

Investigation 5.B: Using Chromatography to Separate Plant Pigments

Question: Which pigments can you identify in a green leaf?

Prediction

Predict at least three pigments that you will observe.

Safety Precautions



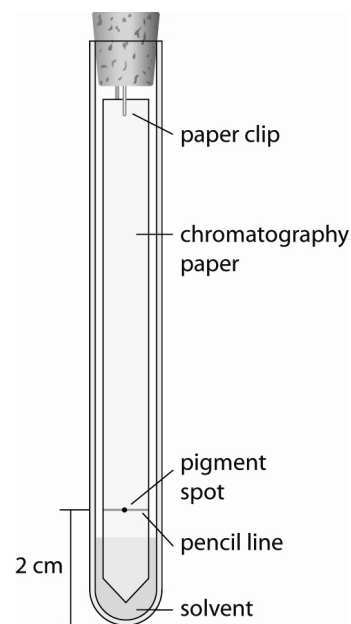
The solvent is volatile. Ensure that there are no flames in the classroom, and avoid breathing the vapours from the solvent. The classroom must be well-ventilated.

Materials

- coleus or spinach leaves (or pigment mixture supplied by your teacher)
- isopropanol (solvent)
- chromatography paper
- paper clip
- retort stand
- test-tube clamp
- cork stopper
- watch glass
- large test tube

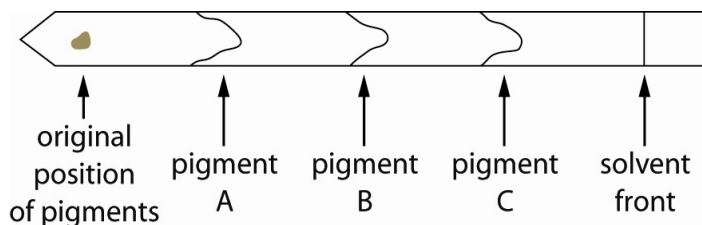
Procedure

1. Attach the large test tube to a retort stand.
2. Set up the cork stopper and the paper clip as shown.
3. Measure a piece of chromatography paper so that it is long enough to hang from the paper clip but not so long that it touches the bottom of the test tube. (Refer to the diagram.) Cut the paper to a point at one end.
4. Place a coleus or spinach leaf over the pointed end of the chromatography paper. Run the edge of a watch glass over the leaf, about 2 cm up from the tip of the paper. Use the watch glass to squeeze out the pigment mixture. Repeat this at least 10 times in the same spot to ensure that enough pigment mixture has been deposited onto the paper.
5. Place 5 mL to 10 mL of solvent in the test tube.
6. Hang the chromatography paper from the stopper in the test tube so that the tip of the paper is in the solvent but the pigment mixture is not.



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- Wait until the solvent has travelled up to about 2 cm from the top of the paper.
- Remove the paper from the test tube. Immediately, before the solvent evaporates, mark the location of the solvent front with a pencil. Also mark the edges of each pigment, as shown in this diagram.



- Measure distance that each pigment travelled, starting from where you applied the pigment mixture to where each pigment stopped moving up the paper strip. Record the measurements in the following table.

Observations and Data for Chromatography of Plant Pigments

Pigment colour	Distance travelled by pigment (cm)	Distance travelled by solvent (cm)	R_f (reference flow value)	Name of pigment

- Measure and record the distance that the solvent front travelled.
- Calculate the R_f (reference flow) value of each pigment, using the following formula:

$$R_f \text{ value} = \frac{\text{distance travelled by pigment (solute)}}{\text{distance travelled by solvent}}$$

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Analysis

1. Sketch your paper strip, using different colours to show the different pigments and their positions. Your sketch is called a chromatogram.
2. Which pigment is (a) most soluble and (b) least soluble in the solvent you used? Explain how you decided.
3. Compare your observations and R_f values with those of your classmates. Identify sources of error in this investigation that might account for any differences.

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4. Use the following chart as a guide to help you complete the last column in your data table. (You may not have observed all the pigments in this chart, or you may have observed other pigments.)

Examples of Plant Pigments and Their Colours

Pigment or pigment group	Colour
chlorophyll <i>a</i>	bluish-green
chlorophyll <i>b</i>	yellowish-green
carotenoids	orange
pheophytin	olive-green
xanthophylls	yellow
phycocyanin	blue
phycoerythrin	red

Conclusion

5.a) Which pigments did you identify in your leaf?

b) Do you think additional pigments could still be present? Hypothesize how you could find out.