

Youtube link: <https://www.youtube.com/watch?v=ej2zXOwASVI&list=WL&index=18>

Aim

Extract and separate photosynthetic pigments by paper chromatography and calculate their Rf values.

Material Required

- Chromatography chamber
- Spinach leaves
- Mortar and pestle
- Scissors
- Ether acetone solvent
- Acetone
- Capillary tube
- Pencil
- Spatula
- Scale
- Filter paper strips
- Stapler
- Thread
- Watch glass

Theory

Plants carry out the process of photosynthesis, during which light energy from the sun is converted into chemical energy (food). The capturing of light energy is carried out by molecules known as pigments, which are present within the plant cells. Pigments are chemical compounds, which are able to reflect only a particular range of wavelengths of visible light. Leaves of plants primarily contain different types of pigments within their tissues. The four different types of pigments are Chlorophyll A, Chlorophyll B, Xanthophylls, Carotenoids. In order to view and distinguish the primary four plant pigments, a simple technique known as chromatography can be used.

Chromatography is a technique that is used to distinguish between different molecules. This differentiation is based on these attributes-shape, size, charge, mass, adsorption and solubility. In paper chromatography, the interaction between three components is involved – solid phase, separation of a mixture and a solvent. The principle of separation is mainly partition rather than adsorption. Substances are distributed between a stationary phase and mobile phase. Cellulose layers in filter paper contain moisture which acts as stationary phase. Organic solvents/buffers are used as mobile phase. The developing solution travels up the stationary phase carrying the sample with it. Components of the sample will separate readily according to how strongly they adsorb onto the stationary phase versus how readily they dissolve in the mobile phase.

Procedure

- Pick a few fresh and green leaves of spinach and wash it.
- Cut out small pieces of spinach using scissors. Add them to the mortar.

- Accurately measure 5ml 30% ethyl alcohol using a measuring cylinder and add it into the mortar.
- With the help of mortar and pestle, grind the spinach leaves into a smooth paste.
- Shift the prepared paste of spinach into the watch glass with the help of a spatula.
- Place a Whatman filter paper strip (15x15 cm) with a tapering notch towards one ending of the strip.
- Horizontally trace a line with a scale and a pencil that is 2 to 3 cm apart from the notch's tip.
- Using a capillary tube, add 1 drop of the extract of the pigment in the point marked on the line.
- Let the drop dry. Repeat the same process of adding a drop and allowing it to dry for 25-30 times.
- In the chromatographic chamber, pour the petroleum ether: acetone (9:1) solvent.
- Suspend the strip in the chamber without touching the side walls.
- The loading spot remains about 1 cm above the level of the solvent.
- Let the chamber remain uninterrupted for a while.
- Once the solvent reaches 3/4th of the strip, carefully take the strip off.
- Allow the strip to dry.

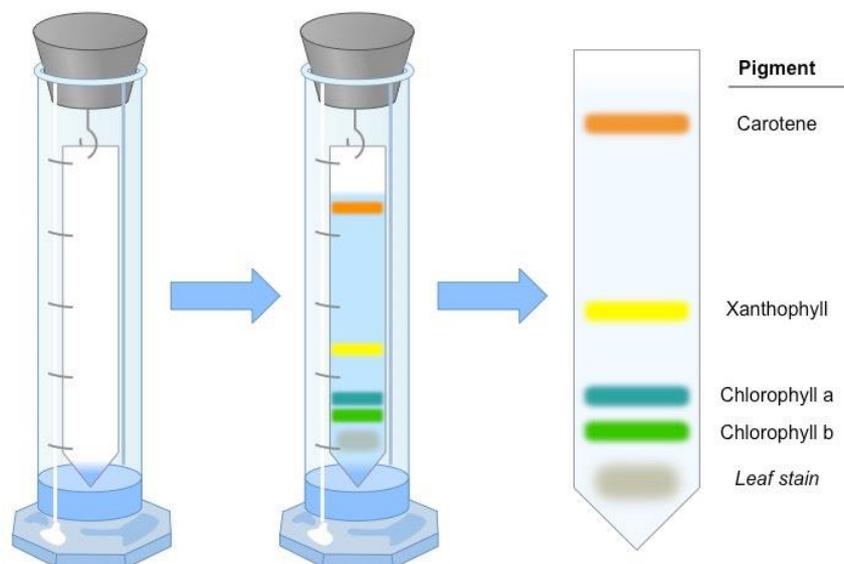
Observation

The dried paper strip displays four different bands. Discrete pigments can be distinguished with the help of colours.

Calculation

The Retention factor or R_f value applies to chromatography to make the technique scientific. It is defined as the distance travelled by the compound divided by the distance travelled by the solvent.

R_f value = Distance travelled by the compound / Distance travelled by the solvent.



Conclusion

1. The Carotene pigment is observed at the topmost as an orange-yellow band of pigments distinctively.
2. Just below this band, a yellowish band appears which indicates the pigment xanthophyll.
3. The third band appearing dark green indicates chlorophyll-a pigment.
4. The yellowish-green band present at the bottom is the chlorophyll b pigment.

Precautions

- The leaves that are selected should be green and fresh spinach leaves
- From the tip of the notch, the loading spot needs to be 2 to 3 cm apart
- While suspending the filter paper strips in the chamber, one need to ensure that the loading spot needs to be set up above 1 cm from the level of the solvent.