

Chlorophyll

Developers:

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Topic Area:

Life Science

Grade Level:

Grades 6 through 8

Goals:

- 1) To teach students how scientists use TLC separations to identify substances.
- 2) To use TLC to separate the various pigments found in plants.

Objectives:

Students will use TLC to separate and identify pigments found in various plants.

Vocabulary:

CAROTENE - reddish-orange pigments found in plants and vegetables.

CHLOROPHYLL - the green pigment found in the chloroplast which is used in the process of photosynthesis.

CHLOROPLAST - a specialized body found in a plant cell that contains chlorophyll.

CHROMATOGRAM - a developed TLC plate.

CHROMATOGRAPHY - the process of separating a substance into its components

CHROMOPLAST - a specialized plastid containing yellow and orange pigments.

SILICA GEL - a white or colorless porous substance. Similar forms are found in sand and glass.

SOLVENT - a liquid that causes a substance to dissolve, producing a solution.

SOLVENT FRONT - line seen as solvent moves up TLC plate.

TLC - chromatography using a thin layer of silica gel coated onto a plastic or glass plate.

XANTHOPHYLL - a yellow chloroplast pigment.

Background:

THIN LAYER CHROMATOGRAPHY

Thin Layer Chromatography is a technique used for the separation of substances into its components. This technique involves the use of a stationary (solid) phase and a mobile (liquid) phase. The stationary phase consists of a thin layer of silica gel coated onto plastic or glass plates. The mobile phase consists of a single solvent or a mixture of solvents.

The substance to be separated is adsorbed onto a TLC plate and the plate is placed into a solvent. The separation occurs as the solvent moves up the plate. The solvent interacts with both the liquid and the solid phases. The solubility of the substance in the liquid phase and its ability to adhere to the solid phase determines the separation.

PLANTS AND PIGMENTS

The chloroplasts of green plants contain pigments called chlorophyll. There are two major types of chlorophyll: blue-green chlorophyll and yellow-green chlorophyll. Green plants also contain other pigments called carotenoids. Examples of carotenoids are carotene and xanthophyll.

During the process of photosynthesis sunlight “excites” the chlorophyll which is used in making organic food for the plant. The carotenoids play an important part in photosynthesis as they also absorb sunlight and pass the energy onto the chlorophyll.

Materials:

sand	disposable plastic pipets (1-2ml)
mortar and pestle	marking pens
melting point capillaries (both ends open)	glass stirring rods
silica plates (TLC plates) Aldrich #Z12,277-7	vials/jars (2-oz, 4-oz, 8-oz)
25-ml and 250-ml graduated cylinders	single-edged razor blade in holder
goggles	

Chemicals:

paint thinner (100% mineral spirits)-hardware store
isopropyl alcohol (99% rubbing alcohol)- drug store
hexane-Fisher Scientific Co.- cat# H291

Plants:

red leaf lettuce	spinach
red cabbage	beet leaves (if available)
leaves from any tree	carrots (baby food)
red and green peppers	

Teacher Procedure:

- 1) To extract pigments from plants and vegetables, tear leaves into small pieces. Place into mortar and sprinkle with some sand. Grind leaves into a pulp with pestle. As you grind you are breaking down the cell walls. Add isopropyl alcohol in small amounts with stirring until a colored solution is formed. (Caution: a concentrated solution works best.)
- 2) Pipet solution into a small vial (2-oz), cap and label. Solutions are sensitive to light and air. Therefore, fresh solutions give the best results (no more than 1 day old).

Note: Peppers and red cabbage show best results when crushed in blender. Blend first, then remove pulp to another container and add isopropyl alcohol to pulp to extract pigments. **Since most blenders are not explosion-proof do not place any solvents into them.**

- 3) Cut silica plates into approximately 1 cm x 8 cm strips by using a single edged razor blade. Cut on the plastic side to avoid damaging the silica coating. Only touch edges of silica plates as fingerprints will affect the separations.

Note: Since TLC plates will pick up moisture from the air, it is best to keep them in an airtight container for long-term storage. Placing Dry Rite® inside the container will keep them even drier.

- 4) Prepare solvent solution:
 - a) pour 100 ml of paint thinner into a jar
 - b) add 7 ml of hexane to the jar
 - c) add 3.5 ml of isopropyl alcohol to the jar

Note: This solution is volatile. Keep stored in a closed container.

- 5) Using plastic or glass disposable pipets, place a small amount, about 1/2 cm depth, of above solution into 4-oz jars and cap (enough jars for each student or group).
- 6) Distribute silica plates and 4-oz jars of solvent solution to students.

***Student
Procedure:***

- 1) Use melting point capillary tubes to spot the TLC plate. Dip one end of the capillary tube into the plant extract solution (the tube will automatically draw a small amount up into the tube). Quickly touch the end of the tube to the plate to dispense a drop onto the plate. Let dry and repeat a few times to get a concentrated spot. Spot the plate about 2 cm above the bottom of the plate (the spot must be above the level of the solvent in the 4-oz jar).
- 2) Place the spotted silica plate into the solvent solution and quickly replace the cap.
- 3) Observe the pigment spot as it moves up the plate with the solvent front.
- 4) Remove plate from jar when solvent front is approximately 1 cm from the top of the TLC plate.
- 5) Discuss observations.

Discussion:

The pigments in green plants have different solubilities. In the solvent system used in this procedure the least soluble pigments are the xanthophylls. These pigments will stay closest to the origin. Yellow-green chlorophyll B is more soluble than xanthophyll and is seen next. Blue green chlorophyll A is even more soluble therefore it moves further than chlorophyll B. Carotene is the most soluble of the pigments and will end up at the top of the solvent front.

***Discussion
Questions:***

- 1) What colors have been separated?
- 2) What pigments are represented by the colors on the silica plate?
- 3) Why are you not able to see the other pigments in a leaf?
- 4) What is the role of each pigment in photosynthesis?

***Management
Suggestions:***

- 1) Acetone may also be used to extract the pigments from the plants. However, acetone chemically alters some of the chlorophyll and can cause a third green band to appear.
- 2) The carotene spot will fade quickly from being exposed to uv light and air.