

Separation of Plant Pigments Using Chromatography (demo lab)

Purpose: To identify plant pigments by separation and isolation of the pigments using thin layer *paper chromatography.

**Paper chromatography is a useful technique in the separation and identification of different plant pigments. In this technique, the mixture containing the pigments to be separated is first applied as a spot or a line to the paper about 1.5 cm from the bottom edge of the paper. The paper is then placed in a container with the tip of the paper touching the solvent. Solvent is absorbed by the paper and moves up the paper by capillary action. As the solvent crosses the area containing plant pigment extract, the pigments dissolve in and move with the solvent. The solvent carries the dissolved pigments as it moves up the paper. The pigments are carried along at different rates because they are not equally soluble. Therefore, the less soluble pigments will move slower up the paper than the more soluble pigments. This is known as developing a chromatogram.*

**Paper chromatography is useful for identifying unknown compounds - often used in crime scene investigations to match ink, lipstick or colored fibers.*

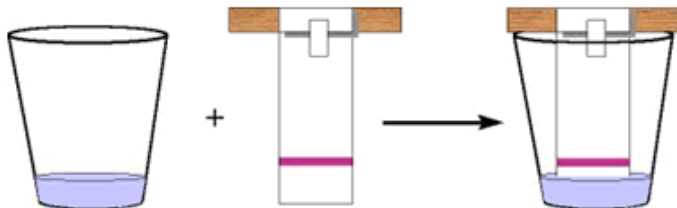
The distance traveled by a particular compound can be used to identify the compound. The ratio of the distance traveled by a compound to that of the solvent front is known as the R_f value; unknown compounds may be identified by comparing their R_f's to the R_f's of known standards.

$$R_f = \frac{\text{distance traveled by the compound from the origin}}{\text{distance traveled by the solvent from the origin}}$$

R_f equation

The different pigments in plants take a long time to separate, so you will need to set this up first and let it run while you are doing the other two experiments.

1. Cut a strip of coffee filter. Draw a horizontal line with a pencil (not pen) about half an inch from the bottom. Place a spinach leaf on the line and roll a penny over it so that you get a line of green pigment on the filter. Using a different part of the leaf, roll the penny again over the same line. Repeat this process until the line is fairly dark.
2. Put about an inch of isopropanol (acetone, fingernail polish remover will work) in a beaker.
3. Tape the top of the coffee filter strip to a pencil and balance the pencil across the top of the beaker. See the image below for the set-up.
4. It is very important that the bottom of the filter strip is in the isopropanol, but the green spot is not in the liquid. If the isopropanol touches the spot directly, the pigment will just dissolve away.
5. Set the cup aside. The isopropanol will move up the filter paper slowly and deposit the pigment components along the way.



You can try this with other pigments: lipstick, felt tip pens, etc. - this is how CSI would determine a suspect from a lipstick mark on a glass.

Analysis

1. Assign a band number for each pigment band - you should see greens, yellows, oranges..etc.
2. Copy the table onto your open paper and fill in the values

Band Color	Plant Pigment	Distance (mm)	R _f (use formula)
Yellow to Yellow-orange	Carotene		
Yellow	Xanthophyll		
Bright Green to Blue Green	Chlorophyll a		
Yellow Green to Olive Green	Chlorophyll b		

3. This experiment can be repeated using other pigments - try lipstick, felt tip pens..etc