

SEPARATION OF DYES BY PAPER CHROMATOGRAPHY LAB

Purpose: Like all chemicals, dyes have different physical properties like boiling points, melting points, and color. These properties can include the affinity of a dye to a particular chemical. If a chemical “sticks” to another, it has a high affinity. If a chemical doesn’t want to stick to another, it has a low affinity. If we have several dyes with different affinities to a single liquid, we can separate them using a technique called paper chromatography. In this lab, we will be separating colored food dyes using salt water and comparing them to dye standards.

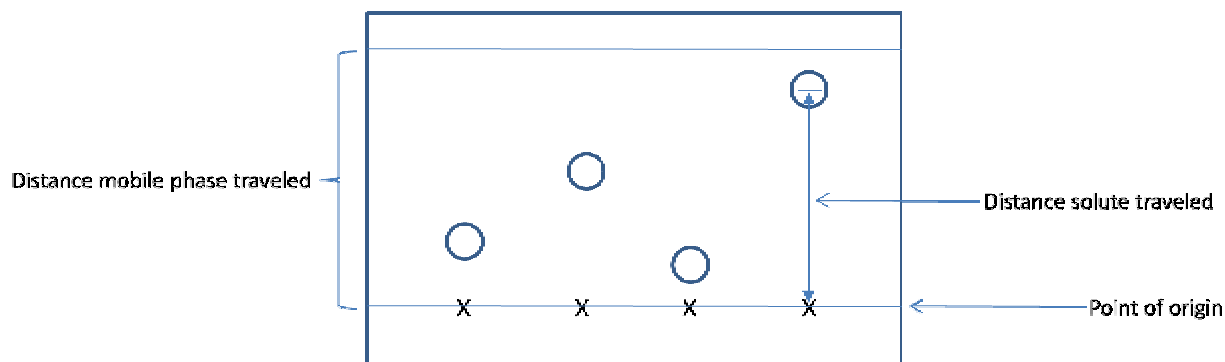
Background: There are a few new definitions you need to know before doing this lab.

1. **Solvent**—a solvent is a liquid that you can dissolve a chemical in. If the chemical is not soluble in the liquid, it can’t be a solvent. Water, for example, is a good solvent for both sugar and salt, because they dissolve completely in it.
2. **Solute**—a chemical that is being dissolved in a solvent is the solute. In the water-sugar example, sugar is the solute.
3. **Solution**—a solute dissolved in a solvent creates a solution. That is, a liquid with other chemicals dissolved in it. In the water-sugar example, the water with the dissolved sugar is the solution.
4. **Paper chromatography**—a method chemists use to separate one chemical from another using their differences in physical properties. Paper chromatography has several components:
 - a. **Stationary phase**—In order to separate chemicals, we have to have a platform on which to separate them. In our case, the platform is paper. The paper does not move in the process of chromatography, therefore, we call it stationary.
 - b. **Mobile phase**—A mobile phase is the solvent that carries the chemicals through the stationary phase. Chemicals don’t move on paper alone, but if we add a solvent, it can draw the chemicals up through the paper.
 - c. **Point of origin**—the spot where you put your chemicals on the stationary phase.
 - d. **Developing**—after putting chemicals on the point of origin, the chromatogram can be “developed”, that is, the mobile phase can be pulled through the stationary phase.
 - e. **Retention factor (R_f)**—The distance our chemicals move during chromatography is typically less than the distance the mobile phase moves. We can measure the difference in distance by using the R_f value. This is the distance a chemical moves on our paper (stationary phase) during our separation divided by the distance the mobile phase moves. If I use water as a mobile phase, sugar as my solute, and paper as my stationary phase, my R_f value will always be the same in this system. Changing the mobile phase to alcohol will change the R_f for the sugar, because sugar has a different affinity for alcohol than it does water. The following formula is used to determine R_f :

$$R_f = \frac{\text{distance solute travels}}{\text{distance mobile phase travels}}$$

5. Chromatograph—The finished result from chromatography. The separated solute(s) on the stationary phase after the mobile phase has dried. Figure 1 shows an example of a chromatograph like what you will produce in this lab.

Figure 1: An example chromatograph depicting the areas that need to be measured to determine R_f . The round spots are the separated solutes, and the large square is the stationary phase.



It is no surprise to you that we used a large number of dyes in our everyday life. Plastics, resins, paints, and clothing are full of pigment from a variety of dyes. Synthetic chemical dyes are used to color the powders and crèmes we use as makeup and the coatings on pills we take when we are sick. Another place we use dyes is in food production. Kool-aid, popsicles, hard candies, and sodas are good examples of food products that contain dyes. Believe it or not, that sour apple gummy worm has to be dyed that bright neon green color! Our government regulates the types of dyes that are used for food, drugs, and cosmetics. They are tested extensively for short term and long term safety before they are approved for use. If you have ever looked on a list of ingredients, you have no doubt seen numbered colors near the end, like red #3. You may have even seen FD&C listed before these dyes. This stands for food, drug, and cosmetic dyes. These are pure colors, only made of one colored chemical. In contrast, most of the uses of these dyes are in mixing them together to create new colors and shades. Food coloring, for example, may look red, but in actuality, it is a mixture of several differed FD&C dyes that give it just the right shade of red. Many foods, drinks, and candies have several FD&C dyes that are combined in different ratios to give different colors.

The purpose of this lab is to experimentally determine which FD&C dyes are in food coloring and Kool-aid samples. You will determine this by taking a chromatograph of the FD&C dyes, then taking two other chromatographs of the food coloring and Kool-aid samples. You will be able to compare the R_f values of the FD&C dyes to those in the food coloring and Kool-aid samples to determine which dyes were used to color the products. Remember to draw a distinction between FD&C dyes (pure, single chemical used to color many products) and food coloring (mixture of several FD&C dyes used in the home to tint icing, cookies, and other baked goods).

Materials:

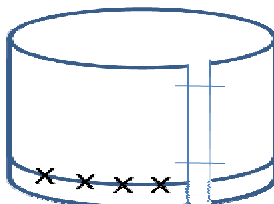
FD&C red #3, red #40, yellow #5, and blue #1 dyes
Red, green, blue, and yellow food dyes
Sodium chloride
Saran wrap
Three flavors of Kool-Aid
Pencil

Rubber bands
Large beakers
Stapler
Filter paper
Toothpicks
Ruler

Procedure:

- I. Chromatography of FD&C dyes
 - a. Take a 9.5 cm X 16 cm filter paper and draw a line (long ways) about 2 cm from the bottom using a *pencil* (a pen contains dyes that will ruin your results!!!). Put 4 small X's on the line about 4 cm from the edges, about 3 cm apart. Use Figures 1 and 2 as a guide.
 - b. Prepare your mobile phase. Add 0.1 g NaCl to 100 mL of water and allow it to dissolve. Pour 25 mL of this salt-water solution into a large beaker (400 or 600 mL) and cover with saran wrap. Throughout the experiment if your mobile phase level gets low, add more of this solution.
 - c. Take a small piece of scrap filter paper and practice "spotting". You will need to use one of the available FD&C dyes and a toothpick. Aim for spots that are about 1-2 mm in diameter. The smaller spots are better! When you are comfortable with your spotting technique, you may continue.
 - d. Make sure you do this BEFORE forming the cylinder below! On your large piece of filter paper (with the line and X's on it) you will be spotting a different standard dye onto each X, using a different toothpick for each dye. You will want to write in pencil under the X which dye is spotted there.
 - e. Shape the paper into a cylinder so that the X's with the dye spots facing out. Place 2 staples to secure the cylinder shape, but DO NOT overlap the end of the paper. Leave a small gap so the edges do not quite meet (Figure 2).

Figure 2: Stapled paper forming a cylinder. Notice that the staples leave a small gap between the ends of the paper.



- f. Place the filter paper in the beaker with your mobile phase in it. Your spots need to be above the solvent level. If they look like they will be below the level, empty some of your solvent out. Be careful to not let the paper touch the sides of the beaker. Replace the saran wrap and secure it with a rubber band so the solvent doesn't evaporate out.

SEPARATION OF DYES BY PAPER CHROMATOGRAPHY PRE-LAB

Name: _____

1. What is our stationary phase in this week's lab? _____
2. What is our mobile phase in this week's lab? _____
3. What is an example of a solution that you consume? _____
4. What is an example of a non-edible solution? _____
5. What is the solute in salt water? _____
6. What is the solvent in sugar water? _____
7. Look around your kitchen to find to find 5 products that have FD&C dyes in them. List the products below and which dyes are present in them.

Food product:	Dyes present:

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8. Look in your bathroom and find 5 products with FC&C dyes in them. Write down the name of the product and which dyes are present.

Product:	Dyes present:

SEPARATION OF DYES BY PAPER CHROMATOGRAPHY DATA SHEET

Name: _____

Partner: _____

*** Show all your calculations for full credit***

I. Chromatography of FD&C dyes

1. Distance mobile phase moved _____mm

2. Distance Red #3 moved _____mm

3. Red #3 R_f _____

4. Distance Red #40 moved _____mm

5. Red #40 R_f _____

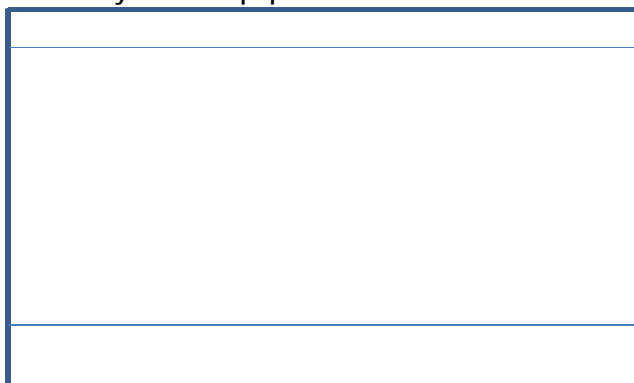
6. Distance Yellow # 5 moved _____mm

7. Yellow #5 R_f _____

8. Distance Blue #1 moved _____mm

9. Blue #1 R_f _____

10. Draw an illustration of your filter paper



11. Food coloring: Calculate the R_f values for the different spots in the food dyes, and use them to determine the FD&C dyes present. Which dyes are present by a visual inspection?

Distance mobile phase moved _____mm

Green food coloring

Distance spots moved:	
Green R_f :	
FD&C dyes present by R_f :	
FD&C dyes present by sight:	

Yellow food coloring

Distance spots moved:	
Yellow R_f :	

FD&C dyes present by R_f :	
FD&C dyes present by sight:	

Red food coloring

Distance spots moved:	
Red R_f :	
FD&C dyes present by R_f :	
FD&C dyes present by sight:	

Blue food coloring

Distance spots moved:	
Blue R_f :	
FD&C dyes present by R_f :	
FD&C dyes present by sight:	

12. Draw and illustration of your filter paper



13. Kool-Aid Pigments: Calculate the R_f values for the different spots in the 3 Kool-Aid flavors and identify the FD&C dyes present. Which dyes are present by a visual inspection?

Distance mobile phase moved _____mm

14. Using the information from the standards you tested in (I) above, identify the spots that separated out from the different Kool-Aid flavors.

Strawberry

Distance spots moved:	
Strawberry R_f :	
FD&C dyes present by R_f :	
FD&C dyes present by sight:	

Grape

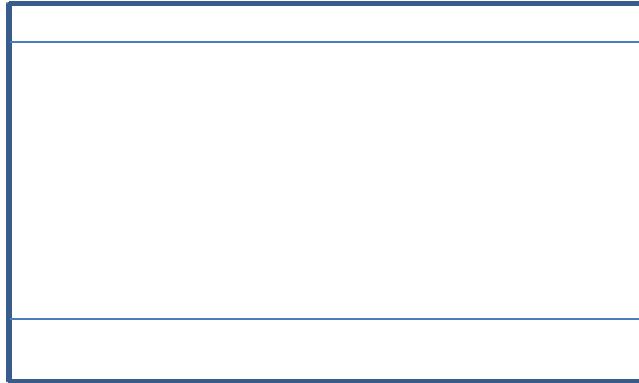
Distance spots moved:	
Grape R_f :	

FD&C dyes present by R_f :	
FD&C dyes present by sight:	

Lemon-lime

Distance spots moved:	
Lemon-lime R_f :	
FD&C dyes present by R_f :	
FD&C dyes present by sight:	

15. Draw an illustration of your filter paper



Post-lab questions:

16. Which of your results based off your R_f values were contradictory to your visual results?

17. How do you think you could get a better result for those that were contradictory?

18. There are people who prefer not to use artificial colors in their daily life. How do you feel about the use of artificial colorings in your food? What are the pros and cons to using artificial colors? _____
