Abstract

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 in a pure state. They are almost always produced with impurities including reaction byproducts and leftover reactants. As a result, a major focus of research in chemistry is designing methods of separating and identifying the various components of mixtures.

Many of these separation methods rely on physical differences between the components of a mixture. Undoubtedly, you are already familiar with several means chemists use to effect separations based on physical differences. These techniques include: Filtration, where separation may be effected because substances are present in different states (solid vs. liquid); Centrifugation, where separation is effected by differences in density; and Distillation, where separation is effected by taking advantage of differences in boiling temperatures of the various components. In this laboratory exercise, we will effect a separation of a mixture of food dyes using paper chromatography.

All chromatography techniques have three important components: the analyte or mixture of species being separated, a mobile phase, and a stationary phase. The mobile phase is a flowing liquid or gas used to push the analyte over or through a stationary porous material (the stationary phase). Because of physical interactions between the analyte and the stationary phase, the analyte moves through or over the stationary phase more slowly than the mobile phase. Furthermore, because physical interactions between the analyte and the stationary phase can be different for each component of the mixture, the different components transit the stationary phase at different speeds. Those that strongly interact with the stationary phase lag behind those that interact only weakly. As a result, the components of the mixture may be separated.

Figure 1 shows the separation of a two-component mixture using a typical liquid column chromatography apparatus. The analyte, dissolved in a liquid, is injected into a flowing mobile phase continuously pumped through the apparatus. The mobile phase sweeps the mixture into a chromatography column containing the stationary phase. As the two components are pushed through the column by the mobile phase, one interacts with the stationary phase more strongly and lags behind. As a result, the two components come out or elute from the column at different times allowing them to be collected in separate vials.

There are several different types of physical interactions that can occur between the analyte and the stationary phase which slow the progress of the analyte across the stationary phase. Under the right experimental conditions, each component of a mixture will exhibit a unique binding interaction with the stationary phase and, therefore, elute from the chromatography column at a unique time.
Paper chromatography is a form of liquid chromatography using a piece of paper as the stationary phase rather than a packed chromatography column. In paper chromatography, analyte is applied directly onto the bottom of the stationary phase (the chromatography paper) which is then placed on edge in a developing tank containing the mobile phase so that the bottom edge of the paper is submersed. Capillary action draws the mobile phase up the sheet of paper carrying along the different components of the mixture. Due to physical interactions between the different components and the stationary phase, the components move up the paper at different speeds. The paper is removed from the tank before the solvent front reaches the top of the paper and the position of each component of the mixture is marked as well as the position of the solvent front itself. Unlike in liquid column chromatography, the components of the mixture are not allowed to elute off of the top of the stationary phase. Rather, each component is characterized by the distance it traveled up the paper. The ratio of this distance to the distance the solvent front traveled, denoted $R_f$, is characteristic of a particular substance and remains constant regardless of the other components present in the mixture and, therefore, can be used to qualitatively identify the substance. The $R_f$ of each component is dependent on the type of stationary phase used and the composition of the mobile phase.

Figure 1 - Liquid Column Chromatography. A two component mixture is injected into the flowing mobile phase and swept into a chromatography column containing the stationary phase. Because the ● molecules interact more strongly with the stationary phase than the ○ component, they lag behind. As more mobile phase is pumped through the column, the two components are eventually separated and removed from the column.
Identification of the components of a mixture is aided if each substance in the mixture exhibits a unique color (as with most food dyes). However, for dyes with similar colors or for substances exhibiting no color, identification may be aided by examining the fluorescence exhibited under illumination with an ultraviolet (UV) lamp. Fluorescence is the phenomenon in which molecules absorb and immediately re-emit light. The emitted light is usually a different color than the absorbed light due to loss of energy between the absorption and emission events. Some substances re-emit absorbed radiation on longer time scales, seconds or minutes after the absorption event. Such substances are said to be phosphorescent. As with fluorescence, phosphorescence can be used to identify substances appearing similar under normal lighting.

In this laboratory, you will use liquid chromatography to determine $R_f$ values of several common food dyes; you will then run a second chromatogram to separate dyes used in the colorful coatings of M&M candies. By comparing $R_f$ values, color, and fluorescence characteristics of the dyes you will identify the individual dyes used in the mixtures found in the M&M coatings.

**Purpose**

To use a physical separation technique to separate and identify the food dyes used to give M&M’s their color.

**Materials**

- Drying oven at 100°C
- Hand-held ultraviolet lamp with long and short wavelength bulbs
- 2 pieces of 10.2 cm x 13.3 cm Whatman cellulose chromatography paper
- 20 mL of 0.10% wt/v solution of NaCl in H$_2$O
- 6 mL of 50/50 water/ethanol solution
- 0.5% wt/v solution of each of the four standard dyes
- 1 beaker, 400 mL
- 2 beakers, 50 mL
- 5 plain M&M’s of each color
- Parafilm
- Pencil
- Ruler
- 2 Micropipets
- Stapler

<table>
<thead>
<tr>
<th>F,D&amp;C Dye</th>
<th>Common Name</th>
<th>CAS number</th>
</tr>
</thead>
<tbody>
<tr>
<td>F,D&amp;C Blue No. 2</td>
<td>Indigo carmine</td>
<td>860-22-0</td>
</tr>
<tr>
<td>F,D&amp;C Green No. 3</td>
<td>Fast green FCF</td>
<td>2353-45-9</td>
</tr>
<tr>
<td>F,D&amp;C Red No. 3</td>
<td>Erythrosine</td>
<td>16423-68-0</td>
</tr>
<tr>
<td>F,D&amp;C Yellow No. 5</td>
<td>Tartrazine</td>
<td>1934-21-0</td>
</tr>
</tbody>
</table>
**Procedure**

**A. Characterization of Standard Food Dyes.**

1. Prepare a developing tank by pouring 20 mL of the mobile phase (0.10% wt/v solution of NaCl in H$_2$O) into a 400 mL beaker and covering the beaker with Parafilm. It is important that the air above the mobile phase become saturated with solvent vapor so that solvent does not evaporate from the stationary phase as the chromatogram develops. Therefore, be sure to keep the developing tank covered at all times.

2. With a **pencil**, draw a horizontal line 1.5 cm from the bottom edge of the chromatography paper. Draw vertical tick marks along this line every 2 cm (see fig 2(a)).

3. Using a capillary and one of the standard dye solutions, make a spot on the chromatography paper at one of the marks. Try to keep your spots less than 4 mm in diameter. Allow the dye to dry and reapply the same dye in the same spot 1 or 2 times or until a sufficiently dark spot has been achieved. With a pencil, note the name of the dye below the spot.

4. Repeat step 3 for the remaining dyes across the bottom of the chromatogram.

5. When the spots have been applied, put your name in pencil across the top of the chromatography paper, form the chromatography paper into a cylinder, and staple the edges of the paper together making sure to leave a gap between the edges as shown in fig 2(b). If the edges come into contact, solvent will not travel at a uniform speed up the chromatography paper and the components of the mixture will not move in a straight line.

6. Place the chromatography paper into the developing tank, do not let it touch the sides of the tank and quickly replace the Parafilm cover. Make sure the level of the mobile phase is below the line of dyes on your paper. Allow the chromatogram to develop.

7. When the solvent front is approximately 1 cm from the top of the chromatography paper, remove the chromatogram and lay it flat on a paper towel. **Immediately** mark the position of the solvent front with a pencil. The front will continue to move as the paper dries so it is important that you mark this position now. Measure and note the distance the solvent front traveled ($D_{\text{solvent}}$, see fig 3.).

*Figure 2* Paper Chromatogram. (a) Schematic for laying out spots. (b) Chromatogram ready for developing tank.
8. Draw an ellipse around each spot on the developed chromatogram and draw a horizontal line through the center of each spot. If a spot shows significant “tailing” make your horizontal line through the darkest part of the spot (see fig 3). Use the distance from the starting line (not the bottom of the paper!) to these horizontal lines to determine $D_{\text{dye}}$ for each dye. Record distances and $R_f$ values in your notebook. Recall:

$$R_f = \frac{D_{\text{dye}}}{D_{\text{solvent}}}$$

9. Place the chromatogram on edge, in the drying oven for 5 min or until dry. Take your dry chromatogram to the student lounge and observe the spots under illumination with a hand-held UV lamp on long-wavelength irradiation. Note the color of the observed fluorescence.

WARNING: UV light can damage your eyes. Always point lamps away from you. Do not look into the UV lamps

B. Separation and Identification of Dyes Used to Coat M&M Candies.

1. Do the M&M candies contain any of the standard dyes above? To begin to answer this question, take one M&M of each color to the UV lamp and see if they fluoresce. Note the color of any fluorescence you observe in your notebook.

2. To separate the dyes used to coat M&Ms, you must first prepare a solution of the coating dyes. To do this, place 5 M&M’s of the same color into a small beaker. Add 3 mL of a 50/50 mixture of water and ethanol and swirl the solvent until the candy coating has dissolved. Remove the M&M’s from the solvent before the chocolate center is exposed.

3. Prepare a second chromatogram like that in Part A using the 2 M&M solutions. The dye solutions prepared with the M&M candies are not as concentrated as the solutions of the standard dyes. You will need to spot these dye mixtures several (>5) times to obtain a sufficiently dark spot. Again, dry each spot between applications of the mixture to maintain as small and concentrated a spot as possible.
4. Develop the chromatogram. Characterize the $R_f$ value, color, and fluorescence characteristics of each dye observed. Remember that unlike the previous chromatogram, you are now separating mixtures of dyes and there may be as many as four components each with its own $R_f$ value, color and fluorescence characteristics. Use all three characteristics to identify all components found in each M&M coloring.

5. Are any of the fluorescing substances you observe on your chromatogram actually phosphorescent? Place your chromatogram on a table and place the UV lamp, on “short-wavelength” on top of your chromatogram. Allow your eyes to adjust to the dark room. Quickly draw your chromatogram out from under the UV lamp and note any substances that continue to glow once out from under the lamp. (hint: pay close attention to the solvent front!)

Figure 3 Developed Chromatogram. Immediately mark solvent front when paper is removed from the developing tank. The spot on the right exhibits significant tailing and the distance the spot has traveled has been correctly identified.
Observations

Physical Separation Of a Mixture

Characterization of Standard Dyes

Distance solvent front traveled ($D_{\text{solvent}}$) (mm):

Table 1: Characterization of Standard Dyes

<table>
<thead>
<tr>
<th>Dye</th>
<th>Color</th>
<th>Distance Dye Traveled ($D_{\text{dye}}$)(mm)</th>
<th>$R_f$</th>
<th>Fluorescence Color/Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indigo Carmine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fast Green</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrosine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tartrazine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brilliant Blue</td>
<td>Blue</td>
<td>N/A</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>Allura Red</td>
<td>Red</td>
<td>N/A</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Sunset Yellow</td>
<td>Yellow</td>
<td>N/A</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>Unidentified Orange</td>
<td>Orange</td>
<td>N/A</td>
<td>0.35</td>
<td></td>
</tr>
</tbody>
</table>
Characterization of Dyes Used to Coat M&M Candies

Distance solvent front traveled ($D_{\text{solvent}}$, mm): ____________

Beside each M&M color note the color, $D_{\text{dye}}$, $R_f$ value, and fluorescence color / intensity for each spot observed. If possible, identify each spot as one of the standard dyes above. (Most mixtures will not have four components.)

Before identifying dyes based on $R_f$ values it is useful to have a good idea what the relative error is in your determination of $R_f$ values. Using what you know about how difficult it is to identify the center of a dye spot, estimate the error in your determination of $R_f$ values.

*Table 2: Characterization of Dyes Used to Coat M&M Candies*

<table>
<thead>
<tr>
<th>M&amp;M Color</th>
<th>Dye Color</th>
<th>$D_{\text{dye}}$ (mm)</th>
<th>$R_f$</th>
<th>Fluorescence Color/Intensity</th>
<th>F, D&amp;C Food Dye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green</td>
<td></td>
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</tbody>
</table>
Questions
1. In chromatography what do the mobile and stationary phases do?
2. a. What is the mobile phase in this experiment?
   b. What is the stationary phase in this experiment?
   c. Why was a pencil used to mark the paper rather than a pen or marker?
3. Why is it important for the initial dye “spots” to be small?
4. Define fluorescence.
5. Define phosphorescence.
6. In chemistry or other sciences, what is chromatography used for?
7. What is the possible range of values for $R_f$ (Lowest possible to highest possible)?

Conclusion
1. Which dyes are present in the green M&M’s? The brown M&M’s?