Introduction to Chromatography

Student Study and Analysis Sheet

Introduction

One of the main tasks of chemists and biochemists is to unravel the complexities of chemical compounds and reduce them to their simple individual components. Paper chromatography is an important technique used in the separation, isolation and identification of chemical compounds. The term chromatography is derived from the Greek word "chromat," which means color and the word "graphon" which means to write. Chromatography was originally used to separate pigments in leaves, berries and natural dyes. Over the last 50 years chromatography has been applied to the separation of a series of organic and biological compounds. Paper chromatography has had an impact in the advancement of knowledge and understanding of every field of biology or chemistry.

Chromatography separates liquid compounds into individual components based on their specific affinity for a solid surface and specific solubility for different developers. In paper chromatography, the solid surface is the cellulose fibers in the chromatography paper and the developer is the solution that's placed in the bottom of the developing chamber. The separation takes place through a process of absorption and capillary action. A minute drop of the mixture to be separated is placed at the bottom of a strip of chromatography paper, which holds the substance by absorption. The chromatography paper is then placed in a developing chamber with a developer. The paper, which acts as a wick, pulls the developer up the paper by capillary action, and dissolves the mixture as it passes over it. The components of the spotted mixture move upward at differing rates. A compound with greater solubility will travel farther than one with less solubility. The result is that the different pigments in the mixture show up as colored streaks. The separated substances on the chromatography paper form a pattern called a chromatogram.

To establish the relative rate of migration for each pigment, the R_f value for each pigment is calculated. The R_f value represents the ratio of the distance a pigment moved on the chromatogram relative to the distance the developer front moved. It is calculated using the following formula:

$$R_f = \frac{\text{Distance substances (pigments) traveled}}{\text{Distance developer traveled}}$$

Any molecule in a given developer matrix system has a uniquely consistent R_f. The R_f value is used by scientists to identify molecules.

In **Part I** of the investigation, you will use the paper chromatography method to separate the polar and nonpolar components of a dye mixture, using polar and nonpolar developers. You will use the following developers: a highly polar compound - water, a relatively nonpolar compound - isopropyl, and a mixture of isopropyl and water. In general, nonpolar substances and polar substances are insoluble in one another. Polar liquids, such as water can function as solvents for many ionic compounds. The dye mixture used in this investigation is a mixture of both polar and nonpolar compounds. The polar dyes are made up of ionic compounds which readily dissolve in water, while the nonpolar dyes do not. When water, a polar compound, is used as the developer, the polar dye components in the mixture attach themselves to the developer and separate as it moves through the paper front. The nonpolar components of the dye mixture will remain at the point of origin. When isopropyl, a relatively nonpolar compound, is used as the developer, the polar dyes which had separated using water, will remain at the point of origin and only the nonpolar dyes will attach to isopropyl and migrate through the paper front. The third developer, which is a mixture of a polar and nonpolar compounds, will produce a series of bands consistent with all of the substances contained in the mixture.

In **Part II**, you will use a specially formulated developer to separate and observe the individual plant pigments (chlorophyll a, chlorophyll b, carotenes, and xanthophylls) that give leaves their color, determine the R_f value of each pigment, and learn their function during photosynthesis. Chlorophyll, the main coloring of green plants, is a chemical pigment needed for photosynthesis. It is the most abundant and important photosynthetic plant pigment and exists in two forms - chlorophyll a and chlorophyll b. Both chorophylls absorb blue and red light and reflect green light quite well. However, chlorophyll a and b have their maximum absorption at different wavelengths of light. Chlorophyll a is the main photosynthetic pigment and its primary purpose is to convert light energy to chemical energy. The two other pigments that are found in plants are carotenes, which are orange, and xanthophylls, which are yellow. These two pigments, along with chlorophyll b absorb light in a region of the spectrum different from chlorophyll a and transfer the energy to chlorophyll a.

The presence of chlorophyll, being the predominant pigment of green plants, hides the color of carotenes and xanthophylls in leaves. However, during autumn chlorophyll starts to break down, allowing the carotenes and xanthophylls to show their brilliant colors of red, orange and yellow.

Part I

Objective

In Part I of the investigation, you will use the paper chromatography method to separate the polar and nonpolar components of a dye mixture, using polar and nonpolar developers.

Materials needed per group of 3 students

3 Chromatography reaction chambers
3 Chromatography paper strips
1 Sample loading micropipet
3 Student Study and Analysis Sheets

Protective Equipment (per student)

Safety goggles
Lab apron
Gloves

Common Materials

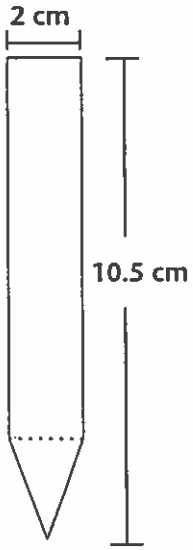
Chromatography dye mixture
Chromatography developer I (Water) [not provided in kit]
Chromatography developer II (Isopropyl)
Chromatography developer III (Isopropyl/Water mixture)

Procedure

CAUTION: The chemicals you will be using are poisonous and irritant. Avoid any skin/eye contact; do not ingest. Flush spills or splashes with water for 15 minutes. Call your teacher.

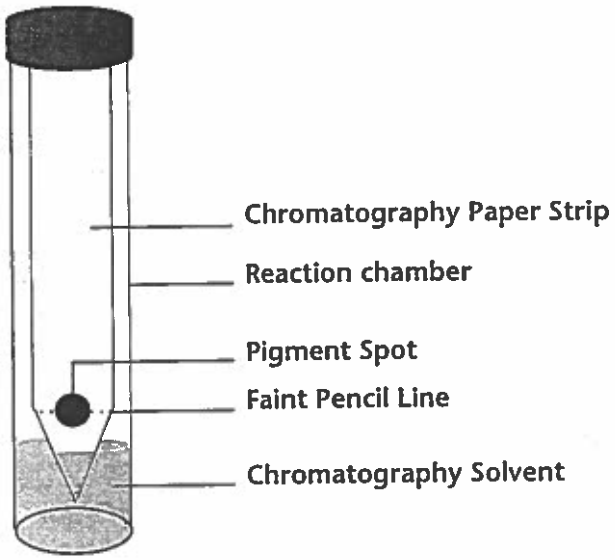
SAFETY: Put on your safety goggles, lab apron and gloves.

1. Use scissors to cut the bottom end of the three chromatography paper strips to a tapered end, as shown below.



2. Draw a faint pencil line to each strip, a few millimeters above the pointed end. Using a micropipet apply a minute drop of the dye mixture on the center of the pencil line to each strip.

3. Pour about 5 ml of the chromatography developer I (water) into your reaction chamber. Place one of your chromatography paper strips into the chamber and adjust the length of the strip so that a small portion of the end tip is immersed into the developer. Do not immerse the pigment spot into the developer. The top end of the reaction chamber is tapered so that it will hold the chromatography paper strip at the desired distance.



4. Ensure that the paper strip is level and it does not touch the walls of the reaction chamber. Place the screw cap over the chamber and allow the developer to advance up the paper without agitating the reaction chamber.

5. Within 8-10 minutes you will notice bands of colors have separated. Remove the chromatogram from the chamber when the developer front reaches near the top of the paper, and allow to air dry.

How many different bands of colors were produced when Developer I (water) was used as the developer?

6. Save your chromatogram for comparison to the ones produced with developers II, and III.

7. Repeat Steps 3 through 6, using developer II and III. Within 15 minutes you will notice certain bands of colors that have separated in each of the chromatograms. Remove the chromatograms from the chromatography chambers, when the developer front reaches the midpoint of the paper strip, and allow them to air dry.

How many different bands of colors were produced when Developer II (isopropyl) was used as the developer?

How many different bands of colors were produced when Developer III (isopropyl/water) was used as the developer?

Explain what accounts for the difference in separation of the individual substances which make up the dye mixture.

Compare your three chromatograms. Which of the three developers produced the best separation of the dye mixture? Why?

Part II

Objective

In Part II of the investigation, you will use a specially formulated developer to separate and observe the individual plant pigments (chlorophyll a, chlorophyll b, carotenes, and xanthophylls) which give a leaf its color, determine the R_f value of each pigment, and learn their function during photosynthesis.

Materials needed per group of students

1 Chromatography reaction chamber
1 Chromatography paper strip
1 Sample loading micropipet
1 Student Study and Analysis Sheet

Common Materials

Plant pigments extract
Chromatography developer IV

Procedure

CAUTION: The chemicals you will be using are poisonous and irritants. Avoid any skin/eye contact; do no ingest. Flush spills or splashes with water for 15 minutes. Call your teacher.

SAFETY: Put on your safety goggles, lab apron and gloves.

1. Use scissors to cut the bottom end of your chromatography paper to a tapered end, as shown in the Procedures section of Part I.

2. Draw a faint pencil line a few millimeters above the pointed end of the paper strip. Using a micropipet apply a minute drop of the plant pigment extract on the center of the pencil line. Air dry for 1-2 minutes and reapply another minute drop. Allow it to air dry for an additional 2-3 minutes.

3. Pour about 5 ml of the chromatography developer IV (methanol/water solution) into your reaction chamber. Place the chromatography paper strip into the chamber and adjust the length of the strip so that a small portion of the end tip is immersed into the developer. Do not immerse the pigment spot into the developer.

4. Ensure that the paper strip is level and it does not touch the walls of the reaction chamber. Place the screw cap over the chamber and allow the developer to advance up the paper without agitating the reaction chamber.

5. Within 20 minutes you will notice the bands of different colors: orange, yellow, and two shades of green. Remove the chromatogram from the reaction chamber when the developer front reaches the midpoint of the chromatography paper.

6. Mark the position of the developer front and the center of each of the separated pigments with a pencil. Then measure the distance of the developer front from the starting point and the distances traveled by the different pigments from the starting point to the center of each band. Record your measurements in the data table and make a sketch of the chromatogram. **Note:** The chromatogram of the plant pigment extract may fade over time.

7. Calculate the R_f value as a decimal fraction for each pigment and record your answers in the data table.

8. Clean up your work area and equipment used and wash your hands before leaving the lab.

Data Table

Band Number	Pigment	Color	Migration Distance in mm	R_f Value
1 (top)				
2				
3				
4				
Solvent				

Questions

1. Describe what happened to the original spot of plant pigments extract.

2. How do your R_f values compare with those of your classmates?

3. List some other uses of chromatography.

4. Which of the 4 pigments migrated the farthest and why?

5. Which of the two chlorophyll forms is more soluble?

6. Explain why leaves change color in the fall.

7. What is the function of these plant pigments in photosynthesis?

FURTHER INVESTIGATION

To learn more about chromatography, you may want to experiment separating pigments found in substances such as fruit juices and writing ink and dyes, using various polar and nonpolar solvents such as water, vinegar, alcohol, salt solution, etc. Determine which solvents are more capable of bringing about a separation and calculate the solvent specific R_f values of the separated pigments.