Color Me Chromatography
Teacher Resource

Grade Level 5-12

Objectives

1. The student will acquaint themselves with chromatography as a technique useful in separating mixtures.
2. The student will perform a paper chromatography separation of a pigment mixture.
3. The student will separate the various pigments found in plant leaves.
4. The student will employ simple equipment and tools to gather data.
5. The student will demonstrate the use of 21st century technology with a document camera.

National Standards

NS.5-8.1; NS.9-12.1	Science as Inquiry
NS.5-8.2; NS.9-12.2	Physical Science
NS.5-8.3; NS.9-12.3	Life Science
NS.5-8.5; NS.9-12.5	Science & Technology
NT.K-12.1	Basic Operations and Concepts
NT.K-12.3	Technology Productivity Tools
NT.K-12.6	Technology Problem-Solving & Decision-Making Tools

Teacher Background Information

Chromatography is a laboratory technique for the separation of a mixture in a solution by passing the mixture dissolved in a 'mobile phase' and through one at a 'stationary phase'. Chromatography simply is the physical separation of a mixture into its individual components. The components to be separated are dispersed between two phases: a stationary phase and a mobile phase. There are a number of different types of chromatography including paper, liquid, high pressure liquid, thin layer, and gas. Chromatographs are used for two basic purposes: 1) to separate the components of a mixture for further use such as obtaining a purified form of a molecule; or 2) it involves smaller amounts of original material and is used to separate component molecules in order to measure relative concentrations of molecules in a mixture.

In this activity, the student will learn how to set up a chromatograph and extract plant pigments. They will discover the three different types of plant pigments: anthocyanins, carotenoids, and chlorophyll. Each of these absorbs light at different levels of the visible light spectrum and has a different function for the plant. Based on the bands formed on the chromatograph paper, the retention factor R_f, can be calculated for each band of pigment.

MATERIALS

Document Camera

Personal Computer

Water Soluble Pens of various colors

Large Test tube (20 to 25mm X 100 to 150mm) with rubber stopper

250mL Beaker

Large paper clip

Chromatograph paper or Filter paper

Hair dryer

Fresh leaves or spinach

Sand

Fine mesh strainer and/or layers of cheesecloth.

Glass Pipette—*heated end point extruded out to narrow opening or use a hypodermic syringe with small needle (28-36 gauge)*

8% acetone--*Acetone can be obtained as nail polish remover or from a paint store as a paint thinner.*

Ethyl alcohol

PROCEDURE

Activity 1a *Learning How to do a Chromatograph*

1. Wear safety goggles and gloves at all times.
2. Bend the paper clip to form a hood for 'hanging' chromatograph inside of test tube.
3. Draw a very light pencil line about 19 mm from the bottom of the chromatograph paper – this will mark the starting point of the chromatograph.
4. Take one of the water soluble pens, and lightly touch the point to the filter paper, allow the spot to dry (or use hair dryer to dry faster). The smaller, more concentrated the dot is, the better the chromatograph will work.
5. Repeat process 3-4 more times, color should be well developed.
6. Using a different color, repeat making another dot along the same pencil line, (will allow comparing mobility of different colors) OR make multiple dots of same color, and then make several such chromatographs each of a different color for comparison.

Activity 1b *Running & Recording the Chromatograph*

1. Wear safety goggles and gloves at all times.
2. Place 5-10mL of water (the solvent) into a large test tube container. Solvent must be high enough that when chromatograph paper is placed into test tube, solvent comes up to the pencil line. *DO NOT overfill the test tube!*
3. Focus a document camera directly on the hanging chromatograph paper.
4. Place the chromatograph paper with extract into the solvent.
5. Start the camera recording. *This chromatograph should run relatively quickly so continuous recording is fine.*
6. Record the chromatograph until it appears the movement of color up the paper has stopped.
7. Stop the chromatograph before the solvent runs off the top of the chromatograph.

DISCUSSION QUESTIONS *Activity 1*

1. Does one color move further then another?

2. Did one color move on the chromatograph faster then another?
3. Did any color break into a number of different pigments?
4. Does the combination of pigments present on the chromatograph make sense in terms of what we know about mixing colors? (yellow and blue make green; red and yellow make orange, etc.)
5. Why do molecules "come out" (adhere) on the chromatograph at different points?
6. Can you think of practical applications for this technique?
7. Have you ever seen this used on CSI or a similar TV show, and what was it used for?

Activity 2a Chromatograph of Plant Pigment Extraction

1. To make the leaf extract, chop up fresh leaves and place in a mortar, or other dish that will tolerate grinding.
2. Add some sand, 5mL ethyl alcohol, and grind the leaves with the pestle.
3. Filter this extract through filter paper or multiple layers of cheesecloth to get rid of the sand and leaf particles, leaving only a green juice.
4. To prepare the solvent make a 50/50 acetone and water solution.

Activity 2b Running the Plant Extract Chromatograph

1. Bend paper clip to form a hook for 'hanging' chromatograph inside of the test tube.
2. Draw a very light pencil line about ¾ inches from bottom of chromatograph paper – this will mark the starting point of the chromatograph.
3. Using an extruded pipette or a small hypodermic syringe and needle put a small dot of leaf extract onto the filter paper. Air dry or use a hairdryer to dry the spot of the leaf extract.
4. Repeat process – object is to get fair amount of extract onto the paper, and keep the spot as small as possible. *Note: multiple, very small applications are preferred over 1-2 large ones.*
5. Place about 5-10mL of solvent into a large test tube container. Solvent must be high enough that when chromatograph paper is placed into test tube, solvent comes up to pencil line.

6. Focus a document camera on the chromatograph paper.
7. Start a time-lapse recording and set it to take 1 image per 3 minutes.
8. Place filter paper with extract into solvent.
9. Record the chromatograph until the pigment molecules appear to stop moving. DO NOT let solvent run 'off' the top of the filter paper.

DATA COLLECTION

Any single image that was recorded can be used to make very precise measurements.

1. Use a calibration tool with the computer software or a metric ruler to determine the retention factor for Activity 1b's chromatograph.
    a. Measure the distance from the pencil line to the point where the solvent stopped going up the paper. (D_f)
    b. Make at least 3 repeat measurements and average them to get a precise measurement.
    c. Measure the distance from the pencil line to the maximum point of migration of one of the bands of extracted molecules (D_s).
    d. Make at least 3 repeat measurements and average them to get a precise measurement.
2. Repeat steps #1a-d for each color band.
3. Have students measure D_f (distance solvent travels from original color dot), D_s (distance each color travels from origin) and then calculate R_f (retention factor) for each color:

$$\mathbf{R_f = D_s/D_f}$$

4. Run several separate leaf chromatograms (or collect class data) to show that an R_f value is a constant under controlled conditions.

QUESTIONS *Activity 2*

1. Why do some leaves look green and others look purple?
2. Why do leaves turn color in the fall?
3. Why should the R_f value be a constant for each color band?
4. Does the R_f value vary with the amount of extract placed on the filter paper? Why or why not?

5. Will the R_f value vary seasonally?
6. Will the number of bands extracted by the chromatograph vary seasonally? (i.e. same species of leaves, different bands in spring and fall) Why might this happen or why not?
7. Would paper towels work as chromatograph paper? Why or why not? (Fairly sophisticated question if pursued because filter paper has a directional fiber structure, while standard toweling has little in the way of directionality, so chromatograph tends to become very diffuse).

PRESENTATION

Have students prepare a lab report including the data, images, and video to give a presentation on the interactive white board or projector for the class.

EXTENSION

- Try other kinds of pens
- Try other kinds of paper for use as a chromatograph, such as paper towels, and different kinds of toweling.
- Try other solvents – turpentine, paint thinner, clear colored sodas such as 7-Up or Sierra Mist.
- Ink mixtures from ball point or other common pens will produce good chromatographs. Depending upon the ink, some will work in water, all will work in the pet ether/acetone solvent.
- Distinguish between the types of chromatography used in modern day analysis: paper, liquid, thin layer, column and gas.
- Role-play medical applications for chromatography by analyzing a chromatogram of ingredients of different headache remedies. Compare this to a patient's blood sample to determine possible headache remedies an unconscious patient may have used.