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# ADVANCED SEPARATION TECHNIQUES (702)

## Introduction:-

following are the separation techniques

### 1) Solvent Extraction:-

Solvent extraction is a separation technique that utilizes an organic solvent to separate the components of a mixture.

### 2) Distillation fractional Distillation:-

Fractional distillation is the process of separating crude oil into groups of hydrocarbons with similar numbers of carbon atoms.

### 3) Chromatography:-

A Physical method of separation that distributes components two separate between two phases, one stationary (stationary phase), the other (the mobile phase) moving in a definite direction.

#### 4) Crystallization:-

- Crystallization is a technique used for the purification of substances. A separation technique to separate solids from a solution.
- It is a physical change.

#### 5) Filtration:-

Filtration separates a solid from a liquid using a porous material that allows only the liquid to pass

- Separating sand from water
- Preparing coffee
- Removing tea leaves from tea.

#### History:-

Advanced separation techniques have a history that includes foundational texts from the 1970 and 1980, like the separation processes by C. Judson King (1980), and more modern

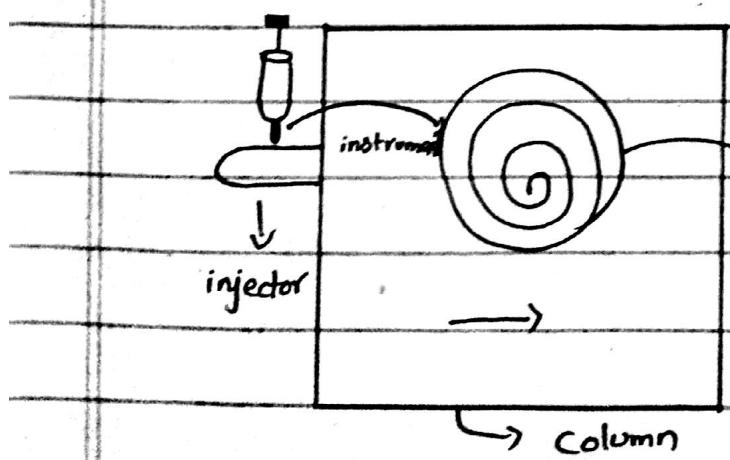
overviews that trace developments through the late 20th and early 21st centuries. Key periods include a 1965 joint meeting of the AIChE and IChemE that

Published Papers on "Advance in Separation Techniques" and the "Century of separation science" Project which documented advancements up to 2001.

## Important Terms Used in Chromatography

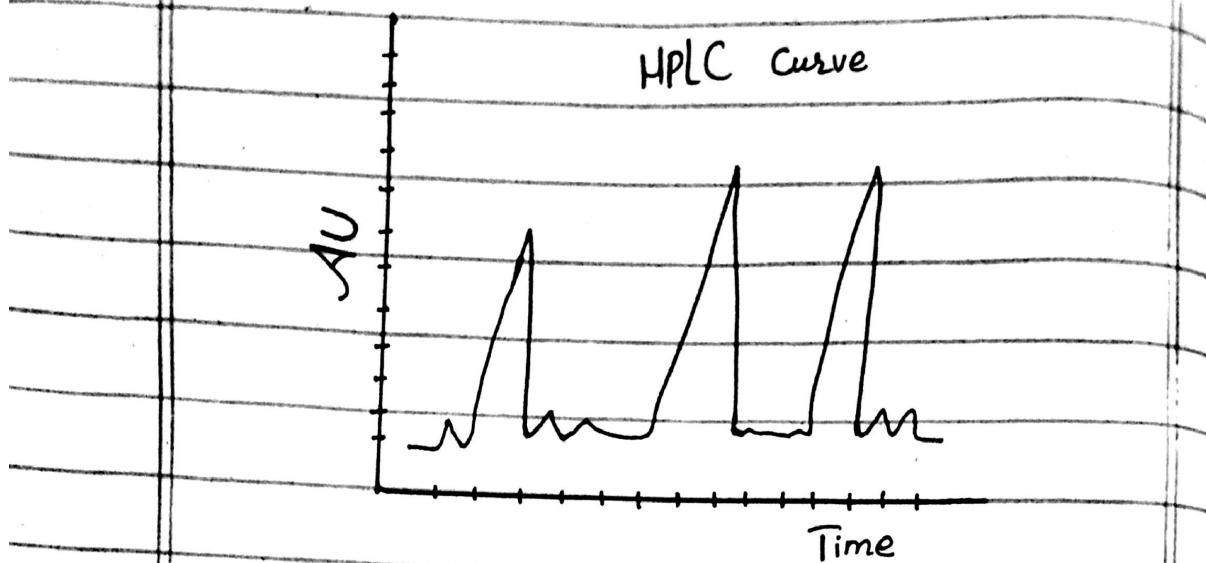
### 1) Chromatograph:-

Chromatograph is an instrument used to carry out chromatography which consists of an injector, a column and detector. Beside these basic components systems for handling the mobile phase, system for controlling temperatures, and devices for recording and processing the detector output are also present in some chromatograph.



### 2) Chromatogram:-

A chromatogram is the resulting chart or pictures that show how different substances in a mixture are separated during chromatography.



### 3) Analyte:-

The analyte is the substance to be separated during chromatography. It is also normally what we want to separate from the mixture.

### 4) Eluate:-

The eluate is the term (in chromatography) used for the mobile phase leaving the column.

### 5) Eluent:-

The eluent is the term (in chromatography) used for the solvent that carries

### 6) Eluotropic Series:-

An eluotropic series is a list of solvents ranked on the basis of their eluting power.

## Classification of Chromatography

Chromatographic methods can be classified in various ways as follows

### 1) Adsorption Chromatography:-

**Definition:-** Adsorption chromatography is a type of chromatography that separates components based on their adsorption to a stationary phase.

The stationary phase is typically a solid or a liquid supported on a solid.

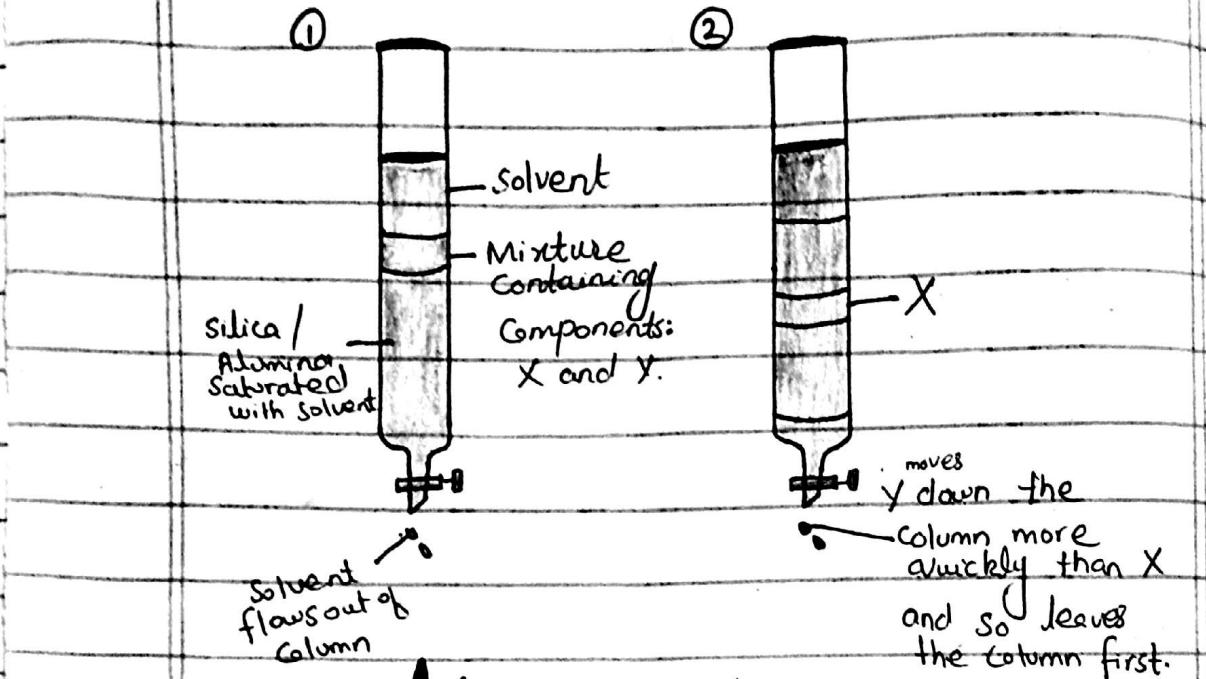
### Principle / Working

Adsorption chromatography is a technique for separation/separating and analyzing mixture components.

It operates on the principle of differential adsorption of molecules on to a stationary phase.

The stationary phase is typically a solid material with a high surface area like Silica or alumina.

Diagram:-



## Adsorption Chromatography

Adsorption Chromatography Advantages:-

- 1) High Selectivity:- Separates complex mixtures effectively.
- 2) High Sensitivity:- Detects trace amounts of components.
- 3) Purification:- Effective for purifying substances.

Adsorption Chromatography Disadvantages:-

- 1) Non-linear adsorption:- Can cause peak tailing and broadening.
- 2) Potential for irreversible adsorption:- Some components may bind irreversibly to the stationary phase.

### Limitation:-

- Low resolution:- May not be able to separate compounds with very similar properties accurately.
- Time Consuming:- Separations can take a long time, especially for some solutes.

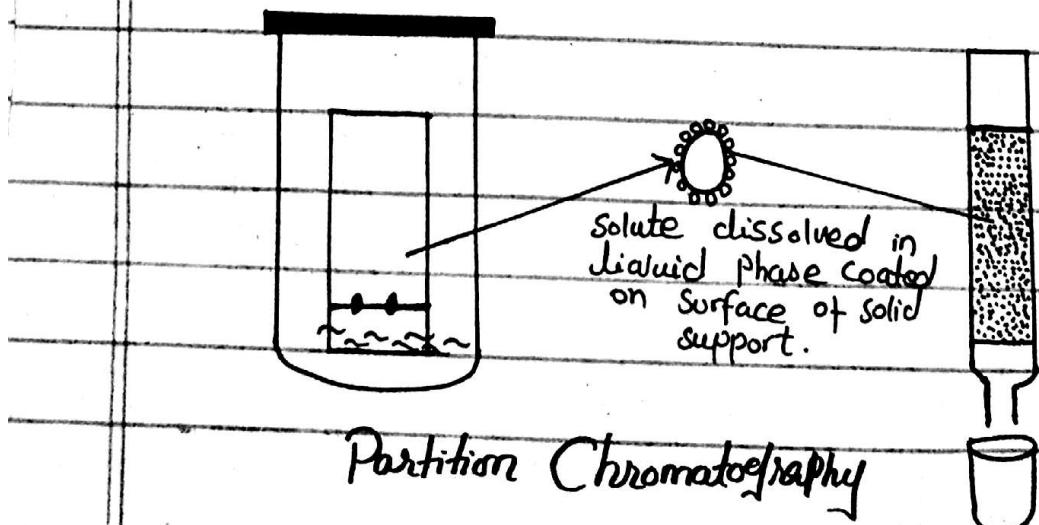
## 2) Partition Chromatography:-

Partition chromatography is a technique used to separate mixtures into individual components.

### Principle / Working:-

The principle of Partition chromatography is based on the distribution of solutes between two phases, where the solutes partition themselves between the two phases based on their relative affinities.

### Diagram:-



Partition Chromatography

### Types:-

There are two types of partition chromatography.

#### 1) Liquid-Liquid Chromatography (LLC):-

Both Phases are liquids.

#### 2) Gas-Liquid Chromatography (GLC):-

Stationary Phase is a liquid, mobile Phase is a gas.

### Advantages:-

The Partition chromatography technique can isolate both organic and inorganic compounds.

It provides high efficiency.

This technique provide accurate result.

### ① Disadvantages:-

The disadvantage of Partition chromatography are as follows.

Data cannot be stored in certain types of Partition chromatography.

In gas-liquid chromatography, only volatile Compounds can be separated.

### 3) Ion Exchange Chromatography:-

Definition:-

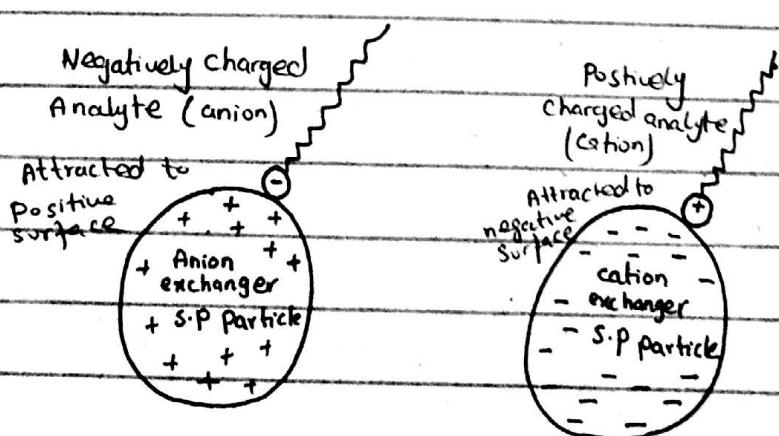
Separation of organic and inorganic ions by their partitioning on to ionic Stationary Phases bonded to a Solid Support.

Principle / Working:-

Ion exchange chromatography works on the principle of electrostatic attraction between charged molecules (ions) in a Sample and the oppositely charged groups on the Stationary Phase (resin).

- Cations (Positively charged ions) are attracted to negatively charged resins.
- Anions (negatively charged ions) are attracted to positively charged resins.

(1) Diagram:-



Ion Exchange Chromatography

## Types of Ion Exchange Chromatography:-

There are two types of ion exchange chromatography.

1) Cation Exchange Chromatography:-  
Separates positively charged ions  
(Cations)

2) Anion Exchange Chromatography:-  
Separates negatively charged ions  
(Anions).

## Advantage:-

The advantage of ion-exchange chromatography are as follows.

- Using this method the inorganic ions can also be separated.
- Resins have a long life.
- It has cheap maintenance.

## Disadvantage:-

The disadvantage of ion-exchange chromatography are as follows.

- The buffer requirement is the major disadvantage of ion-exchange chromatography.
- This method can only be used to isolate charged molecules.

#### 4) Size Exclusion Chromatography:-

Definition:-

A mixture of components with different molecular sizes are separated by using gels which act as sieve.

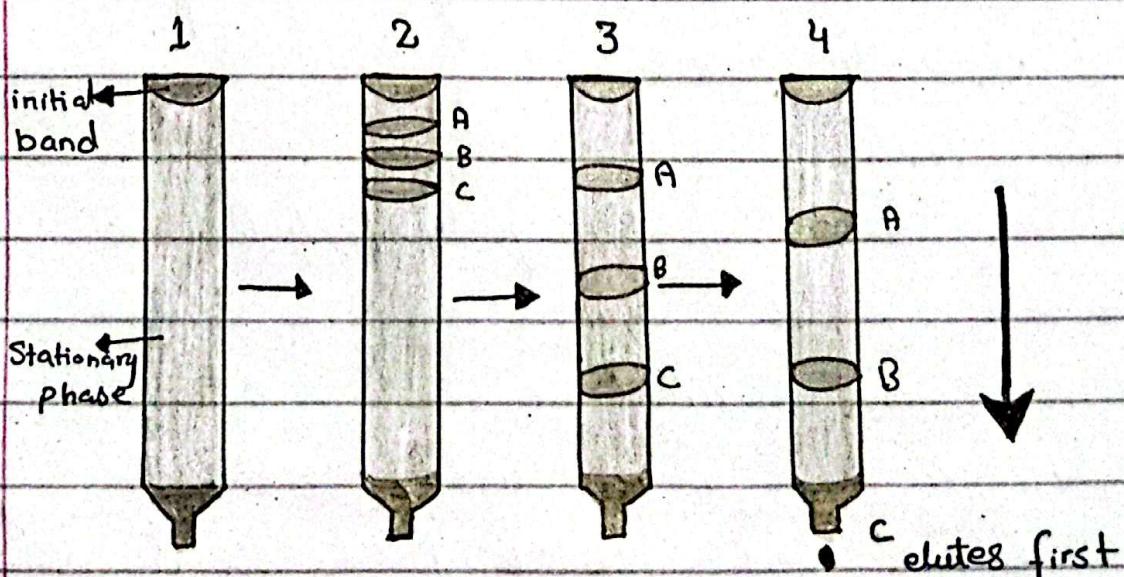
Principle / Working:-

Size exclusion chromatography (SEC) separates molecules based on size.

Also known as gel filtration chromatography.

The stationary phase consists of porous beads made from materials like dextrane, agarose. Smaller molecules can enter the pores, while larger molecules cannot.

Diagram:-



Size Exclusion Chromatography

Types of Size Exclusion Chromatography  
There are two types of size exclusion chromatography

- 1) Gel filtration Chromatography:  
Uses aqueous mobile phases, ideal for biomolecules.

- 2) Gel permeation Chromatography:  
Uses organic solvents, often used for synthetic polymers.

#### Advantages:-

- Ease of scale up.
- Low chances of sample loss.
- less time of analysis.
- Simplicity & reliability.

#### Disadvantages:-

- low resolution compared to other chromatographic techniques.
- low sample handling capacity.

### 5) Electrophoresis:-

#### Definition:-

"Migration of charged particles or molecules under the influence of electric current."

Literally:- greek word means transport by electricity.

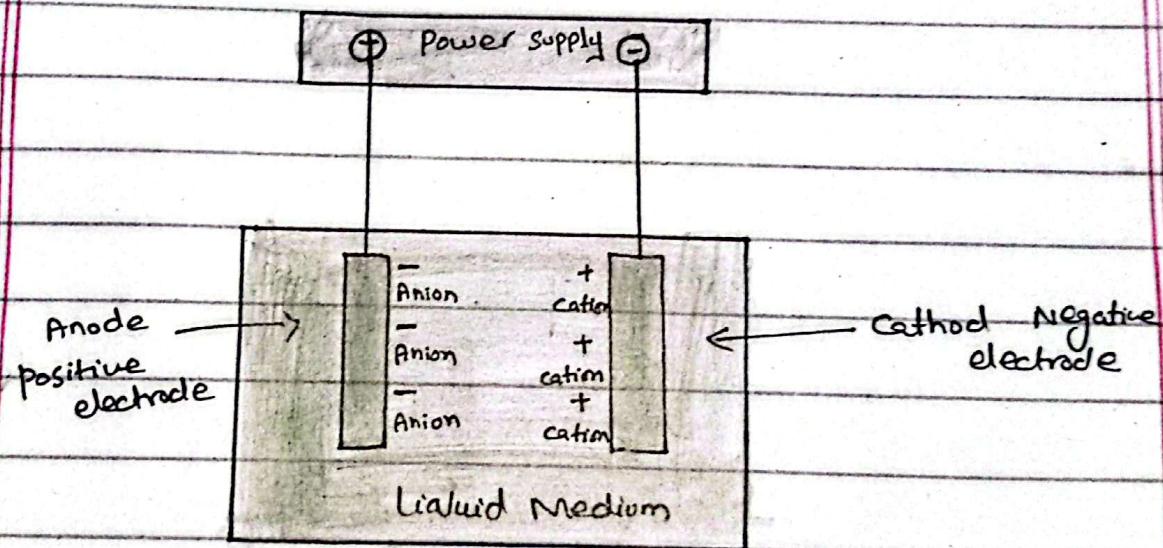
## Principle / Working:-

Electrophoresis is based on the movement of charged particles (ions or molecules) in a liquid medium under the influence of an electric field.

When an electric current is applied:

- Positively charged particles (cations) move toward the cathode (-).
- Negatively charged particles (anions) move toward the anode (+).

## Diagram:-



## Electrophoresis

### Types of Electrophoresis:-

There are three types of electrophoresis

- 1) Pulsed field Gel electrophoresis:-

Separates large DNA molecules.

- 2) Capillary Electrophoresis:-  
Separate molecules in a narrow capillary tube.
- 3) Agarose Gel Electrophoresis:-  
separate DNA and RNA molecules based on size.

#### Advantage:-

- Simple technique, easy to implement.
- Only a small amount of sample is required to perform the experiment.
- Allows for quick analysis of samples.

#### Disadvantage:-

- Electro osmosis is high.
- Resolution is less compared to polyacrylamide gels.

- 6) Affinity Chromatography:-

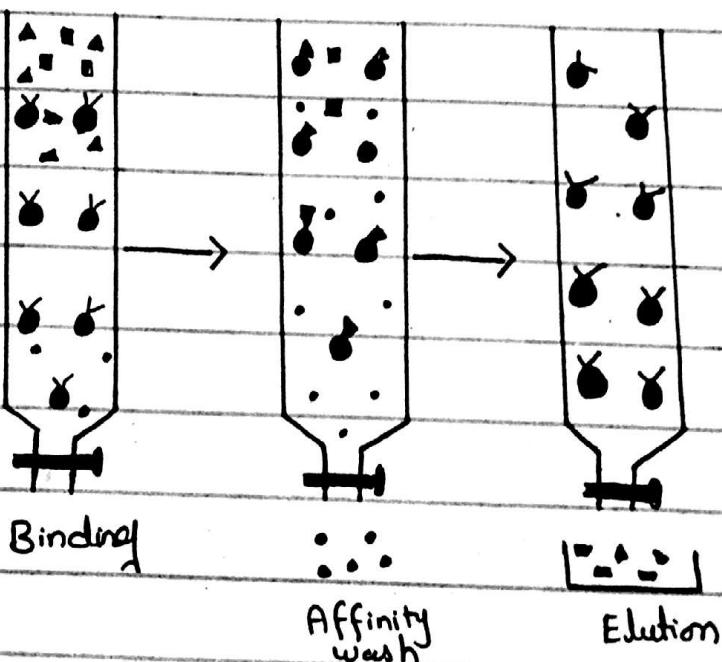
#### Definition:-

Affinity chromatography is very selective and provides high resolution with an intermediate to high sample loading capacity.

## Principle / Working:-

Affinity chromatography works on the principle of specific binding between a molecule and its ligand. A ligand is attached to a stationary phase. When a mixture passes through, only the molecules with a specific affinity for that ligand binds, while others are washed away.

## Diagram:-



▲ → ligand

● → Target Protein

○ → Affinity matrix

:: → unbounded material

## Types of Affinity Chromatography

There are different types of affinity chromatography.

### 1) Lectin Affinity chromatography:-

Uses Lectins to bind carbohydrates.

### 2) Dye- Ligand Affinity chromatography:-

Uses dyes to bind Proteins.

### 3) Protein A/G Affinity chromatography:-

Uses protein A or G to bind antibodies.

## Advantages:-

1) Affinity chromatography is used to study enzymes and other Proteins.

2) Affinity chromatography is used in genetic engineering.

3) Protein or Enzyme Purification by affinity chromatography.

4) High specificity with affinity chromatography.

## Disadvantages:-

1) Expensive ligands

2) leakage of ligand

3) Limited lifetime

4) Non-specific adsorption

5) Relatively low productivity.

# THEORETICAL CONSIDERATION OF CHROMATOGRAPHY

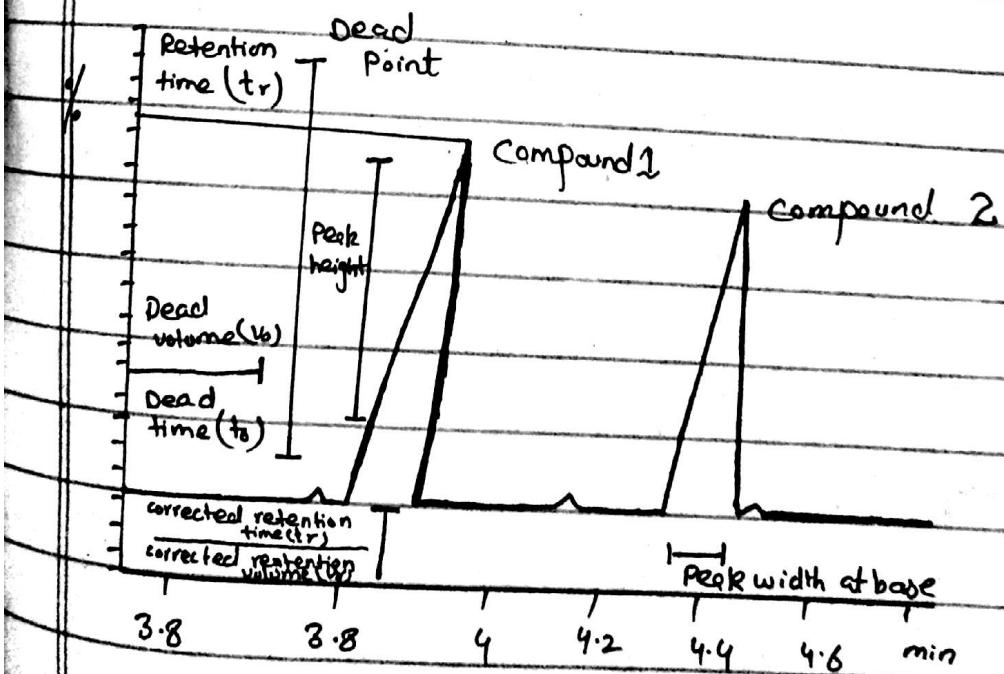
## Chromatographic Parameters / Terms:-

### i) The Chromatogram:-

The chromatogram is representation of variation in the amount of sample present in mobile phase with time when it moves out

from chromatographic system. A detector can show the response of the separation process as a function of elution time in the form

of chromatogram (figure 2.1)



### 2) Baseline:-

The baseline corresponds to the point of chromatogram at which only mobile phase is move out from the column.

### 3) Peak Maximum:-

Peak maximum is the highest point of the peak appears in chromatogram.

### 4) Injection Point:-

Injection Point is that point in response time / position in chromatogram at which the sample is injected in the column.

### 5) Dead Point:-

The position that represents the peak maximum for an un-retained sample is called dead point.

### 6) Dead Time:-

Dead time ( $t_0$ ) is the time from the injection point of sample to the dead point.

## HPLC Instrumentation

HPLC instrumentation mainly consists of different types of pumps, injectors,

columns, detectors, mobile phase reservoir, Pulse damper and Solvent Proportioning value.

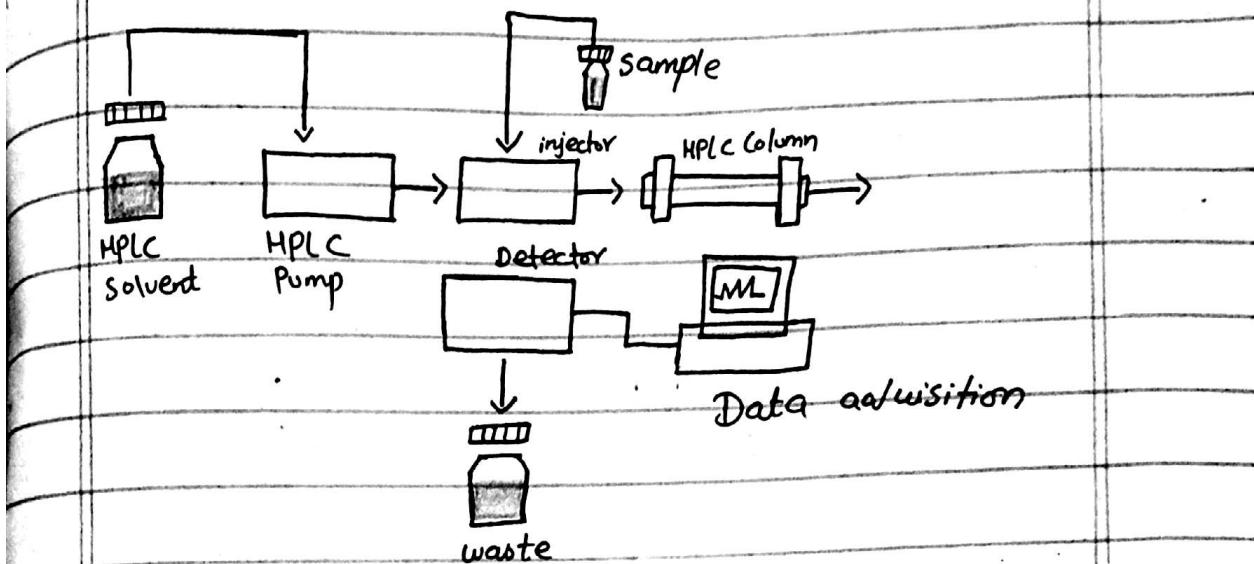


Diagram of a typical HPLC System  
Showing its Components

### Types of HPLC:-

#### 1) Pumping System:-

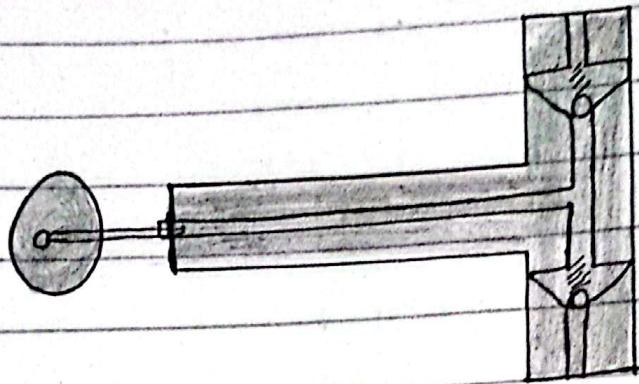
The Pumping system in HPLC is used to deliver the mobile Phase (Solvent) through the column at a constant and accurate Pressure and flow rate, even when the resistance inside the column is high.

#### 2) Constant flow reciprocating Pump:-

A constant flow reciprocating pump is the most commonly used pump in modern HPLC systems.

It is designed to deliver the mobile phase at a constant flow rate, even under high pressure (up to 6000 Psi or more).

(Diagram:-

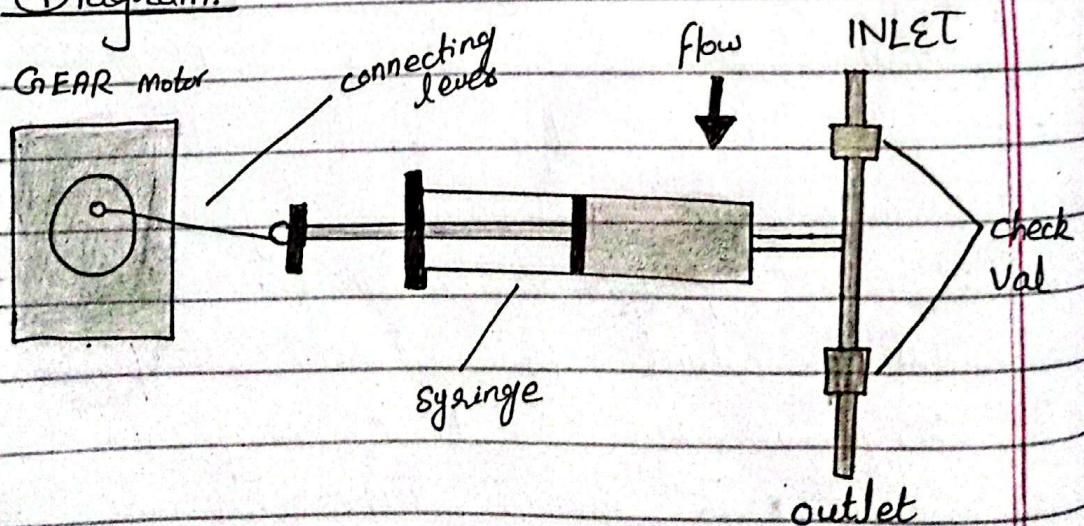


Constant flow reciprocating Pump.

3) Syringe type Pump:-

A syringe-type pump also known as a syringe pump or infusion pump, is a device that delivers precise volumes of fluid at controlled rates.

(Diagram:-



#### 4) Constant Pressure Pump:-

A constant Pressure Pump maintains a consistent Pressure output, regardless of flow rate or System resistance.

#### 5) Injectors:-

The injector is an essential part of the HPLC instrumentation.

It is used to introduce a small, precise volume of sample into the flowing mobile phase that enters the column.

#### 6) Manual Injection:-

Manual injection in an HPLC system involves using a 6-port valve with a syringe to load a sample into a loop and then inject it onto the column.

#### 7) Automatic Injection:-

Automatic injection also known as autoSampling, uses a device to automatically inject sample into the HPLC system.

### Separation Process

#### 1) Normal Phase Chromatography:-

Stationary Phase is polar

(Hydrophilic) and mobile Phase is non-Polar (Hydrophobic). Chromatography, the Stationary Phase is Strongly Polar in nature and the mobile Phase is non-Polar.

## 2) Reverse Phase Chromatography:-

Stationary Phase is non-Polar (hydrophobic) and mobile Phase is Polar (hydrophilic).

→ Polar-Polar bonds and non-Polar - non-Polar bonds have more affinity than Polar-Non Polar bonds.

→ Reverse Phase chromatography is more commonly used as drugs are usually hydrophilic.