## History of Chromatography

Chromatography is derived from the Greek word **'chroma'** means **'color'** and **'graphein'** means **writing** or **recording**.

In 1890, Mikhail Tsvet, a Russian Italian Botanist invented an earliest form of true chromatography technique for the separation of plant pigmentation.

But later, evolution of paper chromatography stroked and improved by Raphael E Liesegang in 1927. Archer Martin and Richard Synge again popularized it and further developed gas chromatography in collaboration with Anthony James.

It separates a chemical mixture into an individual component and helps in analysis of the particular compound.

Chromatography is generally carried out by organic chemist and biochemists for analysis, isolation and purification.

## Definition

Chromatography separates a component of mixture which is dissolved in a substance called the mobile phase and is carried out by a second substance called the stationary phase.

Chromatography is a method of separation in which the components to be separated are distributed between two phases, one of these is called a stationary phase and the other a mobile phase which moves on the stationary phase in a definite direction.

## Basic working principle of Chromatography

Chromatography is a method of physical separation in which components of mixture gets separated on two phases.

One of the phase is the immobile porous bed bulk liquid which is called stationary phase and the other phase is the mobile fluid that flows over the stationary phase under gravity.

During the movement of the sample, a separated result is formed by the repeated desorption and sorption in the direction of the mobile phase migration.

Several key factors are responsible on the separation process like partition between liquid-liquid, affinity between molecular weight and characteristics related to liquid-solid adsorption.

An interaction between the molecules are physical and involves weak chemical bonds like dipole-dipole interaction and hydrogen bond formation and adhere to the stationary components.

Components that adhere strongly to the stationary phase moves slowly than those who adhere weakly.

| **Short Question/blank** | |
|---|---|
| **1. Who inveted Chromarography?** | Russiaan botanist Tswellt |
| **2. Chromatography is ____** | Separation technique |
| **3. Chromatography types of _____ separation technique** | Physical |
| **4. First used chromatography technique name_____** | Column chromatography |
| **5. Chromatography consist two phase name?** | Stationary and mobile phase |

## Classification of Chromatography

If we look into the definition of chromatography and the situation spelt out above,

chromatography can be classified on the basis of the following:

• The shape of the solid support (particle/bed shape).

• The nature of the mobile phase.

• The mechanism responsible for separation.

## Classification of Chromatography based on the Chromatographic Bed/particle Shape

**Column Chromatography:** In this type of chromatography, the stationary phase of the setup is placed inside a tube. Then, the particles of the stationary phase (which is in the solid state) are made to fill the inside with the tube. An unrestricted, open path is then prepared for the mobile phase (somewhere along the middle of the tube).

**Planar Chromatography:** In this type of chromatography, the stationary phase of the apparatus usually has a planar shape. Different subcategories of planar chromatography include paper chromatography (where the stationary phase is a special type of paper) and thin layer chromatography (usually abbreviated as TLC).

## ➢ of Chromatography based on the Physical State of the Mobile Phase

**Gas Chromatography:** In this type of chromatography, the mobile phase is a substance that exists in the gaseous state. It can be noted that gas chromatography is also known as gas-liquid chromatography, and is often abbreviated to GLC. This type of chromatography almost always involves the use of a packed column.

**Liquid Chromatography:** This type of chromatography involves the use of a mobile phase that exists in the liquid state. Liquid chromatography, often abbreviated to LC, can be carried out either on a plane or in a column. It can be noted that there exist many subcategories under liquid chromatography such as high-performance liquid chromatography and reversed phase liquid chromatography.

## ➢ Classification of Chromatography based on the Mechanism of the Separation

**Ion Exchange Chromatography:** This type of chromatography is also known as ion chromatography. Ion exchange chromatography involves the separation of the components of the mixture via an ion exchange mechanism. Differently charged components of the mixture are separated with the help of different ions in this separation technique.

**Size Exclusion Chromatography:** This type of chromatography involves the separation of different components of the mixture based on their sizes. In size exclusion chromatography, components of the mixture are filtered based on their hydrodynamic volume or hydrodynamic diameters. It can be noted that size exclusion chromatography is also known as gel permeation chromatography or gel filtration chromatography.

| **Short Question/blank** | |
|---|---|
| **6. Which type include Column Chromatography?** | Adsorption |
| **7. Which type include Paper Chromatography?** | Partition |
| **8. GLC means?** | Gas Liquid Chromatography |
| **9. LC include Chrmotagraphy name ?** | PC and GLC |
| **10. TLC separate component of mixture based on_____** | Adsorption |

## What is Column Chromatography ?

In chemistry, Column chromatography is a technique which is used to separate a single chemical compound from a mixture dissolved in a fluid. It separates substances based on

differential adsorption of compounds to the adsorbent as the compounds move through the column at different rates which allow them to get separated in fractions. This technique can be used on a small scale as well as large scale to purify materials that can be used in future experiments. This method is a type of adsorption chromatography technique.

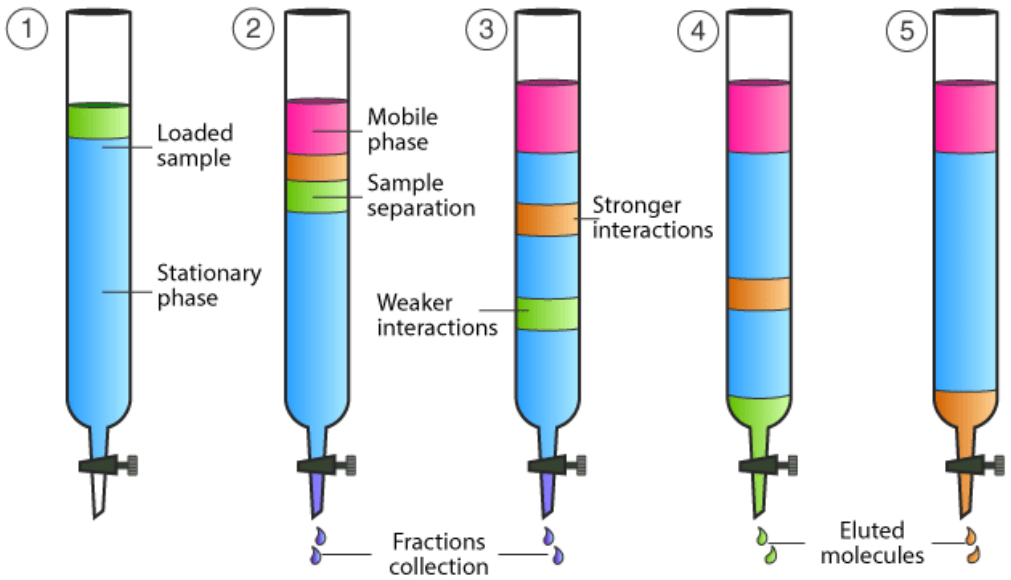
## Column Chromatography Principle

When the mobile phase along with the mixture that needs to be separated is introduced from the top of the column, the movement of the individual components of the mixture is at different rates. The components with lower adsorption and affinity to stationary phase travel faster when compared to the greater adsorption and affinity with the stationary phase. The components that move fast are removed first whereas the components that move slowly are eluted out last.

The adsorption of solute molecules to the column occurs in a reversible manner. The rate of the movement of the components is expressed as:

Rf = the distance travelled by solute/ the distance travelled by the solvent

Rf is the retardation factor.



## Column Chromatography procedure

The stationary phase is made wet with the help of solvent as the upper level of the mobile phase and the stationary phase should match. The mobile phase or eluent is either solvent or mixture of solvents. In the first step the compound mixture that needs to be separated, is added from the top of the column without disturbing the top level. The tap is turned on and the adsorption process on the surface of silica begins.

Without disturbing the stationary phase solvent mixture is added slowly by touching the sides of the glass column. The solvent is added throughout the experiment as per the requirement.

The tap is turned on to initiate the movement of compounds in the mixture. The movement is based on the polarity of molecules in the sample. The non-polar components move at a greater speed when compared to the polar components.

For example, a compound mixture consists of three different compounds viz red, blue, green then their order based on polarity will be as follows blue>red>green

As the polarity of the green compound is less, it will move first. When it arrives at the end of the column it is collected in a clean test tube. After this, the red compound is collected and at last blue compound is collected. All these are collected in separate test tubes.

## Characteristics of Adsorbent/(stationary phase)

- ✓ **Shape and size of particle:** Particles should have uniform shape and size in the range of 60 – 200μ in diameter.
- ✓ **Stability and inertness of particles:** high mechanical stability and chemically inert. Also, no reaction with acids or bases or any other solvents used during the experiment.
- ✓ It should be colourless, inexpensive and readily available.
- ✓ Should allow free flow of mobile phase
- ✓ It should be suitable for the separation of mixtures of various compounds**.**

## Column Chromatography Applications

- ✓ Column Chromatography is used to isolate active ingredients.
- ✓ It is very helpful in Separating compound mixtures.
- ✓ It is used to determine drug estimation from drug formulations
- ✓ It is used to remove impurities.
- ✓ Used to isolation metabolites from biological fluids.

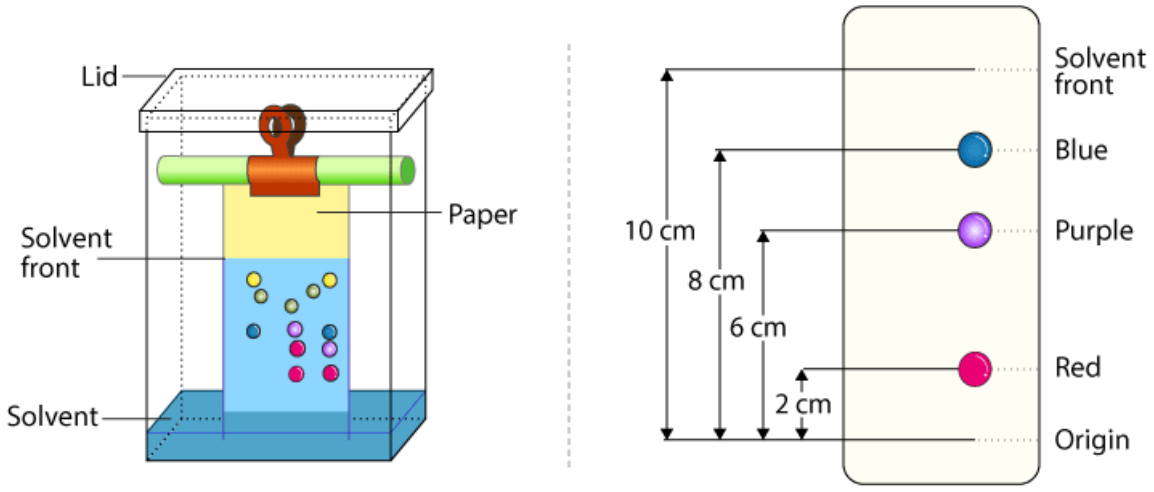
## What Is Paper Chromatography?

Chromatography technique that uses paper sheets or strips as the adsorbent being the stationary phase through which a solution is made to pass is called paper chromatography. It is an inexpensive method of separating dissolved chemical substances by their different migration rates across the sheets of paper. It is a powerful analytical tool that uses very small quantities of material. Paper chromatography was discovered by Synge and Martin in the year 1943.

## Paper Chromatography Principle

The principle involved can be partition chromatography or adsorption chromatography. Partition chromatography because the substances are partitioned or distributed between liquid phases. The two phases are water held in pores of the filter paper and the other phase is a mobile phase which passes through the paper. When the mobile phase moves, the separation of the mixture takes place. The compounds in the mixture separate themselves based on the differences in their affinity towards stationary and mobile phase solvents under the capillary action of pores in the paper. Adsorption chromatography between solid and liquid phases, wherein the solid surface of the paper is the stationary phase and the liquid phase is the mobile phase.

### Paper Chromatography Diagram



## Types of Paper Chromatography:

**1. Ascending Paper Chromatography** – The techniques goes with its name as the solvent moves in an upward direction.

**2. Descending Paper Chromatography** – The movement of the flow of solvent due to gravitational pull and capillary action is downwards, hence the name descending paper chromatography.

**3. Radial or Circular Paper Chromatography** – The sample is deposited at the centre of the circular filter paper. Once the spot is dried, the filter paper is tied horizontally on a Petri dish which contains the solvent.

**4. Two Dimensional Paper Chromatography** – Substances which have the same rf values can be resolved with the help of two-dimensional paper chromatography.

## Paper Chromatography Procedure

Below we have explained the procedure to conduct Paper Chromatography Experiment for easy understanding of students.

- **Selecting a suitable type of development:** It is decided based on the complexity of the solvent, paper, mixture, etc. Usually ascending type or radial paper chromatography is used as they are easy to perform. Also, it is easy to handle, the chromatogram obtained is faster and the process is less time-consuming.
- **Selecting a suitable filter paper:** Selection of filter paper is done based on the size of the pores and the sample quality.
- **Prepare the sample:** Sample preparation includes the dissolution of the sample in a suitable solvent (inert with the sample under analysis) used in making the mobile phase.
- **Spot the sample on the paper:** Samples should be spotted at a proper position on the paper by using a capillary tube.
- **Chromatogram development:** Chromatogram development is spotted by immersing the paper in the mobile phase. Due to the capillary action of paper, the mobile phase moves over the sample on the paper.
- **Paper drying and compound detection:** Once the chromatogram is developed, the paper is dried using an air drier. Also, detecting solution can be sprayed on the chromatogram developed paper and dried to identify the sample chromatogram spots.

## Paper Chromatography Application

There are various applications of paper chromatography. Some of the uses of Paper Chromatography in different fields are discussed below:

- ✓ To study the process of fermentation and ripening.
- ✓ To check the purity of pharmaceuticals.
- ✓ To inspect cosmetics.
- ✓ To detect the adulterants.
- ✓ To detect the contaminants in drinks and foods.
- ✓ To examine the reaction mixtures in biochemical laboratories.
- ✓ To determine dopes and drugs in humans and animals.

| **Short Question/ blank** | |
|---|---|
| **11. Who developed paper chromatography?** | Martin and Synge |
| **12. Silica gel as _____ in column chromatography** | Strong adsorbent |
| **13. Sugar and starch are examples of _____** | Weak adsorbent |
| **14. Amino acid and metal ion mixtures separate _____ use chromatography** | Paper chromatography |
| **15. What is full form of Rf ?** | Retardation factor |

## What is Thin Layer Chromatography?

Thin Layer Chromatography is a technique used to isolate non-volatile mixtures. The experiment is conducted on a sheet of aluminium foil, plastic, or glass which is coated with a thin layer of adsorbent material. The material usually used is aluminium oxide, cellulose, or silica gel.

On completion of the separation, each component appears as spots separated vertically. Each spot has a retention factor (Rf) expressed as:

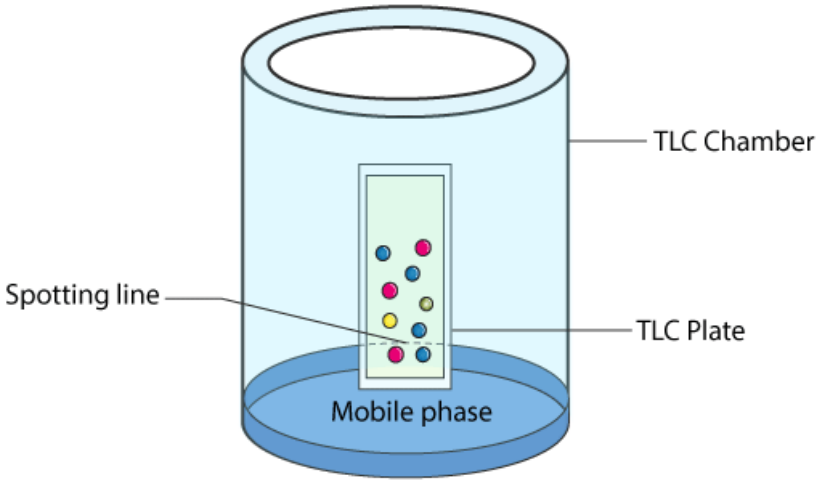
Rf = dist. travelled by sample / dist. travelled by solvent

The factors affecting retardation factor are the solvent system, amount of material spotted, absorbent and temperature. TLC is one of the fastest, least expensive, simplest and easiest chromatography technique.

## Thin Layer Chromatography Principle

Like other chromatographic techniques, thin-layer chromatography (TLC) depends on the separation principle. The separation relies on the relative affinity of compounds towards both the phases. The compounds in the mobile phase move over the surface of the stationary phase. The movement occurs in such a way that the compounds which have a higher affinity to the stationary phase move slowly while the other compounds travel fast. Therefore, the separation of the mixture is attained. On completion of the separation process, the individual components from the mixture appear as spots at respective levels on the plates. Their character and nature are identified by suitable detection techniques.

## Thin Layer Chromatography Diagram




## Thin Layer Chromatography Procedure

Before starting with the Thin Layer Chromatography Experiment, let us understand the different components required to conduct the procedure along with the phases involved.

**1. Thin Layer Chromatography Plates** – ready-made plates are used which are chemically inert and stable. The stationary phase is applied on its surface in the form of a thin layer. The stationary phase on the plate has a fine particle size and also has a uniform thickness.

**2. Thin Layer Chromatography Chamber** – Chamber is used to develop plates. It is responsible to keep a steady environment inside which will help in developing spots. Also, it prevents the solvent evaporation and keeps the entire process dust-free.

**3. Thin Layer Chromatography Mobile phase** – Mobile phase is the one that moves and consists of a solvent mixture or a solvent. This phase should be particulate-free. The higher the quality of purity the development of spots is better.

**4. Thin Layer Chromatography Filter Paper** – It has to be placed inside the chamber. It is moistened in the mobile phase.

## Thin Layer Chromatography Applications

- ✓ The qualitative testing of various medicines such as sedatives, local anaesthetics, anticonvulsant tranquilisers, analgesics, antihistamines, steroids, hypnotics is done by TLC.

- ✓ TLC is extremely useful in Biochemical analysis such as separation or isolation of biochemical metabolites from its blood plasma, urine, body fluids, serum, etc.
- ✓ Thin layer chromatography can be used to identify natural products like essential oils or volatile oil, fixed oil, glycosides, waxes, alkaloids, etc.
- ✓ It is widely used in separating multicomponent pharmaceutical formulations.
- ✓ It is used to purify of any sample and direct comparison is done between the sample and the authentic sample.
- ✓ It is used in the food industry, to separate and identify colours, sweetening agent, and preservatives
- ✓ It is used in the cosmetic industry.
- ✓ It is used to study if a reaction is complete.

## What Is Gas Chromatography?

Gas Chromatography or Gas Liquid Chromatography is a technique applied for separation, identification and quantification of components of a mixture of organic compounds by selective partitioning between the stationary phase and mobile phase inside a column followed by sequential elution of separated components.

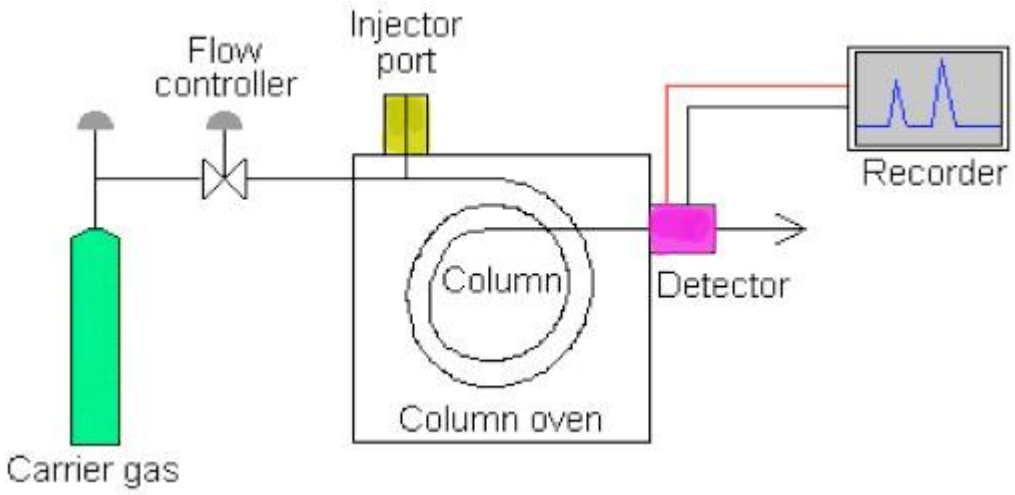
The technique is suitable for separation of compounds having following characteristics:

- ✓ High volatility
- ✓ Thermal stability
- ✓ Low molecular weights

## Principle of Gas Chromatography (GC)

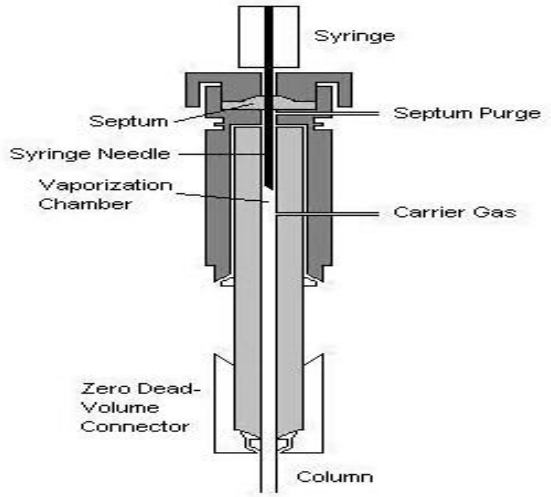
The sample solution injected into the instrument enters a gas stream which transports the sample into a separation tube known as the "column." (Helium or nitrogen is used as the so-called carrier gas.) The various components are separated inside the column. The detector measures the quantity of the components that exit the column. To measure a sample with an unknown concentration, a standard sample with known concentration is injected into the instrument. The standard sample peak retention time (appearance time) and area are compared to the test sample to calculate the concentration.

## Gas Chromatography diagram




## Sample Injection

A sample port is necessary for introducing the sample at the head of the column. Modern injection techniques often employ the use of heated sample ports through which the sample can be injected and vaporized in a near simultaneous fashion. A calibrated micro syringe is used to deliver a sample volume in the range of a few microliters through a rubber septum and into the vaporization chamber. Most separations require only a small fraction of the initial sample volume and a sample splitter is used to direct excess sample to waste. Commercial gas chromatographs often allow for both split and splitless injections when alternating between packed columns and capillary columns. The vaporization chamber is typically heated 50 °C above the lowest boiling point of the sample and subsequently mixed with the carrier gas to transport the sample into the column.




## Carrier Gas

The carrier gas plays an important role, and varies in the GC used. Carrier gas must be dry, free of oxygen and chemically inert mobile-phase employed in gas chromatography. Helium is most commonly used because it is safer than, but comparable to hydrogen in efficiency, has a larger range of flow rates and is compatible with many detectors. Nitrogen, argon, and hydrogen are also used depending upon the desired performance and the detector being used. Both hydrogen and helium, which are commonly used on most traditional detectors such as Flame Ionization(FID), thermal conductivity (TCD) and Electron capture (ECD), provide a shorter analysis time and lower elution temperatures of the sample due to higher flow rates and low molecular weight. For instance, hydrogen or helium as the carrier gas gives the highest sensitivity with TCD because the difference in thermal conductivity between the organic vapour and hydrogen/helium is greater than other carrier gas. Other detectors such as mass spectroscopy, uses nitrogen or argon which has a much better advantage than hydrogen or helium due to their higher molecular weights, in which improve vacuum pump efficiency.

## Column Oven

The thermostatted oven serves to control the temperature of the column within a few tenths of a degree to conduct precise work. The oven can be operated in two manners: isothermal programming or temperature programming. In isothermal programming, the temperature of the column is held constant throughout the entire separation. The optimum column temperature for isothermal operation is about the middle point of the boiling range of the sample. However, isothermal programming works best only if the boiling point range of the sample is narrow. If a low isothermal column temperature is used with a wide boiling point range, the low boiling

fractions are well resolved but the high boiling fractions are slow to elute with extensive band broadening. If the temperature is increased closer to the boiling points of the higher boiling components, the higher boiling components elute as sharp peaks but the lower boiling components elute so quickly there is no separation.

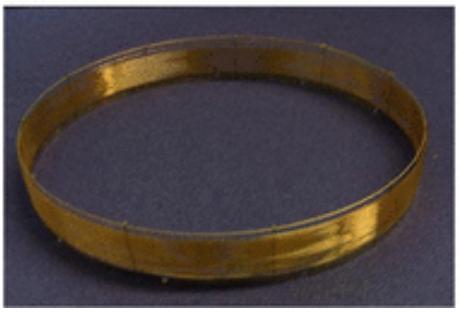
Two types of columns are used in gas chromatography**: packed columns** and **capillary columns.**

**Packed columns**

- Inner diameter of 2 to 4 mm
- Column is completely filled with particles
  Adsorbent → GSC
  Support coated with liquid stationary phase → GLC
- Column length is restricted due to high flow resistivity (< 10 m)
- Column material: copper, stainless steal, glass, quartz

**Capillary columns**

- Inner diameter <1 mm
- Stationary phase is only coated to the inner column wall
- Column length: 5 to 100 m
- Column material: glass (fragile), fused silica (quartz) made from ultra pure $SiO_2$ with an outer protective coating of polyimide (flexible), fused silica coated stainless steal (high temperature resistant)



## Detector

The detector is the device located at the end of the column which provides a quantitative measurement of the components of the mixture as they elute in combination with the carrier gas. In theory, any property of the gaseous mixture that is different from the carrier gas can be used as a detection method.

In GC different types of detector use such as Flame Ionization (FID), thermal conductivity (TCD) and Electron capture (ECD). Each detector has two main parts that when used together they serve as transducers to convert the detected property changes into an electrical signal that is recorded as a chromatogram. The first part of the detector is the sensor which is placed as close the column exit as possible in order to optimize detection. The second is the electronic equipment used to digitize the analog signal so that a computer may analyze the acquired chromatogram.

## Gas Chromatography Application

- ✓ Gas chromatography is a physical separation method in where volatile mixtures are separated. It can be used in many different fields such as pharmaceuticals, cosmetics and even environmental toxins.
- ✓ Since the samples have to be volatile, human breathe, blood, saliva and other secretions containing large amounts of organic volatiles can be easily analyzed using GC. Knowing the amount of which compound is in a given sample gives a huge advantage in studying the effects of human health and of the environment as well.

- ✓ Air samples can be analyzed using GC. Most of the time, air quality control units use GC coupled with FID in order to determine the components of a given air sample. Although other detectors are useful as well, FID is the most appropriate because of its sensitivity and resolution and also because it can detect very small molecules as well.

- ✓ GC/MS is also another useful method which can determine the components of a given mixture using the retention times and the abundance of the samples. This method be applied to many pharmaceutical applications such as identifying the amount of chemicals in drugs. Moreover, cosmetic manufacturers also use this method to effectively measure how much of each chemical is used for their products**.**

| **Short Questions/Blanks** | |
|---|---|
| **16. What is full form of TLC?** | Thin Layer Chromatography |
| **17. Give name column used in GC?** | Packed and Capilary column |
| **18. GSC or GLC more efficient?** | GLC |
| **19. What is GSC?** | Gas Solid Chromatography |
| **20. Detector used in GC?** | TCD,ECD and FID |

## What Is Ion-exchange Chromatography?

Ion chromatography (or ion-exchange chromatography) separates ions and polar molecules based on their affinity to the ion exchanger. It works on almost any kind of charged molecule—including large proteins, small nucleotides, and amino acids. However, ion chromatography must be done in conditions that are one unit away from the isoelectric point of a protein.

## Principle

This chromatography distributes the analyte molecule as per charge and their affinity towards the oppositively charged matrix. The analytes bound to the matrix are exchanged with a competitive counter ion to elute. The interaction between matrix and analyte is determined by net charge, ionic strength and pH of the buffer. For example, when a mixture of positively charged analyte (M, M+, M-1, M-2) loaded On to a positively charged matrix, the neutral or positively charged analyte will not bind to the matrix where as negatively charged analyte will bind as per their relative charge and needed higher concentration of counter ion to elute from matrix.

The matrix used in ion-exchange chromatography is present in the ionized form with reversibly bound ion to the matrix. The ion present on matrix participitate in the reversible exchange process with analyte. Hence, there are two types of ion-exchange chromatography

**1.Cation exchange chromatography-** In cation exchange chromatography, matrix has a negatively charged functional group with a affinity towards positively charged molecules. The positively charged analyte replaces the reversible bound cation and binds to the matrix. In the presence of a strong cation (such as Na+) in the mobile phase, the matrix bound positively charged analyte is replaced with the elution of analyte. The cation exchangers used are given examples.

**2. Anion Exchange chromatography-** In anion exchange chromatography, matrix has a positively charged functional group with a affinity towards negatively charged molecules. The negatively charged analyte replaces the reversible bound anion and binds to the matrix. In the presence of a strong anion (such as Cl-) in the mobile phase, the matrix bound negatively charged analyte is replaced with the elution of analyte. The anion exchangers used are given examples.

| Type | Functional Group | Examples |
|---|---|---|
| strong acid cation exchanger | sulfonic acid | $-SO_3^-$<br>$-CH_2CH_2SO_3^-$ |
| weak acid cation exchanger | carboxylic acid | $-COO^-$<br>$-CH_2COO^-$ |
| strong base anion exchanger | quaternary amine | $-CH_2N(CH_3)_3^+$<br>$-CH_2CH_2N(CH_2CH_3)_3^+$ |
| weak base anion exchanger | amine | $-NH_3^+$<br>$-CH_2CH_2NH(CH_2CH_3)_2^+$ |

## Properties of ion exchange resins

- **Swelling**: Ion exchange resins are hygroscopic. The amount of moisture hydrated by a resin is determined by the cross-linking and the type of functional group. Low cross-linking gel resins with functional groups of sulfonic acid or quaternary ammonium contain large amounts of water resulting in swelling. Frequent swelling and contraction reduce the resin life.
- **Capacity**: Capacity is a number of chemical equivalents of ions that can be taken up by a unit amount of the resin (dry weight/wet weight/wet volume). Cross-linking decreases the capacity measured on the dry basis (fewer functional groups may be attached to highly cross-linked polymer molecules). However cross-linking also decreases hydration of the resin therefore the capacity measured on the wet basis increases with an increase of the cross-linking level.
- **Particle size:** Ion exchange resins are available in different particle (bed) size. Common ion exchange resins are manufactured in form of polydispersed spherical beds with the size distributed within the range 0.01-0.05" (0.25-1.25mm) or in form of uniform particle size (UPS). Smaller particles improve the kinetics of the ion exchanging reaction but cause increase of the water pressure drop and decrease of the flow rate.
- **Stability:** Mechanical (physical) stability of ion exchange resins is determined mainly by the toughness of the polymer structure (cross-linking) and by the frequency of swelling-contraction cycles. Chemical degradation of ion exchange resins may be caused by fouling the resin pores by precipitates (e.g., iron hydroxide), breaking polymer structure, loss of ion exchange capacity due to a modification of the functional groups.

## Ion exchange chromatography Applications

- ✓ Softening of hard water.
- ✓ Pharmaceuticals.
- ✓ Foods and Beverages.
- ✓ Clinical Studies.
- ✓ Separation of lanthanide and actinide element.
- ✓ Separation of alkaloids.
- ✓ Separation of amino acids.

| **Short Questions/Blanks** | |
|---|---|
| **21. Write the types of Ion exchange Chromatography** | Cation and Anion exchange Chromatography |
| **22. Cationic exchanger resin attached _____ functional group.** | Carboxylic, Sulphonic and Phenolic group |
| **23. Anionic exchanger resin attached _____ functional group.** | Chloride, hydroxide and Quarternary amine. |
| **24. Softening of hard water which Chromatography more useful?** | Ion-exchange |
| **25. Who Scientist introduced modern Ion exchange Chromatography?** | Adams and Holms in 1935 |

# Shree H.N.Shukla College of Science Rajkot

**B.Sc. (Sem- 6) (CBCS)**

**CHEMISTRY: [603]**

## Unit-4 Chapter-6: Chromatography