Paper Chromatography

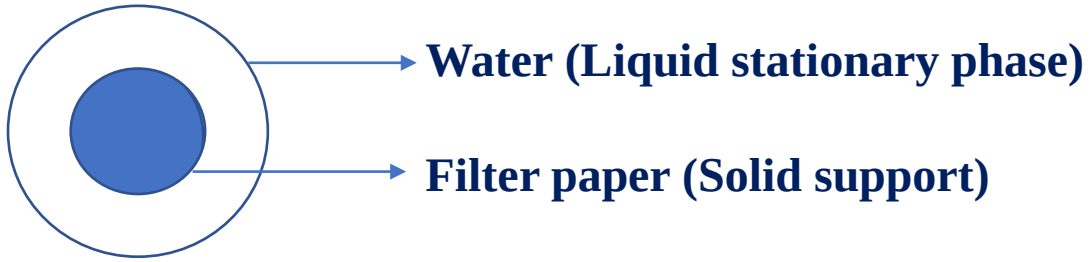
Separation based on Solubility of molecules

Paper Chromatography

- Paper chromatography (PC) is a type of a planar chromatography whereby chromatography procedures are run on a specialized paper
- PC is considered to be the simplest and most widely used of the chromatographic techniques because of its applicability to isolation, identification and quantitative determination of organic and inorganic compounds.
- It was first introduced by German scientist Christian Friedrich Schonbein (1865)
- Also known as Paper Partition Chromatography
- Moisture / Water present in the pores of cellulose fibers present in filter paper acts as stationary phase & another mobile phase is used as solvent

Principle of Paper Chromatography

- The principle of separation is mainly based on partition between the sample components due to differential solubility
- 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 mobile solution travels up the stationary phase carrying the sample with it
- Components of the sample will separate readily according to how readily they dissolve in the mobile phase



Instrumentation of Paper chromatography

1. STATIONARY PHASE AND PAPERS

- Whatman filter papers of different grades like No.1, No.2, No.3, No.4, No.20, No.40, No.42 etc
- In general the paper contains 98-99% of α-cellulose, 0.3 – 1% β -cellulose

Other modified papers

- Acid or base washed filter paper
- Glass fiber type paper.
- Hydrophilic Papers – Papers modified with methanol, formamide, glycol, glycerol etc.
- Hydrophobic papers – acetylation of OH groups leads to hydrophobic nature, hence can be used for reverse phase chromatography.

2. MOBILE PHASE

- Pure solvents, buffer solutions or mixture of solvents can be used

1. Hydrophilic mobile phase

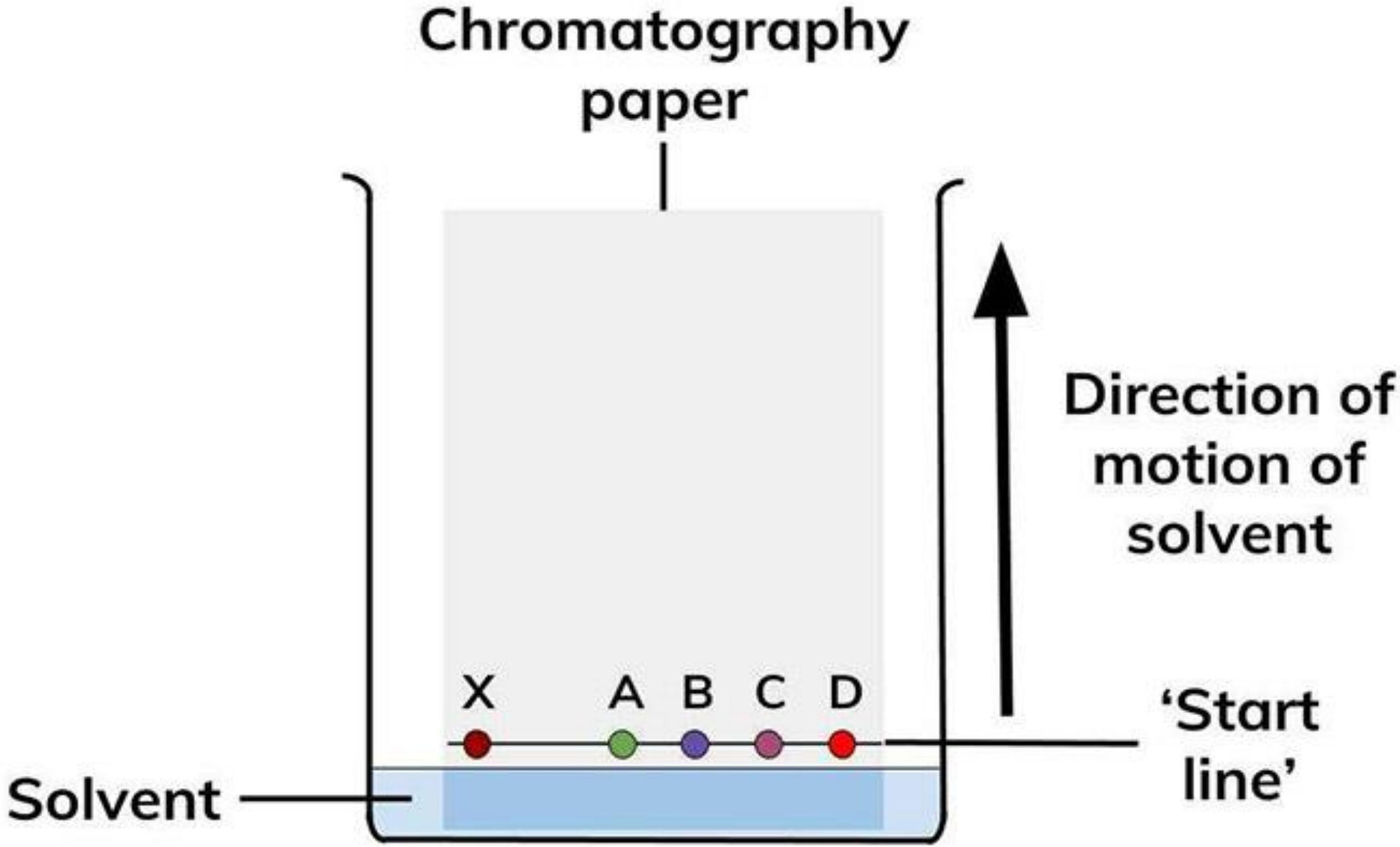
- Isopropanol: ammonia : water 9:1:2
- Methanol : water 4:1
- N-butanol : glacial acetic acid : water 4:1:5

2. Hydrophobic mobile phases

- dimethyl ether: cyclohexane kerosene : 70% isopropanol
- The commonly employed solvents are the polar solvents, but the choice depends on the nature of the substance to be separated
- If pure solvents do not give satisfactory separation, a mixture of solvents of suitable polarity may be applied

3. CHROMATOGRAPHIC CHAMBER

- **The chromatographic chambers are made up of many materials like glass, plastic or stainless steel**
- **Glass tanks are preferred most**
- **They are available invarious dimensional size depending upon paper length and development type**
- **The chamber atmosphere should be saturated with solvent vapor**


Chromatography paper
Direction of motion of solvent
X
A
B
C
D
'Start line'
Solvent

Steps in Paper Chromatography

In paper chromatography, the sample mixture is applied to a piece of filter paper, the edge of the paper is immersed in a solvent, and the solvent moves up the paper by capillary action. The basic steps include:

1. Selection of Solid Support

- Fine quality cellulose paper with defined porosity, high resolution, negligible diffusion of sample and favouring good rate of movement of solvent.

2. Selection of Mobile Phase

- Different combinations of organic and inorganic solvents may be used depending on the analyte.

Example - Butanol: Acetic acid: Water (12:3:5) is suitable solvent for separating amino-acids

3. Saturation of Tank

- The inner wall of the tank is wrapped with the filter paper before solvent is placed in the tank to achieve better resolution.

4. Sample Preparation and Loading

- If solid sample is used, it is dissolved in a suitable solvent. Sample (2-20ul) is added on the base line as a spot using a micropipette and air dried to prevent the diffusion.

5. Development of the Chromatogram

- Sample loaded filter paper is dipped carefully into the solvent not more than a height of 1 cm and waited until the solvent front reaches near the edge of the paper

Different types of development techniques can be used:

a. ASCENDING DEVELOPMENT

- Like conventional type, the solvent flows against gravity.
- The spots are kept at the bottom portion of paper and kept in a chamber with mobile phase solvent at the bottom.

b. DESCENDING TYPE

- This is carried out in a special chamber where the solvent holder is at the top.
- The spot is kept at the top and the solvent flows down the paper.
- In this method solvent moves from top to bottom so it is called descending chromatography.

c. ASCENDING – DESCENDING DEVELOPMENT

- A hybrid of above two techniques is called ascending-descending chromatography.
- Only length of separation increased, first ascending takes place followed by descending.

d. CIRCULAR / RADIAL DEVELOPMENT

- Spot is kept at the centre of a circular paper.
- The solvent flows through a wick at the centre & spreads in all directions uniformly.

6. Drying of Chromatogram

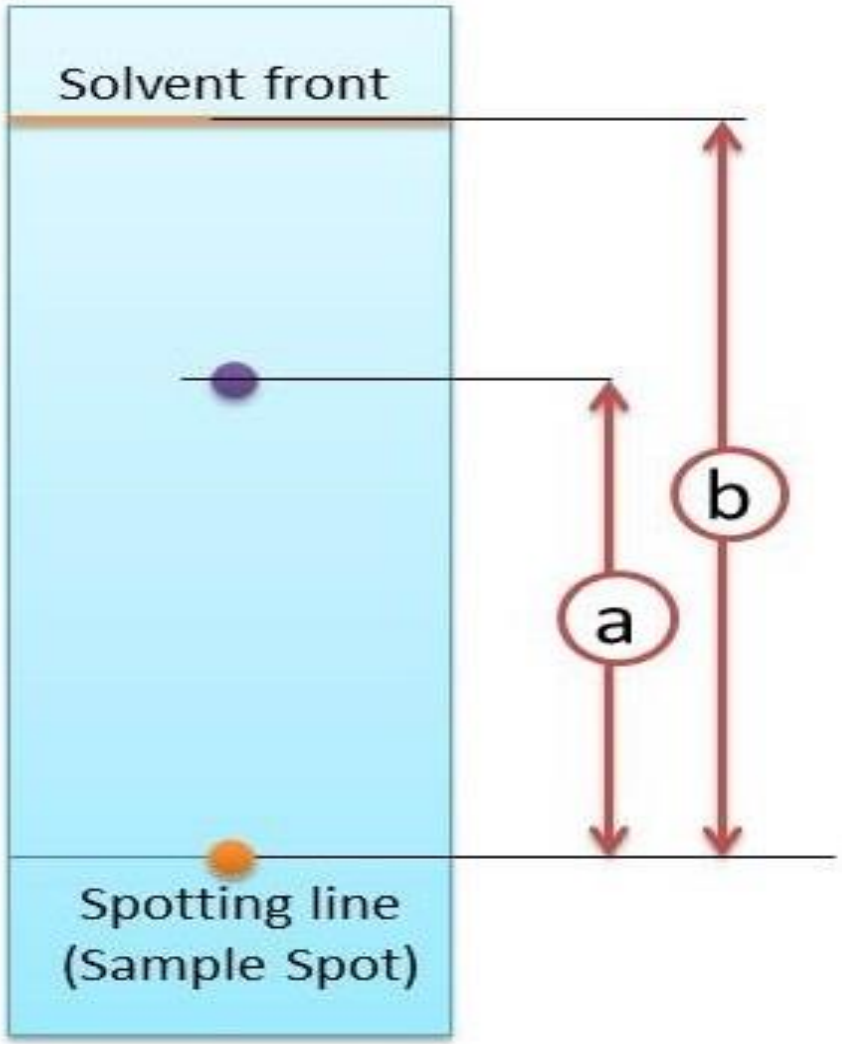
After the development, the solvent front is marked and the left to dry in a dry cabinet or oven

7. Detection

- Colourless analytes detected by staining with reagents such as iodine vapour, ninhydrin etc.
- Radiolabeled and fluorescently labeled analytes detected by measuring radioactivity and florescence respectively.

Rf values

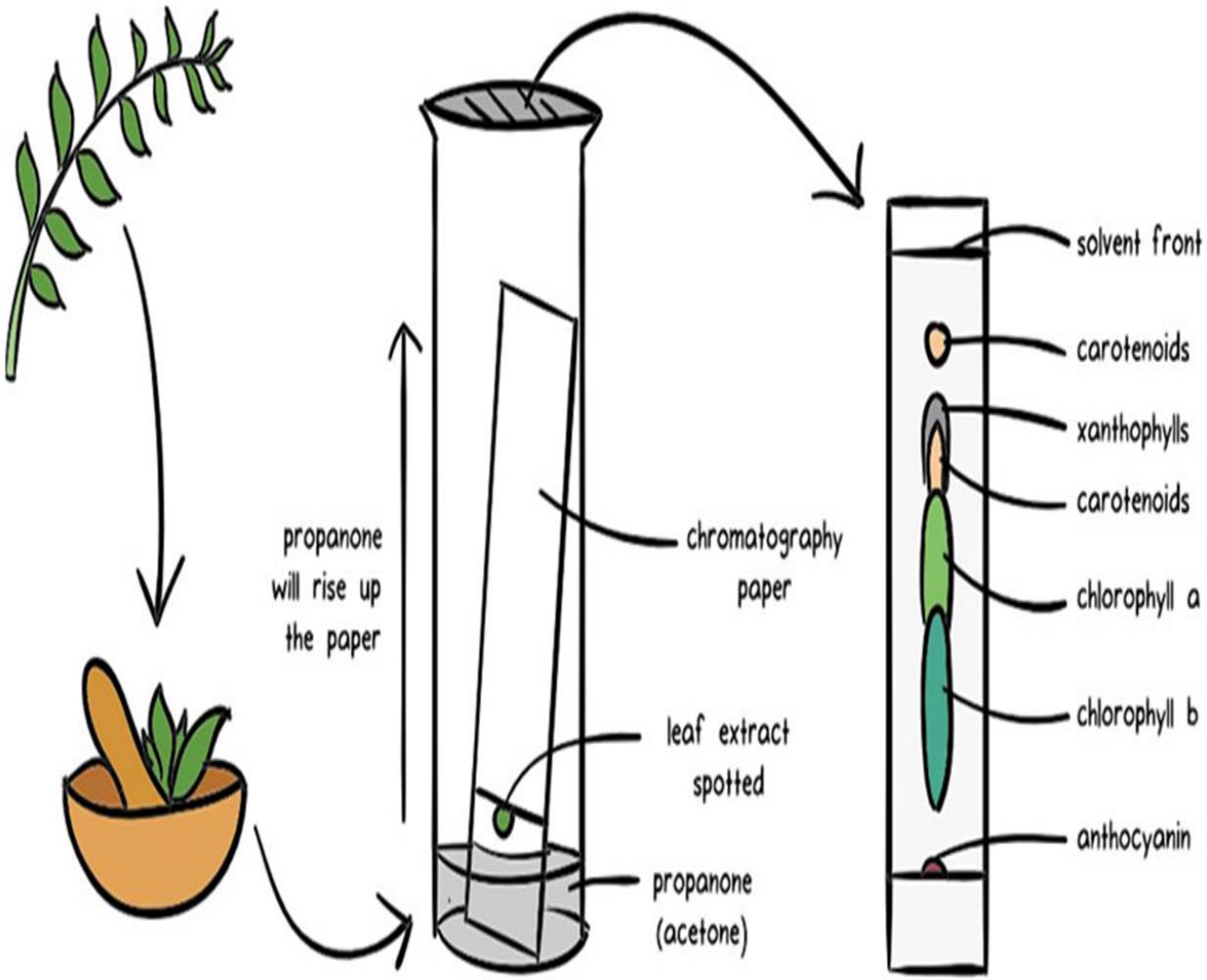
- Some compounds in a mixture travel almost as far as the solvent does; some stay much closer to the base line
- The distance travelled relative to the solvent is a constant for a particular compound as long as other parameters such as the type of paper and the exact composition of the solvent are constant
- The distance travelled relative to the solvent is called the Rf value

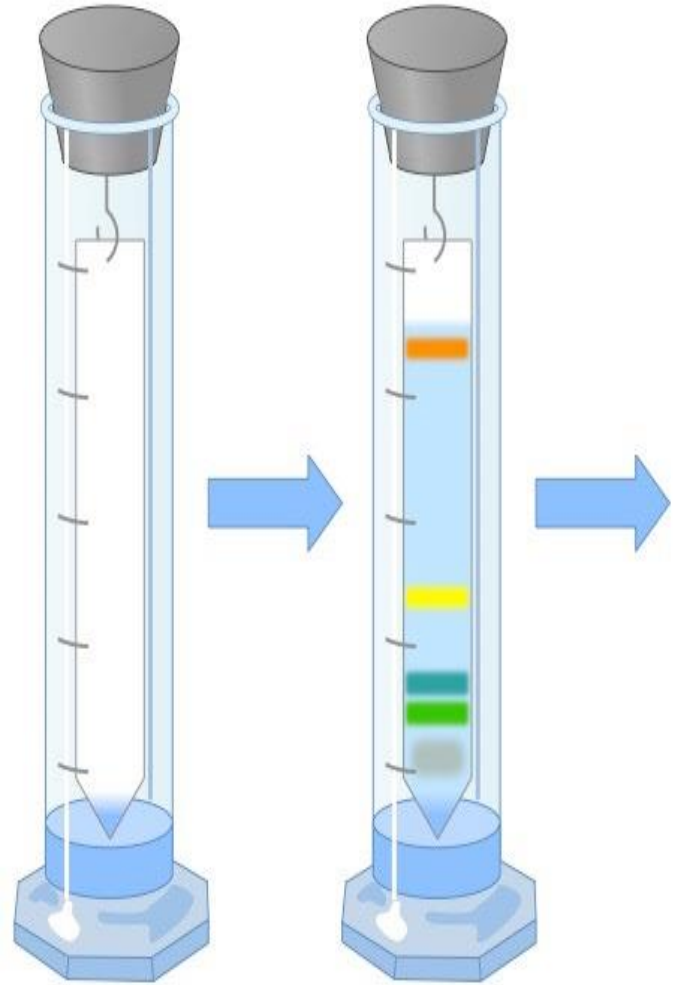


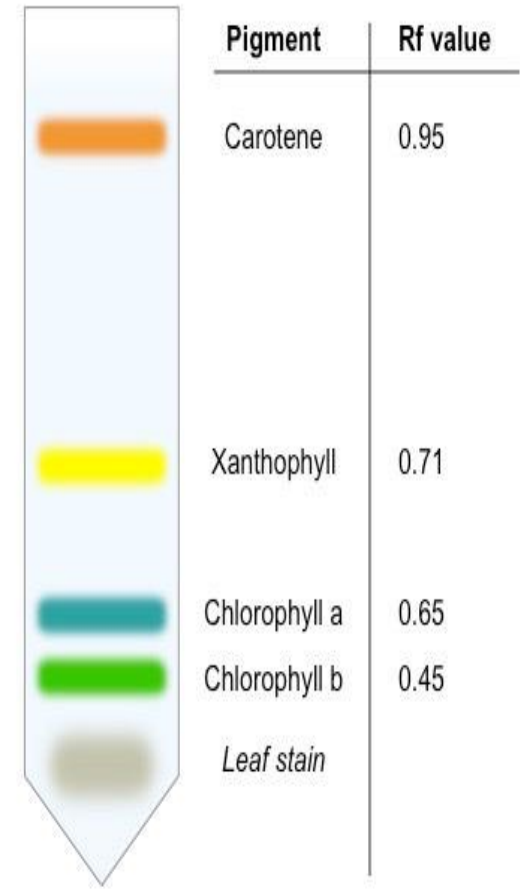
$$Rf = \frac{\text{distance travelled by the component}}{\text{distance travelled by the solvent}}$$

$$= \frac{a}{b}$$

Calculating the Retention Factor Value







Pigment	Rf value
Carotene	0.95
Xanthophyll	0.71
Chlorophyll a	0.65
Chlorophyll b	0.45
Leaf stain	

Applications of Paper Chromatography

- To check the control of purity of pharmaceuticals
- For detection of adulterants
- Detect the contaminants in foods and drinks
- In the study of ripening and fermentation
- For the detection of drugs and dopes in animals & humans
- In analysis of cosmetics
- Analysis of the reaction mixtures in biochemical labs

Advantages of Paper Chromatography

- Simple
- Rapid
- Requires very less quantitative material
- Cheaper compared to other chromatography methods
- Both unknown inorganic as well as organic compounds can be identified by paper chromatography method
- The technique does not occupy much space compared to other analytical methods or equipments
- Excellent resolving power

Limitations of Paper Chromatography

- Large quantity of sample cannot be applied on paper chromatography
- In quantitative analysis paper chromatography is not effective
- Complex mixture cannot be separated by paper chromatography
- Less Accurate compared to HPLC or HPTLC