Chromatography is a process of separating small quantities of a substance in a mixture (often a solution) through selective absorption. For example, the components of solutions of metals, dyes, blood, urine, and antibiotics are separated effectively by chromatography. Once separated, the components can be identified. The process is fast, simple, and generally yields good results.

In conducting a separation, a small amount of the mixture is placed on a strip of adsorbent paper. The solvent, as the carrier, is allowed to pass through the substance. As the carrier passes through the mixture, those particles held loosely on the adsorbent will be picked up by the solvent and be moved away from the point of application. Colors or color bands will appear if the mixture was colored. The paper can also be treated with certain chemicals that will produce characteristic color bands on the adsorbent.

The ratio or quotient R_f compares the average distance traveled by an ion in solution to the distance traveled by the solvent. R_f is calculated for each ion by dividing the average of the distance traveled by each ion or substance, D_s by the distance traveled by the solvent, D_f .

$$R_{\rm f} = \underline{D}_{\underline{s}} \\ D_{\rm f}$$

In this experiment, you will separate various substances found in dye by the proper use of paper chromatography. You will also calculate R_f values for each substance separated and compare ratios to determine if two samples are from the same mixture.

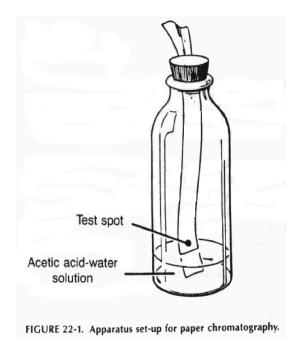
Equipment:

filter paper or chromatography paper (2.5 cm x 20 cm) collecting bottle or Erlenmeyer flask (250 cm³) stopper for bottle or flask stirring rod or pipet metric ruler food coloring (red and blue) Black Cherry Kool-Aid spot plate graduated cylinder toothpicks or micropipette tap water pencil (regular lead)

Procedure:

- 1. Obtain some filter paper or chromatography paper and cut three strips about fifteen centimeters long and 2.5 cm wide.
- 2. Place 20 cm³ of tap water into each of three 250 cm³ collecting bottles or Erlenmeyer flasks, stopper, and let stand for 3-5 minutes.
- 3. Use a toothpick to place a small spot of food coloring (red on one, blue on another, Kool-Aid on the third) about three centimeters from the end of each strip of paper. The dots must be small enough to allow the dyes to separate. Let them dry. Mark the center of the spot on the edge of the paper with a regular pencil. This is the origin.
- 4. Place the paper (spot down) into a bottle (see Figure 22-1), allowing the paper to touch the solution, but not

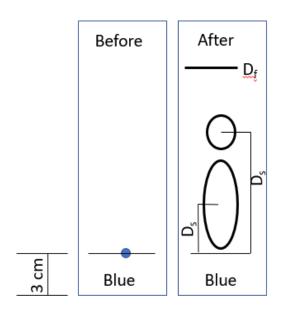
the bottom of the bottle. *Do not let the spot touch the solution*. Carefully reinsert the stopper in the bottle to hold the paper in place.



- 5. Observe the color bands as they travel up the chromatography paper and when the solution has nearly, but not completely reached the stopper, remove the paper and immediately place a mark on the edge of the paper that indicates the farthest distance the solvent traveled.
- 6. Allow the paper to air dry.

Data and Analysis:

- 1. In order to determine the average distance traveled in millimeters for each strip of color, find the middle point of each color band by marking the edge closest to the origin (start location) and the edge farthest (end location) from the origin. Measure to a point halfway between these two marks and make an average distance mark.
- 2. In order to determine the D_s for each color band, measure from the center of the starting spot (origin) to the center of each colored band marked in step 1.
- 3. Determine D_f for each substance by measuring the total distance traveled by the solvent from the center of the starting spot (origin) to the solvent end mark.
- 4. The data table should contain the name of the substance being separated, the color bands, the D_s values for each color band and the D_f value for the solvent.
- 5. In the graph section, the lab report should include a *sketch* or *actual strip* of each of the chromatography papers with the color bands labeled with the color names, D_s and D_f values. There should also be an accompanying paragraph for your chromatograph.
- 6. In the calculations section of the lab report, determine the R_f value for each color band by dividing D_s (average distance traveled by each color) by D_f (the total distance from the spot to solvent end mark). There should be at least six (6) Rf values for this experiment as there are at least 6 total color bands.



7. Determine the percent discrepancy between the expected R_f value in food coloring and the achieved R_f value in Kool-Aide for each identified dye. There will be a percent discrepancy for every color band in the Kool-Aid.

Conclusions:

- 1. Why do color bands appear at different positions on the chromatography paper?
- 2. Which of the dyes has the greatest attraction for the paper? the solvent?
- 3. Why is it necessary to have a saturated atmosphere in each bottle before inserting the substance?
- 3. How do the three papers compare? Do they share any colors in common?
- 4. Can you identify the names of the dyes used in Black Cherry Kool-Aid?
- 5. Explain how R_f values could be used to determine whether two samples are from the same mixture. Why can't you use D_s values to compare two color bands?

Resources:

| Product and Color Blue | FD and C Dye Names |
|-------------------------------|------------------------------|
| Durkee | Blue #1 |
| Schilling McCormick | Blue #1 Red #40 |
| Green Durkee | Blue #1 Yellow #5 |
| Schilling McCormick | Blue #1 Yellow #5 |
| Red Durkee | Blue #1 Red #3 Red #40 |
| Schilling McCormick | Red #3 Red #40 |
| Yellow Durkee | Yellow #5 |
| Schilling McCormick | Red #40 Yellow #5 |