Almost all substances we come into contact with on a daily basis are impure; that is, they are mixtures. Similarly, compounds synthesized in the chemical laboratory are rarely produced pure. As a result, a major focus of research in chemistry is designing methods of separating and identifying components of mixtures.

Many separation methods rely on physical differences between the components of a mixture. For example, filtration takes advantage of substances being present in different states (solid vs. liquid); centrifugation relies on differences in density; and distillation makes use of differences in boiling points of the various components. Chromatography exploits differences in solubility and adsorption.

The word *chromatography*, which is derived from two Greek words literally meaning "color writing", was coined at the beginning of this century when the method was first used to separate colored components of plant leaves. Today, the name is a bit misleading, because most forms of chromatography do not depend on color. Several types of chromatography are commonly used, among which are paper chromatography, thin-layer chromatography or TLC, liquid-liquid chromatography, gas chromatography, and high performance liquid chromatography or HPLC.

Chromatography is so useful that some form can be found in most scientific laboratories around the world. For example, in forensic chemistry crime laboratories, the FBI maintains a library of chromatograms of inks that are used commercially. In the first case in which chromatography of inks were used, a man in Miami falsified travel and expense vouchers. However, the ink pen he used had ink that wasn't available commercially until 3 years after the trips had taken place.

The theory behind chromatography is to allow a mixture of different chemicals to be distributed or partitioned between a *stationary phase* and a *mobile phase* (eluent or solvent). The mobile phase may be a liquid or a gas; the stationary phase is typically a solid. As the mobile phase flows over the stationary phase, the components in the mixture are carried along. The more soluble a component is in the mobile phase the faster it will be transported along the stationary phase. Adsorption refers to the ability of a substance to ‘stick’ (or be adsorbed) to a surface. The more strongly a component is adsorbed to the stationary phase, the slower it will be transported by the mobile phase. As the mixture moves over the stationary phase, the components in the mixture move further and further apart into discrete zones.

**Paper chromatography** uses ordinary filter paper (primarily cellulose) as the stationary phase. **Thin-layer chromatography** (abbreviated TLC) uses a thin glass plate coated with either aluminum oxide (alumina) or silica gel as the solid phase. The mobile phase in both is a solvent chosen according to the properties of the components in the mixture.

In paper chromatography, a drop of solution containing a substance or mixture of substances is spotted along a line near one end of a rectangular piece of filter paper. The paper is the stationary phase and the line is called the origin. The lower edge of the paper is placed in a developing solvent as the mobile phase. Capillary action causes the solvent to flow up the paper at a uniform rate creating a "wet" line across the paper. This line is called the solvent front.
When the solvent front reaches a spot, the components of the spot will begin to migrate upward with the mobile phase. Each component will have a characteristic chemical affinity for the paper and a characteristic chemical affinity for the solvent. These affinities are competitive: The component's affinity for the paper tends to hold the component in one place, but its affinity for the solvent tends to make the component follow the solvent as it moves upward. A component with a strong affinity for the paper and a weak affinity for the solvent will move more slowly than a component with a weaker affinity for the paper and a stronger affinity for the solvent. TLC works in similar manner.

The affinity of a substance for the stationary and mobile phases is characteristic of that substance. Different substances will have different competitive affinities. Since each component of a mixture will have its own characteristic affinities, each component will travel up the paper at its own characteristic rate. If the paper is sufficiently large, all the components can be separated by the time the solvent front has reached the top of the paper and each component will appear as a separate spot. The chromatographic paper will now contain a vertical array of colored spots arranged according to their characteristic rates of ascent.

It is possible to describe the position of spots (so the substances that have separated) in terms of their retention factor, the \( R_f \) value (Figure 1). The retention factor is defined as:

\[
R_f = \frac{\text{distance traveled by spot}}{\text{distance traveled by solvent}}
\]

Because the retention factor for a particular mixture varies depending on conditions, a sample of known composition is typically analyzed at the same time on the same sheet of paper or slide. The \( R_f \) value is a characteristic property of a given compound in a given solvent on a particular stationary phase.

**Figure 1**: Example for calculating the \( R_f \) value

\[
\begin{align*}
R_f \text{ (yellow)} &= \frac{5.8 \text{ cm}}{8.5 \text{ cm}} = 0.72 \\
R_f \text{ (cyan)} &= \frac{3.1 \text{ cm}}{8.5 \text{ cm}} = 0.36
\end{align*}
\]

In Part I of this experiment, the dye content of the shell coating of M&M candies will be analyzed using paper chromatography and TLC. The colors of the shells of M & M candies contain food coloring dye that are FDA approved (5 commonly used of the 7 such dyes) organic compounds and therefore display characteristic \( R_f \) values. Some M & M’s use yellow #5 (tartrazine) as color. This dye causes allergic reactions in some people and must therefore be
named as an ingredient in products that contain it. The M&M package states that yellow #5 is one of the colors added. The big question is then: is the dye present in all candies or only in some of the candies of certain colors? The answer to this question is important to the person who is allergic to yellow #5 and wants to know which candies are safe to eat.

In Part II, overhead pen inks will also be analyzed using TLC. Overhead pens come in many different colors but most colors are mixtures of primary color inks.

**Procedure**

**Part I M&M candies**

1. Select the M&M colors to be tested. Using a disposable pipet, place a small drop of distilled water on top of the candy and let it sit for about 10 minutes. This will give a drop of fairly concentrated dye solution.
2. For the paper chromatography, prepare a developing chamber using a 1-L beaker. Add enough 0.1% NaCl, (solvent or eluting solution) so that the bottom of the beaker is covered by a ½ inch. Tightly seal the beaker with aluminum foil. It is important that the air above the mobile phase become saturated with solvent vapors. Prepare an additional chamber for TLC using a 100-mL beaker and Parafilm. Fill with 0.1% NaCl solution about ¼ inch deep.

3. Cut two 12 x 12 cm strips from Whatman #1 filter paper as shown by the instructor. Along one side, 1 inch from the edge, draw a straight pencil line, and make marks at about 1 cm intervals.

4. Dip a round toothpick in water and use it to gently stir the drop on top of one of the M&M prepared in step 1 (held in one hand). Care must be taken not to dissolve away so much of the shell that the chocolate is exposed. Apply the extract to a spot on the pencil line on the paper. Air dry the spot by blowing gently over the paper. Reapply more extract to the same position at least 4 times until the color is clearly visible, but the spot is not too large. Label under the spot with code representing the color M&M used. Repeat the application using a clean toothpick with each different color of M&M. Spot the standard dye solutions in a similar fashion.

5. Fold the filter paper in an accordion fold 3 times, making sure the spots are in a section and not on the crease. Carefully stand the paper in the developing chamber eluting solution, with the sample spots near the liquid surface, but not in the liquid (Figure 2). Tightly seal the beaker with the parafilm and let it stand undisturbed. The solvent will gradually rise by capillary action, carrying the components in the spots along.

6. Take the paper from the beaker when the solvent front is about 2 cm from the top and immediately mark the solvent front and let the paper dry. Note the position of the most intense part of the separated colors and carefully circle it.

7. Measure the distance from the straight line on which you applied the spots to the solvent front. Then measure the distance from the pencil line to the center of each colored spot and calculate the $R_f$ value from the two numbers you obtained. Repeat the procedure for each colored candy spot that you applied.
8. Repeat the procedure using TLC plates. Note the plates are a plastic sheet coated with silica gel (hydrated SiO₂). Be sure to spot the silica gel (not shiny) side. Calculate R_f values as before.

Part II  Felt tip pens

9. Use TLC plates for the red, blue, green and black overhead marking pens. One plate holding all four evenly spaced spots is sufficient.

10. The spots must be very small and not too concentrated to get a good separation. To do this, take a round toothpick and dip it in distilled water. Touch the moistened end to the tip of the marking pen. Dip the toothpick in distilled water again, touch to the marking pen tip and immediately spot on the line of a TLC plate just once. Repeat for each color.

11. Place in the developing chamber and seal with Parafilm. Allow the solvent front to move close to the top of the plate.

12. There are no standards to compare against. Identify the number of dyes, colors and relative positions. In particular, note which dyes in each color pen move the same distance.

Questions

1. Explain in your own words why samples can be separated into their components by chromatography.

2. Why must the spot applied to a chromatography plate be above the level of the developing solvent?

3. If the solvent front moves 8.0 cm and a component in a sample being analyzed moves 3.2 cm from the baseline, what is the R_f value?

Data Treatment

1. Calculate the R_f value for each color dye in M&Ms with the filter paper stationary phase.

2. Calculate the R_f value for each color dye in M&Ms using TLC (silica gel).

3. Identify the dye makeup of each overhead pen color using your own color and number code.

4. What is the result of applying too much sample?

5. Two components have the same R_f value in a chromatogram. Are they necessarily identical in structure?