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MBOTCC-9: Practical2 (Based on MBOTCC 5o 6,7,8 & 9) (5 Credits)

**EXPERIMENT :Separation of chlorophyll pigment by paper chromatography.**

Aim

To distinguish and study the various pigments present in plants through the process of paper chromatography.

Theory

Plants carry out the process of photosynthesis during which light energy from the sun is converted into chemical food energy. The capturing of light energy is carried out by molecules known as [pigments](#) present in plants. These are chemical compounds which are able to reflect only a particular range of wavelengths of visible light.

Leaves of plants primarily contain four pigments –

Pigment	Colour
Chlorophyll A	Dark green
Chlorophyll B	Yellowish-green
Xanthophylls	Yellow
Carotenoids	Orange

In order to view and distinguish the primary four plant pigments, a simple technique known as paperchromatography 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.

Types of chromatography:

- Column chromatography
- Paper chromatography
- Partition chromatography

- Thin layer chromatography

#### Mechanism of Paper Chromatography

In this technique, the interaction between three components is involved – solid phase, mixture to be separated and a solvent.

The mixture is spotted onto the paper and is dried. The solvent is made to flow through the capillary attraction. While the solvent moves through the paper, the various components of the mixture differentiate into varied coloured spots.

Paper is dried, the position of various compounds is viewed. The substance that is most soluble moves further on the paper as compared to the other substances that are less soluble.

#### 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

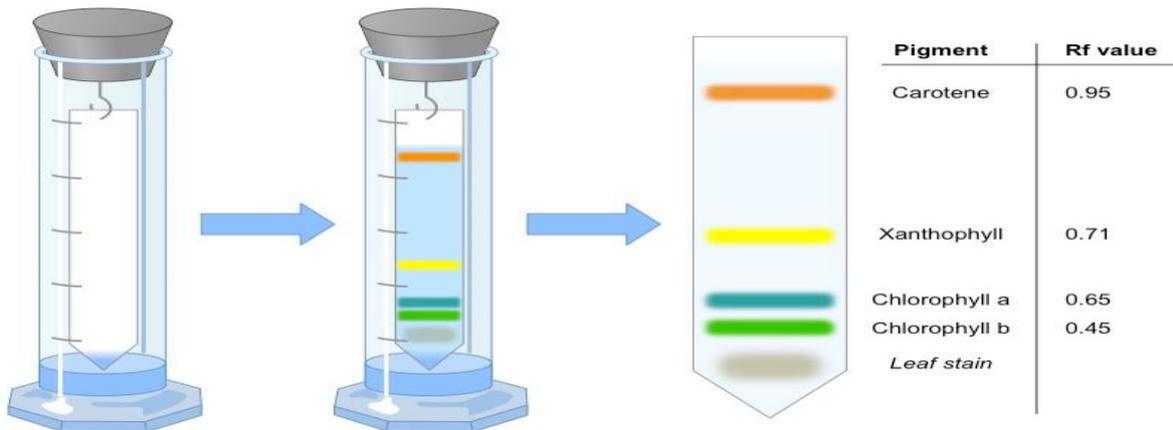
#### Procedure

- In this experiment, spinach leaves are used to separate different pigments, pluck a few fresh leaves.
- Cut out small pieces of spinach using scissors. Add them to the mortar
- Accurately measure 5ml acetone using a measuring cylinder and add it into the mortar

- With the help of mortar and pestle, grind the spinach
- Shift the paste of spinach with the help of a spatula into the watch glass
- Place a filter paper strip with a tapering notch towards one ending of the strip
- Horizontally trace a line with a scale and a pencil that is 2-3cm apart from the notch's tip
- Using a capillary tube, add 1 drop of the extract of the pigment in the midsection of the line
- Let the drop dry. Repeat the same process of adding a drop and allowing it to dry for 4-5 times.
- In the chromatographic chamber, pour the ether acetone solvent
- One ending of the paper should be folded and stapled
- Suspend the strip in the chamber
- The loading spot remains about 1cm above the level of the solvent
- Let the chamber remain uninterrupted for a while
- We notice that the solvent passes along the paper scattering various pigments of the blend to different distances
- Once the solvent reaches 3/4th of the strip, carefully take the strip off. Allow it to dry.
- Calculate the Rf value by the formula

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

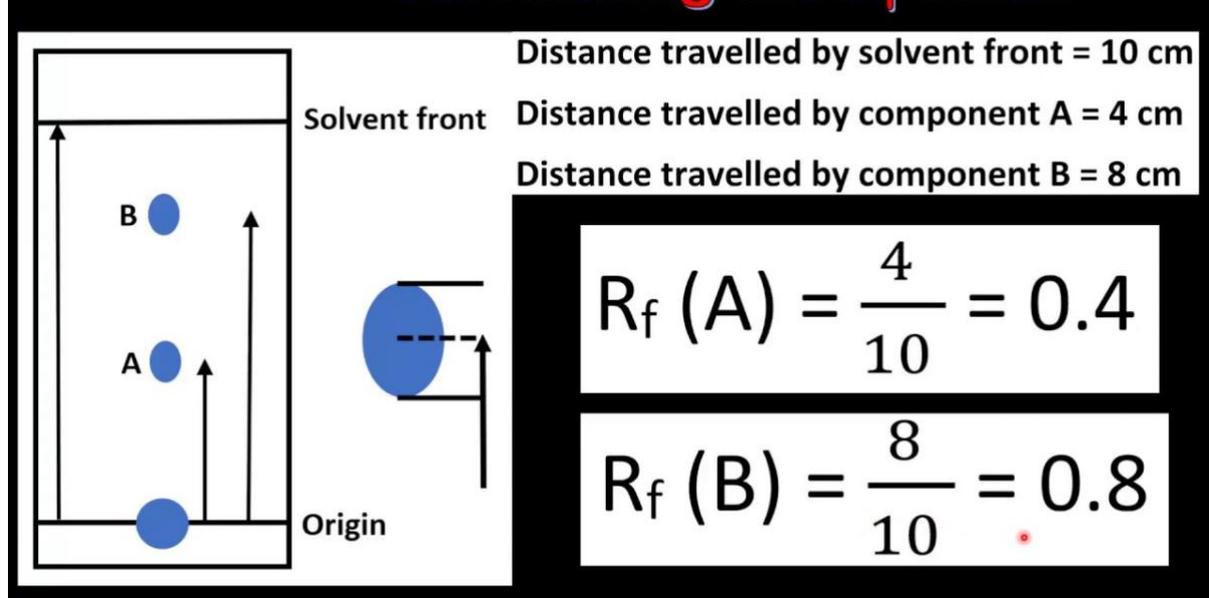
Rf= retardation factor or ratio-to-front value expressed in decimal fraction



EXPERIMENTAL SET UP

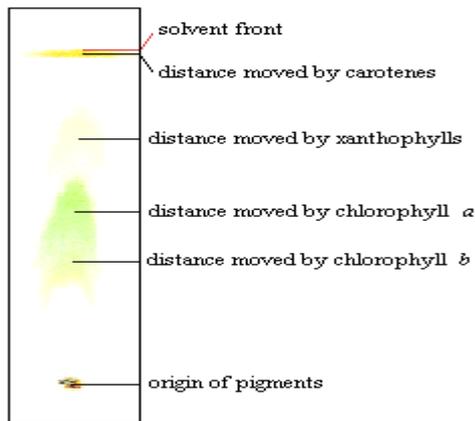
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## Calculating the R<sub>f</sub> value



### Observation

The dried paper strip displays 4 different bands. Discrete pigments can be distinguished with the help of colours.



## Conclusion

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

## Precautions

- The leaves that are picked 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 needs to ensure that the loading spot needs to be set up above 1cm from the level of the solvent.

## Viva Questions

Q.1. What Rf value or retention factor?

A.1. It is a factor that is applicable to chromatography in order to make the technique scientific. It is defined as the distance travelled by the compound divided by the distance travelled by the solvent.

$$R_f = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent}}$$

Q.2. What is phycobilin?

A.2. It is a water-soluble pigment that is found in the chloroplast.

Q.3. What is the significance of pigment in photosynthesis?

A.3. It helps in the absorption of energy from light. The free electrons in the pigments present in their chemical structure transfer their energy to other molecules during photosynthesis when they turn into high energy electrons thereby liberating energy they captured from light. This released energy is then used up by other molecules for the formation of sugars and related nutrients with the use of water and carbon dioxide.

