Pre-lab Assignment

Before coming to lab:

- Read the lab thoroughly.
- Answer the pre-lab questions that appear at the end of this lab exercise.

Purpose

Use paper chromatography to see which dyes are used in the coatings of colored candies.

Background

Food additives are one of the most commonly produced type of chemicals in the United States. The use of additives increased dramatically in the United States in the second half of the nineteenth century. As the economy became more industrial, fewer people lived on farms, city populations grew, and people became more dependent on mass produced foods.

Food dyes were initially used to make food more visually appealing to the consumer and, in some cases, to mask poor-quality, inferior, or imitation foods. For example, meat was colored to appear fresh long after it would have naturally turned brown. Jams and jellies were colored to give the impression of higher fruit content than they actually contained. Some food was colored to look like something else—imitation crab meat, for example. Many food colorings and additives were later discovered to be harmful or toxic. Food colorants were initially added to food with little or no health testing. In 1907, the USDA reduced the number of synthetic food dyes approved for use from 695 to just seven. Only two of the original dyes from 1907 are still accepted for use today. Five others have been added between 1907 and 1971 leading to there only being seven dyes approved for use in the United States today. All of the FD&C approved food dyes are charged, water-soluble organic compounds that bind to natural ionic and polar sites in large food molecules, including proteins and carbohydrates.

Food dyes will be separated and identified in this experiment using chromatography. Chromatography is a technique used to separate the various components in a mixture or solution.

In chromatography there is a mobile phase, which is a fluid the solution is dissolved in, and a stationary phase, which is a material the fluid moves through. For example, in paper chromatography which we are using in this experiment, water is the mobile phase and filter paper is the stationary phase. The mobile phase is also called the solvent. The paper acts as an adsorbent which is capable of attracting and binding the components in a mixture (see Figure 1). The mixture to be separated is "spotted" onto the surface of the paper and a solvent is allowed to flow through the paper by capillary action. If one of the components in the mixture is more strongly adsorbed onto the paper than another, it will move up the paper more *slowly* than the solvent. Components that are not strongly adsorbed onto the paper will move up the paper at a *faster*

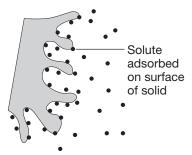


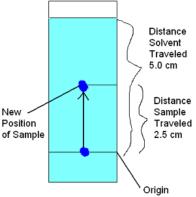
Figure 1. Adsorption of solute particles onto the surface of a solid.

rate. This leads to the components of the mixture separating based on their attraction to the stationary phase and gives rise to different bands or spots. If the components of the mixture are colored, like food dyes or pigments in an ink, the colored bands are easily distinguished.

In paper chromatography, you can identify the components based on how far they travel up the paper. To do this, we calculate the retention factor (R_f value) of each sample. R_f values are calculated by looking at the distance each component travels on the filter paper compared to the distance traveled by the solvent front:

$R_f = \frac{\text{distance travelled by compound}}{\text{distance travelled by solvent}}$

When measuring the distance the compound traveled, you should measure from the **origin** (where the middle of the spot originally was) to the center of the spot in its new location. The distance travelled by the solvent (**solvent front**) is measured from the origin to the farthest distance that the solvent climbs.



For example, if one of the sample components moves 2.5 centimeters (cm) up the paper and the solvent moves 5.0 cm, as shown in the figure to the right, then the R_f value is 0.5.

In this food science project, you will use the Rf value to compare the "unknown" components of colored candy dyes with the "known" components of food coloring dyes. Since there are only a small number of approved food dyes, you should be able to identify the ones used in the candies by comparison to the chromatography results for food coloring.

Procedure:

Safety: The FD&C dyes are slightly hazardous by ingestion, inhalation, eye and skin contact. Red No. 40 may be absorbed through skin and Yellow No. 5 may be a skin contact sensitizer. All are irritating to skin and eyes. Avoid contact with eyes, skin, and clothing. Wear chemical splash goggles. Wash hands thoroughly with soap and water before leaving the laboratory.

Waste: All waste may be disposed of down the sink or in the trash can.

1. Cut a 20 cm \times 18 cm piece of chromatography paper (see Figure 2). Handle the paper by the edges to prevent contamination.

2. Using a ruler and pencil, draw a faint line 1.5 cm from the bottom across the entire width of the paper (see Figure 3).

3. Using the same ruler and pencil, draw eight small dots. Measure 2.0 cm from one edge for the first dot and then add a dot every 2.0 cm across the line. Label the dots (see Figure 3).

4. Obtain the seven pure dye solutions.

5. Using a clean toothpick for each dye sample, spot the chromatography paper by putting the toothpick into the dye sample solution and then touching the tip of the toothpick gently onto a pencil dot. Repeat the procedure as necessary to increase the concentration of the sample but do not increase the size of the dot.

Note: Make sure the initial sample spots are as small as possible. If the spots are too large, your experiment will give poor results.

6. Next you need to extract some dye from each candy you wish to test.

a. Fill the 150 mL beaker with some water.

b. Use a dropper, put a single drop of water into a petri dish. Set one candy in the drop of water.

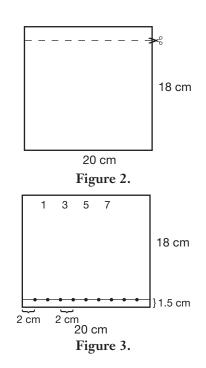
c. Leave the candy in the drop of water for three minutes to allow the dye to dissolve

d. Remove the candy, then dip a clean wooden toothpick into the now-colored drop of water.

e. Spot the candy dye solution onto the chromatography strip by touching the wooden toothpick to marked location on the chromatography paper.

f. Allow the spot on the strip to dry completely (this should take approximately 1 minute). g. Repeat steps 6e to 6f three more times. You want to make sure to have enough dye on the chromatography strip so that you can see the dye components when they separate out on the paper.

7. While the sample is drying obtain a tall-form beaker or Mason jar and a watch glass that will fit over the container.



8. Pour 50 mL of 0.1% NaCl solution into the beaker and cover the top with a watch glass. This is the chromatography chamber. The 0.1% NaCl is the developing solvent.

9. Wrap the chromatography paper into a cylinder, and slightly overlap the blank ends. Staple, being careful not to disrupt the samples (see Figure 4).

10. Remove the watch glass from the beaker and carefully place the paper cylinder into the chamber with the sample end down (as shown in Figure 5).

Do not get any solvent on the upper portion of the chromatography paper. The sample spots must be above the level of the solvent. If the solvent level is too high, the samples will dissolve into the solvent.

11. Place the watch glass back on the beaker and allow the chromatogram to develop (about 15–25 minutes).

12. When the solvent is within 2-3 cm of the top of the paper, remove it from the beaker.

13. With a pencil, lightly draw a line to mark the distance the solvent traveled to the top of the chromatography paper. This is called the solvent front.

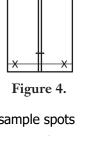
14. Gently remove the staples and lay the paper flat. Place the paper in the oven to dry.

15. When the paper is dry, remove it from the oven and measure the distance from the pencil line at the bottom of the chromatography paper to the solvent front. Record this distance in cm.

16. In pencil, trace the shape of each dye band or spot to mark the location of each separated band. This should be done immediately because the color and brightness of some spots may fade over time.

17. Measure the distance traveled in cm by each dye in each pure solution or mixture. Measure from the line at the bottom of the paper to the center of each band.

18. Calculate the R_f value for each sample.



Paper Developing solvent 0.1% NaCl

Figure 5.

Name _____

Data Table

Solvent Front Distance _____ cm

Sample Number	Identity	Color	Distance to center of spot	Calculated R _f value
1	Yellow #5			
2	Blue #2			
3	Green #3			
4	Red #3			
5	Blue #1			
6	Red #40			
7	Yellow #6			
Unknown Component 1				
Unknown component 2 <i>(if present)</i>				
Unknown component 3 <i>(if present)</i>				

Identity of dye(s) in candy _____

Example calculation for R_f values

Post-Lab Questions

1. Which food coloring had the strongest attraction to the chromatography paper? How do you know this?

2. A student didn't read the instructions and put 100 mL of solvent into the developing tank. The liquid level was above the baseline. Will she still get valid data?

3. Imagine a researcher allowed the paper chromatogram to develop for far longer than necessary in the mobile phase, such that the solvent front ran into the top edge of the paper and the spots continued to move. Would the resulting Retention Factors (Rf) be overestimated, underestimated or remain unaffected? Explain your reasoning.

Name

Pre-lab Assignment for Identification of Food Colors in Candies

Show all work and round all answers.

1. Why should you use a pencil, rather than ink or colored pens, for marking the chromatography paper ?

- 2. Define these terms.
- a. Mobile phase
- b. Stationary phase
- c. Solvent
- d. Chromatography
- 3. In paper chromatography, what is the stationary phase and what is the mobile phase?
- 4. What is the formula for calculating the Rf value?

5. In an experiment the solvent front is measured to be 6.0 cm. If a sample of blue food coloring moves 2.2 cm during a chromatography experiment, what is the R_f value for the blue food coloring?