

## 2. Separation of plant (photosynthetic) pigments by paper chromatography.

Date : / /

### Aim :- Separation of plant (photosynthetic) pigments by paper chromatography.

The plants show presence of many pigments. The different photosynthetic pigments present in the leaf cells are : chlorophylls, carotenes and xanthophylls.

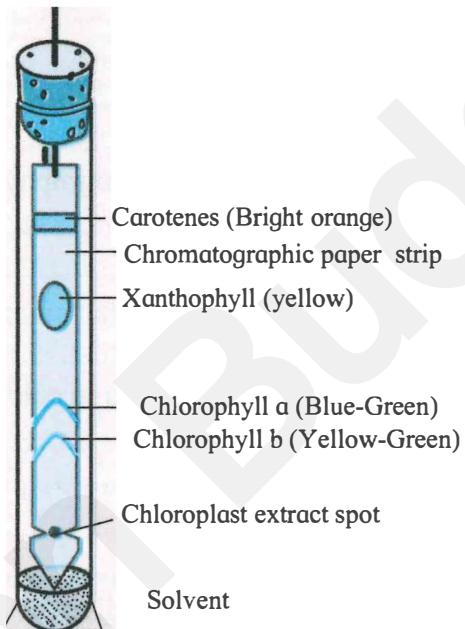
These photosynthetic pigments can be separated using the technique of paper chromatography.

### Principle :-

The separation of solutes (chloroplast pigments) is based on the liquid-liquid partitioning of pigments in paper chromatography. The partitioning takes place between the solvent (water) molecules (static phase) adsorbed to the cellulosic matter of the paper (capillary action) and organic (mobile) phase.

**Requirements :-** Chromatography chamber, chromatography paper (Whatman's filter paper no.1), pestle-mortar, capillary tube, muslin cloth and suitable solvent system, fresh spinach leaves, etc.

### Figure :-



Separation of chloroplast pigments by paper chromatography

### Procedure :

- Prepare solvent system - Petroleum ether : acetone (90:10), and pour it in the chromatography chamber and cover it tightly. Keep it undisturbed for 1 hour for saturation of the solvent system.
- Take few spinach leaves (5gms), wash and keep them dry.
- Grind these leaves in acetone (organic solvent) with a small amount of  $MgCl_2$ . (as a buffer)
- Filter it through muslin cloth and collect the filtrate.
- Cut suitable strip of chromatographic paper and mark the loading point with pencil. With the help of capillary tube, the extract or filtrate is repeatedly loaded at the loading point.
- Allow it to dry.
- Now place the strip in the solvent system in such a way that its lower tip gets dipped in the solvent.

- Take care not to dip it too close to the loaded spot. Cover the chamber and keep it undisturbed to get the chromatogram run.
- Let the solvent travel close to the other end of the chromatography paper. Different pigments carried along with the solvent up to different heights on the basis of their molecular weights.
- Take out the paper, allow it to dry and observe the separation of photosynthetic pigments in the form of separate bands of different colours.

**Caution :Perform the experiment in well ventilated laboratory.**

#### **Observation :**

On the strip we can observe the separation of photosynthetic pigments as bands of different colours in a particular sequence. From loaded spot up to the top the sequence is; chlorophyll-b (yellowish green), chlorophyll-a (bluish green), xanthophylls (yellow) and carotene (orange).

#### **Questions**

1. What is the composition of solvent used for separation of photosynthetic pigments?

The solvent used for the separation of photosynthetic pigments is Petroleum Ether and Acetone in the proportion 9 : 1.

2. Which pigment reaches to the top of chromatography paper?

Carotene reaches to the top of chromatography paper because it is highly soluble in the solvent and forms no hydrogen bonds with the chromatography paper fibres.

3. Which photosynthetic pigment is bluish green in colour?

The bluish green photosynthetic pigment is - chlorophyll-a

4. Why acetone and  $MgCl_2$  are used while grinding the leaves?

Acetone being amphipathic (with both polar and non-polar ends) can dissolve both polar and non-polar substances.  $MgCl_2$  is used to remove water from the leaves.

#### **Activity**

1. Find out the concept of retardation factor and RF value in chromatography.

Rf = distance traveled by spot/distance traveled by the solvent (Note Rf value is always less than 1).

The Rf of component A and B can be determined as:

Rf of component A =  $d_2/d_1$

Rf of component A =  $d_3/d_1$ ,

The Rf value of any substance may be about the same whenever we use that particular solvent at a given temperature. However, the Rf value of a substance differs in different solvents and at different temperatures.

**Remark and Signature of Teacher .....**