

## EXPERIMENT NO: 09

# SEPARATION OF PHOTOSYNTHETIC PIGMENTS THROUGH PAPER CHROMATOGRAPHY

### PRINCIPLE

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. This set-up shows two different pen inks.

Rf. equation:

$R_f = \text{distance traveled by compound} / \text{distance traveled by solvent}$

### MATERIALS REQUIRED

Chromatography paper, Acetone solution, Beaker, Pencil

### PROCEDURE

1. Cut a strip of coffee filter (or filter paper). 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 acetone in the beaker (isopropyl alcohol will also work.)
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 acetone, but the green spot is not in the liquid. If the acetone touches the spot directly, the pigment will just dissolve away.

5. Observe what happens to the liquid in the beaker and the spot on the filter paper. Results will take about 20 minutes.

**ANALYSIS:**

Assign a band number for each pigment band - you should see greens, yellows, oranges...etc.

Band colour	Plant pigments	Distance (mm)	Rf. (use formula)
Yellow to yellow orange	Carotene		
Yellow	Xanthophyll		
Bright green to Blue green	Chlorophyll a		
Yellow green to Olive green	Chlorophyll b		

**CONCLUSION**