T.Y.B.SC. INORGANIC CHEMISTRY

PRACTICAL SEM-II

ONLINE LECTURE NO. 3

CHROMATOGRAPHIC ANALYSIS

DATE:- 18, MAY 2021

TIME: (9.00 A.M.)

Chromatography

Precipitation→ Difference in solubility

Solvent extraction→ Difference in solubility

Distillation→ Difference in volatility

Sublimation→ Difference in vapour pressure

Floatation→ Difference in solubility or wetting tendency

Crystallization→ Difference in solubility at different temperatures

Dialysis→ Difference osmotic flow through membrane

Chromatography→ Difference in solubility or adsorption rate

These traditional methods applied for analysis in those cases where large amount of sample is available, but for very close solubility's and close boiling points chromatography method is used.

Mikhail Tswett in 1906, separated the plant pigments such as chlorophyll and xanthophylls from leaf extract with the column of calcium carbonate then colour bands are obtained.

Chromatography can be defined as a technique for the separation of the mixture of solutes in which separation is brought about by differential movement of the individual solutes through a porous medium under the influence of a moving solvent.

Chromatography is a modern technique used for the separation of components of the mixture or as an analytical technique used for the separation and purification of organic and inorganic substances. This technique is also useful for the fractionation of complex mixtures, separation of closely related compounds such as isomers and in the isolation of unstable substances. Mikhail Tswett (a botanist and physical chemist) Greek –Khromatos-means colour and graphos means writing. i.e. Colour writing. But now colourless components can be analyzed and hence the name is misnomer.

Chromatography is the analytical tool to separate the components of the mixture and provide qualitative and quantitative information of each component. In chromatographic methods, out of two phases used, one is stationary and other mobile. The Separation depends on the relative motion of the two phases.

Advantages: -

1) Non tedious and very perfect method.

2) A mixture of close similarity components in their chemical properties can be easily separated.

3) Detects and estimates very low concentration in micrograms.

4) Simple apparatus is required. For distillation, complicated equipments are necessary and at the time of distillation, substances are likely to be decomposed by heating. But chromatography is carried out at room temperature.

5) Used to monitor the progress of reaction.

6) Used for separation, identification, purification and detection.

7) Requires very small quantity for analysis.

Drawbacks: -

1) Time consuming technique.

2) Skillful operations are very essential.

3) Selection of suitable solvent is a difficult job, which can be done by trial and error method.

4) This technique is generally incomplete, reversible, resulting in poor separation.

5) Chemicals should be available in high state of purity.

6) Adsorption phenomena may decomposers the substances.

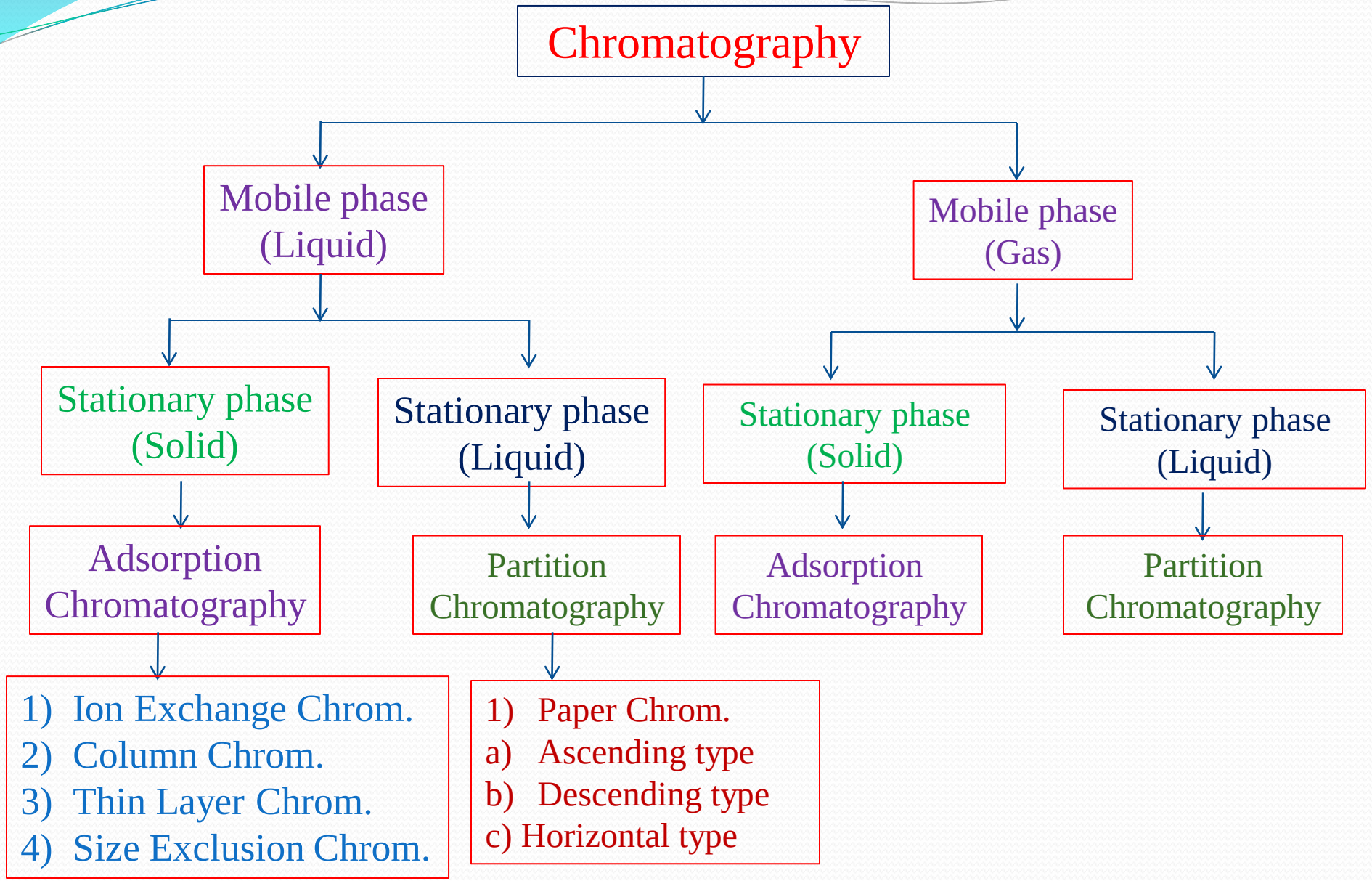
General principle: - Separation is achieved by differential distribution of components of mixture between two phases. i. e. One is held more strongly by the stationary phase than the other, which tends to move on faster in the mobile phase. i. e. Because of the retention of different kind of molecules from the mixture towards one of the phases. Thus, in this technique one of the components of the mixture is retained by the mobile phase and another component is retained by the stationary phase.

Phases in Chromatography: -

1) Stationary (Non mobile) → May be liquid held on suitable inert solid support or solid (adsorption).

2) Moving/ Mobile→ May be liquid or gas (solvent phase).

The chromatographic methods of separation are classified on the basis of stationary and mobile phases used.


Chromatography
Mobile phase (Liquid)
Mobile phase (Gas)
Stationary phase (Solid)
Stationary phase (Liquid)
Stationary phase (Solid)
Stationary phase (Liquid)
Adsorption Chromatography
Partition Chromatography
Adsorption Chromatography
Partition Chromatography
1) Ion Exchange Chrom.
2) Column Chrom.
3) Thin Layer Chrom.
4) Size Exclusion Chrom.
1) Paper Chrom.
a) Ascending type
b) Descending type
c) Horizontal type

The theory of chromatography is based on two physico-chemical concepts. Viz. Adsorption and Partition.

Adsorption chromatography: - The separation of the constituents of the mixture depends on the different adsorption affinities. The constituents which are adsorbed least strongly, move rapidly than those adsorbed more strongly. Hence, the Separation is achieved. The technique in which adsorption is the basic principle is known as adsorption chromatography.

Partition chromatography: - In partition chromatography, the separation of the constituents of the mixture depends on the different solubility's in two different phases. i. e. Stationary phase and moving phase. In paper chromatographic technique, both the phases are liquid. The moisture which is adsorbed on the cellulose paper is a stationary phase while the solvent used for the separation is a moving phase. The constituents which are more soluble in moving phase move rapidly than those least soluble in moving phase.

Chromatographic separation can be carried out by the distribution of components in a mixture between the two phases which are:

1) Stationary phase (Non mobile phase) may be liquid or solid.

2) Moving phage (Mobile phase or solvent phase) may be liquid or gas.

These two phases are responsible for the separation process. The separation of components in the mixture is achieved by the differential distribution of components of the mixture between the mobile and stationary phase. i. e. Separation between two substances begins to take place when one is held more strongly by the stationary phase than the other, which tends to move on faster in the mobile phase. The mobile phase may be known as solvent.

Paper chromatography: - When the separation is carried out on cellulose paper, the technique is known as paper chromatography. Cellulose is so hydrophilic that it normally holds a coating of water, through grossly imperceptible adsorbed from the air. This water layer forms a stationary phase.

The mobile phase is a mixture of one or more organic solvents and water. Whatman paper has a high degree of uniformity.

Paper chromatography: - This is a simple and widely used technique of separation. It is microanalytical technique which is useful for estimation of cations containing less than 10 microgram of the sample. In this case, both mobile and stationary phases are liquid. Filter paper is used as a support for the stationary liquid phase. A thin film of water is adsorbed on the cellulose molecules acting as stationary phase. The mobile phase is also liquid which is moving over the stationary phase by capillary action. The mobile phase is usually a mixture of water and organic solvent. The paper chromatography is partition chromatography. The separation is based on the difference in the distribution i. e. solubility of the solutes in the mixture in two liquid phases. The solutes are distributed between two liquids depending on their distribution coefficient. The two liquids are immiscible and solute which is more soluble in stationary phase moves slowly while which is more soluble in mobile phase moves fastly. Therefore, different cations move at different rates and appear at different distances on the filter paper. The cation is identified by spraying the reagent that form the coloured spot.

The results of chromatographic separation are expressed in terms of RF value. It is defined as the distance travelled by the given cation from the origin to the distance travelled by moving solvent.

RF value is the characteristic of each cation and different for different cations. The RF value is reproducible under identical experimental conditions. There are three types of paper chromatography depending on movement of solvent:

1) Ascending type - solvent moves upward.

2) Descending type - solvent moves downward and

3) Horizontal - solvent moves horizontal.

The steps of for ascending paper chromatography: -

1) Saturating the cylinder with mobile phase.

2) Application of the sample on the paper.

3) Developing the chromatogram.

4) Location of the spots of different cations.

5) Identification of cations.

6) Calculation of RF value

Precautions: - 1) The line of solution drawn should be homogeneous throughout and it should not spread.

2) The process of applying of solution may be repeated for 4 to 5 times in order to increase the concentration of constituents of the solution on the paper.

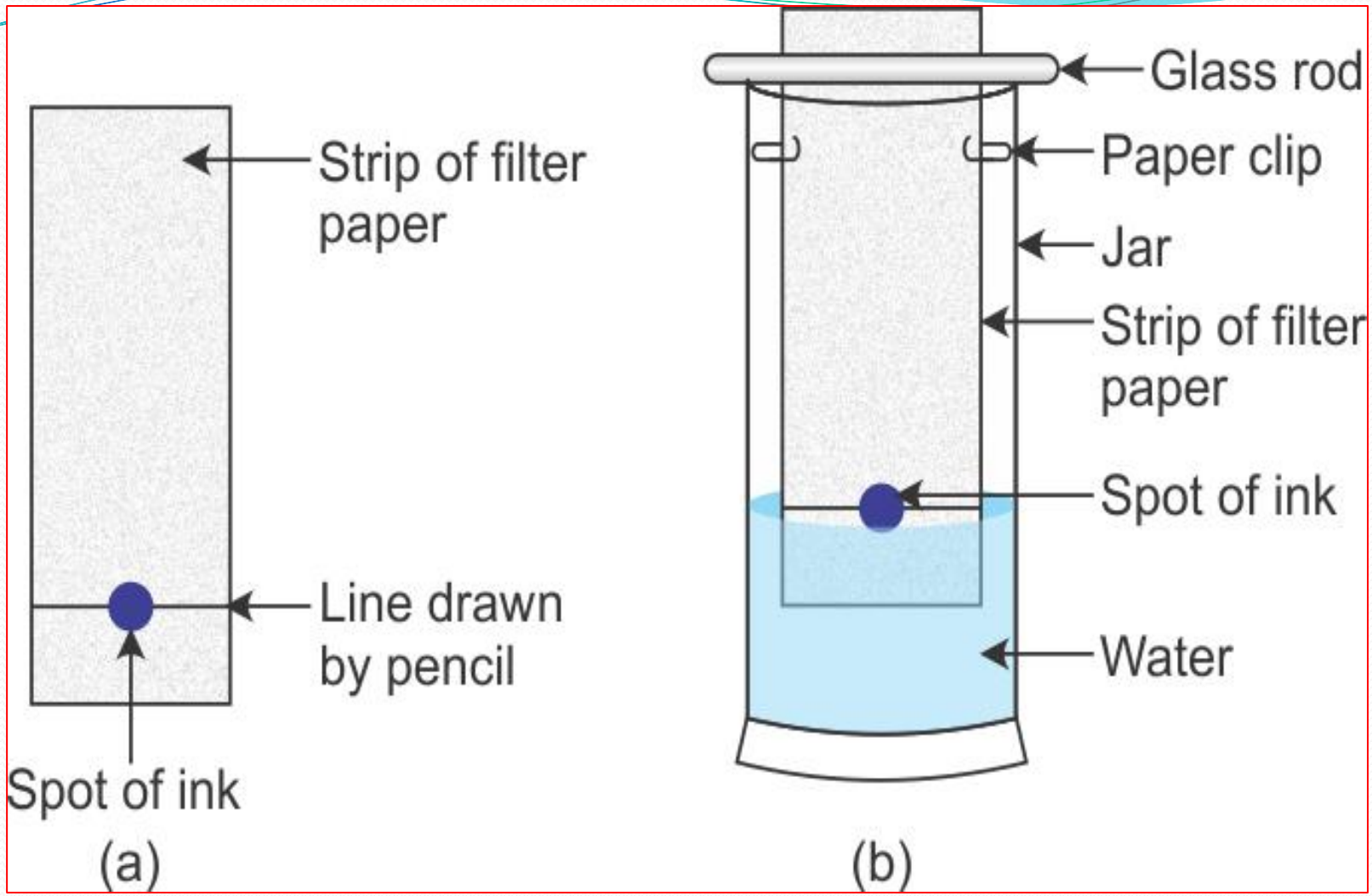
The solvent continuously rises up the paper strip by capillary action, carrying the constituents of the sample along with it at various speeds, according to their partition coefficients.

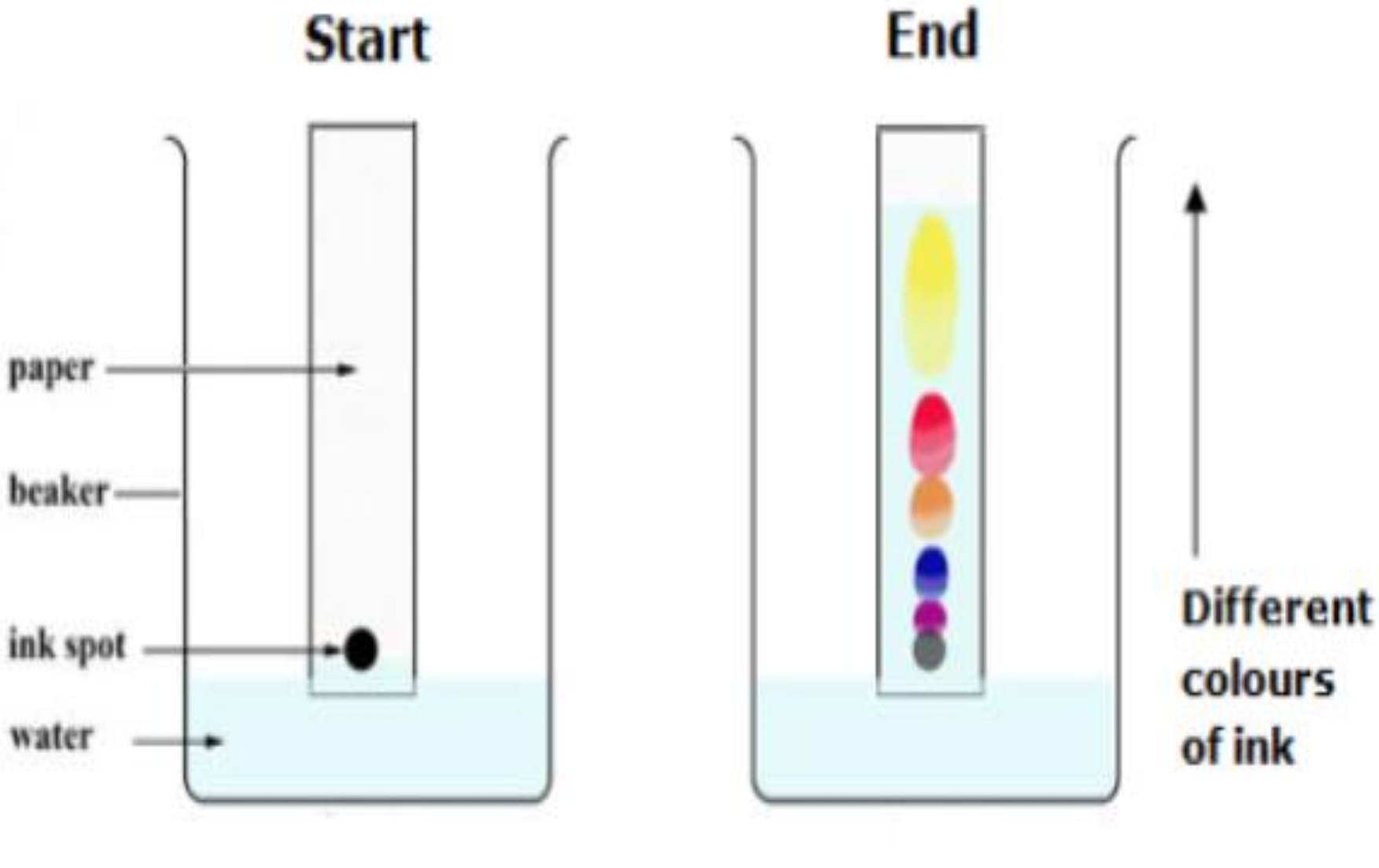
RF value: - (Rate of flow or retardation factor)

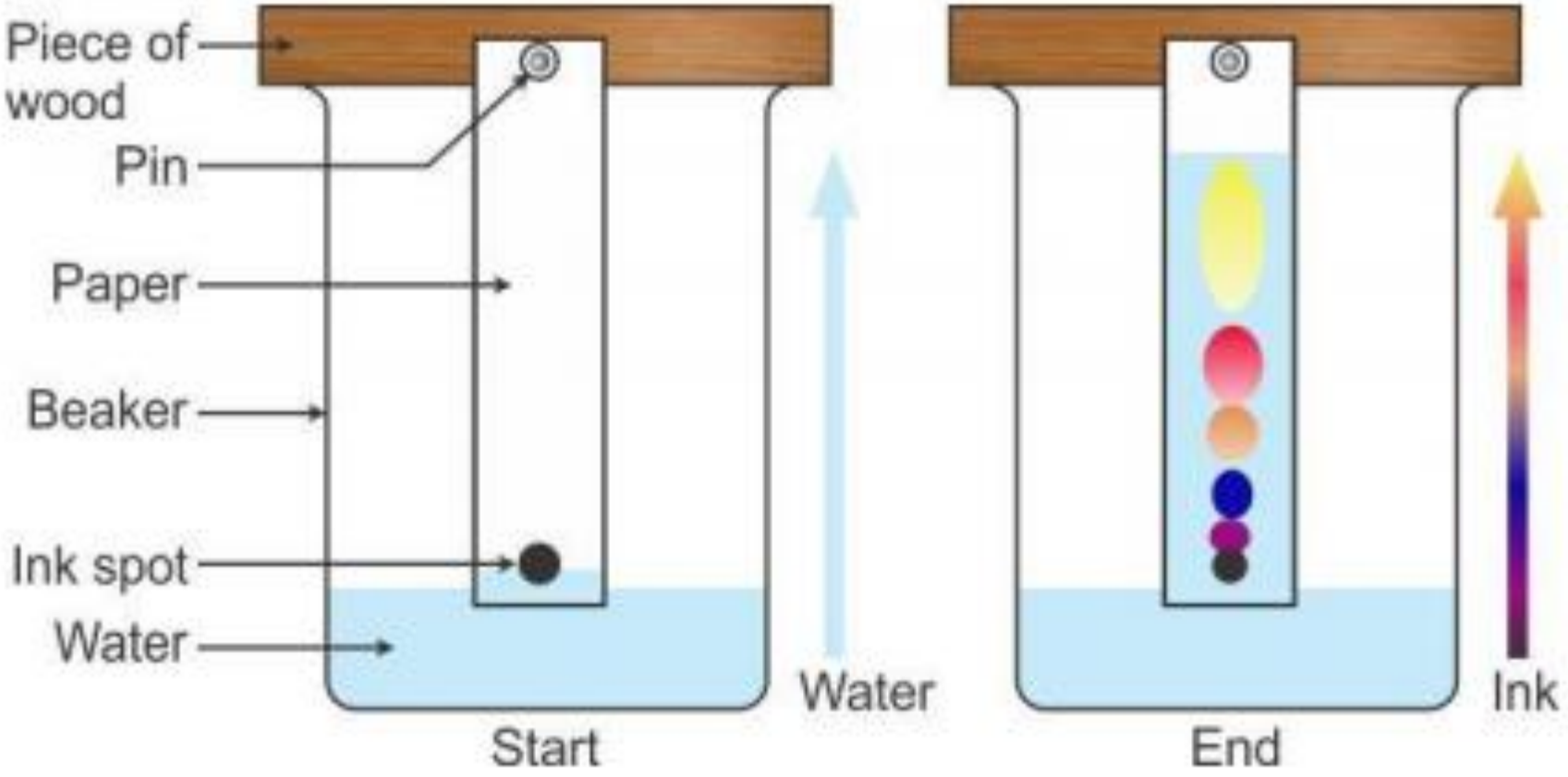
The results are represented by RF values. The movement of any substance relative to the solvent front in a given chromatographic system is characteristic of the substance. The constant is known as RF value. This RF value expresses the relative rate of movement of solute and solvent. RF value is defined as the ratio of the distance travelled by the compound at its point of maximum concentration to the distance travelled by the solvent. Both the distances are measured from the point of application of the sample. RF value has no units.

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

It is to be noted that RF value is always less than Unity. The RF value is the characteristic, reproducible property of a compound. RF values of different compounds are entirely different. RF value is identifying characteristic of the compound.

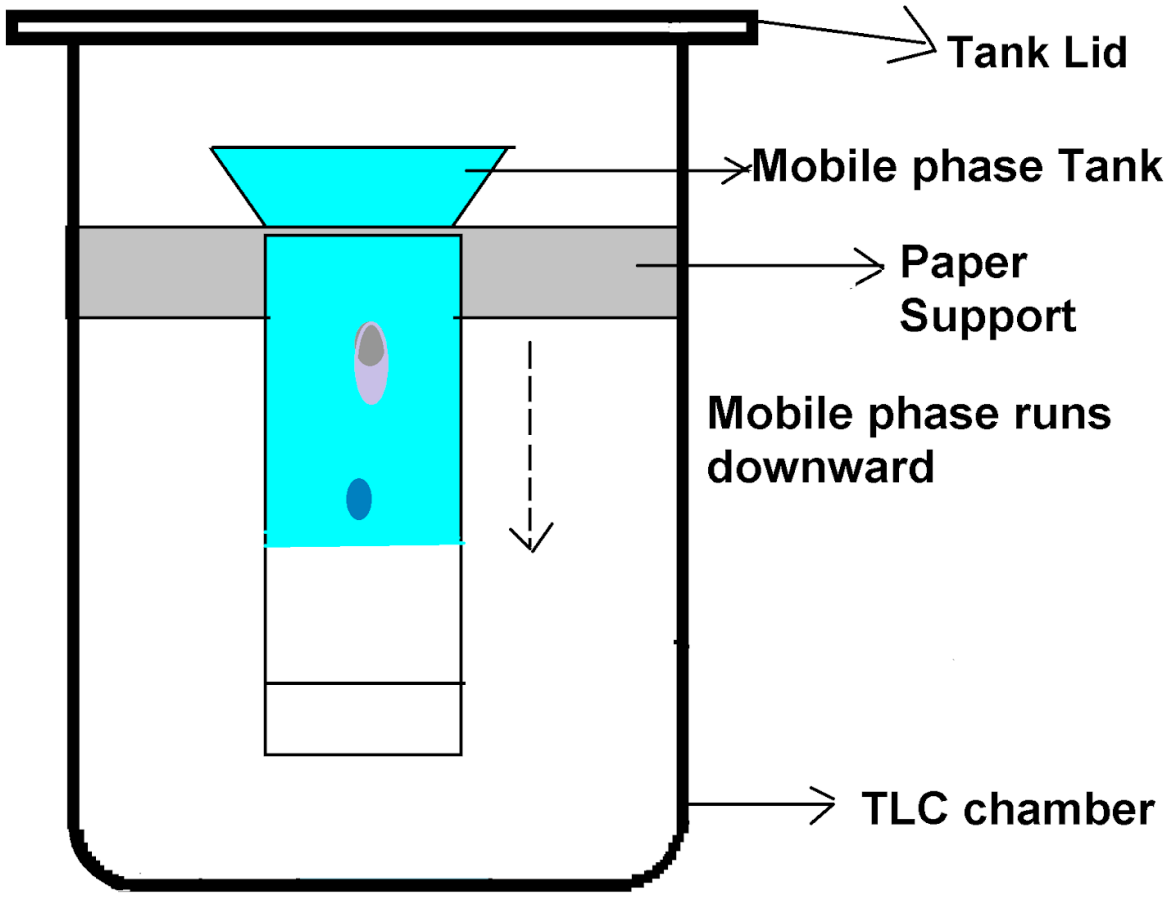

Strip of filter paper
Line drawn by pencil
Spot of ink
(a)
Glass rod
Paper clip
Jar
Strip of filter paper
Spot of ink
Water
(b)

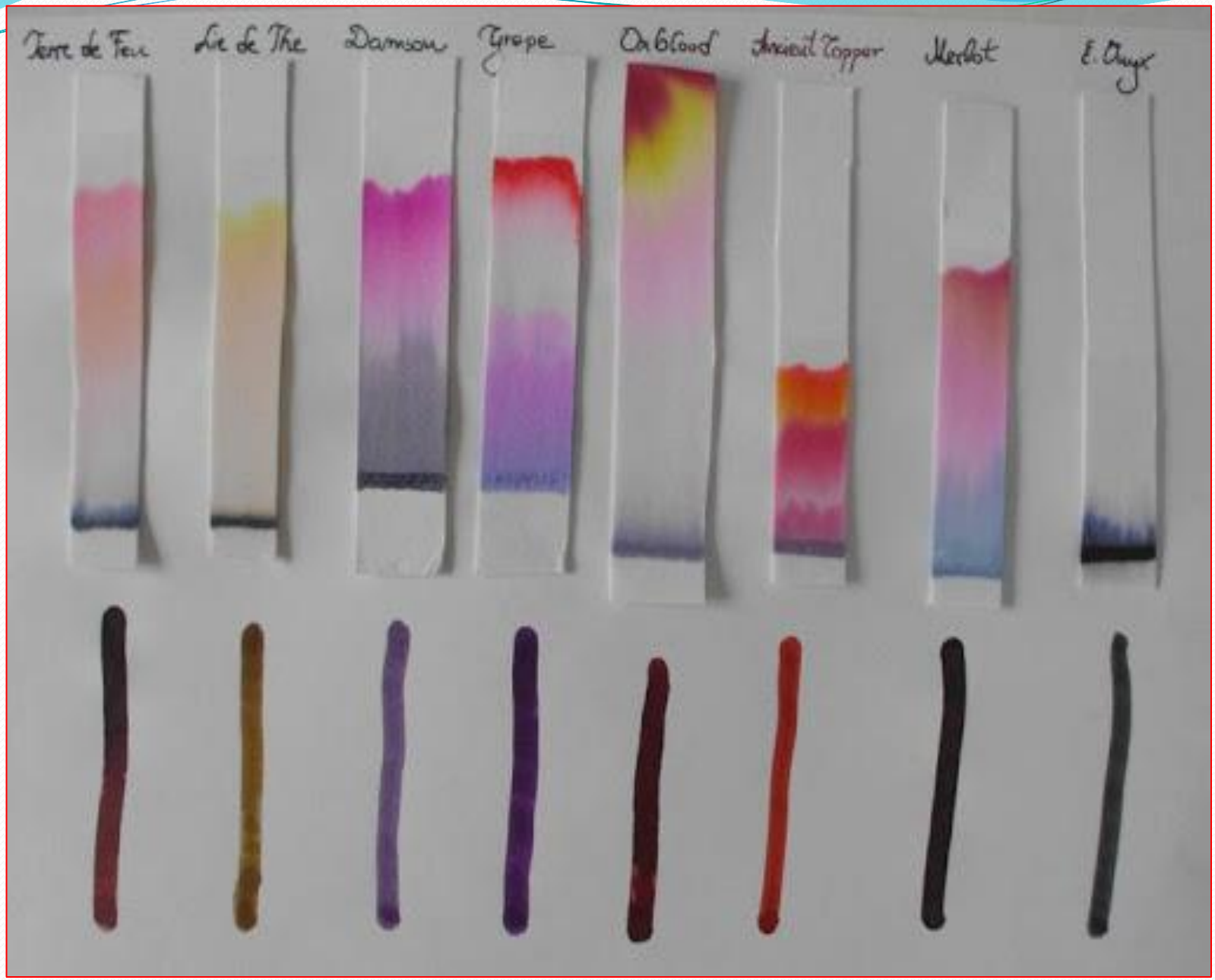

Start
End
paper
beaker
ink spot
water
Different colours of ink

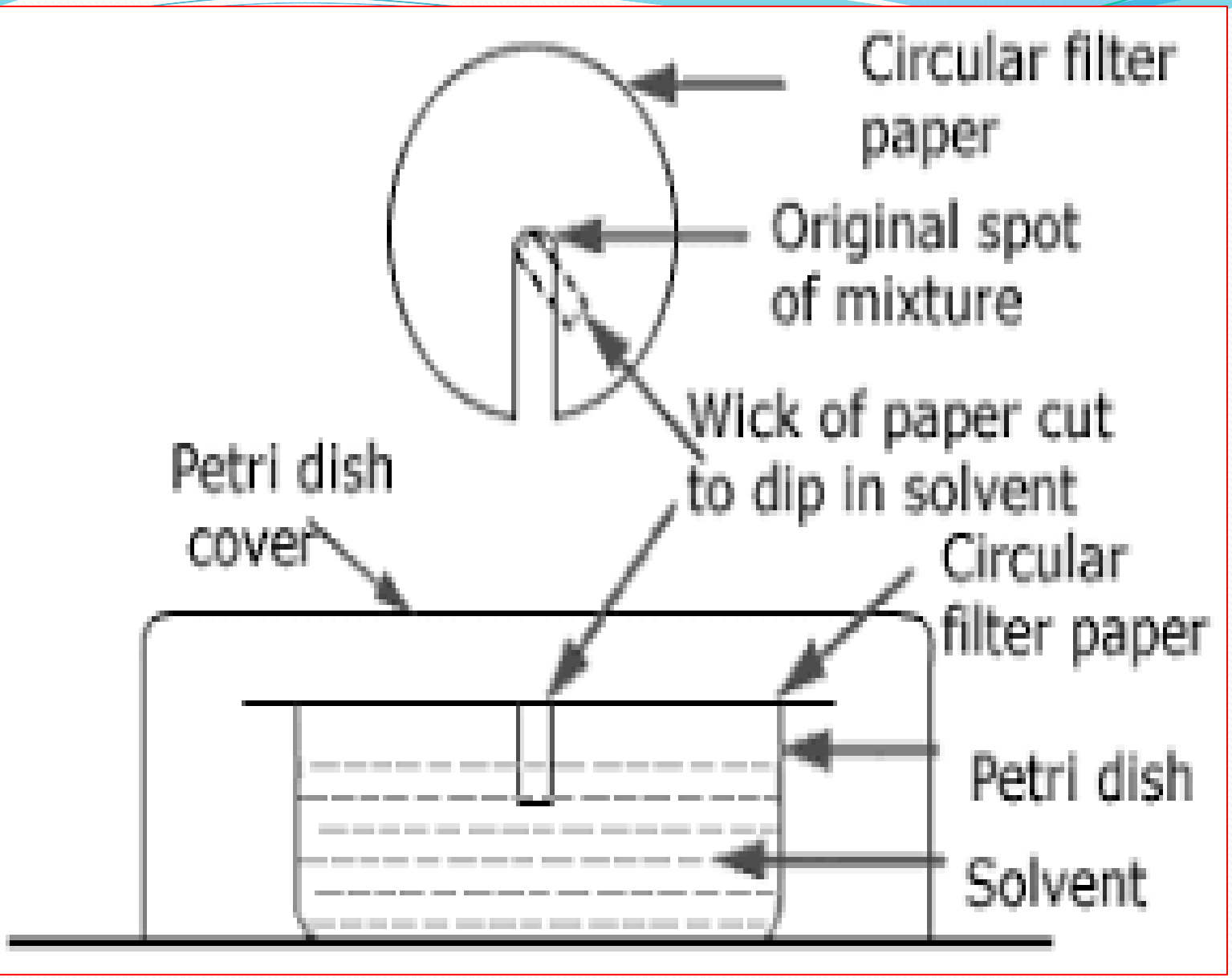


Paper Chromatography

Descending Chromatograpphy




Terre de Feu
Lie de The
Damson
Grape
Ox Blood
Ancient Copper
Merlot
E. Onyx


Circular filter paper
Original spot of mixture
Wick of paper cut to dip in solvent
Petri dish cover
Circular filter paper
Petri dish
Solvent

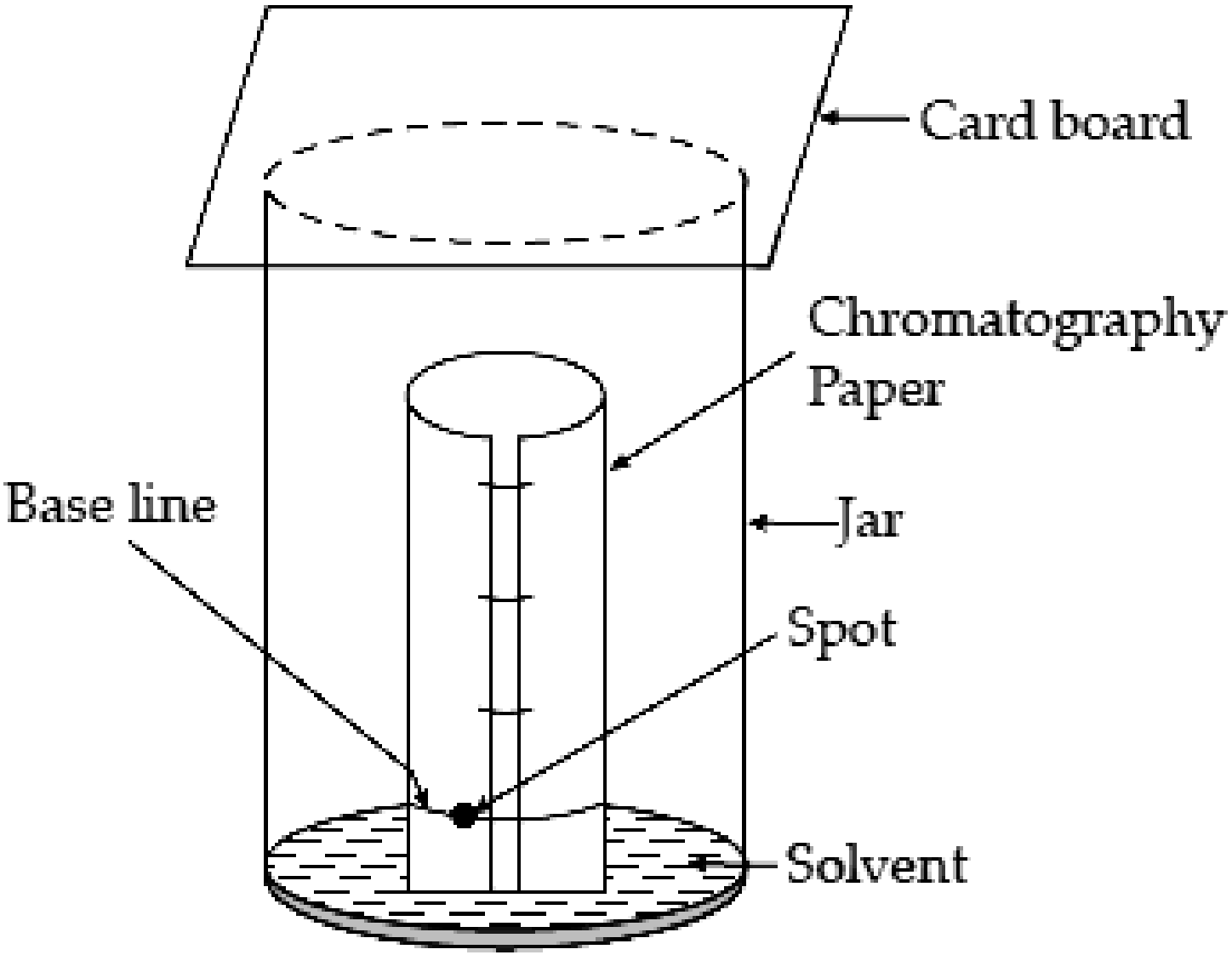
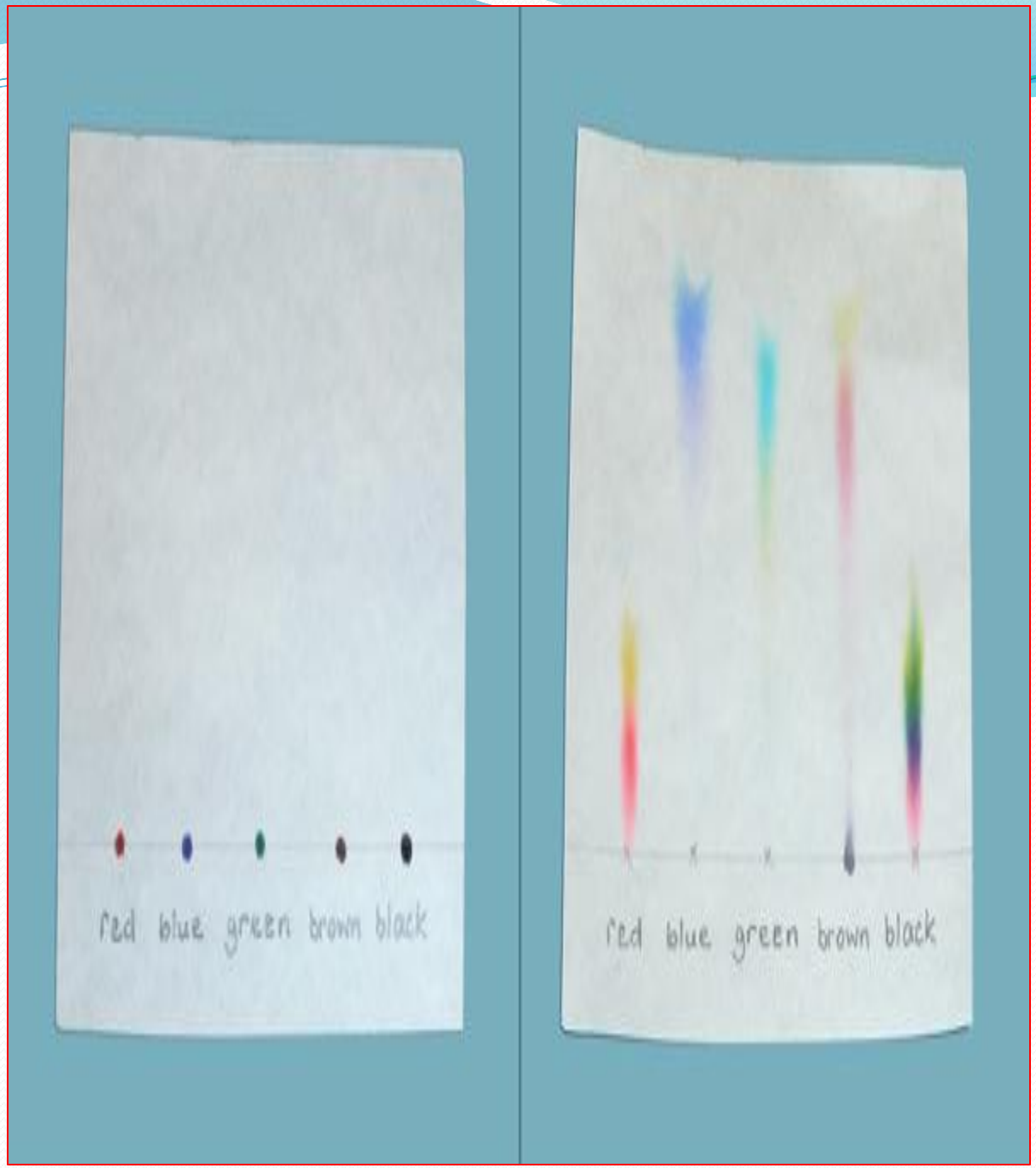
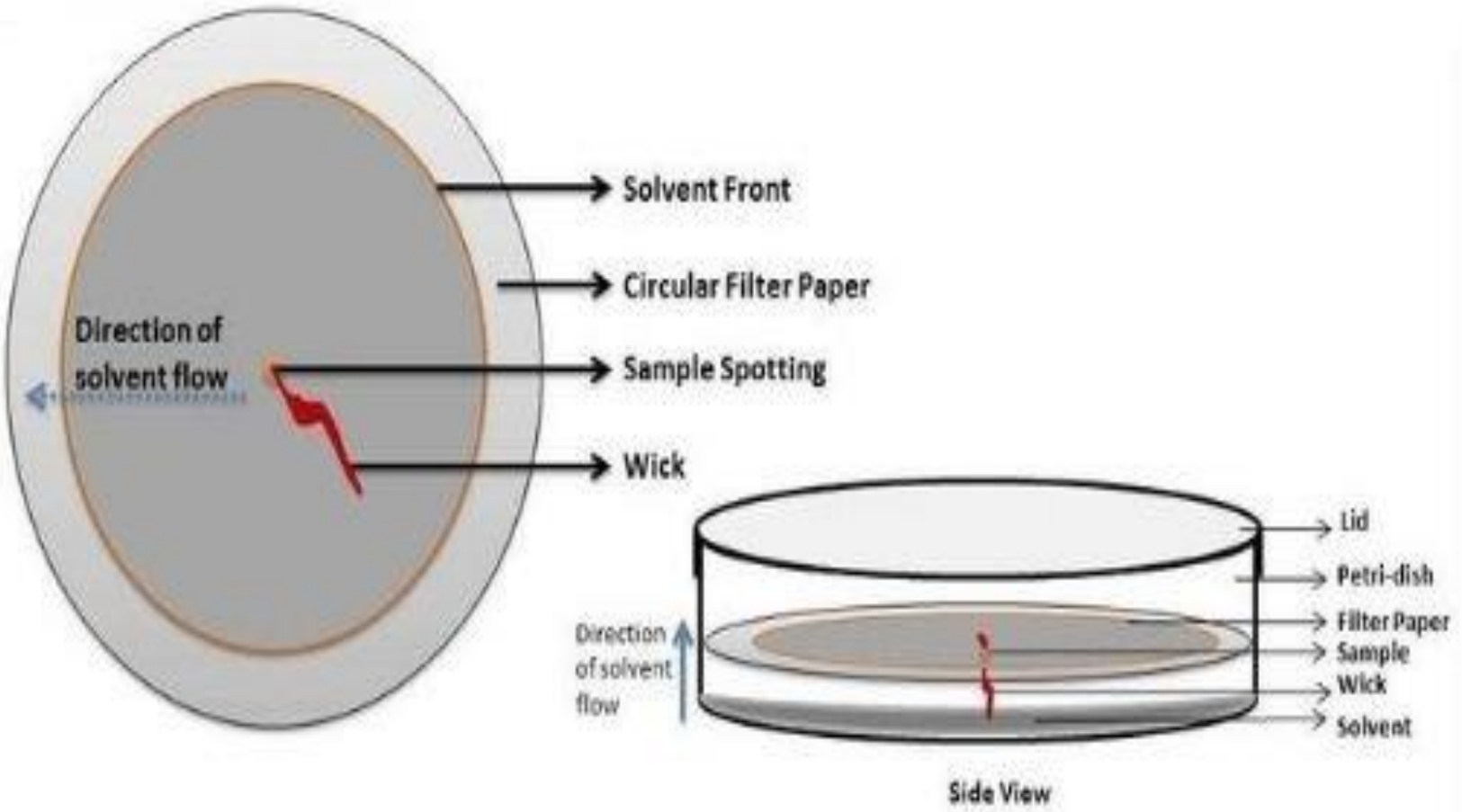
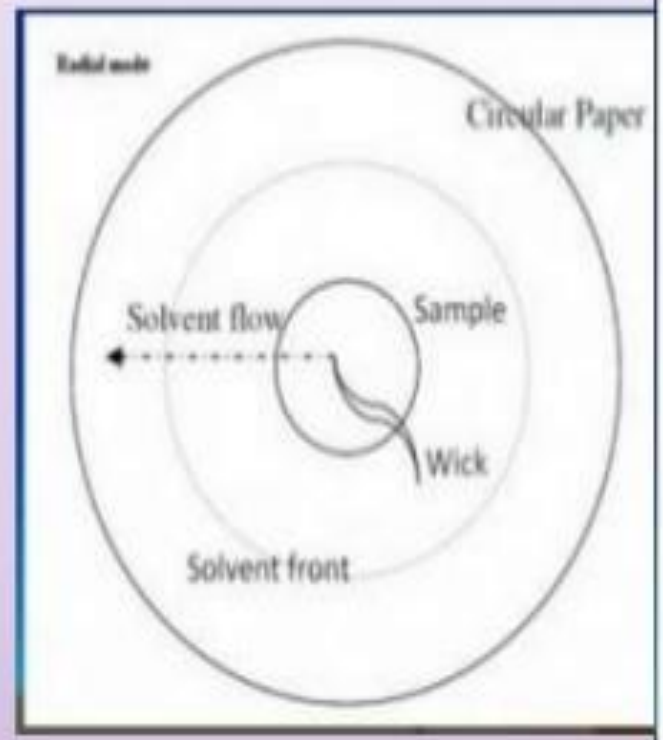


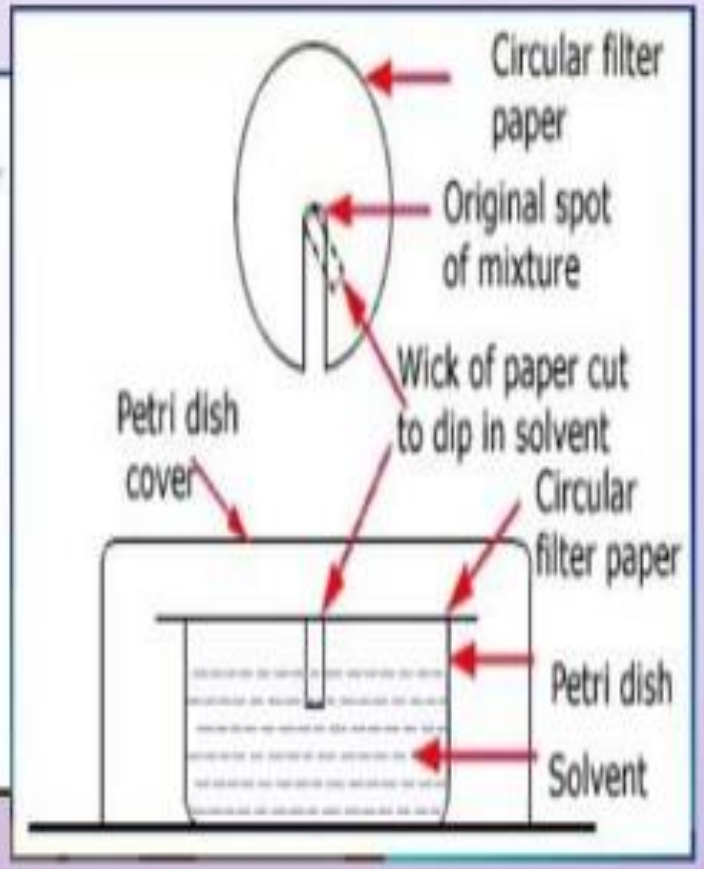
Fig. Paper Chromatography


red blue green brown black
red blue green brown black


Solvent Front
Circular Filter Paper
Direction of solvent flow
Sample Spotting
Wick
Lid
Petri-dish
Filter Paper
Sample
Wick
Solvent
Direction of solvent flow
Side View

Circular/Radial Development





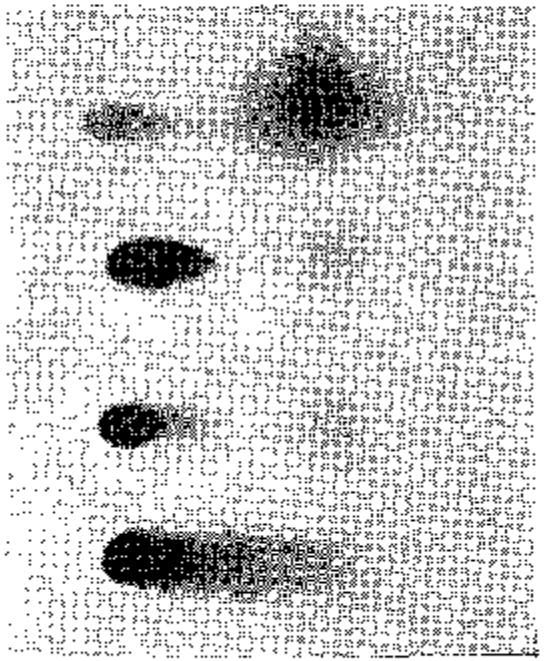


Figure 2

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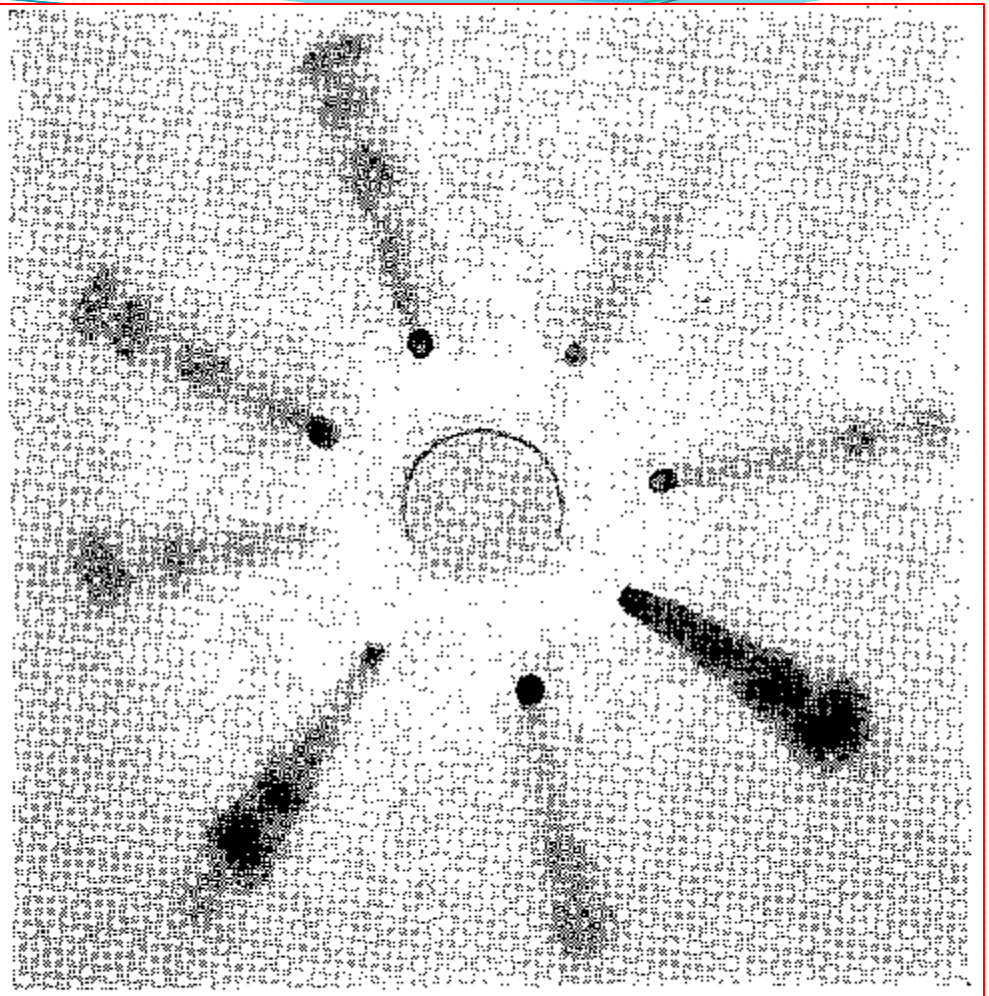

Figure 3

Typical Horizontal Chromatograms of Blue and

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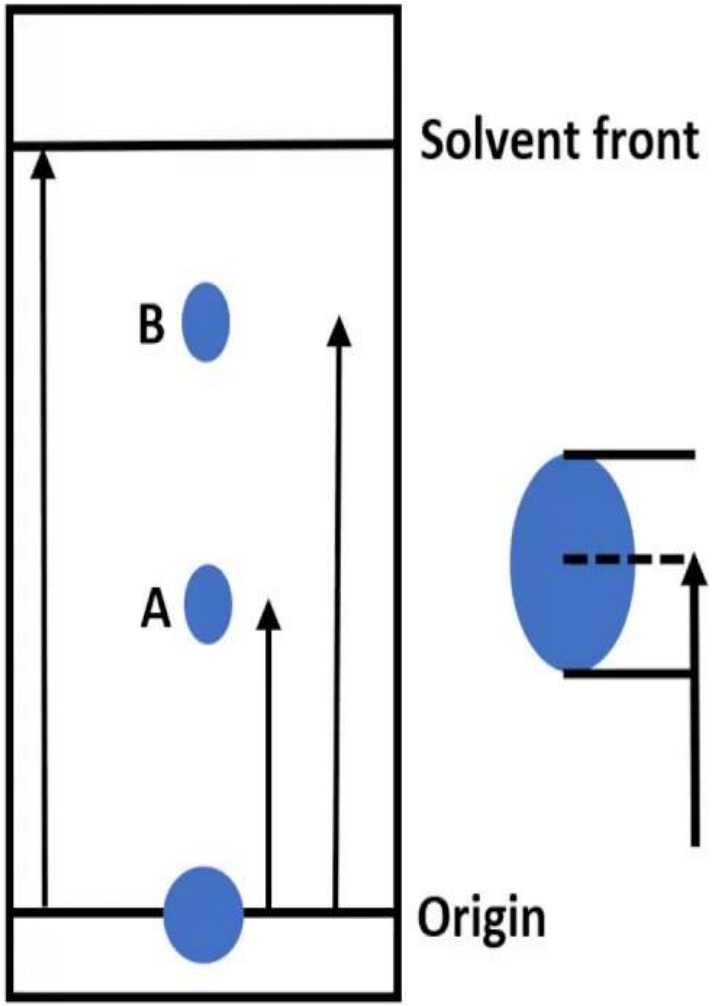
Calculating the R_f value



Distance travelled by solvent front = 10 cm

Distance travelled by component A = 4 cm

Distance travelled by component B = 8 cm

$$R_f\,(A) = \frac{4}{10} = 0.4$$

$$R_f\,(B) = \frac{8}{10} = 0.8$$

Thank You.

STAY HOME,
STAY SAFE