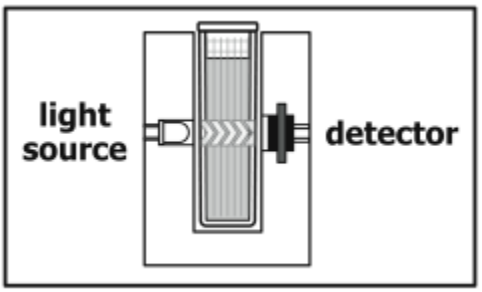
- Investigate the basic principles of spectroscopy.
- Observe the spectra of commercial dyes.
- Conduct a liquid-chromatographic separation.
- Determine the dyes present in a commercial drink.

Materials:

- Graduated cylinder
- 70% isopropanol
- Green Hawaiian Punch & Grape Gatorade
- Stock solution of Yellow #5, Blue #1, and Red #40
- 1-100 mL beaker
- 5-50 mL beakers
- LabQuest 3 and spectrometer
- 6 large test tubes and test tube rack
- 1-400 or 600 mL beaker for waste
- Disposable pipettes pipettes
- C18 cartridge and #5 syringe with plunger
- Spatula

Introduction:

Food dyes are used in soft drinks to simulate natural colors of fruits or vegetables and to make products more appealing to consumers. In this experiment, you will look at three common food dyes approved by the Food and Drug Administration: FD&C Blue No.1, FD&C Yellow No.5, and FD&C Red No.40. You will analyze a common soft drink to determine the food dyes used to give the drink color.



You will use spectroscopy to observe the absorbance spectrum of the soft drink to see what colors (wavelengths) of visible light are absorbed by the dyes in the drink. The spectrometer passes a light beam containing all visible colors of light through the sample of the drink. As the light passes through the sample, some of the colors are absorbed and other colors pass through.

The spectrometer measures the amount of each color of light that is visible and provides a signal that is graphed.

In Part II, you will separate the dyes from a sample of the drink using a technique called column chromatography. In column chromatography, the column contains a material (called the stationary phase) to which food dyes are partially attracted. Some dyes are more attracted to the material in the column than others. The dyes can be moved through the column by various solvents (called the mobile phase). The rate at which a dye passes through the column can be controlled by varying the ratio and concentration of solvents that are injected through the column. Some dyes will move more readily and others will require higher concentrations of a particular solvent to move through the column. In this lab, you will create serial dilution of isopropanol (IPA). This will allow you to expose the sample of food dye to varying concentrations of IPA, separating the individual dyes within the sample.

From the data you will collect, you will be able to view how the dyes contribute to the overall spectrum of the drink. Additionally, you will be able to determine which commercial food dyes were used to make the beverage.

Procedure:

Part I Observe the absorbance spectrum of a soft drink

1. Obtain and wear goggles.

2. Prepare a blank by filling a cuvette 3/4 full with distilled water. To correctly use a cuvette, remember

    - All cuvettes should be wiped clean and dry on the outside with a tissue.
    - Handle cuvettes only by the top edge of the ribbed sides.
    - All solutions should be free of bubbles.
    - Always position the cuvette so the light passes through the clear sides.

3. Turn on LabQuest3 and launch the LabQuest App. Plug the spectrometer into the USB input on the LabQuest3.

4. To calibrate the spectrometer, place the blank cuvette of water in the spectrometer and select SENSORS, CALIBRATE, and USB SPECTROMETERS. Allow the spectrometer to warm up and click FINISH CALIBRATION when the warm up is complete. Then click OK.

5. Obtain solutions of the food dyes you will analyze: Blue #1, Yellow #5, and Red #40.

    a. Remove the blank cuvette, and place the cuvette containing the sample of the first dye into the slot. Make sure to align the cuvette correctly.

    b. Click COLLECT  . The absorbance *vs*. wavelength spectrum will be displayed. Click STOP  .

c. Collect the absorbance spectrum of each dye. Rename the run for each dye you use. To rename go to the data table  , double click on "Run 1" and type a new title.

d. Click Store (). Repeat for all three dyes.

6. Prepare a test sample by filling a cuvette 3/4 full with soft drink.

7. Collect a full spectrum for the soft drink.

   a. Remove the dye-filled cuvette, and place the cuvette containing the sample of drink into the slot. Make sure to align the cuvette correctly.

   b. Click COLLECT  . The absorbance *vs*. wavelength spectrum will be displayed. Click or tap STOP  .

   c. Click STORE (). Rename the data set.

7. Save your data so you can use the data in Part III.

8. Answer the question below.

From the spectrum in Part I, how many dye components do you suspect made up the drink? How did you determine this? After recording your prediction, return to Part II.

Part II Separate the dyes in the soft drink

8. Before separating the dyes in the samples, answer Analysis Question 1. After writing your answer, continue with the next step.

9. Prepare serial dilutions of alcohol.

   a. Place 10 mL of 70% isopropyl alcohol (IPA) in a 150 mL beaker. Label the beaker 70% IPA.

   b. Place 10 mL of 70% isopropyl alcohol into another 150 mL beaker. Add 10 mL of distilled water. Label this beaker 35% IPA.

   c. Remove 10 mL of 35% IPA from the second beaker and place it in a third 150 mL beaker. Add 10 mL of distilled water. Label this beaker 17.5% IPA.

   d. Repeat this process until you have a series of beakers from 70% to 4.38% (five beakers total).

10. Pretreat the cartridge.

   a. Arrange six test tubes in a test tube rack (see Figure 2).

   b. Insert the C18 Sep-Pak cartridge into one hole in the #5 stopper.

c. Using the syringe, **slowly** push 5 mL of 70% alcohol through the cartridge into the first test tube. Repeat with 5 mL of water. **Important**: Never draw liquid backward through the cartridge.

d. Discard the liquid in the test tube.

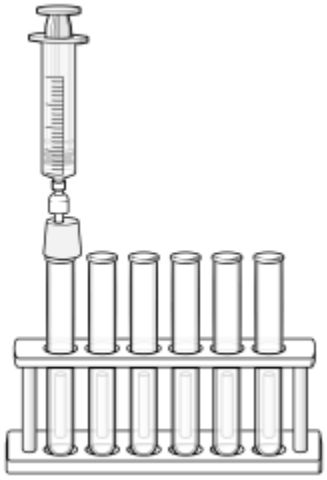


Figure 2

11. Load the cartridge with 5.0 mL of soft drink.

    a. Using the syringe, **slowly** push 5.0 mL of drink through the cartridge into the first test tube.

    b. Note the color of the cartridge and the liquid coming from the cartridge. Record your observations in the data table.

    c. Discard the liquid in this test tube. Clean the syringe with distilled water.

12. To separate the dyes, you will elute the mixture of dyes in the drink with different concentrations of alcohol.

    a. Starting with pure distilled water (0% alcohol), slowly elute (push) 5 mL of solvent through the loaded cartridge with the syringe into the first test tube.

    b. Note the color of the cartridge and the eluted liquid and record your observations in the data table.

13. Next, slowly elute with the 4.38% IPA solution into the second test tube. Note the color of the cartridge and eluted liquid.

14. Repeat Step 12, working your way through all the dilutions of alcohol you made, from the least concentrated to the most concentrated.

15. After the last elution, clean the cartridge by slowly passing 5 mL of distilled water through the cartridge into a waste container or the sink as directed by your instructor.

16. Save these test tubes for Part III.

17. Answer the question below before continuing.

From the separated fractions in Part II, how many dyes made up the drink? What colors did you observe?

Part III Identify the dyes in the soft drink

17. Return to the LabQuest App and open the file you saved at the end of Part I.

18. Click SENSOR and calibrate your spectrometer as you did in Step 4.

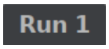
19. Using a clean pipette, remove a sample of separated dye from one of the test tubes from Part II. Fill a cuvette 3/4 full with the dye.

20. Collect spectrum data for the dye from this fraction using the process described in Step 6. Rename the data set with the solvent of the mixture (i.e. 0% alcohol, etc.).

21. Repeat Steps 19 and 20 for the other fractions from the drink sample.

Part IV Compare the dyes in the soft drink

22. Compare the spectrum of the known food dye to the dye components of your drink.

    a. Click 

 in the bottom right hand corner to select the desired spectra graphs.

    b. Click or tap the graph to locate the wavelength of maximum absorbance (highest peak) for each spectrum. Record the values in the data table.

25. Dispose of your solutions as instructed.

Separating Food Dyes Lab Report

Name: **Date:**

Part II Separate the dyes in the soft drink

Elution process step	Color of cartridge	Color of solution eluted into test tube
After loading the cartridge with drink		
After eluting with water (0% alcohol)		
After eluting with 4.38% alcohol		
After 8.75% alcohol		
After 17.5% alcohol		
After 35% alcohol		
After 70% alcohol		

Part III Identify the dyes in the soft drink

Color of dye separated from drink	Wavelength of maximum absorbance (nm)

Color and number of FD&C dye	Wavelength of maximum absorbance (nm)
Red #40	
Yellow #5	
Blue #1	

Analysis Questions:

1. While you were separating the dyes in Part II, at one point, one dye passed through the column while the other did not. Why do you suppose this happened? In other words, why did one color come through the column before the other?

2. In Part II, which dye component was the most soluble in alcohol? How do you know this?

3. From your spectra in Part III, what FD&C dyes made up the dye components of your drink? How did you determine this?