Chromatography

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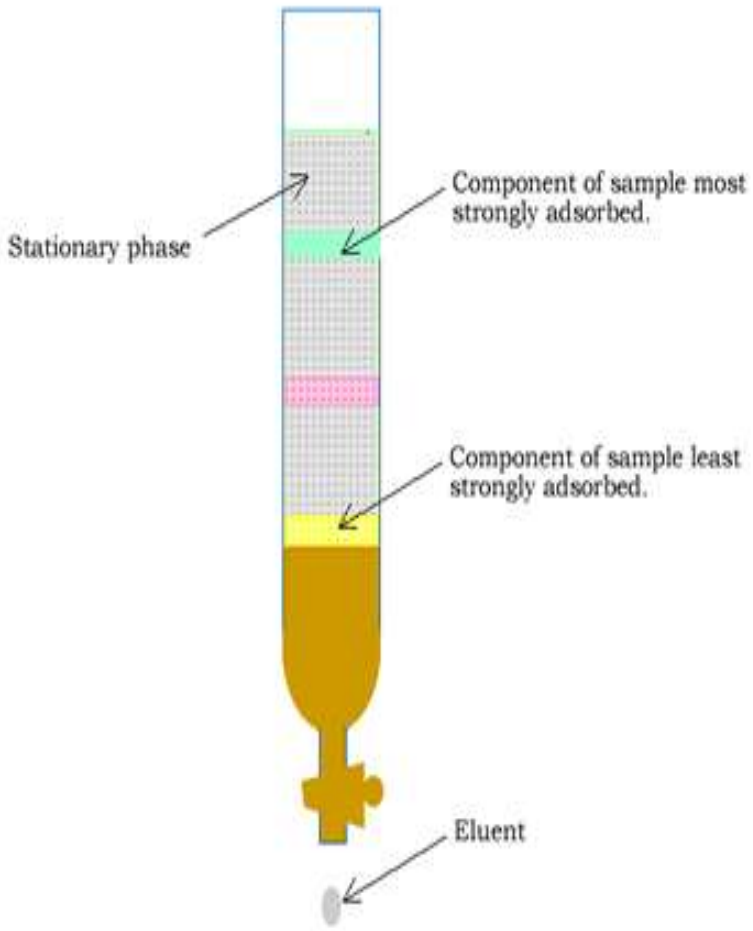
CHROMATOGRAPHY

Chromatography is a very important **analytical technique** used for **the separation, purification and identification** of chemical components in complex mixtures.

Chromatography means 'coloured writing'.

Basic principle of chromatography



- stationary phase -remains fixed
- mobile phase - percolates over the surface of the stationary phase.
- selective adsorption or retention of components from solution

- The movement of the mobile phase causes a differential migration of the sample components.
- i.e., As the various components possess different affinities for a given adsorbent they migrate at different rates as they are being carried through the system by the mobile phase.
- component having the weakest interaction with the stationary phase comes out first.

TYPES OF CHROMATOGRAPHY

There are several ways to classify the different chromatographic technique

Based on the operational technique

Column Chromatography, adsorbent column is used

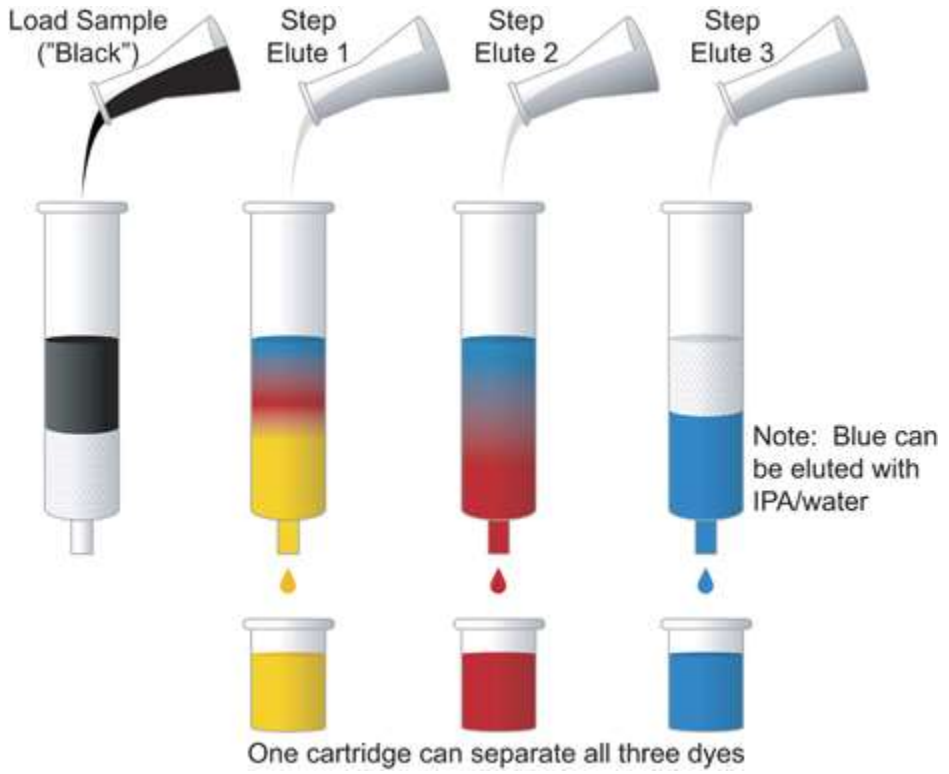
paper chromatography a stationary liquid film held on a paper strip is used

thin layer chromatography (TLC) a stationary film of liquid held on an adsorbent coating on a glass plate is used

Column chromatography

The basic principle

differential distribution of the sample components between two immiscible phases - a stationary phase and a mobile phase.



Liquid- solid adsorption chromatography is based on the selective adsorption of the sample components on an adsorbent column

Separation subsequently occurs because of the different rates at which the components are carried over the stationary phase by the mobile phase.

stationary phase -silica gel, alumina, cellulose
mobile phase - solvent like petroleum ether, acetone, benzene, etc. -

Column Chromatoraphic Process

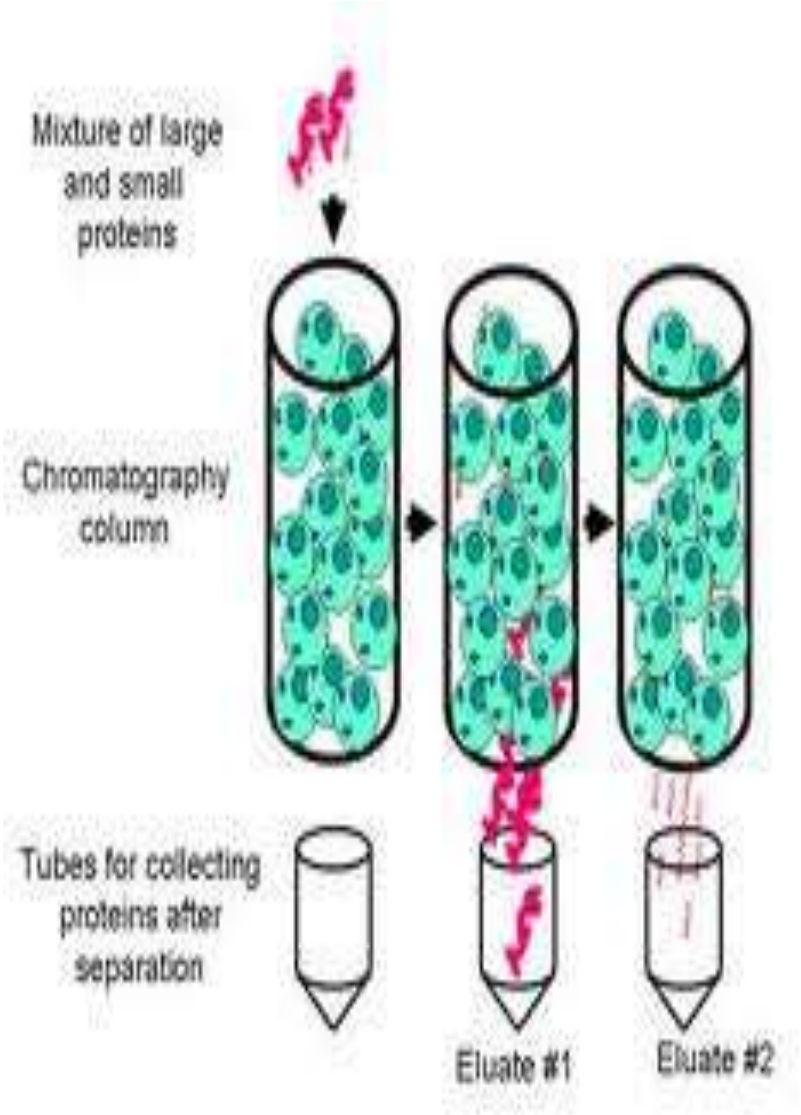
- An adsorbent column is prepared by packing a long glass tube uniformly with a suitable adsorbent.
- A solution of the mixture of the component (say A, B and C) in a suitable solvent is then introduced at the top of the column. As it percolates through the adsorbent column, different components are adsorbed to different extents.

The individual components are extracted from the adsorbent by passing a suitable solvent (the same or a different one) down the column.

The components are then washed down one by one and are collected in separate fractions. The weakly adsorbed component (C) emerges first and the most strongly absorrbed one (A) comes out last.

The process of dissolving out the components from the adsorbent using a suitable solvent is called elution and the solvent used for the purpose is called an eluent.

Pure components are recovered by removing the solvent by a suitable method.



Applications of **column chromatography:**

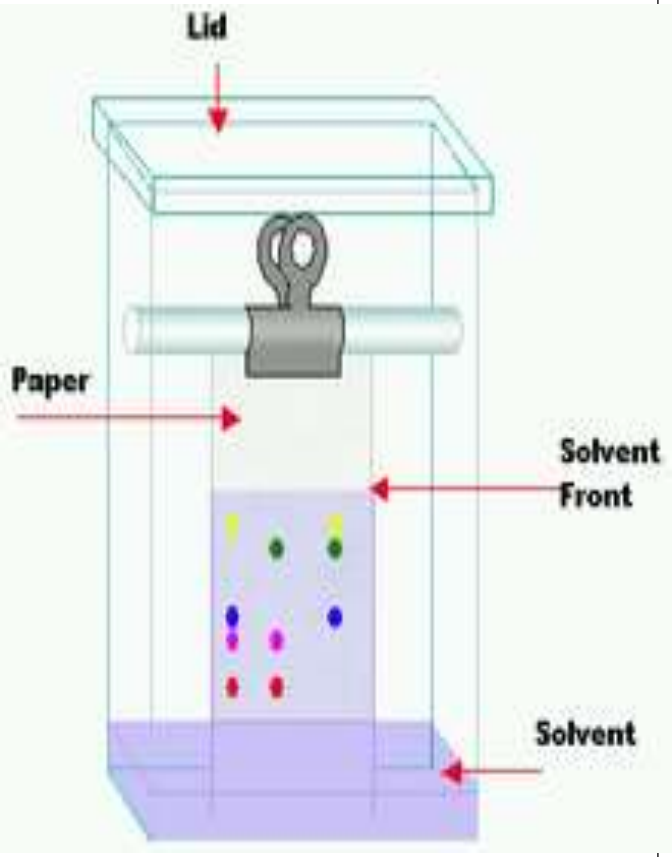
(i) the quantitative separation of components in a mixture of organic or inorganic substances.
(ii) purification of the products of reaction in the laboratory as well as in industry.
(iii) the identification of organic and inorganic components of a mixture

Paper Chromatography

a special field of liquid-liquid partition chromatography.

stationary phase - a film of liquid (*e.g.*, water) adsorbed on a paper mat usually made of highly purified cellulose.

The mobile phase is another liquid percolating over stationary phase



The apparatus

- a support for the paper
- a solvent trough
- and an air-tight glass cylinder to develop the chromatogram.

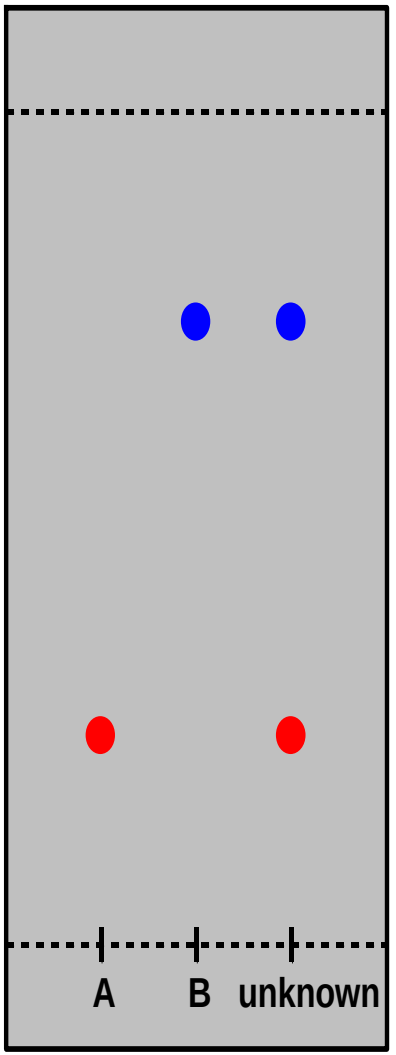
•The lower edge of the paper strip is spotted near its edge with the solution containing the components and dried

•The paper is then suspended in the glass cylinder with its lower end just dipping in the solvent., the mobile phase

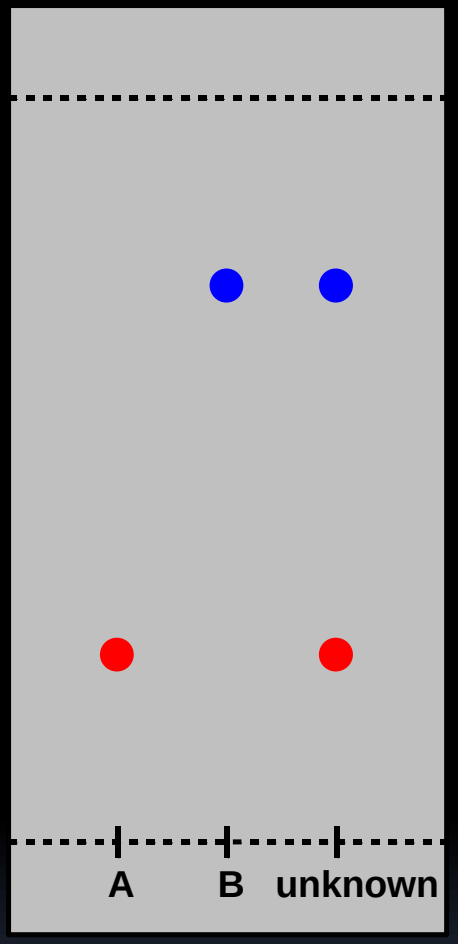
•The spotted position should be above the solvent level.

•As the solvents ascends through the paper by capillary action it carry the various components.

• The components move at different rates due to differential distribution between the immiscible liquids



- After some time, the paper strip is removed, the solvent front marked and dried.
- The coloured components will appear as a number of coloured spots at different distances.
- The positions of colourless components may be determined by spraying the paper with a sensitive reagent capable of forming coloured compounds with them.
- Separation of sample components present even in small quantities is possible by paper chromatography.



Applications

many applications in inorganic organic, biochemical and industrial fields.

for the separation and identification of sample components present in trace quantities (in microgram levels).

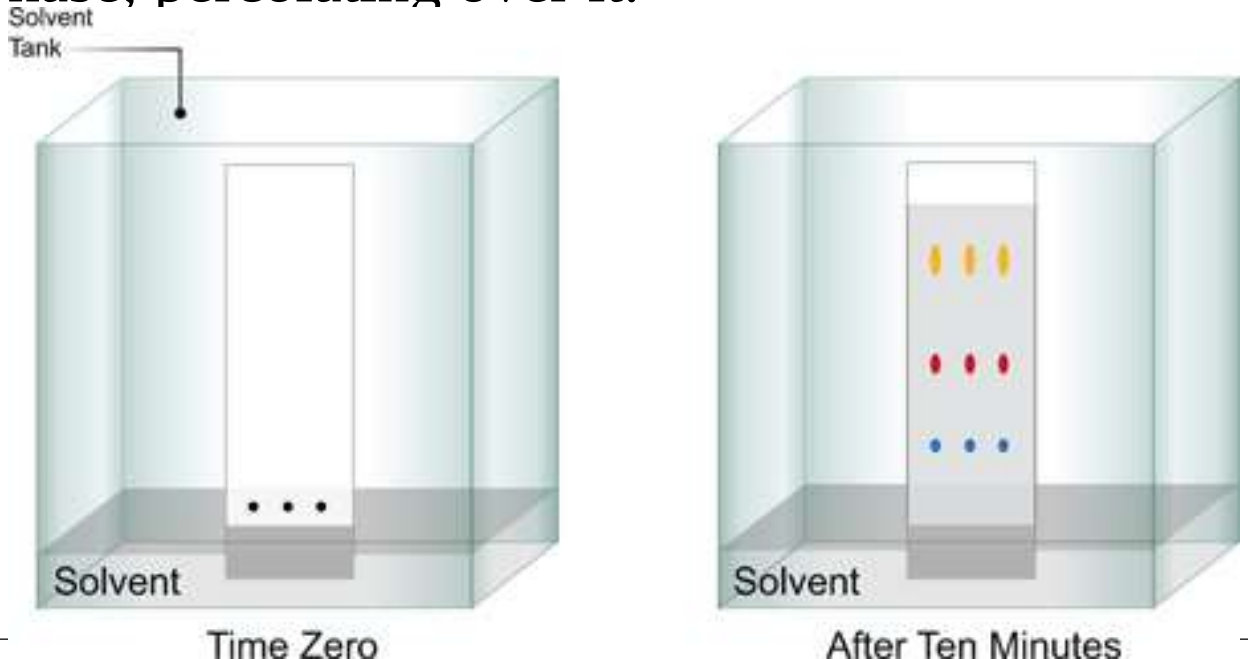
1. in the separation of components which are closely related chemically.
2. used in the laboratory and in industry for quick check of purity of organic chemicals.
3. for the control of the purity of food products and pharmaceuticals.
4. to detect drugs and dopes in animals and humans.
5. used in the analysis of cosmetics.
6. Resolution of isomeric metal complexes can be achieved in the determination of metals in ore samples .

Thin layer chromatography (TLC)

- Special field of liquid-liquid partition chromatography.

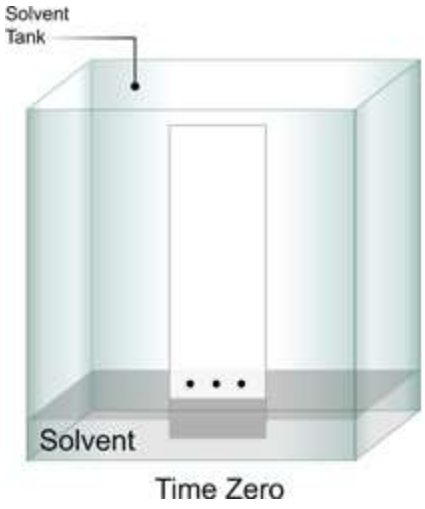
- Stationary phase - a film of liquid (e.g., water), adsorbed on a uniform thin layer of an adsorbent like silica gel, alumina or cellulose coated on a glass (or plastic) plate

- The mobile phase is a suitable solvent, immiscible with the stationary phase, percolating over it.

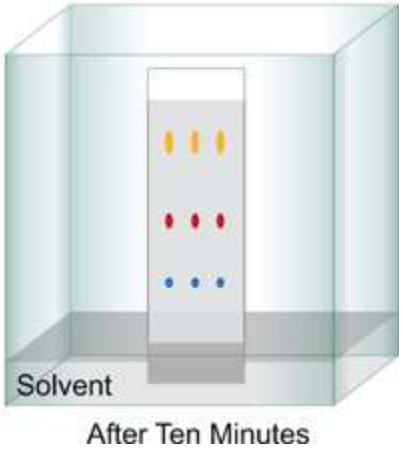


A slurry of the adsorbent in the liquid (e.g., water) selected as the stationary phase is spread on a flat and uniform glass plate.

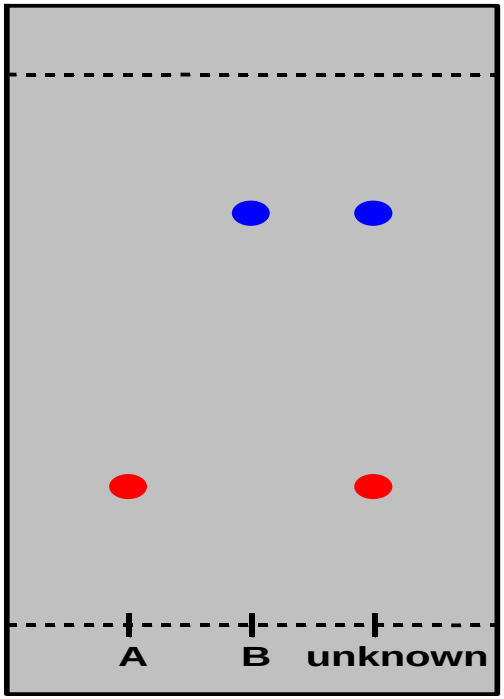
The chromoplate thus prepared is spotted near its edge with the solution containing the components.
It is placed in a slanting manner in a glass tank containing a layer of solvent such that' the sample spots are just above the solvent level. The tank is closed with a lid.



Time Zero



After Ten Minutes

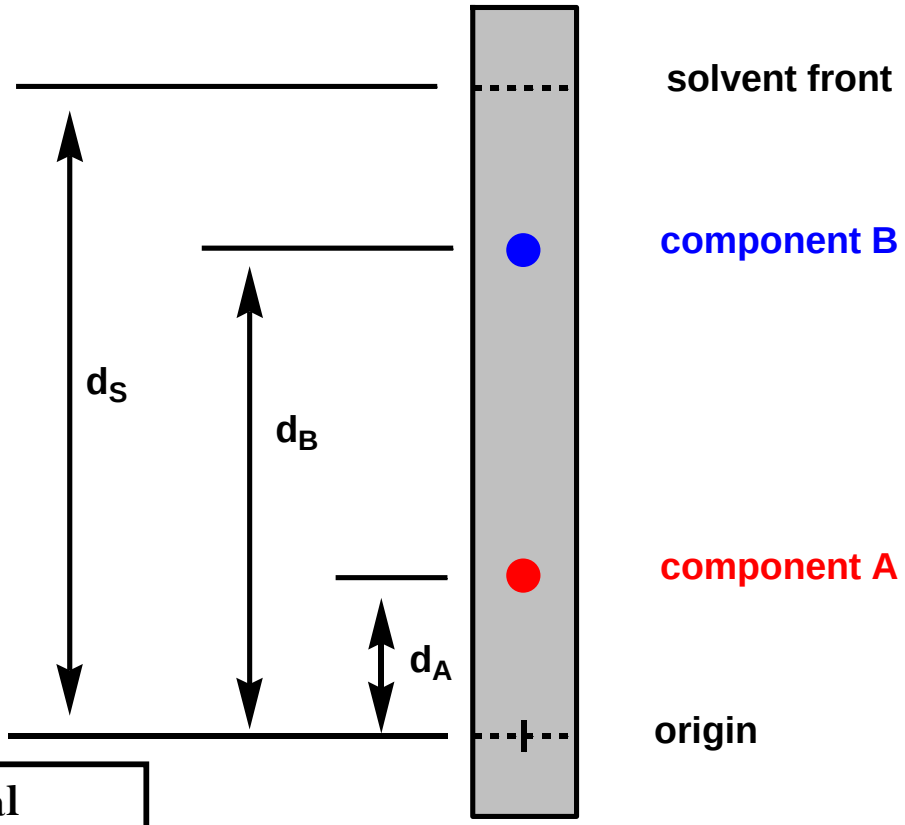


- As the mobile phase, ascends by capillary action carry the various components and yields compact spots within a few minutes.
- The plate is then taken out, the solvent front marked and dried.
- If not coloured, the components can be determined by spraying with suitable colour giving reagents.
- A comparison of the Rf values of the spots will help in the identification of the components

Thin-Layer & Paper Chromatography: Determination of R_f Values

R_f of component A = $\frac{d_A}{d_S}$

R_f of component B = $\frac{d_B}{d_S}$



The R_f value is a decimal fraction, generally only reported to two decimal places

R_f *values*

the **degree of retention** of a particular component is expressed as the 'retention factor' or 'retardation factor' or Rf

"The R_f value is defined as the ratio of the distance travelled by a particular component to the distance travelled by the solvent front during the same time".

$$R_f = \frac{\text{Distance travelled by the component from the origin line}}{\text{Distance travelled by the solvent front from the origin line in the same time}}$$

The R_f value depends upon

- the solvent employed
- the quality of the medium used for separation
- the temperature
- the nature of the substance.

Applications of TLC

- Used for the rapid separation, purification and identification of even those components which are present in very minute quantities.
- Used for the analysis of complex organic mixtures.
- The high sensitivity of TLC is made use of in checking the purity of samples.
- Used for the identification and separation of drugs and plant extracts such as alkaloids.
- TLC is particularly useful in the separation and isolation of biochemical preparations.
- TLC is used for the detection of contaminants or adulterants in chemical samples in the laboratory as well as in industry .

GAS CHROMATOGRAPHY

Two types

Gas-liquid chromatography (GLC)
Gas-solid chromatography (GSC).

mobile phase – gas
stationary phase - liquid immobilized on the surface of an inert solid support.

Principle

The technique is based on the differential partitioning of the components of a sample in the vapour phase between the mobile gas phase and the stationary liquid phase.

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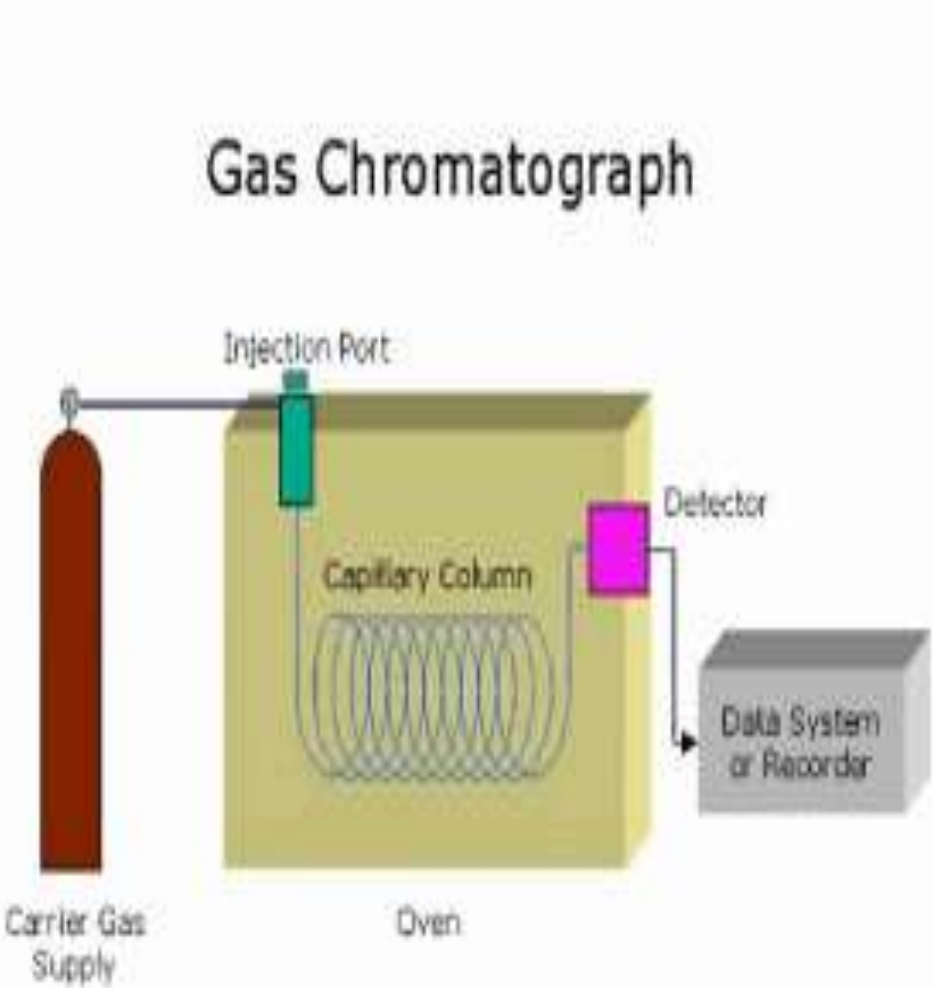
Stationary phase

- The liquid phase immobilized
- **non-volatile (high-boiling) liquid**
- Eg. methyl silicone or polyethylene glycol.
- supported on small particles of an inert porous spongy siliceous material or on a capillary wall of chemically modified glass or fused silica.
- This is contained in a column of coiled tubing made of heat resistant material like stainless steel or Teflon.

Mobile phase

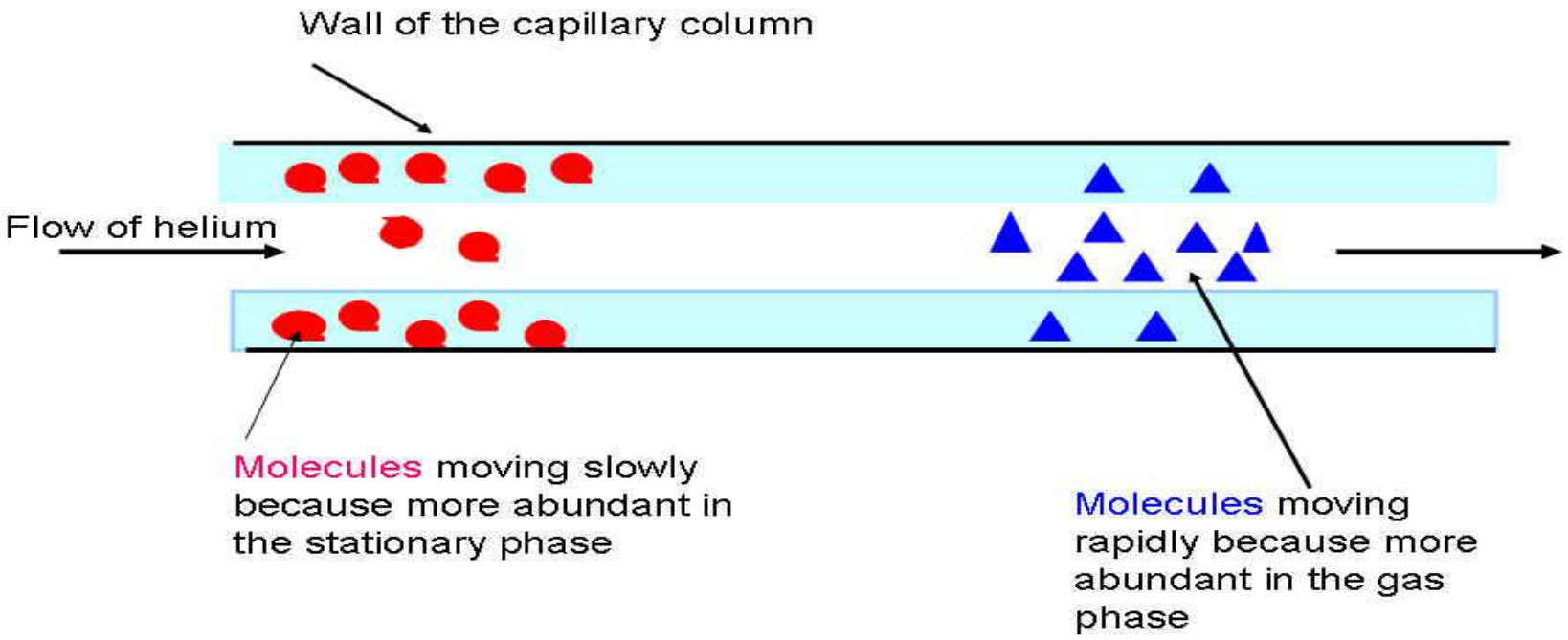
- –pure chemically inert gas like helium argon, nitrogen, co2
- also called carrier gas
- major function is to transport the analytes through the column.

- The carrier gas is supplied into the apparatus from a pressurized cylinder at a controlled flow rate.
- A small amount of the sample is rapidly Injected by. means of a syringe into **a heated chamber.**
- the sample in the vapour state is carried by the carrier gas (mobile phase) into the column
- it is maintained at a constant temperature higher than the boiling point of the least volatile component in the sample mixture:



As the mobile phase (carrier gas) carries the vaporized sample through the column, separation of components occurs; due to their differential partitioning and equilibration between the stationary liquid phase and the carrier gas.

Components differentially retained in the stationary phase reach the column outlet at different times and, hence, separated components emerge from the column one after the other.



Each component that comes out of the column detected automatically by a sensitive detector.

The detector signals are fed to a recording device

the intensities recorded as a function of time to get a gas chromatogram, a graphical chart consisting of separate chromatographic peaks corresponding to different components.

The number of peaks → The number of components in the sample,

The area under each peak → relative concentration of that component

Applications of gas chromatography

1. separation of components In complex organic and biochemical systems.

2. since retention times allow identification of species, It can be used In qualitative analysis of mixtures of organic compounds such as natural products.

3. It provides an excellent means of confirming the presence or absence of a suspected compound in a mixture of organic compounds.
4. Since contaminants present in samples are revealed by the additional peak that they produce, gas chromatograms are used to check the purity of organic compounds.. .
5. Since peak areas are proportional to the concentration of the concerned components and thereby reveal quantitative information