Pigment Chromatography Lab

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Tuesday Mar. 22nd, 2005

Purpose: Separation of plant cell pigments by paper chromatography.

Materials:

- Chromatography paper
- Scissors
- Test tube
- Chromatography tube and solvent
- Pencil
- Fresh leaf

Procedure:

1.) A test tube was obtained containing developing fluid (9 volumes of petroleum ether / 1 vol. acetone) and a stopper with a hook.
2.) A 15 centimeter strip of Whatman chromatography strip was cut.
3.) One end to a point was trimmed with scissors. A pencil line was drawn just above the point.
4.) The strip on a hard clean flat surface was laid. A fresh leaf was put over the paper and the tissue was crushed onto the pencil line by rolling the edge of a test tube along the leaf surface. The leaf was moved and this was repeated several times. The line of pigment which has been crushed onto the paper was kept as thin as possible.
5.) The paper was immediately hooked onto the hook of the tube stopper so that the tip of the paper was immersed in the developing fluid. The paper was hanging vertically and not touching the sides of the tube.
6.) The tube stood for about 10 minutes. The movement of the solvent front was observed. When the solvent front was within one centimeter of the hook the paper was removed and a pencil line was drawn at the edge of the front (This was done quickly before it evaporated!).
7.) The distance that each pigment band traveled was measured from the bottom pencil line to the middle of the pigment band. The distance that the solvent front moved was measured. The migration rate (Rf value) was calculated for each pigment.

Observations:

Pigment Colour	Distance-Pigment	Distance – Solvent	Migration Rate (Rf Value)
orange to yellow	6.0 cm	9.0 cm	0.667
yellow or grayish	4.0 cm	9.0 cm	0.444
green to blue-green	3.5 cm	9.0 cm	0.389
green to yellowish-green	1.5 cm	9.0 cm	0.167

Sample Calculations:

$$Rf = \frac{\text{distance pigment traveled}}{\text{distance solvent traveled}}$$

$$= \frac{3.5\text{cm}}{9.0\text{cm}}$$

$$= 0.389$$

Discussion Questions:

1.) Chromatography separate molecules, because a sample containing two components is applied to a column containing a solid support coated with a given chemical layer (stationary phase); also present is solvent that is being applied continuously to the column (mobile phase); as sample components travel through the column, they interact with the mobile and stationary phases to different degrees; those interacting more strongly with the stationary phase stay in the column longer; results in separation of components as they pass through the column.

2.) **Orange to Yellow (Beta-Carotene):** absorbs light of wavelengths that are not absorbed by chlorophylls and are called accessory pigments.
Yellow to Grayish (Xanthophylls): Yellow pigment in plants that, like chlorophyll, is responsible for the production of carbohydrates by photosynthesis. It absorbs light in different wavelengths from that of chlorophyll a (green to yellowish-green) and pass the absorbed energy to chlorophyll a. This allows maximum absorption of solar energy for synthesis of carbohydrates during photosynthesis.
Green to Blue-green (Chlorophyll *a*): The main pigment that makes up about 75% of the pigmentation in plants. It is the only pigment that can transfer the energy of light to the carbon fixation reactions of photosynthesis.
Green to Yellowish-Green (Chlorophyll *b*): Acts as an accessory pigment, absorbing photon that chlorophyll *a* absorbs poorly, or not at all. It makes up about 25% of the pigmentation.

3.) Orange to Yellow: Beta-Carotene
Yellow or Grayish: Xanthophylls
Green to Blue-green: Chlorophyll *a*
Green to Yellowish-green: Chlorophyll *b*

4.) In the fall, my sample leaf might turn yellow, red, or brown, because plants stop producing chlorophyll molecules due to lower temperatures.

Error Analysis:
Fingerprints on the filter paper may have affected the experiment because the oil from your hands can get on these things and affect the results. The spectrophotometer may have not been calibrated correctly because this was the first time this particular one had been used.

Conclusion:
The many pigments found in chloroplasts are all involved in gathering energy from sunlight. About four different pigments were present in the plant cell: Chlorophyll a, chlorophyll b, xanthophylls and beta-carotene. The spectrum of color displayed on the filter paper showed the pigments and the solubility of each. This indicated that photosynthesis was occurring and at what rate it was occurring.