

Study no. - 08

Name of the study: Study on determination of R_f values of chloroplast pigments by paper chromatography

Paper chromatography: Chromatography is a technique by which the components of a mixture can be separated out and the components thus separated are usually identified by their colours and/or their R_f values. There are two phases of chromatography, namely, the stationary phase and the mobile phase. In paper chromatography, the stationary phase is the chromatographic paper and the mobile phase is the solvent. The components of a mixture are separated out by their relative affinity to the phases. An index showing the relative affinity of a component; is called relative front (R_f), which is the ratio of the distance travelled by the component to the distance travelled by the solvent.

$$R_f \text{ value} = \frac{\text{Distance traveled by the component}}{\text{Distance traveled by the solvent}}$$

The chloroplast of higher plants contains four major types of pigments, these pigments are fat-soluble. These different types of chloroplast pigment are in the following table:

Name	Formula	Color
Chlorophyll a	$C_{55}H_{72}O_5N_4Mg$	Bright green
Chlorophyll b	$C_{55}H_{70}O_6N_4Mg$	Pale green
Carotene	$C_{40}H_{56}$	Orange
Xanthophyll	$C_{40}H_{56}O_2$	yellow

Materials required:

1. Litchi leaf
2. Acetone
3. Ethanol (Ethyl alcohol)
4. Morter
5. Pestle
6. Chromatographic paper
7. Test tube
8. Scissors
9. Test tube stand
10. Beaker
11. Measuring cylinder
12. Filter paper
13. Funnel
14. Scotch tape
15. Measuring scal

Procedure:

- (1) Some litchi leaves were taken in a mortar along with acetone and ethanol (at the ratio of 2:1). Then the leaves were crushed well with the help of a pastle.
- (2) The solution thus obtained was filtered and taken in a test tube. A strip of chromatographic paper was put in the test tube and the apparatus was kept undisturbed.
- (3) After about an hour, the paper strip was picked up and it was dried in the air.
- (4) The distances travelled by the pigments and the solvent along the paper strip were measured to obtain the R_f values.

Calculation:

R_f of chl a =

R_f of carotene =

R_f of chl b =

R_f of xanthophyll =

Results:

R_f of chl a =

R_f of chl b =

R_f of carotene =

R_f of xanthophyll =

Precautions:

- (1) The paper strip must be kept undisturbed during the components moved along it.
- (2) The measurements should be accurate enough.

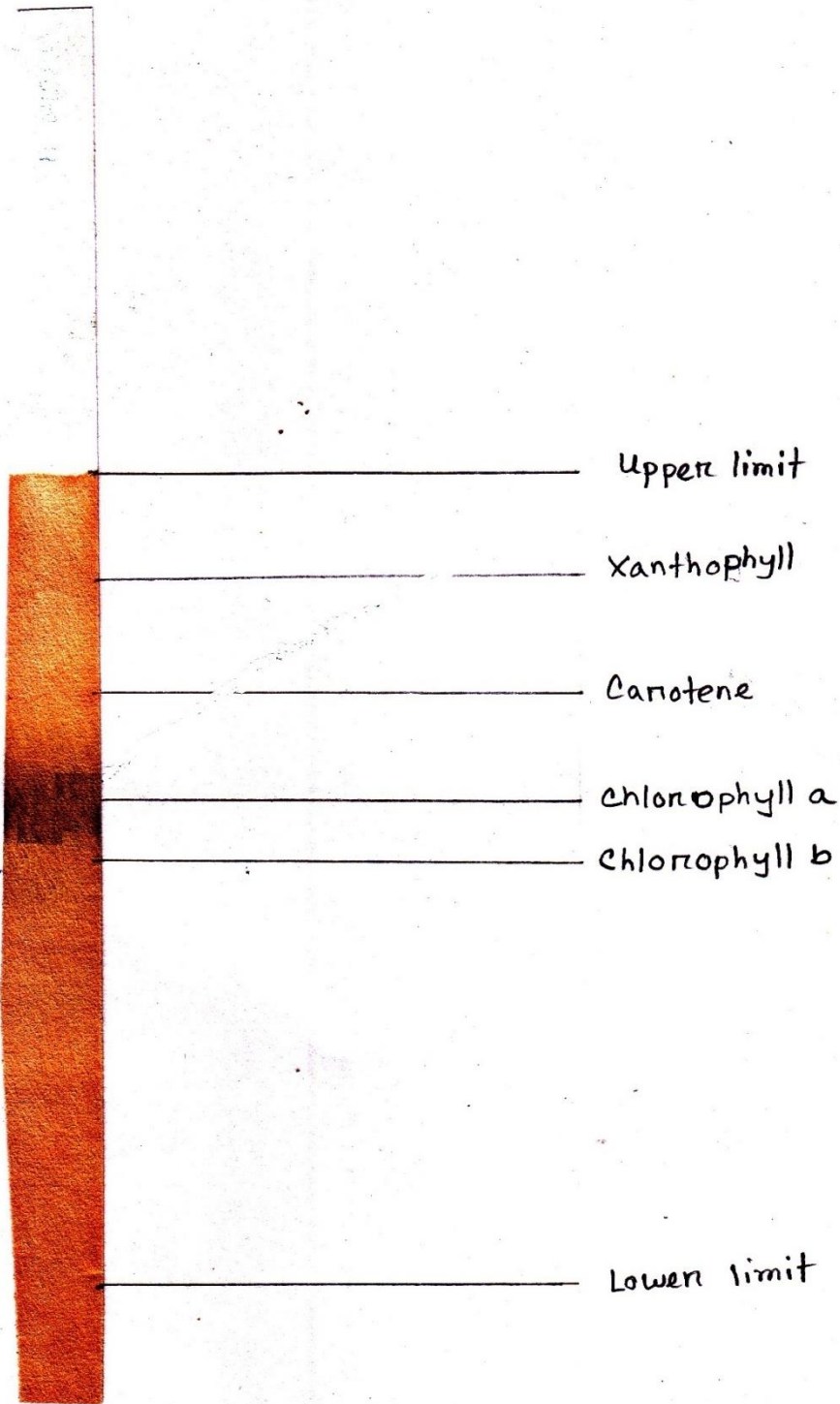


Fig: Determination of R_f values of chloroplast pigments by paper chromatography