CHEM FAX!

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Column Chromatography Kit

Student Laboratory Kit

Introduction

Column chromatography is a popular analytical technique used to separate, isolate, and purify organic and biological compounds. In this activity, pigments from tomato paste and spinach powder will be extracted and separated using column chromatography.

Concepts

- Adsorptivity
- Chromatography
- Separation methods
- Solubility
- Polarity

Background

Chromatography is one of the most useful methods to separate mixtures. *Chromatography* is defined as the separation of a mixture of two or more different compounds or ions by distribution between two phases. The first phase is a stationary phase known as the adsorbent, and the second phase is a mobile or traveling phase called the *eluent*.

In column chromatography the adsorbent phase is carefully packed into a cylindrical column containing a liquid solvent so that there are no channels or gaps through the solid. A thin layer of the mixture to be separated is placed on top of the solid adsorbent. A flow of solvent, the eluent, is slowly 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 and 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 a lower affinity for the solid adsorbent will spend more time traveling down the column with the liquid mobile phase. As the components travel down the column at different rates, they begin to separate into distinct bands. Ideally, each band will contain only a single component in the original mixture. As each band passes through the bottom of the column individually, it can be collected. The nature of the adsorbent and the polarity of the solvent (eluent) must be optimized in order to achieve maximum separation or resolution of the components in a given mixture.

Successful separation in column chromatography is based on the properties of the substances being separated—adsorptivity to the adsorbent and their solubility in an eluent.

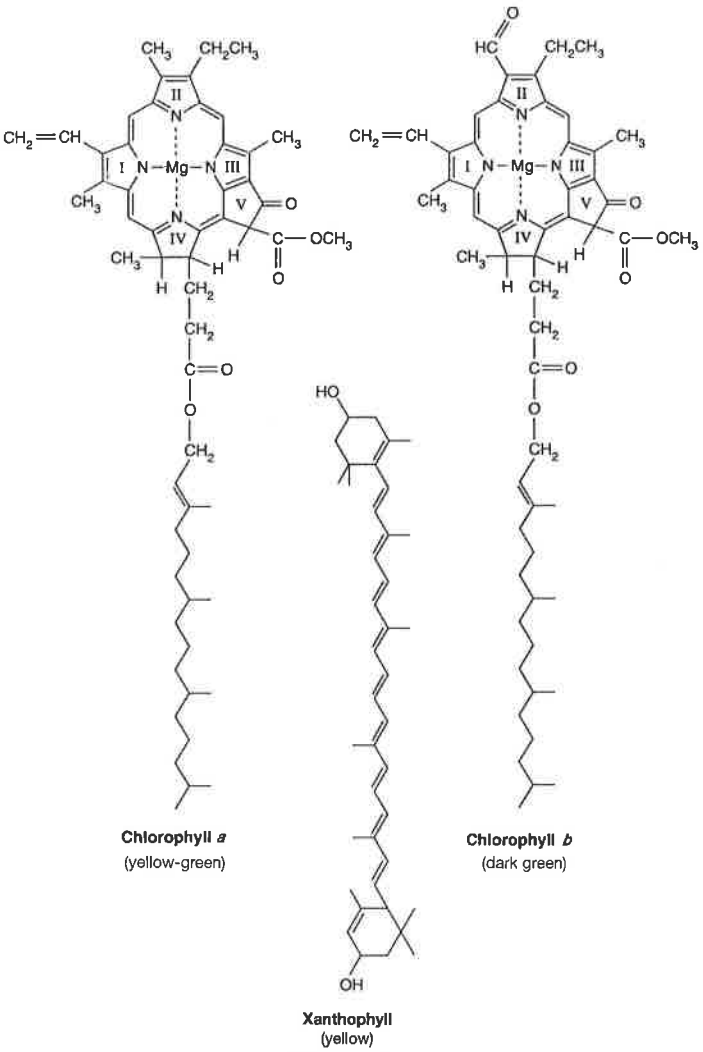

Figure 1.

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 attract and "hold" molecules in the substance 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 in chromatography experiments.

The affinity with which different molecules of the substance being separated will "stick" to the adsorbent particles depends on their 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 molecules and the molecules in the substance being separated. Nonpolar molecules exhibit relatively weak dispersion forces. Polar molecules display weak dispersion forces and stronger dipole–dipole forces. Traditionally, the adsorbent is a relatively polar material and the eluent is rather nonpolar. Therefore, more polar molecules in the structure being separated will exhibit stronger intermolecular forces to the adsorbent, and will therefore move slowly through the column.

The choice of eluent is critical to the success of the separation. Very rarely will a single eluent be able to separate all the components in a mixture. Typically a single eluent will not move the mixture at all or carry it all at once. To compensate, the eluent is varied during the experiment. First a nonpolar solvent is used to remove the nonpolar components. Then solvents with gradually increasing polarity are added to the column until all components have been removed.

Pigment Structure and Photosynthesis

In this experiment, several pigment molecules will be extracted from spinach powder and tomato paste—chlorophyll *a,* chlorophyll *b,* xanthophyll, β-carotene, and lycopene. The structures of these molecules are shown in Figures 1 and 2.

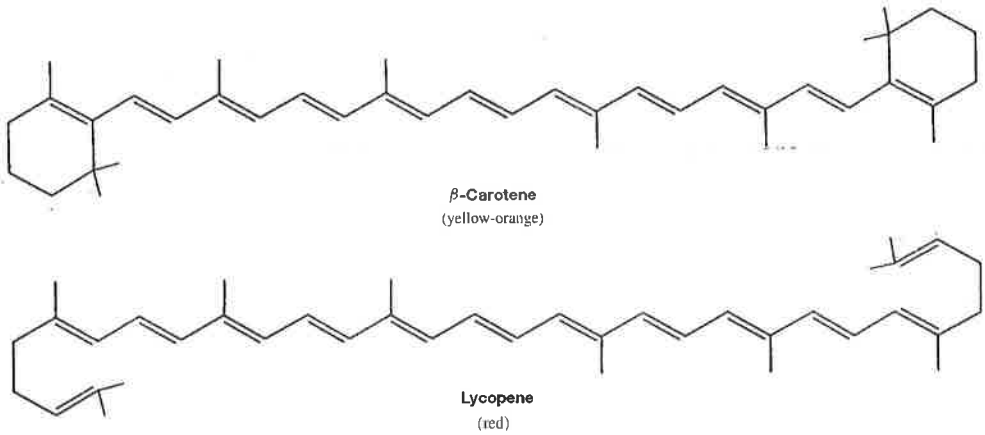

Figure 2.

Chlorophylls are green pigments found in the chloroplasts of green plants and algae. Xanthophyll is a yellow pigment also found in plant leaves, some plants, and algae. Both spinach and tomatoes also contain the yellow-orange pigment β-carotene. Lycopene is a red pigment that gives tomatoes their characteristic red color. Xanthophyll, lycopene and β-carotene are carotenoid pigments. The color of these pigments is important because it is possible to see them separate and exit the chromatography column individually, based on their color. The colors of the pigments are due to their chemical structures. Each pigment molecule contains conjugated double bonds—alternating single and double bonds. Compounds that have eight or more conjugated double bonds absorb light in the visible region of the spectrum, hence we see their color. Chlorophyll *a* and *b,* xanthophyll, β-carotene, and lycopene are different colors because their absorption maxima occur at different wavelengths of visible light. This color difference is due to subtle differences in the molecular structure of each pigment. In each case, the color the pigment absorbs is not the color it appears. Instead, the color the pigment appears is the same as the *complement* of the color of light it absorbs. These complementary colors are the colors the human eye perceives. Table 1 on page 3 lists the absorption maxima for each pigment, the colors of light each pigment absorbs, and the perceived color of each pigment.

Table 1.

Pigment	Absorption Maxima (nm)	Color Absorbed	Color Observed
Chlorophyll *a*	660	red	green
	429	violet	green
Chlorophyll *b*	643	red	green
	453	violet	green
Xanthophyll	481	violet	yellow
	453		
	429		
β–Carotene	497	blue-green	yellow-orange
	466		
Lycopene	504	green	red
	473		
	446		

Thie absorption maxima and pigment colors can also be displayed graphically in Figures 2 and 3 by plotting the absorbance of visible light as a function of wavelength. Figure 2 shows the absorption spectra of visible light by plant pigments. Chlorophyll pigments in spinach leaves absorb primarily at the red and blue ends of the spectrum, so that green light is reflected or observed. Hence, spinach appears green. Figure 3 shows tomato pigments absorb light in the blue and green regions of the spectrum. Therefore, red is reflected and tomatoes appear red.

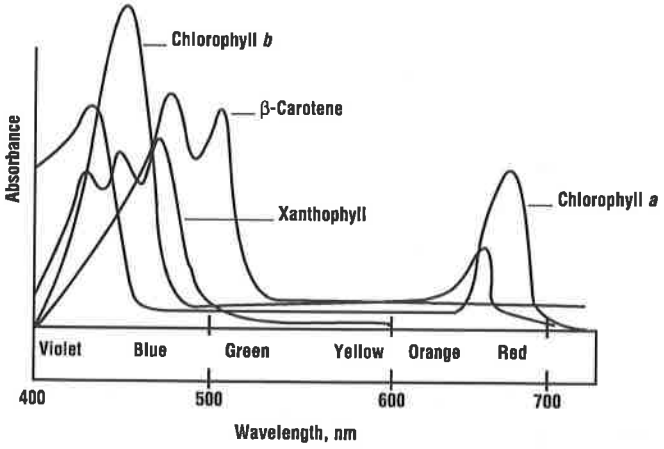

Figure 2. Absorption of visible light by pigments in spinach.

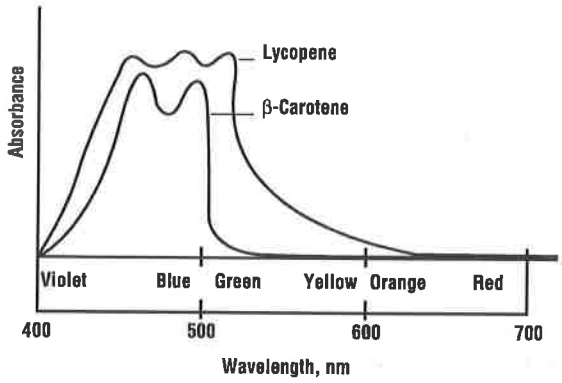

Figure 3. Absorption of visible light by tomato pigments.

Experiment Overview

Each lab group will carry out a column chromatography experiment on either spinach powder or tomato paste. The pigments will be separated by elution with different solvents and collected in separate test tubes.

Pre-Lab Questions

1. What adsorbent will be used in this chromatography experiment?

2. What solvents will be used as eluents in this chromatography experiment?

3. Use a reference source such as a textbook or the Internet to identify the solvents used in this experiment as either polar or nonpolar. Explain what these terms mean.

Materials

Acetone, CH_3COCH_3, 10 mL
Aluminum oxide, Al_2O_3, 2.0 g
Hexane, C_6H_4, 10 mL
Hexane-acetone, 50/50 mixture, 10 mL
Hexane-acetone, 80/20 mixture, 10 mL
Sand, 0.5 g
Spinach powder, 0.5 g
Tomato paste, 1 g
Balance, 0.1-g precision (shared)
Beaker, 50-mL
Beaker, 250-mL
Chromatography column
Clamp
Graduated cylinder, 10-mL
Pipets, thin-stem, 3
Spatula
Stirring rod
Stoppers, size 0, 2–4*
Support stand
Test tubes, 13 × 100 mm, 2–4*
Test tube rack
Weighing dish, small

**Material quantities vary based on experiment performed.*

Safety Precautions

Acetone and hexane are flammable liquids and dangerous fire risks. Acetone is also slightly toxic by ingestion and inhalation. Hexanes are a respiratory irritant. Perform this experiment only in a well-ventilated lab. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Wash hands thoroughly with soap and water before leaving the laboratory.

Procedure

Preparation of the Chromatography Column *(all groups)*

1. Attach a clamp to a support stand. Tighten the clamp so that the wide part of the chromatography column is held snugly by the clamp.
2. Place an empty 250-mL beaker underneath the column.
3. Remove the column from the clamp and place the tip on the bottom of the column. Pour about 2.5 mL of hexane into the column—enough liquid to fill the narrow part of the column (see Figure 4).
4. Slowly pour 2.0 g of aluminum oxide into the column. Fill the narrow part of the column until it is about ¾ full. Tap the tip of the column on the lab bench while adding the aluminum oxide—tapping the column well will ensure there are no holes or channels in the adsorbent layer.
5. Place the column back in the clamp so that it is positioned above the beaker.

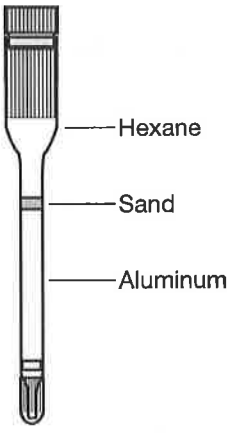

Figure 4.

6. Carefully pour a small amount of sand into the column so it forms a 2-mm layer on top of the aluminum oxide.
7. 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.

Extract Preparation and Separation of Spinach *(spinach groups only)*

1. Obtain 10 mL of each of the following solvents: hexane, 50/50 hexane–acetone, and acetone. Place each solution in a clean 50-mL beaker.
2. Using a 0.1 g balance, weigh 0.5 g of spinach powder and place it in a 13 × 100 mm test tube. Add 2 mL of the 50/50 hexane-acetone mixture.
3. Insert a stopper into the test tube and shake vigorously. Continue shaking until the solution begins to turn a dark green. Place the test tube in the test tube rack.
4. Remove the tip from the chromatography column and allow the hexane in the column to begin draining into the beaker below. Allow it to drain until the hexane level is just above the sand layer. As the hexane is draining, shake the test tube containing the spinach extracts again.
5. Place a clean pipet into the spinach extract and fill it with only the *liquid* portion of the spinach mixture. *Note:* Decant the top liquid into a clean test tube if necessary. Avoid obtaining solid spinach in the pipet as much as possible.
6. Once the hexane in the chromatography column has drained to the point where the meniscus is just above the sand layer, carefully 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 the liquid into the center of the column as this will disturb the sand layer. Allow the extract to soak through the sand and down to the aluminum oxide layer.
7. While the extract is soaking into the column, fill a pipet with hexane. As soon as the extract is absorbed into the sand layer carefully add hexane to the column by running it down the inside of the column in a circular fashion. Add hexane until the entire column, including the wide portion at the top, is full.
8. As the hexane begins to flow 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. Make sure the column is always wet with hexane. Continue to add hexane until the first band has traveled almost all the way down the column. Record observations in the data table.
9. When the first band gets close to the bottom of the column, label a clean test tube "spinach pigment 1."
10. As the band begins to exit the tip of the column, place the test tube underneath the column to collect the pigment. As soon as the band has completely exited the column, remove the test tube, stopper it and set aside. Record observations in the data table.
11. If hexane is remaining above the sand layer of the column insert a pipet into the hexane and carefully draw up the liquid. Remove the liquid only, do not disturb the sand or the aluminum oxide layers. Leave a very thin layer of hexane, just enough so that the column remains wet.
12. Using a pipet, add the 50/50 hexane–acetone mixture. Fill the column as it was filled with the hexane all the way to the top. *Note:* The same pipet that was used with the hexane may be emptied and used with the hexane–acetone mixture.
13. As the hexane–acetone mixture begins to flow down the column, it will carry another pigment band with it. While the band is travelling make sure that the column remains wet. Add more hexane–acetone as needed until the second band has almost reached the bottom.
14. As the second band approaches the bottom of the column, label a small empty test tube "spinach pigment 2." As the band begins to exit through the tip of the column, place the test tube underneath the column to collect the pigment.
15. As soon as the band has completely exited the column, remove the test tube, stopper it and set aside. Record observations in the data table.
16. If any hexane–acetone mixture remains in the column carefully draw it off using a pipet. Leave a thin layer so that the column remains wet.
17. Using a clean pipet add acetone to the column. Fill the column to the top as previously done with the other two solutions.
18. As the acetone mixture begins to flow down the column, it will carry the last spinach pigment with it. While the band is travelling make sure that the column remains wet. Add more acetone as needed until the third band has almost reached the bottom. Record observations on the data table.

19. As the third band approaches the bottom of the column, label a clean test tube "spinach pigment 3." As the band begins to exit through the tip of the column, place the test tube underneath the column to collect the pigment. As soon as the band has completely exited the column, remove the test tube, stopper it and set aside. Record observations in the data table.

Extract Preparation and Separation of Tomato Paste *(tomato groups only)*

1. Obtain 10 mL of 80/20 hexane–acetone solution and 10 mL of acetone. Place each solution in a clean 50-mL beaker.
2. Place an empty 50-mL beaker on an electronic balance and press the tare.
3. Using a spatula, measure 1 g of tomato paste into the beaker.
4. Using a clean pipet add 1 mL of the 80/20 hexane–acetone mixture to the tomato paste. Using a stirring rod, mix the tomato paste with the hexane–acetone mixture *extremely* well until the solution is a deep red color.
5. Remove the tip from the chromatography column and allow the hexane in the column to begin draining into the beaker below. Allow the liquid to drain until the hexane level is just above the sand layer. As the hexane is draining, stir the beaker containing the tomato extract again.
6. Place a clean pipet into the tomato extract and fill it with only the liquid portion of the tomato mixture. Avoid obtaining solid tomato in the pipet as much as possible.
7. Once the hexane has drained to the point where the meniscus is just above the sand layer, carefully add 10 drops of the tomato 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. Allow the extract to soak through the sand and down to the aluminum oxide layer.
8. While the extract is soaking into the column, fill a pipet with the 80/20 hexane–acetone solution.
9. As soon as the extract is absorbed into the sand layer, carefully add the hexane–acetone solution to the column by running it down the inside of the column in a circular fashion. Add solution until the entire column, including the wide portion at the top is full.
10. As the hexane–acetone mixture begins to flow down the column, it will begin to carry one of the pigments with it.
11. When the first band gets close to the bottom of the column, label a small, clean test tube "tomato pigment 1."
12. As the band begins to exit the tip of the column, place the test tube underneath the column to collect the pigment. As soon as the band has completely exited the column, remove the test tube, stopper it and set aside. Record observations in the data table.
13. If any liquid is remaining above the sand layer of the column insert a pipet into the solution and carefully draw up the liquid. Remove the liquid only, do not disturb the sand or the aluminum oxide layers. Leave a very thin layer of liquid on top of the column, just enough so that the column remains wet.
14. Using a clean pipet add acetone to the chromatography column. Fill the column to the top as previously done with the hexane–acetone solution.
15. As the acetone flows down the column it will carry the remaining pigment with it. *Note:* This pigment may be a very faint band. Follow it carefully so it is not lost.
16. When the second band approaches the tip of the column, label a small clean test tube "tomato pigment 2."
17. As the band begins to exit the column, place the test tube underneath the column to collect the pigment. Once the band has completely exited the column, remove the test tube, stopper it and set aside. Record observations in the data table.
18. At this point, the interface between the aluminum oxide and the layer of sand should have no coloration. This indicates all of the pigments have been carried through the column with one of the solvents. Allow any remaining solvent to drain or pipet any excess off the top.
19. To clean the chromatography column, rinse with distilled or deionized water to remove the contents. Do not stick a spatula into the column to dig out the contents. Doing so will scratch the insides of the column and damage it for future use.

Name: _____

Column Chromatography Worksheet

Extract:_____

Note: Spinach extract chromatography will separate three pigments and tomato extract chromatography will separate two pigments.

Pigment Number	Observations	Eluent Used (circle one)	Identification
1		Hexane Hexane–acetone mixture	
2		Hexane Hexane–acetone mixture	
3 Spinach group only		Hexane Hexane–acetone mixture	

Questions

1. Use the observations above and the information included in the *Background* section to tentatively identify the pigments that were separated in this lab.

2. Given the polarity of each eluting solvent (see Question 3 in the *Pre-Lab*) describe the polarity of each pigment relative to the polarity of the other pigments in the mixture.

3. Design an experiment for the separation of pigments in a different colorful vegetable not used in this lab. Outline a procedure and explain your reasoning for each step.

Teacher's Notes

Column Chromatography Kit

Materials Included in Kit (for 15 groups of students)

Acetone, CH_3COCH_3, 500 mL
Aluminum oxide, alumina, Al_2O_3, 75 g
Hexane, C_6H_{14}, 1 L
Sand, 30 g
Spinach powder, 15 g
Tomato paste, 6-oz can
Chromatography columns, 15
Pipets, thin-stem, 90

Additional Materials Needed (for each lab group)

Balance, 0.1-g precision (shared)
Beakers, 50-mL, 3
Beaker, 250-mL
Clamp
Graduated cylinder, 10-mL
**Material quantities vary based on experiment performed.*
Spatula
Stoppers, size 0, 3–4*
Support stand
Test tubes, 13 × 100 mm, 3–4*
Test tube rack
Weighing dish, small

Additional Material Needed (for *Pre-Lab Preparation*)

Erlenmeyer flask, 250-mL
Erlenmeyer flask, 500-mL
Can opener
Graduated cylinder, 100-mL
Stirring rod
Stoppers, 4 *(to fit flask)*

Pre-Lab Preparation

1. Pour approximately 225 mL of hexane into a clean 250-mL Erlenmeyer flask.
2. Pour approximately 225 mL of acetone into a clean 250-mL Erlenmeyer flask.
3. Using a graduated cylinder measure 50 mL of hexane and 50 mL of acetone. Pour into a clean 250-mL Erlenmeyer flask and stir. This is the 50/50 hexane–acetone mixture. *Note:* To be used by spinach groups only.
4. Using a 100-mL graduated cylinder, measure 80 mL of hexane and 20 mL of acetone. Transfer into a clean 250-mL Erlenmeyer flask and stir. This is the 80/20 hexane–acetone mixture. *Note:* To be used by tomato groups only.
5. Insert a rubber stopper in each flask. Place solutions in a central location where they can be accessed by all lab groups.