Basic Column Chromatography

Introduction

Spinach is a leafy green vegetable rich in iron. However, besides green chlorophyll, additional pigments that are not visible to the naked eye are also present in spinach. The presence and identity of additional accessory pigments in spinach may be determined using column chromatography.

Concepts

- Chromatography
- Adsorption
- Eluents
- Polarity

Background

The word *chromatography* is derived from two Greek words meaning color (*chroma*) and to write (*graphein*)—"color writing." The term was coined by the Russian chemist Michael Tswett in 1903 to describe a new technique he had invented to separate the pigments in green plant leaves. Tswett found that in addition to chlorophyll, the main green pigment, plant leaves also contained red and yellow secondary pigments. The results were literally "written in color" when a plant extract was passed through a column containing a clay-like adsorbent solid.

More than 100 years after Tswett's discovery, chromatography has evolved into the most important tool chemists have for separating the components in a mixture. Some examples of different types of chromatography and their uses include:

- Gas chromatography, which is used in forensics and toxicology to analyze drugs and other substances in blood samples.
- Gel-permeation chromatography, which is used to separate and purify proteins.
- Ion-exchange chromatography, which is used to remove ions from water, also known as water softening.

Column chromatography is an example of a more general type of chromatography called *adsorption chromatography*. The column contains a solid, such as aluminum oxide, Al_2O_3, which acts as the *adsorbent*. A thin layer of the mixture to be separated is placed on top of the adsorbent. A flow of a liquid *eluent* or solvent is washed through the column, carrying the components of the mixture to be separated down the solid column.

The rates at which the components travel down the column depend on their relative affinity for the adsorbent versus the eluent. Those components that have a greater affinity for the adsorbent will remain in the column longer, traveling at a slower rate. On the other hand, those components that have a lesser affinity for the adsorbent will not interact with the adsorbent. The components of the mixture that have little affinity for the solid adsorbent will not bind as strongly to the solid phase and will travel through the column at a faster rate with the liquid mobile phase. As the components in the original mixture travel down the column at different rates, they begin to separate into distinct bands. Ideally, each band will contain only a single component from the original mixture, resulting in separation of the mixture.

Successful separation of substances via column chromatography is based on two properties of the substance being separated—its adsorptivity on the solid and its solubility in the eluent. *Adsorptivity* is the adhesion of the molecules in the substance being separated to the molecules on the surface of the adsorbent. The adsorbent gets its name because it has the ability to bind and hold certain molecules in the mixture to be separated. Different materials with different polarities or other chemical properties may be used as the adsorbent. Aluminum oxide, Al_2O_3, and silica gel are commonly used as adsorbents.

The affinity with which molecules in the mixture being separated will "stick" to the adsorbent particles depends on intermolecular forces. *Intermolecular forces* are the relatively weak interactions that occur between molecules. The types of intermolecular forces present depend on the nature of both the adsorbent and the substances in the mixture. Nonpolar compounds generally exhibit only weak dispersion forces. Polar compounds display weak dispersion forces and stronger dipole–dipole forces. Traditionally, the adsorbent is a relatively polar material and the eluent is rather nonpolar. Therefore, the more polar the compound in the mixture being separated, the stronger its intermolecular forces to the adsorbent will be, resulting in the compound being very slow to travel through the column.

The choice of the eluent is critical to the success of the separation in column chromatography. Very rarely will a single solvent be able to separate all the components in a mixture. Typically a single solvent may not move the mixture at all or it

will carry all of the components at once. To compensate, the composition of the eluent is varied during the process. First a nonpolar solvent is used to remove or carry the nonpolar components through the column. Then solvents with gradually increasing polarity are used until all the components have been removed from the column.

Materials

Acetone, CH_3COCH_3, 10 mL
Aluminum oxide, Al_2O_3, 2 g
Hexane, C_6H_{14}, 10 mL
Hexane–acetone mixture, 50/50, 10 mL
Sand, 0.5 g
Spinach leaves
Chromatography column, with tip
Balance, 0.1-g precision
Beaker, 250-mL
Beakers or Erlenmeyer flasks, 50-mL, 3
Clamp
Graduated cylinder, 10-mL
Marker
Mortar and pestle
Pipets, plastic, thin-stem, 4
Spatula
Stoppers, size 0, 4
Support stand
Test tubes, 13 × 100 mm, 4
Test tube rack
Weighing dish, small

Safety Precautions

Acetone and hexanes are flammable liquids and dangerous fire risks. Acetone is also slightly toxic by ingestion and inhalation. Hexanes are a respiratory irritant. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Remind students to wash their hands thoroughly with soap and water before leaving the laboratory. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.

Procedure

Preparation of the Chromatography Column

1. Attach the clamp to the support stand.
2. Place the tip on the bottom of the chromatography column. Pour about 2.5 mL of hexane into the column so that the liquid fills the narrow portion of the column.
3. Slowly pour 2 g of aluminum oxide into the column. Fill the narrow portion of the column until it is about ¾ full. Tap the tip of the column on the lab bench while pouring the aluminum oxide—tapping the column sufficiently will eliminate holes or spaces. Having holes or spaces in the packing of the solid will leave channels for the substances to travel through and lead to poor separation.
4. Place the column in the clamp and tighten until the wide portion of the column is held firmly by the clamp.
5. Place an empty 250-mL beaker underneath the column.
6. Carefully pour a small amount of sand into the column so it forms a 2-mm layer on top of the aluminum oxide (see Figure 1).
7. Remove the column from the clamp and tap the column on the lab bench again to ensure the sand forms an even layer.
8. Place the column back in the clamp so that it is positioned above the beaker.

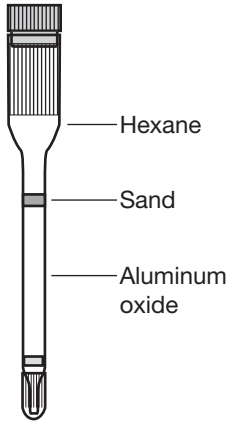

Figure 1.

Preparation and Separation of Spinach Extract

9. Obtain 10 mL each of the following solvents—hexane, 50/50 hexane–acetone, and acetone. Place each solvent in a clean 50-mL beaker or flask.
10. Finely tear or chop two spinach leaf greens. Add this to a mortar and pestle. Add 5–6 mL of 50/50 hexane–acetone mixture to the greens. Grind vigorously for two to three minutes.
11. Place a clean, plastic pipet into the mortar and withdraw only the liquid portion of the extract into the pipet. Avoid obtaining solid in the pipet.
12. Remove the tip from the chromatography column and allow the hexane in the column to drain into the beaker below. Allow it to drain until the liquid level is just above the sand layer. Replace the tip on the column.
13. Remove the tip from the column and add 5 drops of the spinach extract to the top of the column by running it down the inside of the column in a circular fashion. Do not simply squirt it into the center of the column as this will disturb the sand layer.
14. As soon as the extract is absorbed into the sand layer, carefully add a pipet-full of hexane to the column by running it down the inside of the column in a circular fashion. Continue adding hexane in this manner until the entire column is full.
15. As the solvent moves through the column, it will begin to carry one of the pigments with it. This will take several minutes as the pigments interact with the adsorbent phase. Colored bands should become visible in the column.
16. Label three clean test tubes pigment 1, pigment 2, and pigment 3.
17. As the first pigment band begins to exit the tip of the column, place the appropriate test tube underneath the column to collect the solvent containing this pigment (see Figure 2). As soon as the band has completely exited the column, remove the test tube, stopper it and set it aside. Record observations.

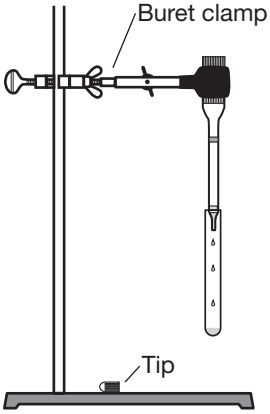

Figure 2.

The same general procedure will be used to collect additional pigments as they pass through the column. Different solvents, first 50/50 hexane–acetone and then acetone, will be needed to collect pigments 2 and 3, respectively. In each case the same general procedure should be followed. *Never allow the top of the column to run completely dry.* Add each solvent carefully by running it down the inside of the column in a circular fashion so as not to disturb the sand layer. Collect each pigment band solution in a different test tube. Replace the test tube with a beaker to collect eluent that does not contain a pigment.

18. Collect remaining hexane in the 250-mL beaker. Do not allow column to go dry.
19. Using a pipet, add the 50/50 hexane–acetone mixture to fill the entire column.
20. As the hexane–acetone mixture begins to flow down the column, it will carry another pigment band with it. Collect the eluent in the 250-mL beaker until the colored band is at the bottom of the column.
21. Collect the solvent containing the second band in the appropriate test tube. Record observations.
22. Allow any remaining hexane–acetone to drain into the 250-mL solvent beaker. Leave a thin layer so the column remains wet.
23. Using a clean pipet, add acetone to fill the column.
24. As the acetone begins to flow down the column, it will carry the last spinach pigment with it. Add more acetone as needed to make sure the column does not dry out until the third band has almost reached the bottom.
25. Collect acetone eluent in the 250-mL solvent beaker until the third band approaches the bottom of the column. Replace the beaker with the third test tube to collect the last pigment band. As soon as the band has completely exited the column, remove the test tube, stopper it and set it aside. Record observations.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. Dispose of remaining acetone or hexane by evaporating in a fume hood. Dispose of the dry spinach powder, sand, aluminum oxide, and the chromatography column in the

regular trash according to Flinn Suggested Disposal Method #26a.

Tips

- Two or more color bands may be collected from the spinach extract. A yellow pigment, a yellow-green pigment, and a dark green pigment may be collected.
- More vibrant and distinct colors may be observed using more concentrated extract, such as a powdered extract.
- Powdered vegetable extract may be found in many health food stores and is also available from online retailers.

Materials for *Basic Column Chromatography* are available from Flinn Scientific, Inc.

Catalog No.	Description
AP7392	Basic Column Chromatography—Student Laboratory Kit
AP7613	Chromatography Columns, Pkg/15
A0009	Acetone, 500 mL
A0161	Aluminum oxide, 100 g
H0002	Hexanes, 500 mL

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.