UNIT-IV

GAS CHROMATOGRAPHY

Points to be covered in this topic

- ❖ INTRODUCTION
- ❖ THEORY
- ❖ INSTRUMENTATION
- ❖ DERIVATIZATION
- ❖ ADVANTAGES
- ❖ DISADVANTAGES
- ❖ APPLICATIONS

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GAS CHROMATOGRAPHY

❖ INTRODUCTION

- **A.T. James and P. Martin** first time used the gas chromatography technique in 1952 for separating **long chain fatty acids**.
- The **gases and vaporisable substances** can also be separated by gas chromatography based on differential adsorption.
- In gas chromatography, **gas is used as the mobile phase** and **solid or liquid is used as the stationary phase**.
- When the **stationary phase is solid, it is known as Gas Solid Chromatography (GSC)** and when the **stationary phase is liquid, it is known as Gas Liquid Chromatography (GLC).**
- Gas Solid In gas chromatography, a moving gas phase is passed over a stationary sorbent to separate the mixture components.
- This technique is similar to that of liquid -liquid chromatography, with the only exception that in the former a moving gas is used as the mobile phase while in the latter it is a liquid. The stationary phase remains the same. i.e. a solid or a liquid.

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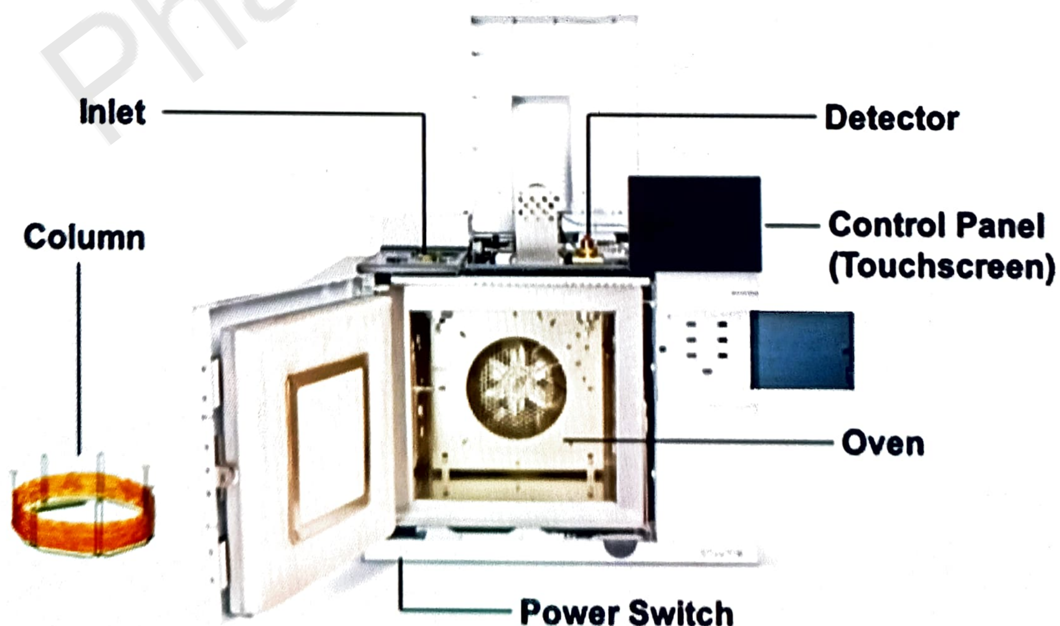

GAS CHROMATOGRAPHY

PRINCIPLE

- In gas chromatography, the substance to be analysed is **partitioned between the mobile and stationary phases.**
- During the separation, the **sample is vaporised and carried through the column** by the mobile gas phase (i.e., the carrier gas).
- The different components get separated **based on their vapour pressure and affinities for the stationary phase.**
- The affinity of a component towards the stationary phase is termed as **distribution constant** (Kc), which is also known as the **partition coefficient.**

Kc = [A]s / [A]m

Where,

[A]s = concentration of component A in the stationary phase

[A]m = concentration of component A in the mobile phase.

- Movement of different components through the column is controlled by the distribution constant (Kc), thus the chromatographic separation occurs based on the differences in distribution constant.

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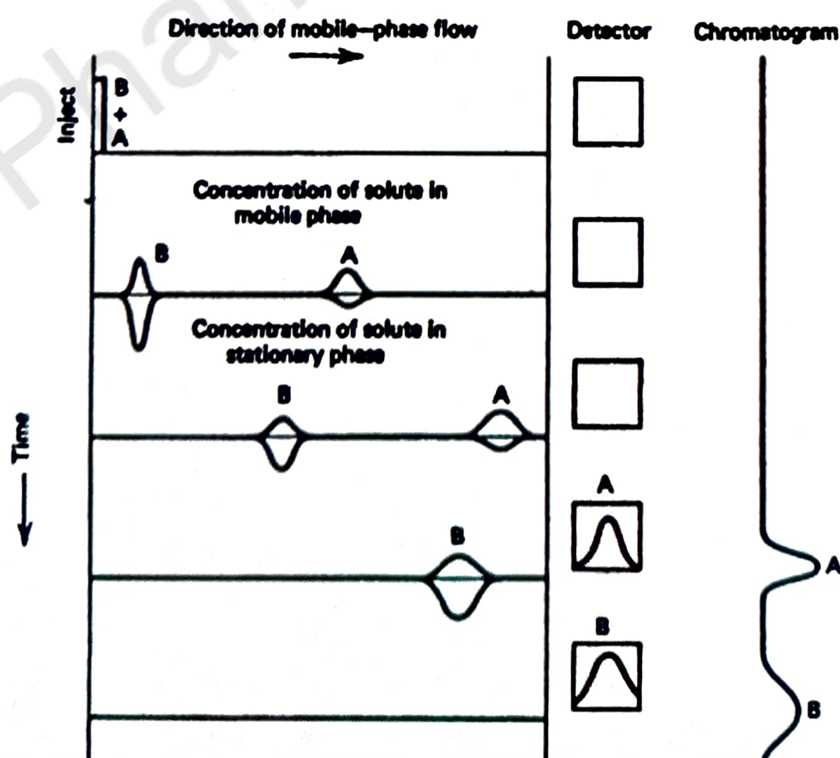
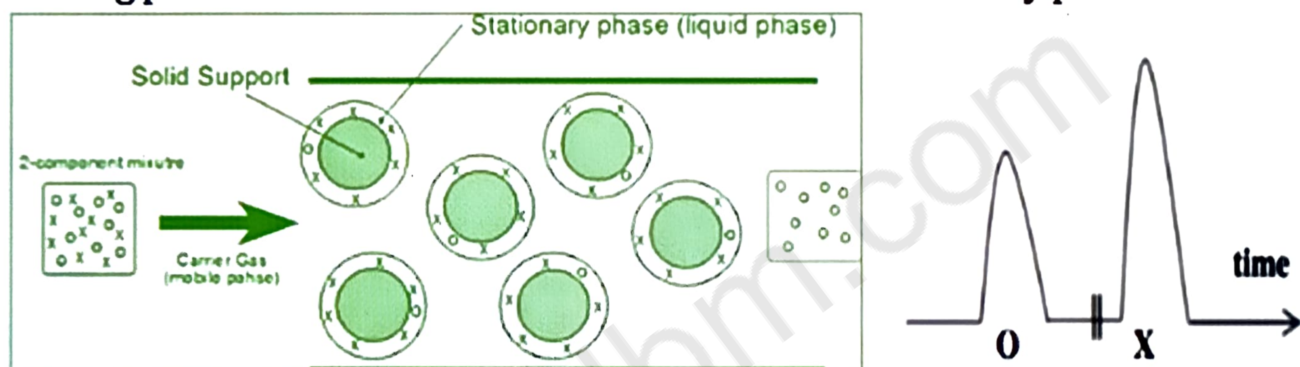


Schematic Representation of the Chromatographic Process

❖ THEORY

- The separation of compounds in a mixture is **based on different polarities** in a direct (interaction with stationary phase i.e., solubility) or indirect way (physical properties i.e., boiling point).
- The gas chromatography column consists of **solid support that is covered with a high-boiling liquid in a thin capillary tube.**
- In the example, **compound "X" has a higher affinity towards the stationary phase compared to compound "O".**
- Compound "O" elutes before compound "X" because it displays a lower boiling point and a weaker interaction with the stationary phase.



❑ Factor that influences the outcome in the GC

➢ Vapor Pressure of the Compound

- The **higher the boiling point of the compound is, the lower the vapor pressure will be.**
- Thus, the compound with **higher boiling point migrates slower through the column** resulting in a longer retention time and peak broadening.
- Low boiling solvents like **diethyl ether, dichloromethane, ethyl acetate, methanol**, etc. are usually used to dissolve the sample because they elute very early from the column.

➢ Polarity of the Compound and the Polarity of the Column

- The **stronger the interaction of the compound with the stationary phase** is going to be increasing the retention time and peak broadening.
- Very polar compounds (i.e., alcohols, amines) often cause tailing on polar columns (or older columns). A variety of column polarities is available for the separation of compounds using gas chromatography.

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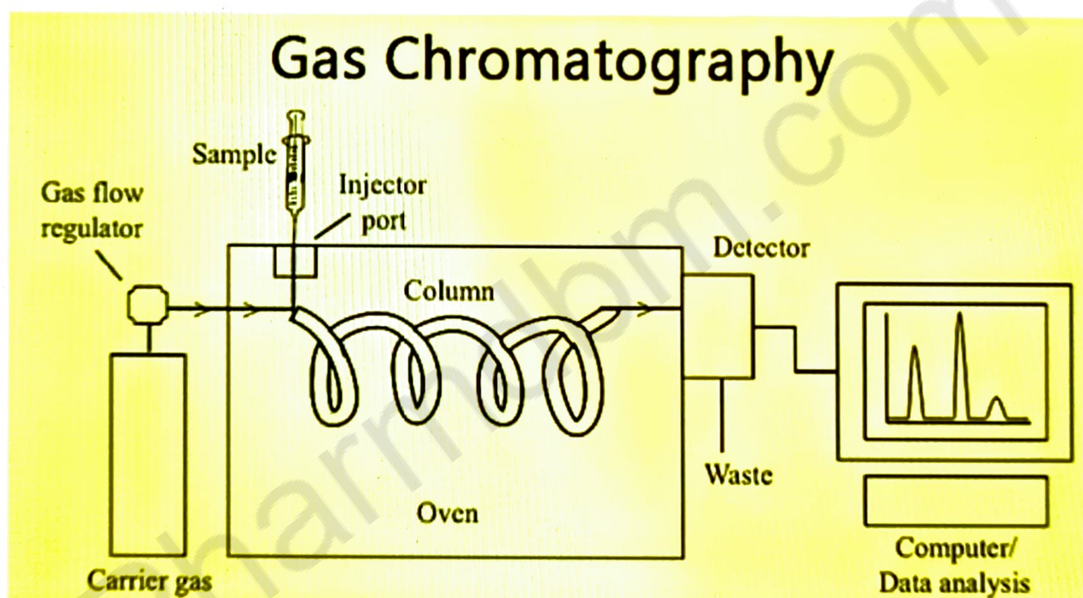
❖ INSTRUMENTATION

The following components make up the instrumentation of gas chromatography;

1. **Carrier gas** maintained at a high pressure and delivered at a rapid and reproducible rate,
2. **Sample injector,**
3. **Separation columns,**
4. **Detectors,**
5. **Thermostated chambers** for regulating the temperature of column and detectors,
6. **Amplifier** and **recorder** system.



❑ Carrier Gas

- **Hydrogen, helium, nitrogen**, and **air** are the most widely used carrier gases.
- **Hydrogen** in comparison to other gases is **more advantageous and also dangerous to use.**
- **Helium** is the next best gas, and is used because of its exceptional **thermal conductivity, inertness, low density, and greater flow rates**; but it is expensive.
- Nitrogen is **inexpensive but reduces sensitivity.**
- Air is used only when the **atmospheric oxygen is useful to the detector** or separation.

✓ The following considerations should be kept in mind while **selecting a carrier gas**:

- **It should be inert**, i.e., it should not react with the sample, stationary phase, or contacted hardware.
- Should be **suitable for the detector** used and the type of sample being analysed.
- It should be available in **high purity**.
- It should give best column performance reliable with **required speed of analysis.**
- It should not be **expensive.**
- It should not cause any **fire or explosion hazard**.

Sample Injector

- The system of sample injector is used for **introducing the sample in a reproducible manner and should vaporise it rapidly** so that the sample enters the column as a single slug.
- Liquid samples are introduced into a **small inlet chamber using hypodermic syringes** through a self-sealing rubber septum.
- The chamber is heated to cause **flash evaporation**, and the temperature should not be very high to **avoid sample decomposition**.
- Solid samples are **either dissolved in volatile liquids** prior to their introduction or are directly introduced if they are liquefiable.
- Gas samples are introduced into the carrier gas stream using a **special gas sampling valves.**

Separation Columns

- The columns used are made of **glass or metal tubing**, and have a diameter of **4.8mm.**
- They may be of any length ranging from a few centimetres to a hundred meters. They may be coiled, bent, or straight.
- The following **six types** of analytical columns are used in gas chromatography:

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- **Packed Columns:**

- These columns are prepared by **packing metal or glass tubing with granular stationary phase.**

- **Open Tubular or Capillary or Golay Columns:**

- These columns are made of **long capillary tubing (30-90m) and have uniform and narrow internal diameter (0.025-0.075cm).**
- They are of **stainless steel (most popular), copper, nylon, glass**, etc.
- The liquid phase is coated over the inner wall of capillary tubing as a thin (0.5-1μ) and uniform film.

- **Support Coated Open Tubular Columns:**

- These columns are prepared by **coating the inner wall of a capillary column with a micron size porous layer of support material**, followed by coating with the liquid phase as a thin film.

- **Wall Coated Open Tubular Columns:**

- These columns are prepared by **coating the unmodified smooth inner wall of the tube** with the liquid stationary phase.

- **Porous-Layer Open-Tubular (PLOT) Columns:**

- These columns are prepared by **coating the inner wall with a porous layer.**
- Porosity can be achieved either by **chemical methods** (e.g., etching) or by **depositing porous particles** on the wall from a suspension.

- **Support-Coated Open-Tubular (SCOT) Columns:**

- In these columns, the **porous layer consists of support particles** and was deposited from a suspension

Detectors

- Gas chromatography employs a wide range of detectors, of which **Flame Ionisation Detector (FID)** and the **Thermal Conductivity Detector (TCD)** are the most common ones.

✓ **Some desirable properties of a detector are:**

- Its **sensitivity should be high** and should not show instability at high sensitivities.

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- Its **volume should be low** so that the compound eluted from the column in a small plug of carrier gas does not undergo further dilution within the detector.
- Its **response should be rapid and linear** with the concentration of compound. It should be calibrated to determine the optimum range.
- Its response **should not be affected by the flow rate of carrier gas** and temperature.

Some commonly used detectors in gas chromatography are discussed below:

➤ **Katharometer:**

- This detector relies on the variation in thermal conductivity of the carrier gas in the presence of an organic compound.
- The platinum wires are heated by electric means and equilibrium conditions of temperature and resistance are attained when the carrier gas passes over them.
- They are mounted in a **Wheatstone bridge arrangement**, and when a compound emerges, the thermal conductivity of the gas surrounding wire changes.
- Also changing the temperature and resistance of the wire along with the associated out-of-balance signal, which is amplified and recorded.

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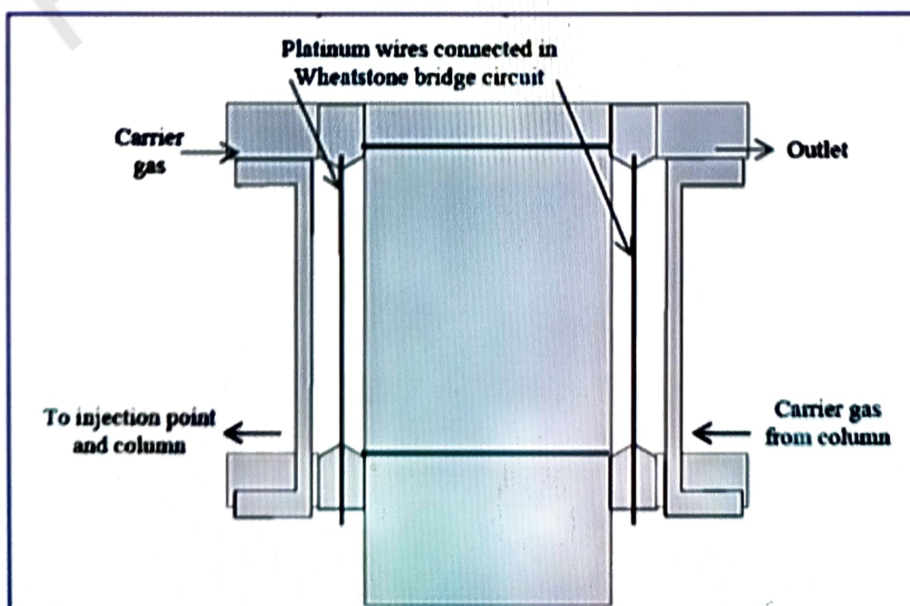
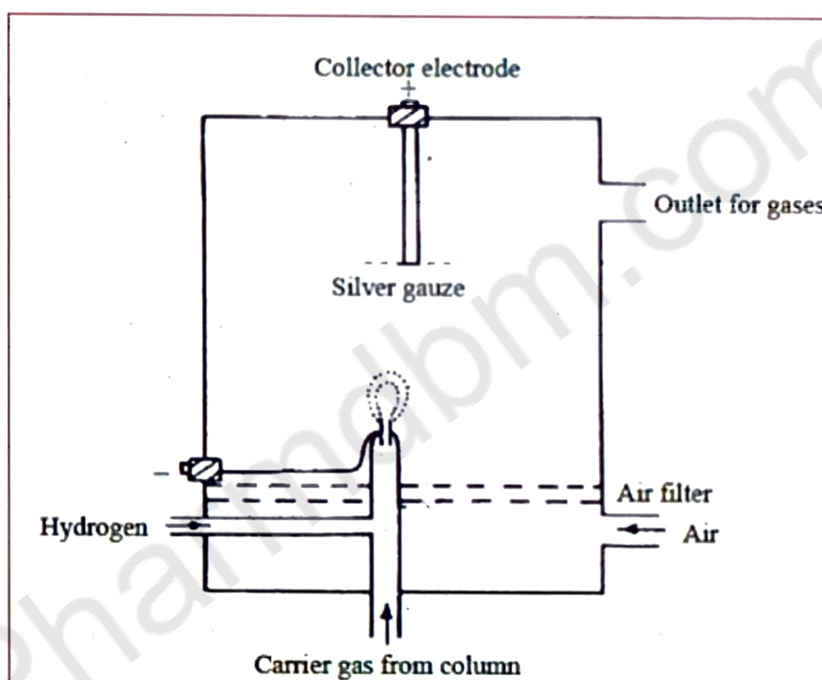


Diagrammatic Representation of Katharometer

➢ Flame Ionisation Detector (FID):

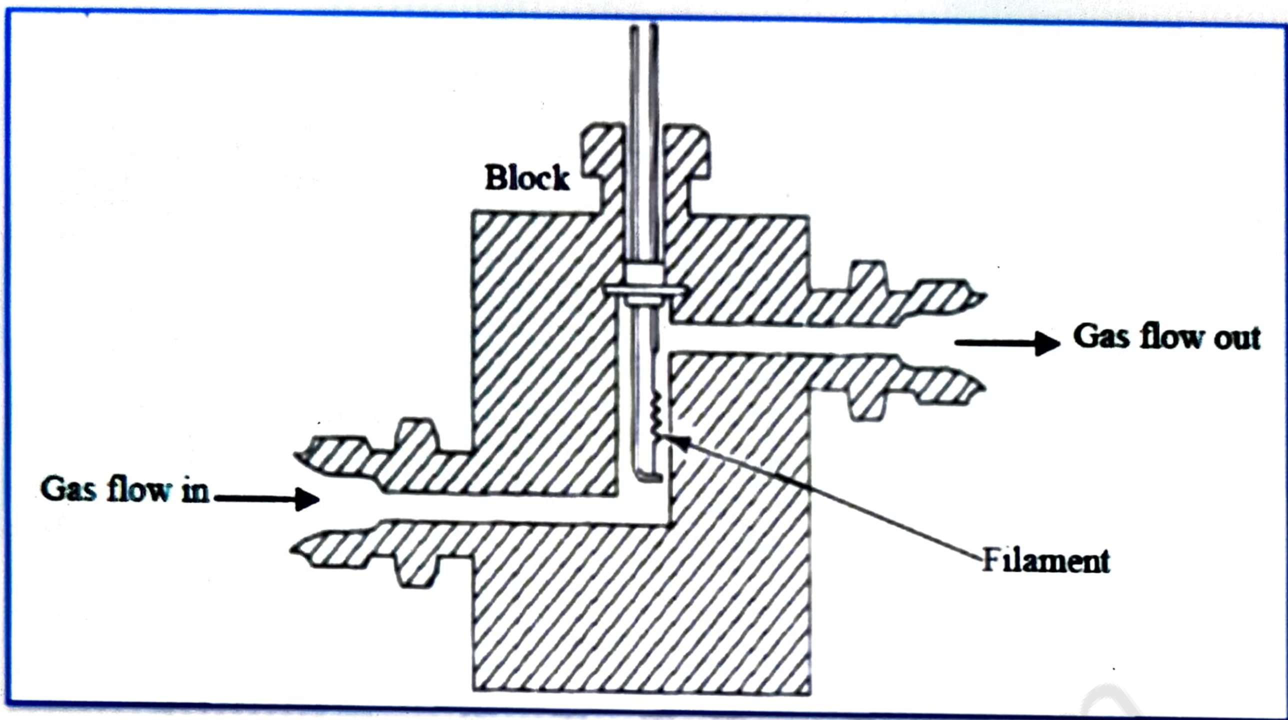
- This detector is simpler in design and relies on the **change in conductivity of the flame as the compound is burnt**.
- The change in flame conductivity is **not because of simple ionisation** of the compounds emerging from the detector.
- The molecule undergoes **partial or complete stripping and gives charged hydrogen-deficient polymers** or aggregates of carbon with low ionisation potential.
- The carrier gas used is **nitrogen or argon mixed with hydrogen** before passing to the burner tip (made of a platinum capillary).



Diagrammatic Representation of Flame Ionisation Detector

➢ Thermal Conductivity Detector (TCD):

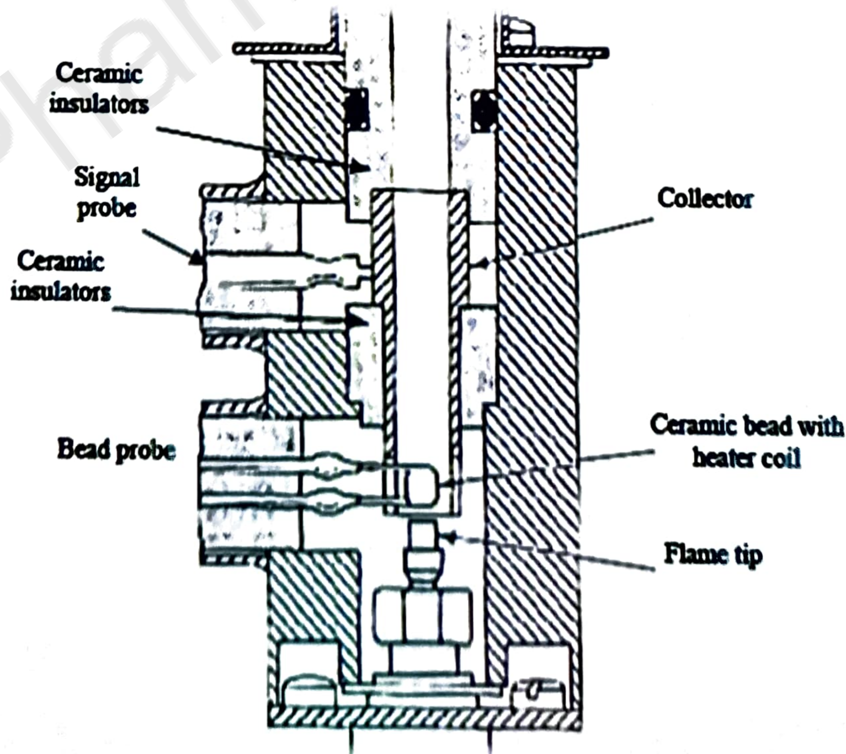
- This detector utilises a **heated filament placed in the emerging gas stream.**
- Thermal conductivity of the gas phase governs the amount of heat the filament loses by conduction to the detector walls.
- Temperature depends on **thermal conductivity (He & H)**of surrounding gas.
- Hydrogen and helium have higher thermal conductivity and carrier gas provide best sensitivity.



Cross-Sectional View of a Thermal Conductivity Detector

- **Thermionic Emission Detector (TED):**

- This detector utilises **fuel-poor hydrogen plasma** and a low temperature flame.
- This flame **suppresses the normal flame ionisation** response of compounds not containing nitrogen or phosphorus.



Diagrammatic Representation of Thermionic Emission Detector

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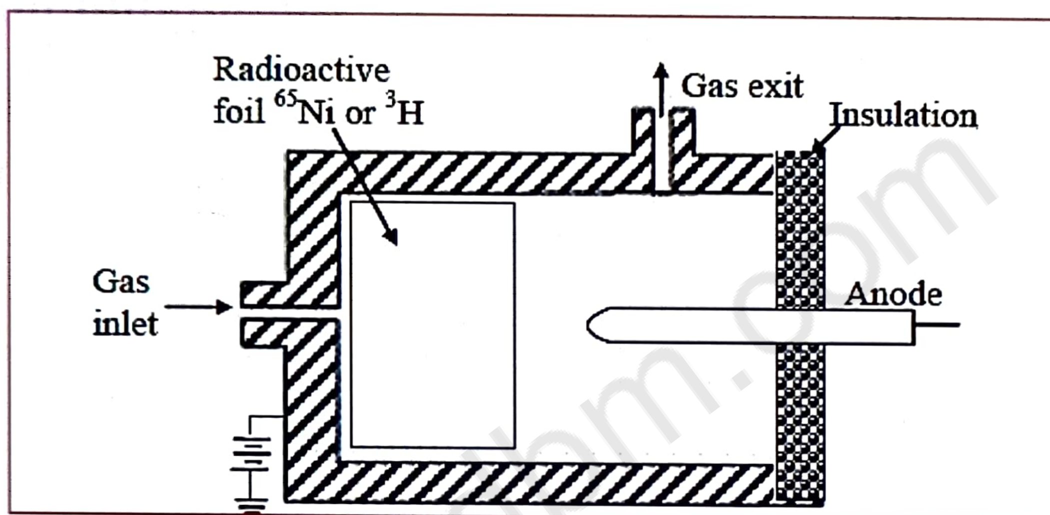
➢ Electron Capture Detector (ECD):

- This detector relies on the **electron affinity of different substances.**
- It responds to compounds whose molecules have electron affinity, **e.g.,** chlorinated compounds, alkyl lead, etc.
- It responds less to hydrocarbons.
- ECD is used for detecting **trace environmental pollutants.**
- It is highly sensitive to halogenated compounds and is used for detecting **herbicides, pesticides**.



Diagrammatic Representation of Electron Capture Detector

❖ DERIVATIZATION

- Derivatisation is a technique of treatment of the sample to **improve the process of separation by column or detection by detector**.
- There are **two types** based upon its need. They are

❑ Pre-column derivatisation

- This is done to **improve some properties of the sample for separation** by column.
- By this technique, the components are converted to **more volatile and thermostable derivatives**. Moreover improve separation and less tailing will be seen after such convert treatment.
- In the following conditions, pre-column derivatisation is done.
  1. The component is less volatile.

2. The compounds are thermolabile.
3. To reduce tailing.
4. To improve separation factor.

- e.g. Carboxylic acids, sugars, phenols, alcohols etc. can be converted to less polar compounds by using reagents like Bis trimethyl silyl Acetamide reagent.

❑ Post column derivatisation

- Post column derivatisation is done to **improve the response** shown by detector.
- The components may be converted in such a way that **their ionisation or affinity towards electrons is increased**.
- Normally this is 'on-line' detection technique where the **flow rate is neither stopped nor altered.**
- Pretreatment of Solid Support is used to **hold the stationary phase liquid as a thin film**. But sometimes due to some **defects, uniformity and stability of the film of liquid stationary phase may not exist.**
- In such cases, tailing of peaks and low separation efficiency can be observed. Therefore to overcome such demerits, it is best to **do pre-treatment of the stationary phase.**
- Generally, while doing the separation of no polar components like esters, ethers etc. tailing of peaks are observed.
- These problems can be overcome by following techniques:
  1. By using more polar liquid stationary phase.
  2. Increasing the amount of liquid phase on the support.
  3. By selecting a less active support.
  4. Pretreatment of the solid support to remove active sites.

❖ TEMPERATURE PROGRAMMING

- Gas chromatographs are usually capable of performing what is known as **temperature programming gas chromatography (TPGC).**
- The temperature of the column is changed according to a **preset temperature isotherm.**

- TPGC is a very important procedure, which is used for the **attainment of excellent looking chromatograms in the least time possible**.
- Temperature programming is a technique in which **column temperature is increased either continuously or in steps as the separation proceeds**.
- In general, **optimum resolution is associated with minimal temperature**.
- Lower temperature results in longer elution times and hence slower analysis.
- Using temperature programming, **low boiling point constituents** are separated.
- As separation proceeds, **column temperature is increased** so that the higher boiling point constituents come off the column with good resolution and at reasonable length of times.
- The elution rate is **proportional to the column temperature**.
- In the beginning, it uses **lower temperature that gives a higher resolution of lighter compounds.**
- With the increasing temperature, the elution rate of **heavier compounds also increases.**
- This gives sharper peaks for heavier compounds.
- Temperature programmed mode refers to a **continual rise of temperature at predetermined rate during the sample analysis.**
- This mode of operation offers several advantages such as
  - ✓ **Improvement of peak shapes**
  - ✓ **Improvement of resolution**
  - ✓ **Completion of analysis in a fraction of time it would take for the isothermal operation.**
- Temperature programming combines the **best results of runs at different temperatures.**
- In this way, the approximate **proper temperature programme** can be **estimated.**

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❖ ADVANTAGES

Gas chromatography has the following advantages:

- It is a reliable technique and provides **rapid analysis**.
- It is highly efficient and **leads to high resolution**.
- It utilises **sensitive detectors**.
- It requires **small samples** (<1 ml).
- It is **non-destructive** as it enables the coupling to mass spectrometers, which measures the masses of individual molecules converted into ions, i.e. molecules that have been electrically charged.
- It provides high **quantitative accuracy.**
- It is a well-established technique with extensive literature and applications.

❖ DISADVANTAGES

Gas chromatography has the following disadvantages:

- It is **limited to volatile samples**.
- It is not suitable for **thermolabile samples**.
- It is not suited to **preparative chromatography**.
- It **requires MS detector** for structural elucidation of the analyte, since most of the non-MS detectors are destructive.

❖ APPLICATIONS

➢ Qualitative analysis:

- It is nothing but **identification of a compound**, This is done by comparing the retention time of the sample as well as the standard Under identical conditions.

➢ Checking the purity of a compound:

- **By comparing the chromatogram of the standard and that of the sample.**
- The purity of the compound can he reported. If additional peaks are obtained, impurities are present and hence the compound is not pure.

➢ Presence of impurities:

- This can be seen by the **presence of additional peaks when compared** with a standard or reference material.

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➢ **Quantitative analysis:**

The quantity of a component can be determined by several methods like

a. **Direct comparison method**

b. **Calibration curve method**

c. **Internal standard method**

➢ **Multicomponent analysis or Determination of mixture of drugs:**

- Similar to the quantification of a **single drug multicomponent analysis can also be done easily.**
- Marketed formulations are available which contain several drugs and each component can be determined quantitatively.

➢ **Isolation and identification of drugs** or metabolites in urine, plasma, serum etc. can be carried out.

➢ **Isolation and identification of mixture** of components like amino acids, plant extracts, volatile oils, etc.

UNIT-IV

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Points to be covered in this topic

- ❖ INTRODUCTION
- ❖ THEORY
- ❖ INSTRUMENTATION
- ❖ ADVANTAGES
- ❖ DISADVANTAGES
- ❖ APPLICATIONS

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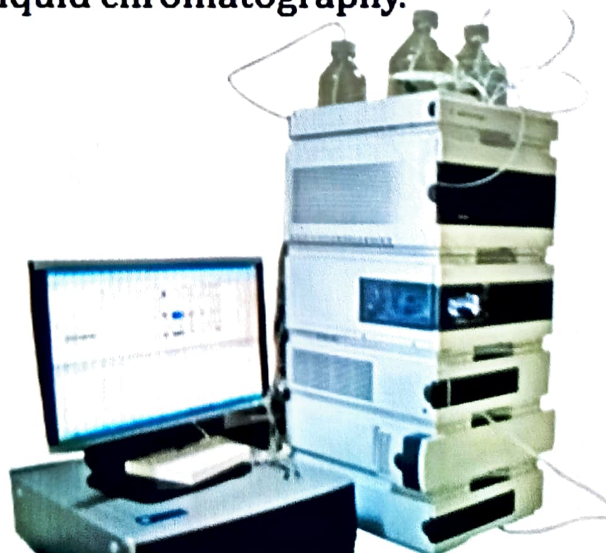
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

❖ INTRODUCTION

- High Performance Liquid Chromatography (HPLC) is used to **separate complex chemical mixtures.**
- Previously, it was known as **High Pressure Liquid Chromatography**, but now the term **performance** is used instead of pressure which indicates that **pressure is not essential for high performance**, and also defines the technique in a better way.
- HPLC is a **highly rapid process** as it involves high resolution and high speed columns.
- Following are the points showing the **advantages of HPLC over gravity-fed (classical) column chromatography:**
  1. The separated substances show better resolution,
  2. Less time is required for separation,
  3. separation with more accuracy, precision, and sensitivity,
  4. Useful for qualitative as well as quantitative analysis.
- The system involves **pumping of mobile phase through the packed column under the influence of high - pressure**, therefore the technique is also named as high-pressure liquid chromatography.
- The high-pressure liquid chromatography separation method involves a **stationary phase contained in one end of the column and mobile phase connected to the other end of the column.**



High performance liquid chromatography

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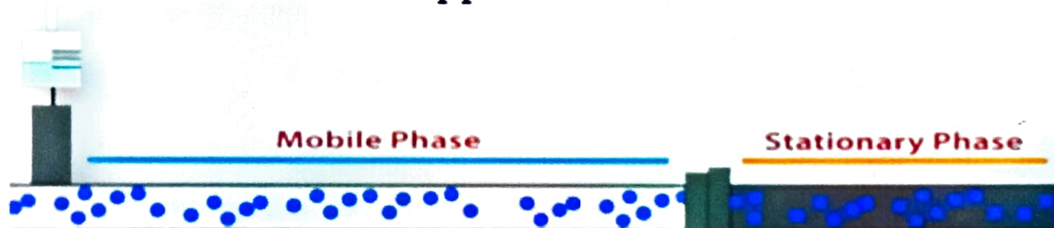
PRINCIPLE

- HPLC is a form of **liquid chromatography** used to separate compounds that are **dissolved in solution**.
- **Particles of small diameter** is used as stationary phase.
- Compounds are separated by **injecting a sample mixture onto the column**. The different component in the mixture pass through the column and differentiates due to **differences in their partition behavior between the mobile phase and the stationary phase.**

> ✓ **The following four methods are used for separating chemical mixtures:**
> 1. **Adsorption,**
> 2. **Partition,**
> 3. **Ion exchange,**
> 4. **Exclusion.**

- Selection of method for the separation process depends on the **stationary phase nature.**
- HPLC involves separation of mixture compounds **on an analytical column that is packed with small particles of stationary phase** (e.g., silica) by elution with a liquid mobile phase.
- A **high pressure is applied to pump** the mobile or liquid phase through the packed columns.
- The working of HPLC is based on the **principle that separation of molecular forms involves elution of a sample from a solid inorganic support by using a mixture of organic solvents.**
- In HPLC, capillary columns packed with **cross -linked dextran or silica** is used for solid support



Mobile phase and stationary phase

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❖ THEORY

- HPLC overcomes the **limitations found in standard liquid chromatography**.
- In classic liquid chromatography, the **separation process is very slow** as the movement of solvent occurs under gravity.
- The limiting factor is **the size of column packing** in liquid chromatography.
- In the HPLC setup, the apparatus should **operate efficiently under high pressure** and should be specialised **at low tolerances.**
- Thus, HPLC is **highly expensive** than other chromatography techniques.
- A mixture of **polar and non -polar liquid components forms the mobile phase of HPLC.**
- The concentration of these liquids depends on the sample composition.
- The solvent should not have **dissolved gases**, which might hinder solution mid -separation and particulates.
- In HPLC column, the components are separated according to their **differing interactions with the column packing.**
- If the interaction between species and stationary phase is weak, it spends a **comparatively less time in the column and reduces the retention time**.
- For homogeneous columns, **silica or alumina can be used as stationary phase**, while a liquid stationary phase is considered as a bonded column.
- **HPLC pump is used to introduce solvent** and sample in the column.
- Use of HPLC pump also helps to maintain a constant, pulse free flow rate.
- The HPLC pump can be a multi - piston pump or syringe pump.
- At the column end, **HPLC detector** is present that registers the **presence of components in the sample** but not the solvent.
- In the HPLC system, a **UV absorption detector** or an **NMR detector** is preferred.

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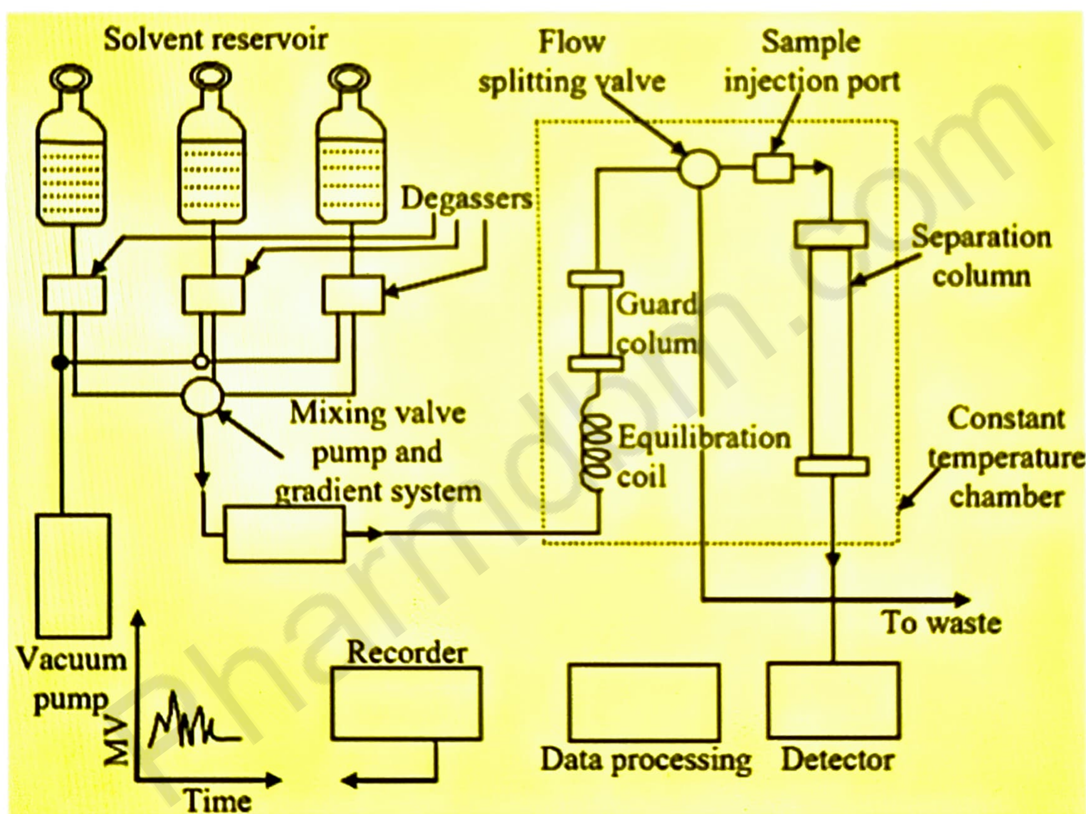
❖ INSTRUMENTATION

Modern HPLC Instrument includes the following components :

1) Solvent reservoir and degassing system,
2) Pumping system,
3) Sample injection system,
4) Columns,
5) Detectors,
6) Stripchart recorder, and
7) Data handling system



Schematic Representation of HPLC Instrument

❑ Solvent Reservoir and Degassing System

- High pressure liquid chromatography makes use of a **single solvent or a mixture of solvent as a mobile phase**, which contained in a reservoir.
- Selection of mobile phase **depends on the chromatographic method and the detector** to be used.
- Commercially available **special grades of solvents** that have been **refined to completely remove the UV -absorbing impurities** and any particulate matter are also used in HPLC

- Prior to using other grades of solvents, **purification** should be performed.
- This is because the **separation may get influenced if the impurities are strongly UV-absorbing,** affect the detector, or are of high polarity (e.g., traces of H_2O or EtOH, commonly included as a stabiliser, in $CHCl_3$).
- Liquid entering the pump should be **free from any impurity** (dust and particulate matter), or else these **impurities will result in irregular pumping action, irregular behaviour of column** owing to its contamination, damage the seals and valves, and ultimately block the column.

Degassing

- Generally, liquids dissolve some amounts of atmospheric gases (e.g., air or suspended air-bubbles) that **cause some major practical problems in HPLC,** specifically affecting the working of pump and the detector.
- These problems can be avoided by **degassing the mobile phase**.
- Degassing is performed by:
  1. Subjecting the mobile-phase under vacuum,
  2. Distillation,
  3. Spurging with a fine spray of an inert gas of low solubility (argon or helium), or
  4. Heating and ultrasonic stirring.

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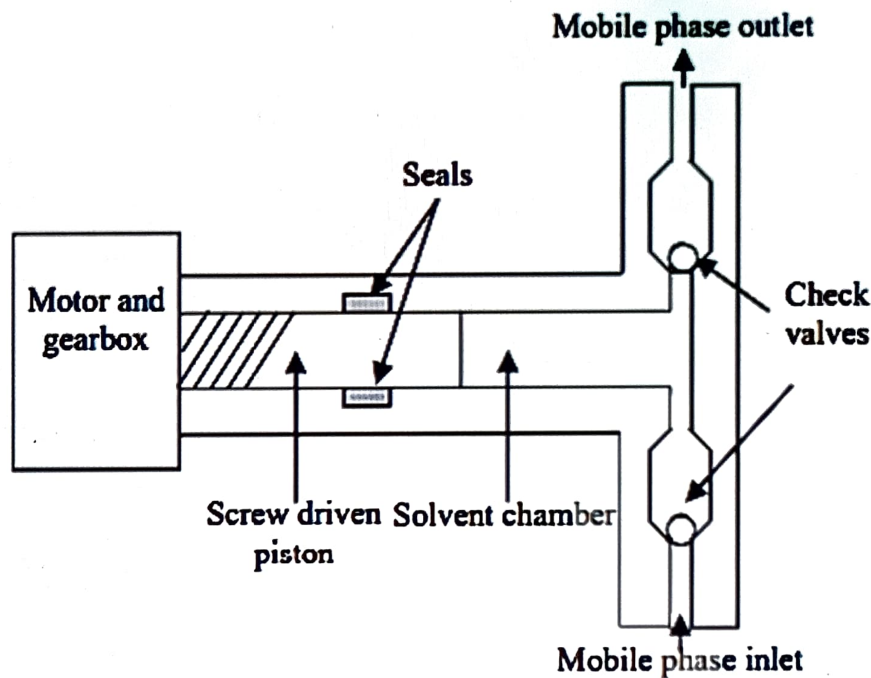
❑ Pumping System

The types of pumps that are used in HPLC are:

1. Screw-driven syringe pump,
2. Reciprocating pump, and
3. Pneumatic or constant-pressure pump.

➢ Screw-Driven Syringe Pump

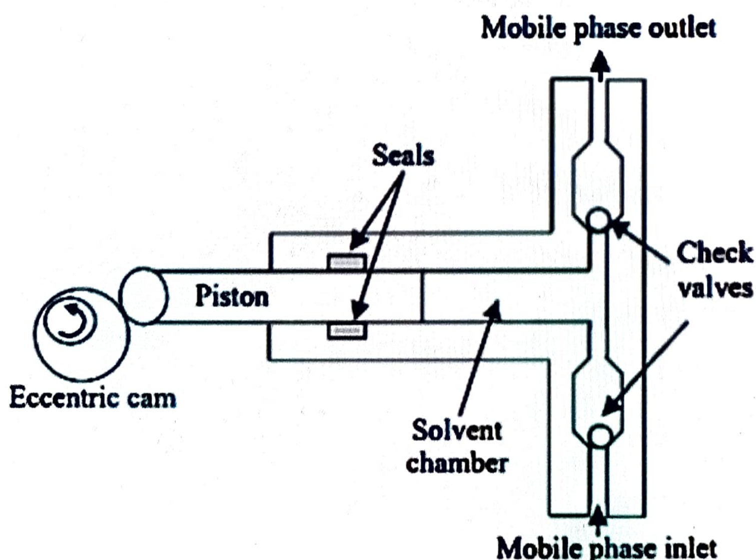
- **A variable speed stepper motor turns a screw** that drives a piston, which **displaces the mobile phase from a chamber** (of 200 -500cm^3 volume).
- The mobile phase **capacity depends on the solvent chamber volume**.
- This volume is quite large for running numerous chromatograms before the chamber is required to be refilled.



Diagrammatic Representation of Syringe Pump

➢ Reciprocating Pump

- In this pump an gear **drives the piston in and out of a solvent chamber.**
- Upon **moving forward**, the inlet check valve closes, the outlet check valve opens, thus pumping the mobile phase into the column.
- Upon **moving back**, the outlet valve closes and the chamber is refilled.
- In comparison to syringe pumps, the reciprocating pumps have **unlimited capacity,** and their internal volume can be adjusted to very low, ranging from 10-100µl.
- The flow rate can be altered by varying the length of piston stroke or the motor speed.
- Access to the valves and seals is direct.



Diagrammatic Representation of Reciprocating Pump

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➤ **Pneumatic or Constant-Pressure Pump**

- This is the simplest form of pump, **consisting of collapsible solvent container inside a vessel** pressurised by a compressed gas.
- These pumps are **inexpensive and pulseless**.
- However, they suffer from **limited capacity, pressure output**, and their pumping rates rely on solvent viscosity.
- They also cannot be **operated in gradient elution** mode.

❑ Sample Injection System

The following **three modes** of sample injection system are used in HPLC:

➤ **Septum Injectors:**

- In this system, the sample is introduced through a **high pressure syringe via self -sealing septum** of elastometer.
- The major **shortcoming** of this system is that the **mobile phase in immediate contact with the septum** , gives rise to a leaching effect that results in **ghost or pseudo peaks**.

➤ **Stop-Flow Septum-Less Injection:**

- In this system, the **flow of mobile phase through the column is stopped** for a few moments,
- When the **column attains ambient pressure**, the column top is opened and the sample is introduced at the top of the packing.

  The first two methods are inexpensive.

➤ **Micro-Volume Sampling Valves:**

- In highly sophisticated modern HPLC apparatus, **micro-volume sampling valves , having good precision** and adaptable for automatic injection used.
- These valves allow sampling to be done **reproducibly into pressurised columns with minimum interruption** of the mobile phase flow.

✓ describes the operation of a sample loop in two varied modes:

i) Sampling mode,

ii) Injection mode.

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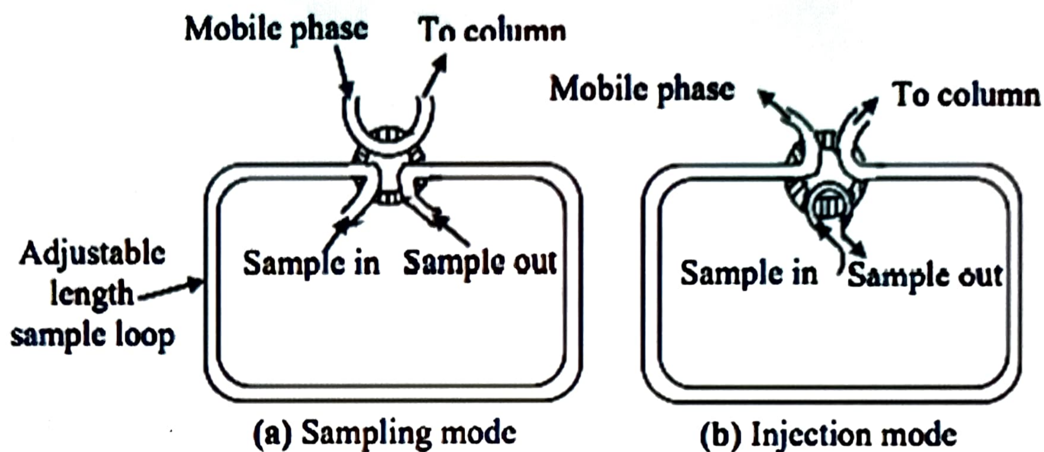


❑ Columns

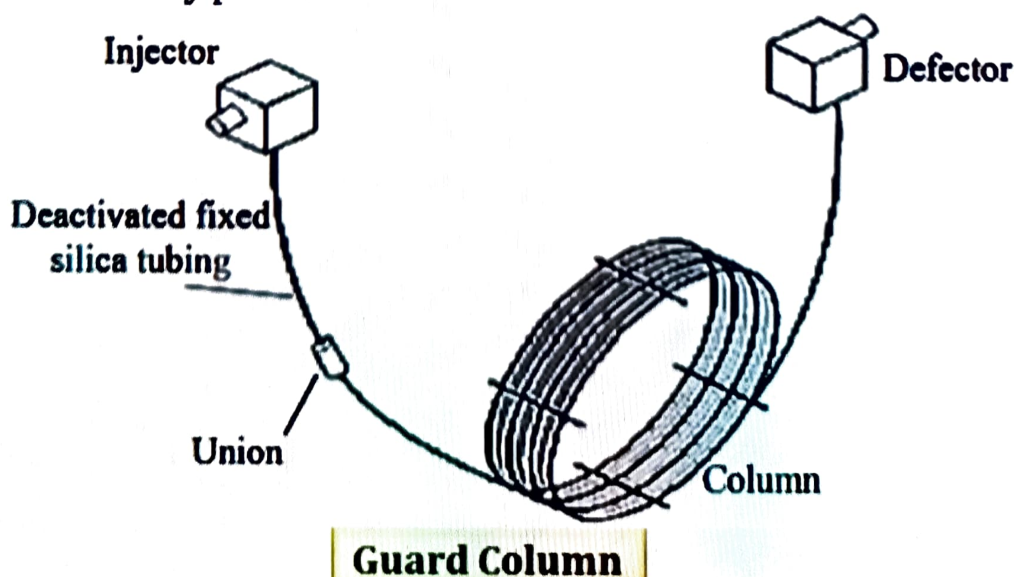
The following two types of columns are used in HPLC:

1) Guard columns,

2) Column thermostats.

➢ Guard Columns

- It is a short column **present between the injector and analytical column**.
- Although the **packing composition** of guard columns and analytical columns is similar, but **particle size is larger in guard columns** to aid in the **reduction of pressure drop**.
- The benefits of guard columns are:
  1. They **eliminate foreign particles and contaminants** from the solvents, thereby improving the life of analytical columns.
  2. In liquid-liquid chromatography, they **minimise the loss of stationary phase from the analytical columns** since the mobile phase is saturated with the stationary phase.



Guard Column

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➢ Column Thermostats

- Chromatographic operations are done at **room temperature without the requirement of sharp control of column temperature.**
- Improved chromatograms are obtained if the column temperature is **maintained constant to the few tenths of a degree Celsius.**
- To achieve a constant and precise temperature control, **water jackets are fitted in the columns.**
- The modern commercial instruments **contain heaters for controlling the column temperature** to a few tenths of a degree from near ambient to 150°C.

❑ Detectors

✓ In HPLC, the **detector monitors the mobile phase passing out of the column**, which further **releases electrical signals directly proportional to the characteristics of the solute** or the mobile phase.

The commonly used detectors in HPLC are:

1) Bulk-Property Detectors

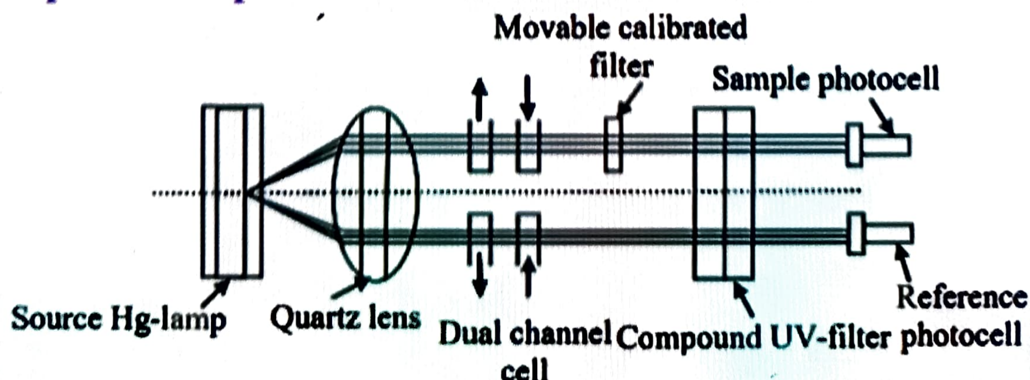
2) Solute-Property Detectors

3) Multipurpose Detectors

4) Electrochemical Detectors

➢ UV-Detector

- **Principle** - An UV -detector works on the principle of **absorption of UV visible light from the effluent emerging out of the column** and passing through a **photocell positioned in the radiation beam.**



Schematic Representation of a Double-Beam UV Detector

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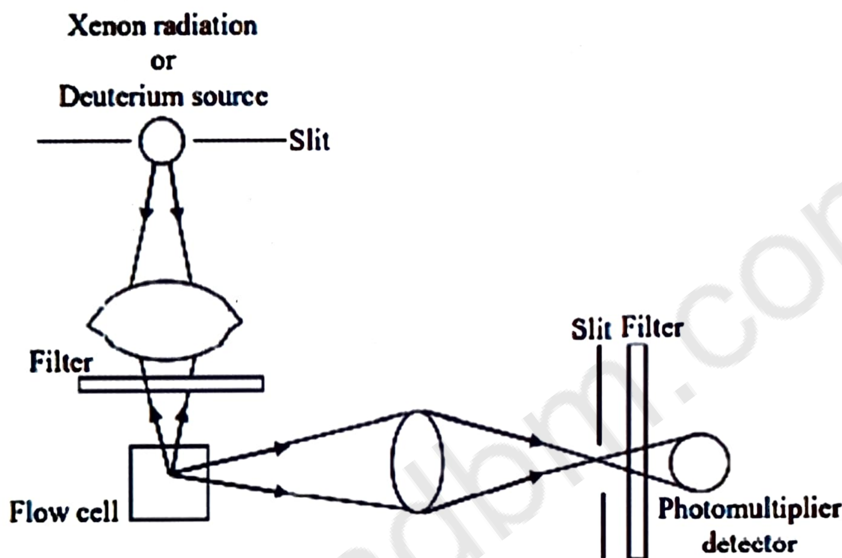
➢ Fluorescence Detector

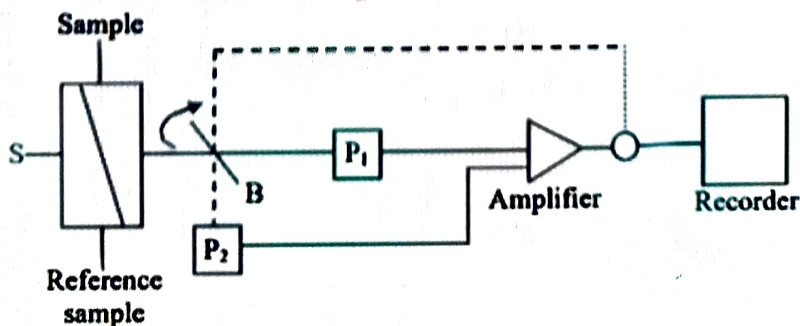
- Many compounds (solutes) are present in the mobile phase. When they are **allowed to pass as column effluent through a cell irradiated with xenon or deuterium source,**
- first UV radiation is absorbed and subsequently radiation of a longer wavelength is emitted in the following two ways:
  1) If instantly, named as 'Fluorescence', and
  2) If after a time-gap, named as 'Phosphorescence'.



Schematic Representation of a Fluorescence Detector

➢ Refractive Index Detector or RI-Detector or Refractometer

- In refractive index detector, **light emitted from the source is concentrated into the cell containing the sample and reference sample.**
- The light passes through the cell and reaches the **beam splitter that diverts the light towards two photocells.**
- **A change in the observed refractive index of the sample results in a difference in their relative output**, and this difference is amplified and recorded.



Block Diagram of a Refractive Index Detector

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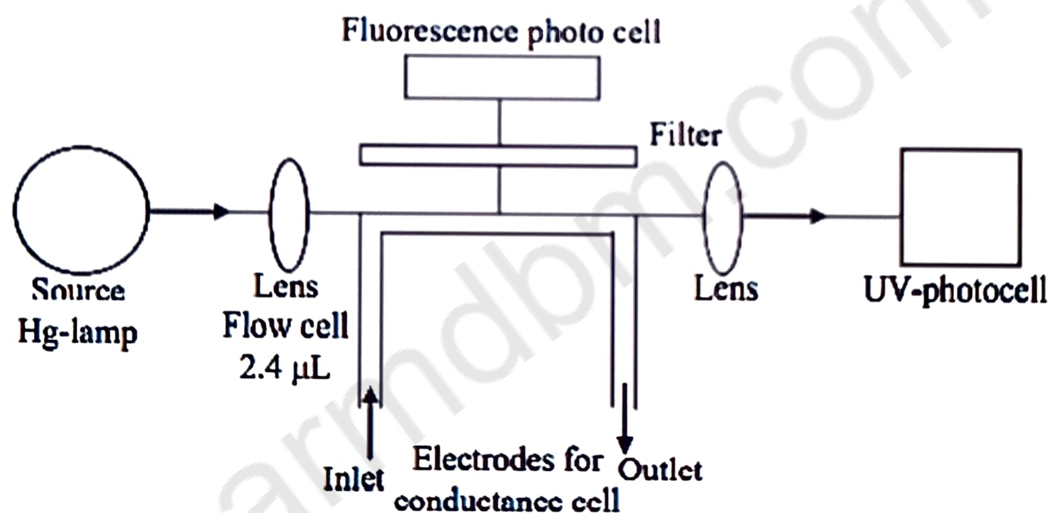
➢ Multipurpose Detector

- A multipurpose detector includes **three detectors that are combined** and kept together in a single unit.
- An example of this type of detector is **Perkin -Elmer 3D System**, developed by Perkin-Elmer.

The three different detectors perform the following functions:

1. **Fluorescence Function:** They monitor emission above 280nm, based on excitation at 254nm.
2. **UV-Function:** It is a fixed wavelength (254nm) detector.
3. **Conductance-Function:** The metal inlet and outlet tubes function like electrodes that measure the conductance of ions.



Block Diagram of Perkin-Elmer Detector

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➢ Electrochemical Detectors

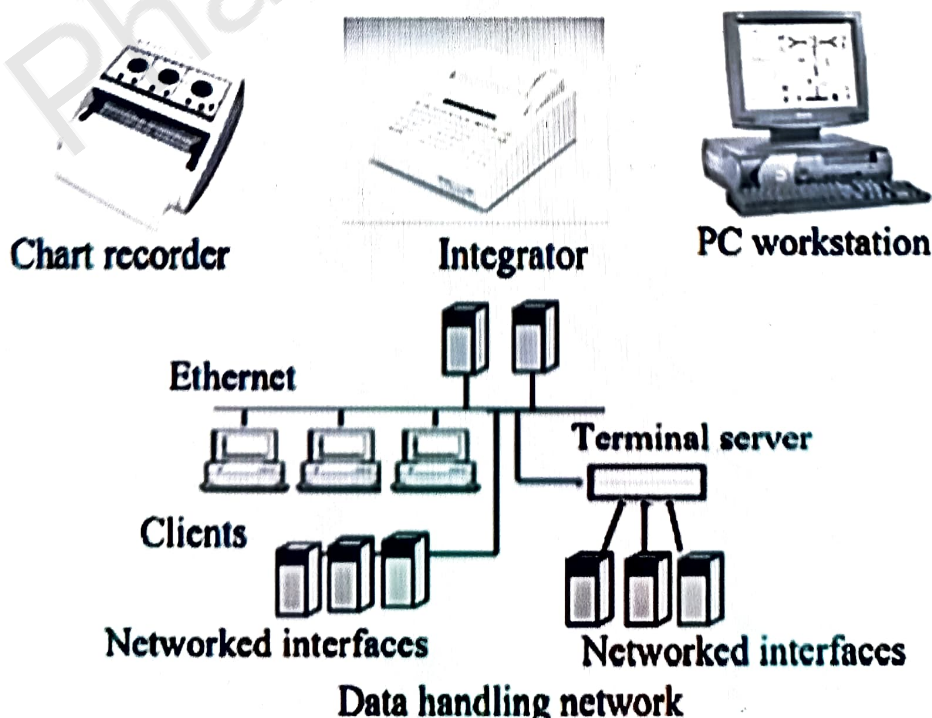
- At the present time, **amperometric detector** is considered the best electrochemical detector and it possesses the following distinguished features:
  1) Small internal cell-volume,
  2) High sensitivity,
  3) Limited range of applications, and
  4) Best for trace analyses as UV-detector lacks adequate sensitivity.
- Practically, it is difficult to utilise the functions of electrochemical reduction as a mean of detection of HPLC.

Strip Chart Recorder

- The signals emerging from the HPLC detector are continuously recorded as function of time.
- For these purposes generally, a potentiometric recorder is used.
- The most efficient recorder is that which records **1 -10mV** full -scale deflection over a **stretch of approximately ten inches** and having a **response -time of one second or less.**
- Therefore, the most preferred recorder is a strip -chart recorder with variable chart speeds ranging between 5-5mm/min.
- A feedback signal arrangement (device) using a servomechanism is used to balance the input signal of a potentiometric -recorder continuously.
- With pre - adjusted attenuation a **pen is attached with this device**, which moves proportionately along the width of the chart paper so that signals can be accurately recorded.

Data Handling System

- In HPLC, there is a tremendous development in the data handling devices that ranges from a strip chart recorder, an electric integrator, and a PC -based workstation to a client - server network system (the latest one).
- The automation and sophistication has also advanced with the time.



Diagrammatic Presentation of Chromatography Data Handling System

❖ ADVANTAGES

HPLC has the following advantages:

1. It is a **simple, rapid, and reproducible** technique.
2. It is **highly sensitive**.
3. It shows a **better performance.**
4. It is a **rapid process** and is less time consuming.
5. Its resolution and **separation capacity is high**.
6. It is **accurate and precise**.
7. It utilises a **chemically inert** mobile and stationary phases.
8. It needs a **small amount of mobile phase** for developing chamber.
9. It involves **early recovery** of separated components.
10. It enables **easy visualisation** of separated components.
11. It shows a good **reproducibility and repeatability**.
12. It is useful in **qualitative and quantitative analysis.**
13. It is used for analytical and preparative purposes.
14. It is used for **validation and quality control** studies of product.

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❖ DISADVANTAGES

The disadvantages of HPLC focus on the **detection systems** available, and include:

1. The most commonly used detectors in HPLC are UV spectrometers; however, the **compound to be analysed should have a UV absorbing chromophore.**
2. Variable wavelength UV spectrometers offer versatility but some steroids and other **drugs must be derivatized before UV detection**.
3. Another slight disadvantage is that the chemically bonded stationary phases applicable in drug analysis should be used **within 3 -7 pH range to ensure long term stability.**

❖ APPLICATIONS

Following are some common applications of HPLC:

- **Stability Studies** Stability of various **pharmaceutical compounds, degradation products** (e.g., stability studies of atropine), and other chemical substances can be studied using the technique of HPLC.
- **Bioassays and its Complementation** HPLC is used in the bioassay test of many **complex molecules** (e.g., peptide hormones and antibiotic molecules).
- **Design of Dosage Form Biopharmaceutics** of the dosage form and the **pharmacokinetic properties** of the drugs are studied with the help of HPLC. These properties are involved in dosage form designing.
- **In Cosmetic Industry** In this industry, HPLC is used for **analysing the quality** of various cosmetic products such as lipsticks, gels, creams, etc.
- **Isolation of Natural Pharmaceutically Active Compounds** HPLC is the most specific and sensitive method used for the **separation of different therapeutically active components present in plant extracts**. HPLC method is used in the isolation of different types of **alkaloids and glycosides.**
- **Control of Microbiological Processes** HPLC is used to analyse antibiotics (e.g., **tetracyclines, chloramphenicol, streptomycin, and penicillins**) produced by various microbiological processes.
- **Assay of Cephalosporins** Many derivatives of cephalosporin class of antibiotics can be **precisely separated** by HPLC.
- **Assay of Furosemide** The study of furosemide and its decomposition products is performed using **fluorescence and UV detection methods** during the HPLC analysis.
- **Assay of Corticosteroids** The mixture of **six corticosteroids** (hydrocortisone acetate, cortisone, deoxycortisone, hydrocortisone, prednisolone, and prednisone) can be assayed by HPLC.

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