

Chromatography

History

Mikhail Tswett, Russian, 1872-1919

Botanist

In 1906 Tswett used chromatography to separate plant pigments

He called the new technique chromatography because the result of the analysis was 'written in color' along the length of the adsorbent column

Chroma means "color" and graphein means to "write"



Importance

Chromatography has application in every branch of the physical and biological sciences

12 Nobel prizes were awarded between 1937 and 1972 alone for work in which chromatography played a vital role



Chromatography is a physical method of separation in which the components to be separated are distributed between two phases

one of which is stationary (stationary phase) while the other (the mobile phase) moves through it in a definite direction.

The chromatographic process occurs due to differences in the distribution constant of the individual sample components.

Chromatography

Is a technique used to separate and identify the components of a mixture.

Works by allowing the molecules present in the mixture to distribute themselves between a stationary and a mobile medium.

Molecules that spend most of their time in the mobile phase are carried along faster.

Classification of chromatography according to mobile phase:

1- Liquid chromatography: mobile phase is a liquid. (LLC, LSC).

2- Gas chromatography : mobile phase is a gas. (GSC, GLC).

Classification according to the packing of the stationary phase:

- 1- Thin layer chromatography (TLC): the stationary phase is a thin layer supported on glass, plastic or aluminium plates.**
- 2- Paper chromatography (PC): the stationary phase is a thin film of liquid supported on an inert support.**
- 3- Column chromatography (CC): stationary phase is packed in a glass column.**

Classification according to the force of separation:

- 1- Adsorption chromatography.**
- 2- Partition chromatography.**
- 3- Ion exchange chromatography.**
- 4- Gel filtration chromatography.**
- 5- Affinity chromatography.**

Thin layer chromatography (TLC)

is a method for **identifying** substances and
testing the purity of compounds.

TLC is a useful technique because it is
relatively **quick** and requires **small**
quantities of material.

Separations in TLC involve distributing a mixture of two or more substances between a **stationary phase** and a **mobile phase**.

The stationary phase:

is a thin layer of adsorbent (usually silica gel or alumina) coated on a plate.

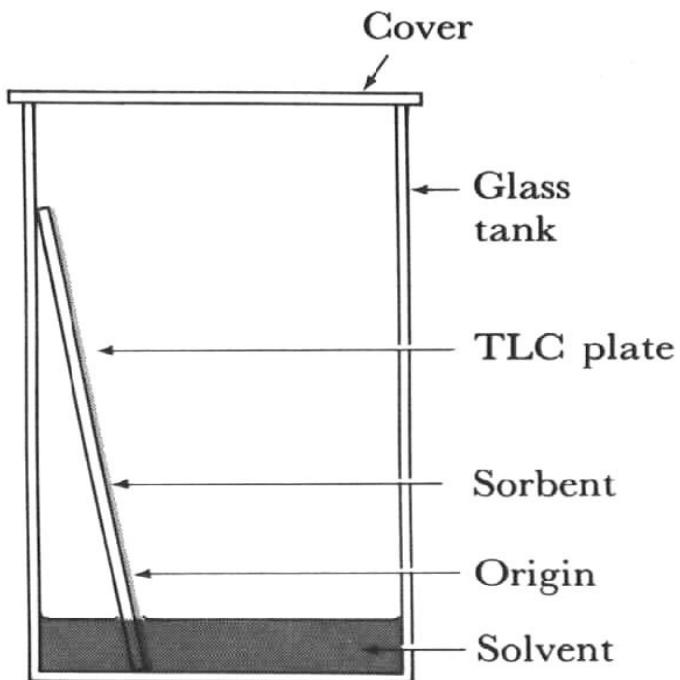
The mobile phase:

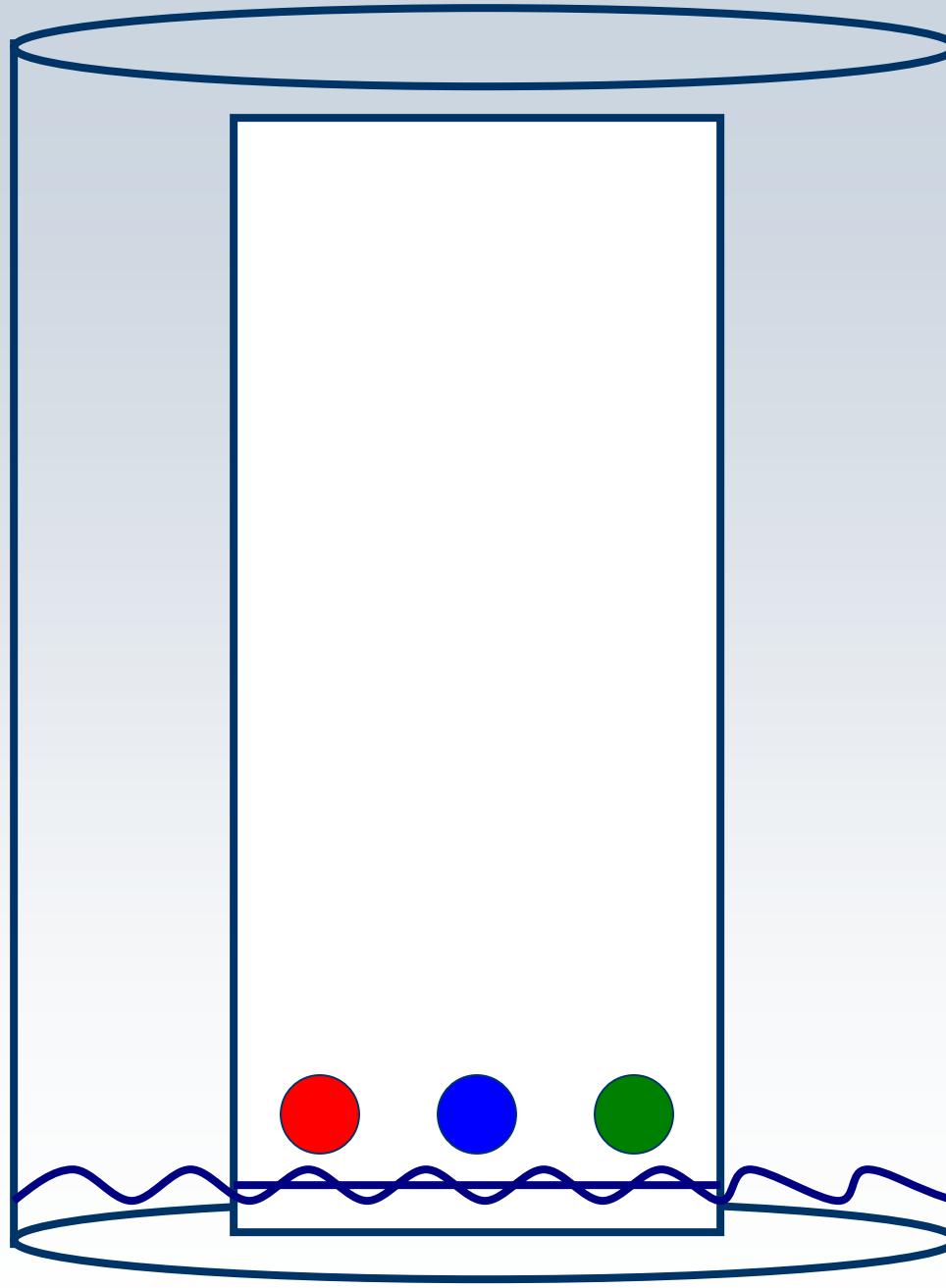
is a developing liquid which travels up the stationary phase, carrying the samples with it.

Components of the samples will separate on the stationary phase according to

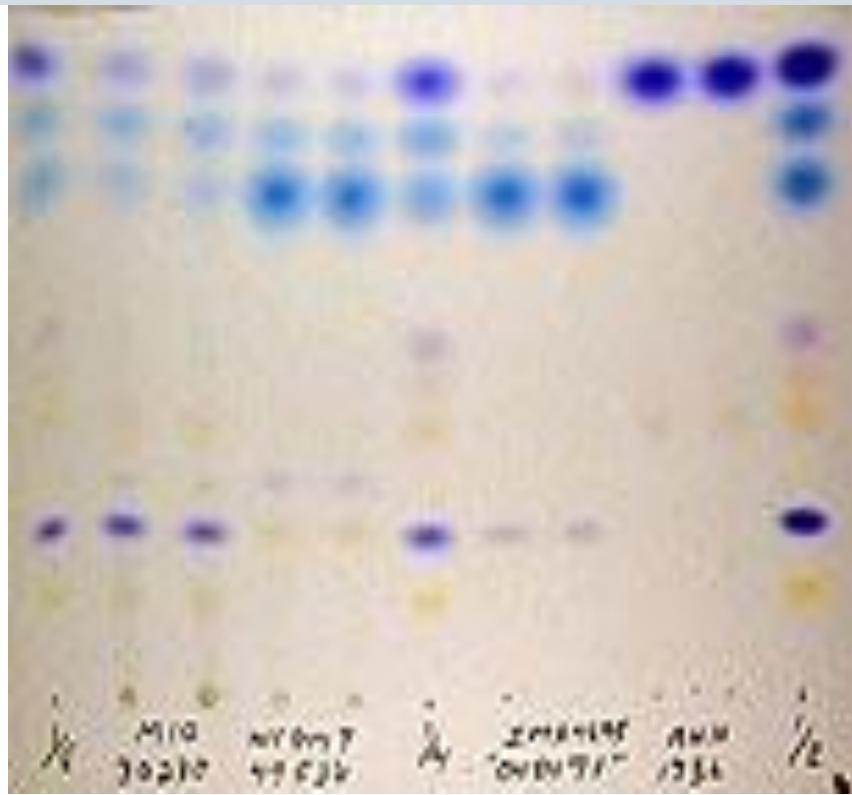
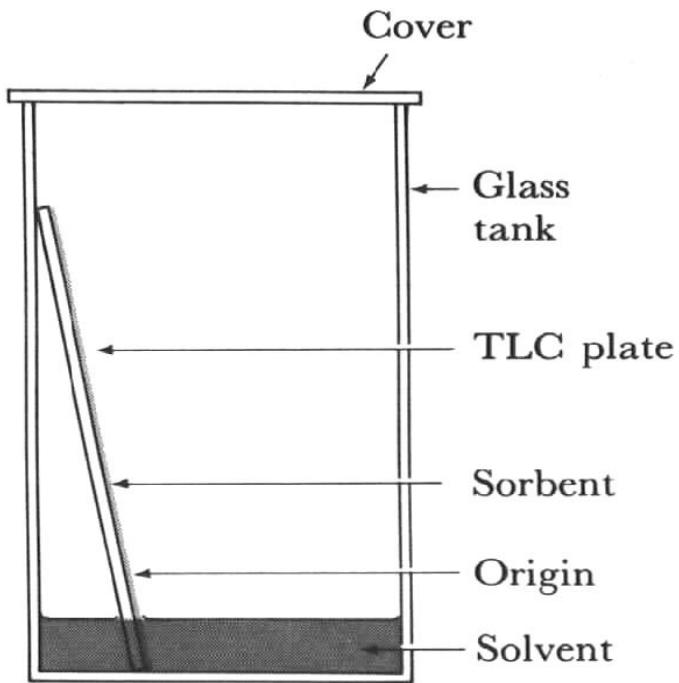
how much they adsorb on the stationary phase versus how much they dissolve in the mobile phase.

Thin Layer Chromatography (TLC)





TLC



Preparing the Chamber

To a jar with a tight-fitting lid add enough of the appropriate developing liquid so that it is 0.5 to 1 cm deep in the bottom of the jar.

Close the jar tightly, and let it stand for about 30 minutes so that the atmosphere in the jar becomes saturated with solvent.

Preparing the Plates for Development

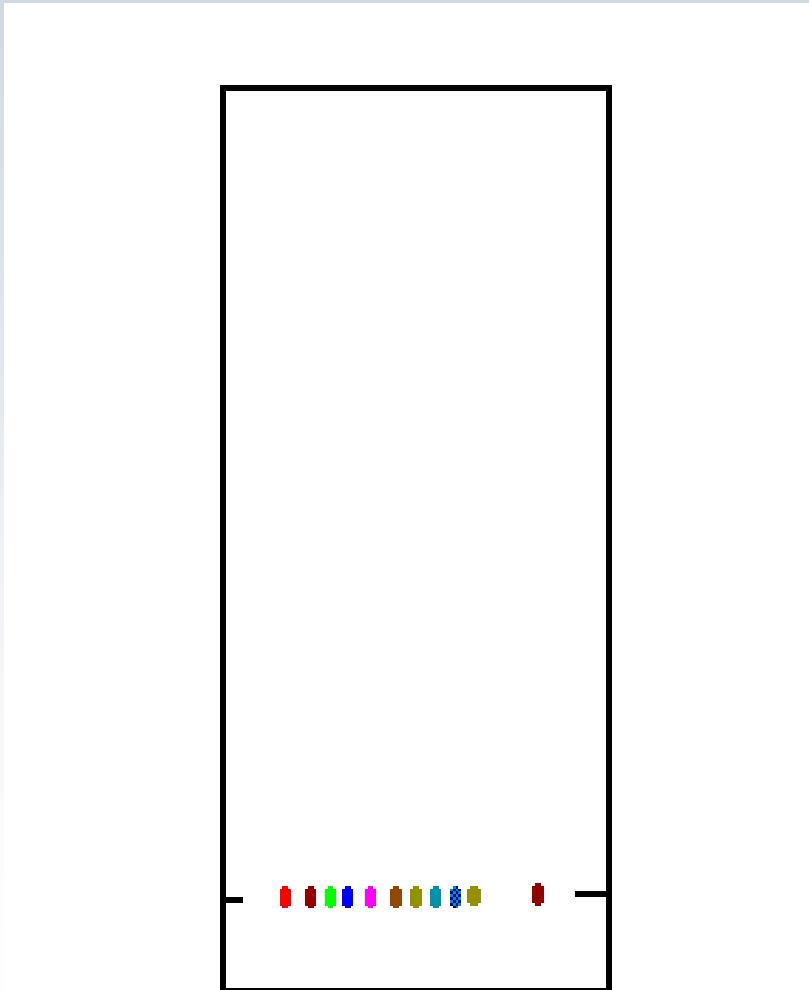
With a pencil, etch two small notches into the adsorbent about 2 cm from the bottom of the plate.

The notches should be on the edges of the plate, and each notch should be the same distance up from the bottom of the plate.

The notches must be farther from the bottom of the plate than the depth of the solvent in the jar.

Using a drawn-out capillary tube, spot the samples on the plate so that they line up with the notches you etched.

Question: What is wrong with the plate shown below?



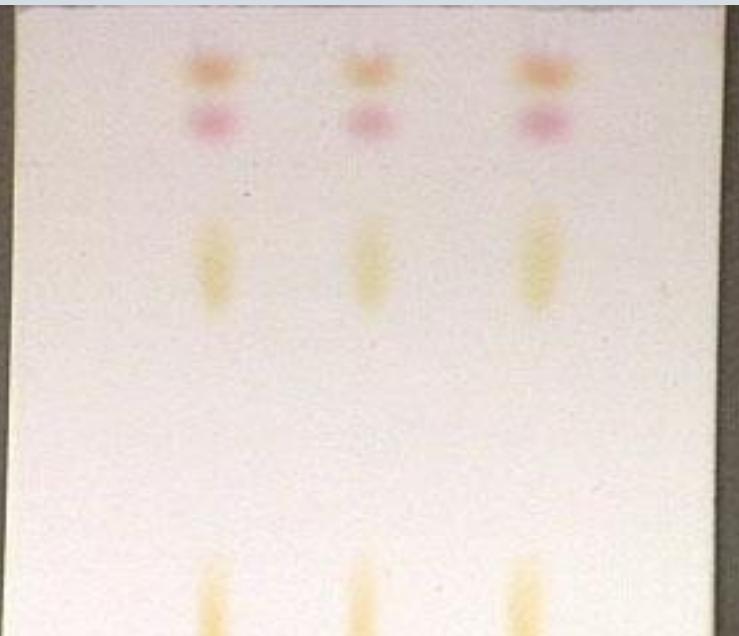
Developing the Plates

After preparing the development chamber and spotting the samples, the plates are ready for development.

Be careful to handle the plates only by their edges, and try to leave the development chamber uncovered for as little time as possible.

When the plates are removed from the chamber, quickly trace the solvent front (the highest solvent level on the plate) with a pencil.

Identifying the Spots (visualization)



If the spots can be seen, outline them with a pencil.

If no spots are obvious, the most common visualization technique is to hold the plate under a UV lamp.

Many organic compounds can be seen using this technique, and many commercially made plates often contain a substance which aids in the visualization of compounds.

Visualizing Agents

Alkaloids: Dragendorff's reagent

Cardiac glycosides: Antimony trichloride

Sugar: Aniline phthalate

Amino acids: Ninhydrin

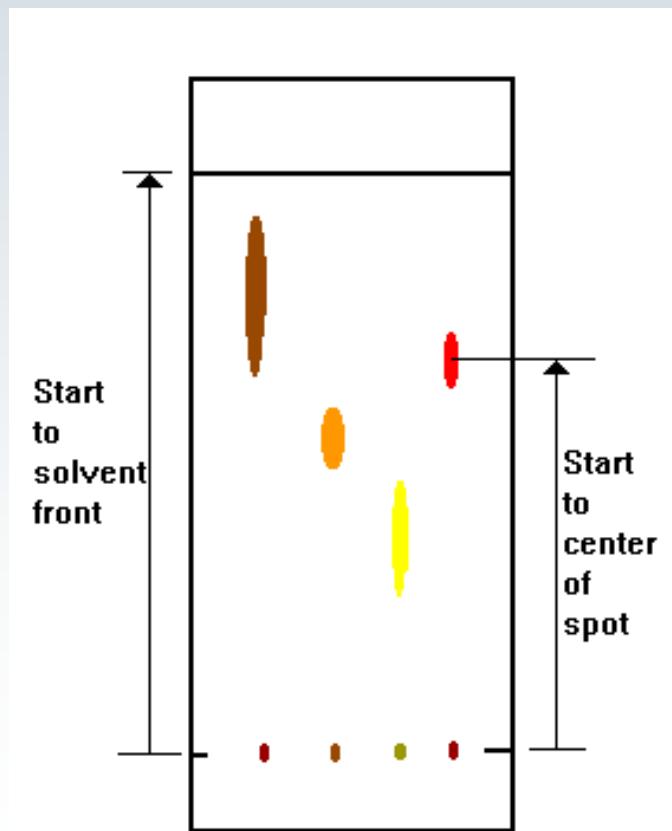
Interpreting the Data

The R_f (retention factor) value for each spot should be calculated.

It is characteristic for any given compound on the same stationary phase using the same mobile phase for development of the plates.

Hence, known R_f values can be compared to those of unknown substances to aid in their identifications.

$$R_f = \frac{\text{Distance from start to center of substance spot}}{\text{Distance from start to solvent front}}$$



(Note: R_f values often depend on the temperature and the solvent used in the TLC experiment.

the most effective way to identify a compound is to spot known substances – authentic - next to unknown substances on the same plate.)

In addition, the purity of a sample may be estimated from the chromatogram.

An impure sample will often develop as two or more spots, while a pure sample will show only one spot

Summary

A TLC plate is a sheet of glass, metal, or plastic which is coated with a thin layer of a solid adsorbent (usually silica or alumina).

A small amount of the mixture to be analyzed is spotted near the bottom of this plate.

The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid.

This liquid, or the eluent, is the mobile phase, and it slowly rises up the TLC plate by capillary action.

As the solvent moves past the spot that was applied, an equilibrium is established for each component of the mixture between the molecules of that component which are adsorbed on the solid and the molecules which are in solution.

In principle, the components will differ in solubility and in the strength of their adsorption to the adsorbent and some components will be carried farther up the plate than others.

When the solvent has reached the top of the plate, the plate is removed from the developing chamber, dried, and the separated components of the mixture are visualized.

If the compounds are colored, visualization is straightforward. Usually the compounds are not colored, so a UV lamp is used to visualize the plates.

PAPER CHROMATOGRAPHY

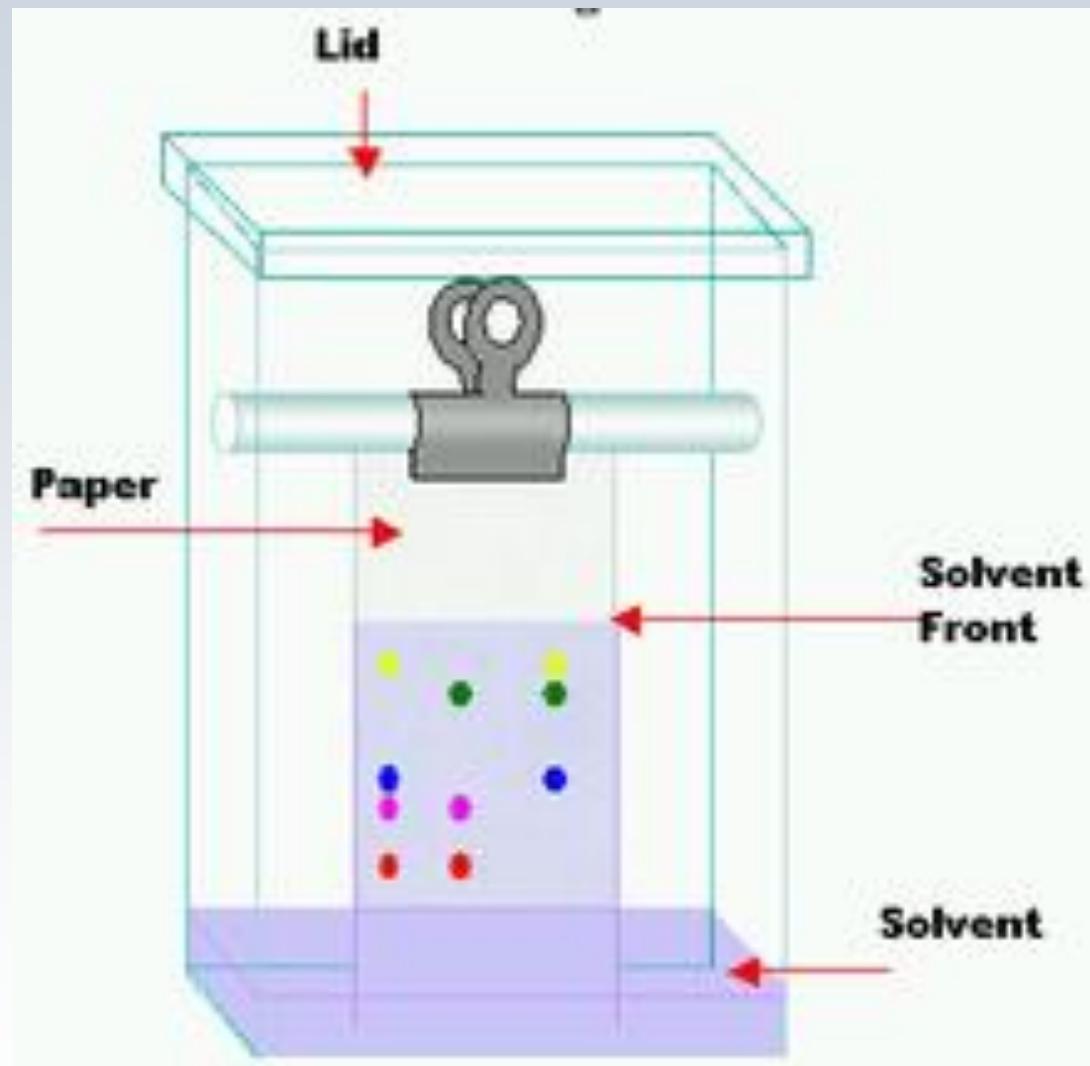
PAPER CHROMATOGRAPHY

A method of partition chromatography using filter paper strips as carrier or inert support.

The factor governing separation of mixtures of solutes on filter paper is the **partition between two immiscible phases**.

One is usually water adsorbed on cellulose fibres in the paper (stationary phase).

The second is the organic solvent flows past the sample on the paper (stationary phase).



Partition occurs between the mobile phase and the stationary aqueous phase bound by the cellulose.

The isolation depends on partition coefficient of the solute.

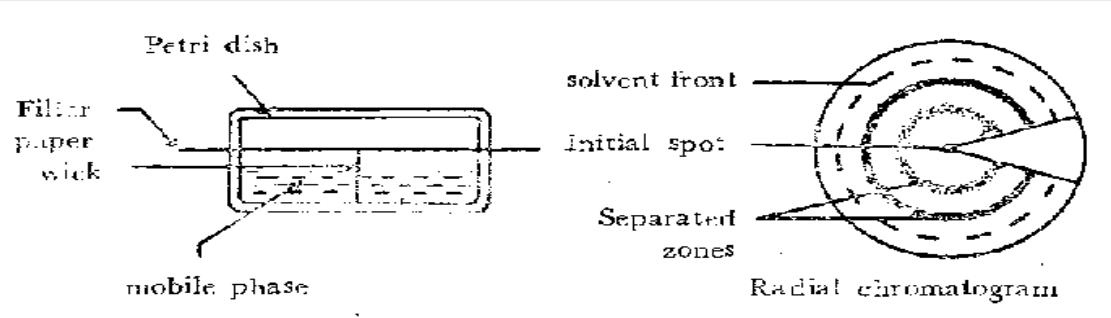
$$K = \frac{c(\text{stationary})}{c(\text{mobile})}$$

General Procedure

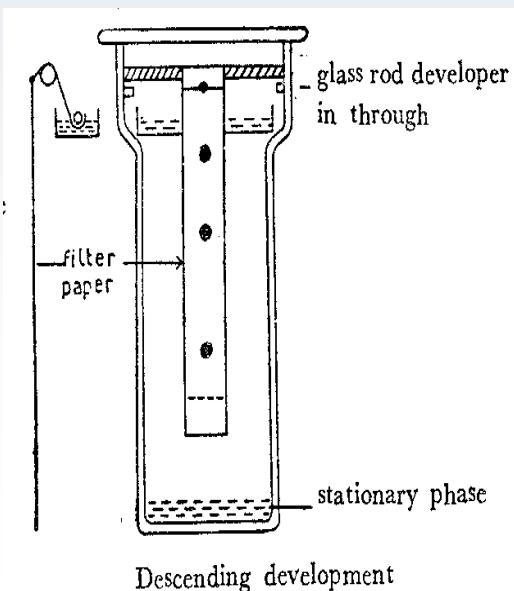
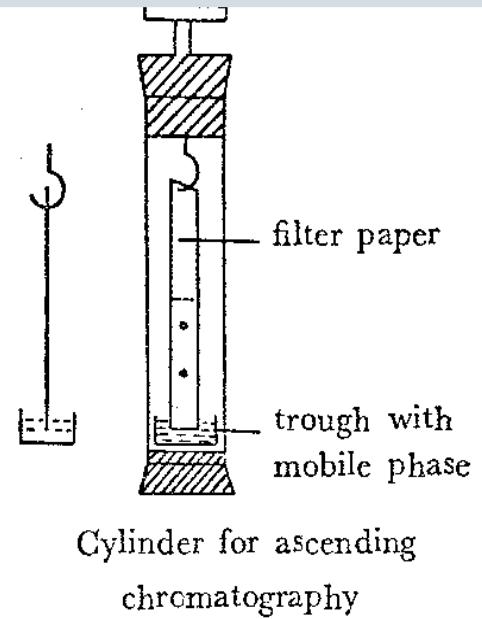
- 1- Choice of paper and solvent to be used.
- 2- Desalting of sample.
- 3- Application of the sample.
- 4- Equilibration of paper.
- 5- Development.
- 6- Detection.
- 7- Identification of substances.

Techniques of development with various flow directions

Radial development



Ascending development



Descending development

Multiple chromatography

Multiple chromatography includes all procedures in which the development is repeated after one development is completed.

A- multiple development: the chromatogram is repeatedly developed in the same direction and thus the complete resolution of two or more substances which have R_f values close together can be obtained.

As the mobile phase one can use either the same solvent system or different solvent systems.

B- two-dimensional chromatography:

When large numbers of substances are to be separated on a single chromatogram.

Development in a direction perpendicular to the first, and with a solvent system different from that used initially is often necessary.

The sample is applied on one corner of a square piece of paper and after development with the first solvent, the paper is dried , rotated 90° and developed in the second direction.

Usually, different types of solvents systems are used in each direction. It is essential that the first solvent be completely volatile.

