Experiment 3 - Paper Chromatography: A Technique of Separation and Identification

One of the problems encountered most frequently in chemistry is that of separating a mixture into the pure substances which compose it. Most natural materials, such as seawater, air, oil, coal, and so on are mixtures. In order to study these materials chemically, we must first treat the mixture in some way to separate it into single, pure substances. (A "pure substance" consists entirely of just one thing; meaning one kind of molecule.) Because it does consist of just one thing, it always behaves in the same way when tested. For example, pure water always boils at 100°C (212°F), provided that the test is done when atmospheric pressure is normal.

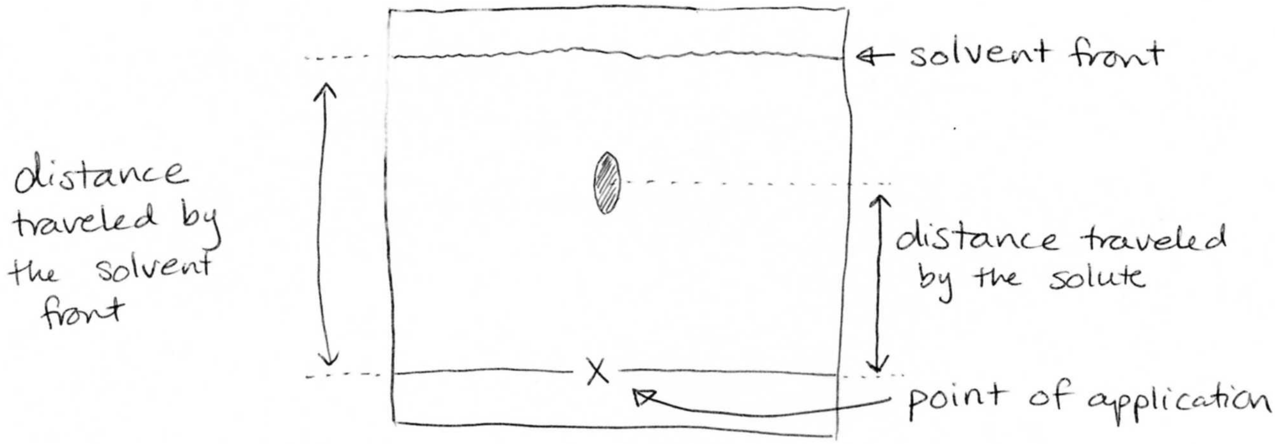
Many different methods have been devised for separating mixtures into their components. In the present experiment, we will use a method called paper chromatography. It is used quite widely for making small-scale separations and identifications. The method works because of the differences in the ways various components of a mixture are absorbed and redissolved from a piece of paper. First, the mixture that is to be separated is dissolved (if it is not already in liquid form.) Then, a small drop of the solution is applied to a piece of special chromatography paper, which is a porous paper similar to filter paper. This drop makes a tiny spot on the paper. Many such spots (of different materials) may be placed side by side on the same piece of paper. Next, the spotted paper is made to stand in a small amount of some special solvent (liquid), in such a way that only the bottom edge of the paper is submerged in the liquid, not the spots. The solvent then slowly rises up the paper, reaches the spots, and begins to dissolve them and carry the substances up the paper with it.

The key to the separation is that the different components of the mixture in each spot will rise at different rates, and so will be found (after a few minutes) to have reached different heights on the paper. This happens because some components are more strongly attracted to the paper (and so move more slowly), while others are more strongly attracted to the solvent. Any particular substance always moves at the same rate, no matter what else it may have been mixed with originally. For that reason, it can always be recognized and identified, because it will always rise to the same height, relative to the heights that the other components rise.

The movement of any spot on the paper can be quantified by calculating its R_f (*retention factor*) value after you have stopped the experiment and the paper has dried.

$$R_f = \frac{\text{distance traveled by the solute}}{\text{distance traveled by the solvent front}}$$

The distances used in calculating R_f values are measured as shown in the following figure. To determine the *distance traveled by the solute*, measure from the point at which you originally applied the spot to the center or densest part of the spot. The *distance traveled by the solvent front* is measured from the original point of application of the spot to the limit of movement of the solvent front (which must be marked immediately after the paper is removed from the beaker, because it may be nearly invisible after the solvent evaporates).



If all conditions could be maintained constant, R_f values would be constant. However, either variations in temperature or in the composition of the solvent phase or changes in the paper can alter the R_f value. The R_f value is useful mainly for expressing the relative mobility of two or more solutes in a particular chromatographic system. The absolute R_f values may change from day to day, but their values in relation to each other remain nearly constant.

In this experiment, you will test ordinary food colors, as used in cooking and baking, to find out:
(1) which colors are mixtures and which are single substances
(2) whether different manufacturers use any of the same substances in their food colors.

Follow the procedure below, testing 3 colors of one manufacturer and the same 3 colors of a second manufacturer.

Safety Precautions:
- Wear your safety goggles. The solvent used in this lab may give off fumes, so keep the beaker containing solvent covered with plastic wrap when it is not in the hood.

Waste Disposal:
- The solvent used should be disposed of in the **organic waste** bottle (which has a pink label) in the hood. The chromatography paper should be dried and attached to your report.

Procedure

1. Obtain a rectangular piece of chromatography paper. Use gloves to handle it. If gloves are not available, touch it only at the edges. (Oils from your hands can affect the separation.)
2. Make sure that the chromatography paper will fit into the beaker you plan to use when it is rolled into a cylinder. Make sure that the paper will not touch the sides of the beaker. If the paper is too large, cut it down to a smaller size.
3. Use a pencil (not a pen) to make a straight line all the way across the long way of the paper, at a distance of about one centimeter above the bottom edge.

4. You will be testing three colors of one manufacturer and the same three colors of a different manufacturer. (There will be a total of 6 spots.) For each material that you test, make a small, light circle in pencil on the line, spacing the circles no closer than 2 cm apart. Next to each circle put an identifying letter or code so that you will know what the spot contained.
5. Apply a tiny drop of each liquid to be tested to the appropriate spot on the pencil line. This can be done by repeatedly dipping a toothpick into the liquid and then touching it to the paper. The darker and smaller your spot is, the better results you will get. (You may wish to practice one spot first on a scrap of paper towel before spotting your chromatography paper. The chromatography paper is expensive, so please don't waste it.)
6. Roll the spotted paper into a cylinder with the spots on the outside and staple the ends together. Use two staples, placing them about one-third of the way from the upper and lower edges. Staple the paper so that the ends of the paper do not touch each other, because if they do, it will affect the flow of the solvent at that point.
7. Add a few milliliters of solvent to a clean, dry beaker. (A 400-mL beaker is a good size for this experiment.) Check the beaker size against your paper cylinder size first, before adding solvent. Use just enough solvent so that it will wet the bottom edge of the cylinder of paper, but will not be deep enough to touch the spots.
8. Carefully stand the paper cylinder in the beaker that has the solvent in it. Make sure that the paper does not touch the sides of the beaker. Remember that the spots must not be immersed in the solvent. Cover the beaker with plastic wrap or aluminum foil.
9. Let the beaker stand undisturbed. Do not pick it up and walk around with it, because that will cause sloshing. Watch the solvent rising up the paper (it looks wet where solvent is), and watch the original spots spreading out into different-colored components.
10. A chromatogram is finished when the separations are complete. However, since separations are not always perfect, you should stop yours when the solvent has risen about three-fourths of the way toward the top of the paper, even if the results are not absolutely perfect. (Sometimes it is necessary, in actual scientific research, to try a different solvent or use a larger piece of paper.)
11. Stop the chromatography by lifting the paper out of the beaker. Immediately mark (with a pencil) the height reached by the solvent. (This is called the solvent front, and is useful as a reference point.) Dispose of the solvent in the ORGANIC WASTE container in one of the fume hoods.
12. Let the paper dry. You may use a hair dryer if you wish to speed up the process.

Calculations and Questions

1. For each spot, measure the distance traveled by the spot and the distance traveled by the solvent front.
2. Calculate R_f values for each of the spots.
3. Which food colors consist of mixtures? Which are pure substances?

4. Compare the same colors of different manufacturers. Which ones appear to be the same? Which appear to be different?

Additional Questions:

1. Why use a pencil and not a pen to mark where to put the food coloring spots?

2. Why can't the chromatography paper touch the sides of the beaker?