Advanced

Lab 10 Separation by Paper Chromatography

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Learning Objectives

- Interpret the results from a paper chromatography separation experiment
- Determine the solvent which will most effectively separate the analytes
- Calculate the R_f for colored dyes found in candy-coated chocolates

Introduction

How do green M&Ms get their color? Is the dye a mixture of blue and yellow dyes, or is it a pure green dye? What dye colors are used to make an orange M&M? These are questions that can be answered through the use of a chemical technique called chromatography.

Figure 1: M&Ms were developed in 1941. The red, green, and yellow colors were added in 1960, orange was added in 1976, and blue was added in 1995.

Chromatography

Chromatography refers to a broad range of techniques used to separate or identify individual analytes (components) within a complex mixture. In each case, the mixture is added to an eluting solvent, and the analytes within the mixture migrate along an adsorptive material at different rates. The physical and chemical properties of each analyte affect the rate of migration. These different rates, consequently, separate the analytes.

Chromatography can be applied to any chemical or bioprocessing industry. In these industries, the need to separate or purify a substance is significant. One industry in which substance purification is essential is forensics, where chromatography is used to identify or isolate biological substances and develop evidence. It is also used to isolate proteins in many biotechnology industries, such as pharmaceuticals or drug synthesis (Figure 2). The

petrochemical industry also uses chromatography to determine fuel or fuel additive purity. Chromatography is further used in environmental arenas to isolate trace pesticides or detrimental chemicals from ground water. Chemists often use paper chromatography to separate colored or dyed mixtures. The separated chemicals can then be used in more advanced chemical preparations or simply identified.

Figure 2: One type of chromatography used in laboratories is gas chromatography (GC). This is a picture of an GC autosampler.

Mobile Phase and Stationary Phase

Chromatography is based on two phases: the mobile phase and the stationary phase. The **mobile phase** (eluting solvent) is the phase that moves up the chromatography paper. The mixture of analytes is placed in the mobile phase.

The **stationary phase** is the material held in place for the chromatography procedure. A good separation results when the components of a mixture have varying levels of affinity for the mobile and stationary phases. Think of the mobile phase as a moving stream and the stationary phase as the stream bed. If you were to toss in a leaf, a stick, and a large rock, what would happen? Each unique component would travel at different rates along the stationary phase, using the mobile phase as a vehicle. Many properties affect the affinity of a substance for the mobile or stationary phase, including polarity, solubility, particle size, and electrical charge. Chemists can use their knowledge of these properties to separate a mixture effectively.

There are some additional terms needed to completely understand a chromatography experiment. Some of these terms include:

- **Analyte:** An individual component within a mixture that is of interest in analytical chemistry. Analytes are separated out during the mobile phase. They are carried up the stationary phase in the eluting solvent (the carrier).
- **Eluent or Eluting Solvent:** The solvent which the mixture of analytes is placed in. This chemical acts as the carrier molecules within the mobile phase.
- **Immobilized Phase:** A mobile phase that has become immobilized (movement is halted) on the stationary phase.
- **Mobile Phase:** A mixture of the eluting solvent and the analyte components. This phase moves (usually up) the stationary phase. The physical and chemical properties of the analytes suspended in the mobile phase allow the analytes to separate during the mobile phase's movement.
- **Solvent Front:** The edge of the mobile phase after it has completed its migration up the stationary phase (see Figure 3 for example)
- **Stationary Phase:** A fixed substance or material. Used as a foundation for the mobile phase to travel upon. Chromatography paper is the stationary phase in paper chromatography.

R_f Value

One way to compare the movement of the analyte is to calculate the retention factor value, or **R_f value**. Assuming that the same mobile and stationary phase substances are used, the R_f value for a chemical will not change. Therefore, R_f values allow for individual analyte identification within a compound. The R_f value is determined by taking the distance travelled by the analyte and divide it by the distance travelled by the mobile phase. This is mathematically expressed as:

$$R_f = \frac{\text{Migration Distance of Substance}}{\text{Migration Distance of Solvent Front}}$$

Where:

- **Migration Distance of Substance:** The distance the analyte travels up the stationary phase
- **Migration Distance of Solvent Front:** The total distance which the mobile phase travels up the stationary phase.

For example, suppose an analyte travels up the stationary phase 3.2 cm, but the mobile phase travels a total of 7.5 cm. Calculate the R_f value:

$$\text{R}_\text{f} = \frac{3.2\ \text{cm}}{7.5\ \text{cm}}$$

$$\text{R}_\text{f} = 0.43$$

Chromatography Factors

Different types of materials used for mobile and stationary phases lead to different types of chromatography. Some of these include paper, ion-exchange, gas, high performance liquid, column, affinity, and thin layer chromatography. In paper chromatography, the type of paper used controls how fast the mobile phase moves.

There are also factors which influence how effective an eluting solvent is at separating the analyte components. For example, salt is an ionic eluting solvent, isopropyl alcohol is a non-polar eluting solvent, and water is a polar eluting solvent. By changing the concentration of salt, you change the ionic characteristics of the solvent. By altering the concentrations of water and alcohol in the solution, you change the polarity of the solution. All of these factors affect the overall affinity and specificity of the analyte separation.

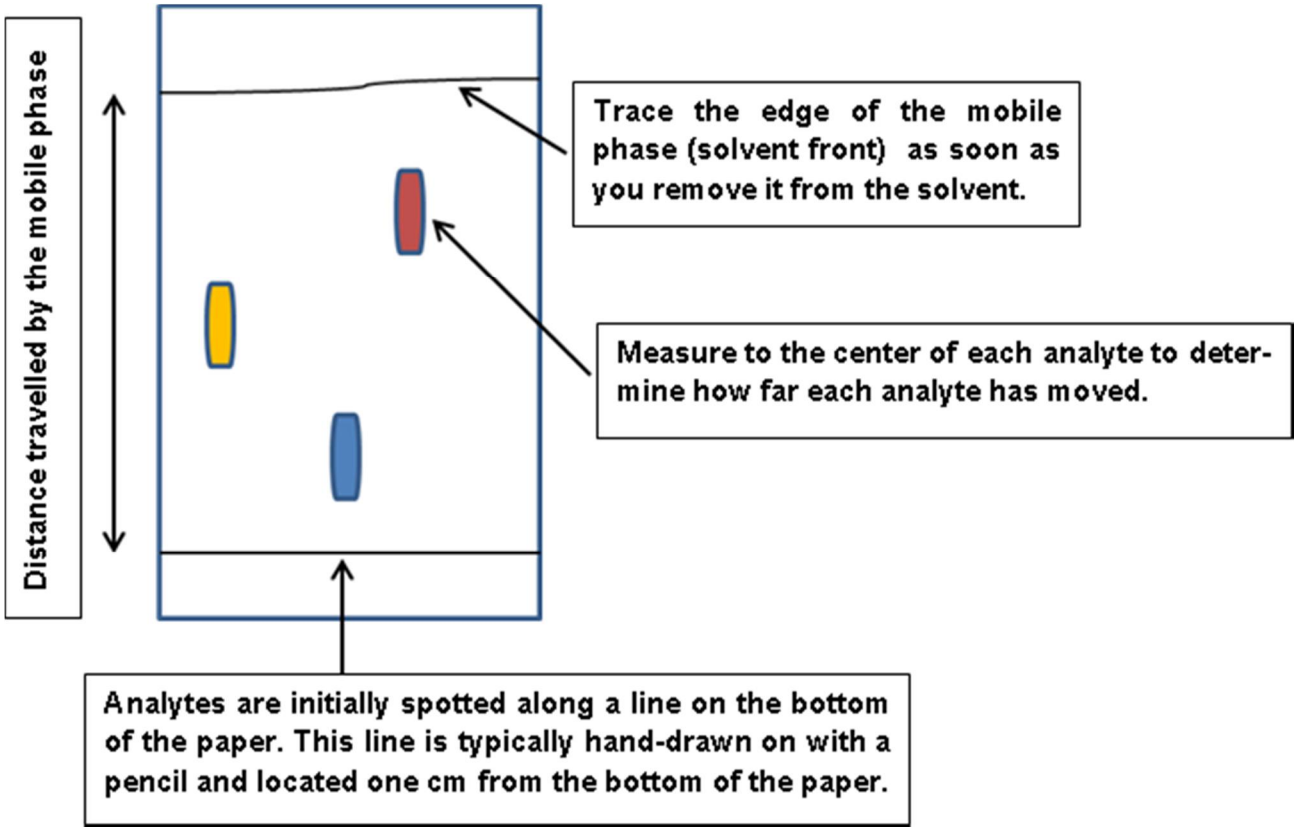

Figure 3: **Sample paper chromatography results.**

Pre-Lab Questions

1. Rank the following solutions from least polar to most polar:
    a. 50% isopropanol/H_2O
    b. 25% isopropanol/H_2O
    c. Pure water
    d. 70% isopropanol/H_2O
2. Identify the analyte, eluting solvents (mobile phases), and stationary phase used in Experiment 1.

3. Why would it be important to know which food dyes are in food?

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Experiment 1: Paper Chromatography

In this experiment, you will use paper chromatography to determine the R_f value for the dyes found in candy-coated chocolate pieces. The chromatography paper acts as the stationary phase for the procedure, and a variety of mobile phases will be tested. Multiple tests with different eluting solvents must be run to determine the best eluting solvent to separate the food dyes.

Materials

(3) 50 mL Beakers

500 mL Beaker

3 Capillary Tubes (packaged inside of a test tube)

(2) 11 x 11 cm Pieces of Chromatography Paper

100 mL Graduated Cylinder

*6 M&Ms® (2 blue, 2 green, and 2 red)

Ruler

3 Wooden Stir Sticks

30 mL of each of the following eluting solvents:

30 mL 0.5% Sodium Chloride, NaCl solution

30 mL 0.2% Sodium Chloride, NaCl solution

*Pencil

*50 mL Isopropyl Alcohol, C_3H_8O

*30 mL Distilled Water (eluting solvent)

*30 mL Isopropyl Alcohol, C_3H_8O

*Camera/Smart phone is Sufficient

*Computer/Internet Access

*Scissors

*Tap Water

*You must provide

*Note: The individual candies have been repackaged by eScience Labs. Do not eat .

Procedure

1. Put on your safety glasses and gloves (provided in your safety box).
2. Gather three 50 mL beakers, one for each color candy you will test.
3. Place 2 M&Ms® candies of one color into a 50 mL beaker.
4. Repeat for each color of the candy you will test. You should have two green candies in one beaker, two red candies in a second beaker, and two blue candies in the third beaker.

Part 1: Preparation of the Analyte

1. Use a pipette to add 1 mL of isopropyl alcohol to each 50 mL beaker.
2. Stir with a wooden stir stick for one minute (the colors may not appear to be very dark). Remove the candies. Be sure to use a clean stir stick each time you change beakers.
3. Allow the solutions to sit and concentrate while the stationary phase is prepared.

Part 2: Preparation of Stationary Phase

1. Cut each piece of chromatography paper in half. Direction doesn't matter, but keep it consistent for all papers.
2. Set up the chromatography paper according to Figure 3.
3. Using a pencil, mark the paper 1 cm from the bottom edge. Refer to Figure 4.

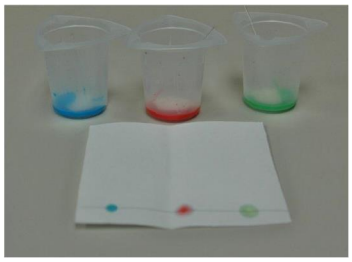

Figure 4. ***Chromatography set up.***

4. Using a capillary tube, place small spots of the analyte equal distance apart on the marked line. Since there are three colors to be tested, there will be three spots on the line (use one capillary tube per color; save the tubes for the additional trials).

   Note: Capillary tubes are extremely thin tubes. They are useful when working with very small amounts of a sample, and collect liquid samples through capillary action. To use the capillary tube, simply place the open end of the tube in the sample. The liquid molecules will be drawn into the tube and stick to the inner walls. Figure 5 provides a references for this process.

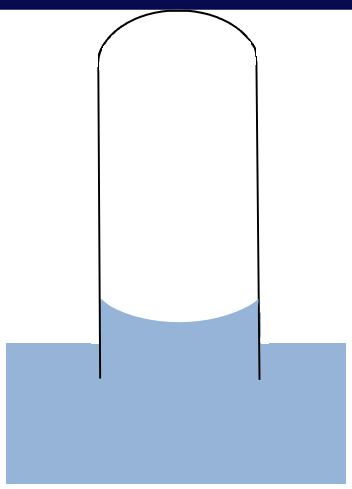

Figure 5 ***Capillary tubes use capillary action to pull up liquid.***

5. Allow the spot to dry, and re-spot the analyte in the exact same area as done in Step 9. Repeat this process at least five times, or until the colored dots appear on the paper.
6. Use a 100 mL graduated cylinder to pour 20 mL distilled water (your eluting solvent) into the 500 mL beaker.
7. Place the paper vertically with the line-side down in the 500 mL beaker with the eluting solvent. Let it stand for 3 - 5 minutes (Figure 6).

Figure 6. ***Eluting solvent set up.***

8. Use a pencil to mark the edge of the solvent front (the edge of the mobile phase) and the location of the analytes with a pencil (see Figure 3 for reference). Measure the solvent and dye fronts based on farthest location from origin line. Record your data and any additional observations in Table 1.
9. Use tap water to rinse the 500 mL beaker and then repeat steps 4-13 for the remaining eluting solvents (0.5% NaCl, 0.2% NaCl, and isopropyl alcohol, record your data in Table 1.

10. Line up the results of all your chromatography experiments and use your camera (or smart phone) to take a picture of the results. Be sure to correctly label the picture and send it to your instructor along with the answers to the lab questions.

Table 6: Paper Chromatography Data and Observations

Solvent	Distance Traveled by Mobile Phase (mm)	Distance Traveled by Each Analyte (mm)	R_f Value	Additional Observations
1: Distilled Water		**Green:** **Blue:** **Red:**	**Green:** **Blue:** **Red:**	
2: 0.5% NaCl Solution		**Green:** **Blue:** **Red:**	**Green:** **Blue:** **Red:**	
3: 0.2% NaCl Solution		**Green:** **Blue:** **Red:**	**Green:** **Blue:** **Red:**	
4: 70% Isopropyl Alcohol		**Green:** **Blue:** **Red:**	**Green:** **Blue:** **Red:**	

Post-Lab Questions

1. Which solvent provided the best separation?

2. Explain which characteristics of the solvent were used to effectively separate the analytes.

3. Some children have reactions to Yellow 5 or Yellow 6 dye. Yellow 5 is a pale yellow color and Yellow 6 is more orange. Use the colors seen on the chromatograms to determine which M&Ms® candies you tested contain Yellow 5.

4. Chromatography has many applications. Research one application of chromatography and explain how it is used and what characteristic is utilized for the separation of the analyte(s).