UNIT-IV – GAS CHROMATOGRAPHY

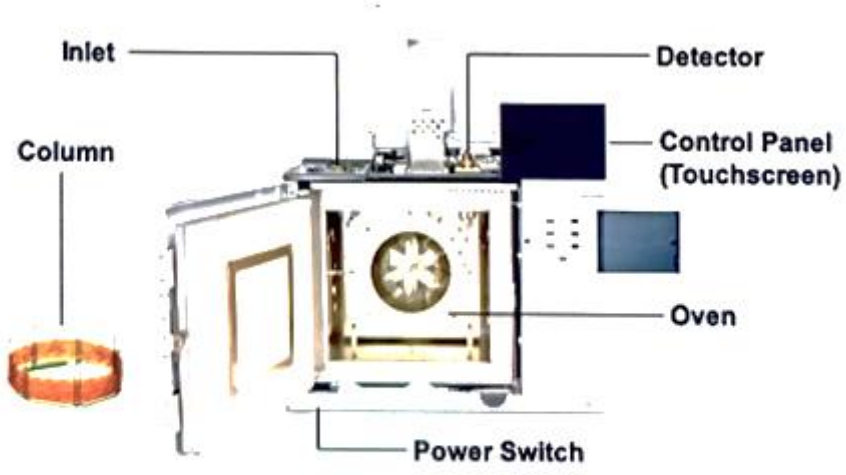
Points to be covered in this topic

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

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)**.
- In gas chromatography, a **moving gas phase is passed over a stationary sorbent** to separate the mixture components.
- The technique is similar to **liquid-liquid chromatography**, except that the mobile phase is gas instead of liquid.
  The stationary phase remains solid or liquid.



GAS CHROMATOGRAPHY

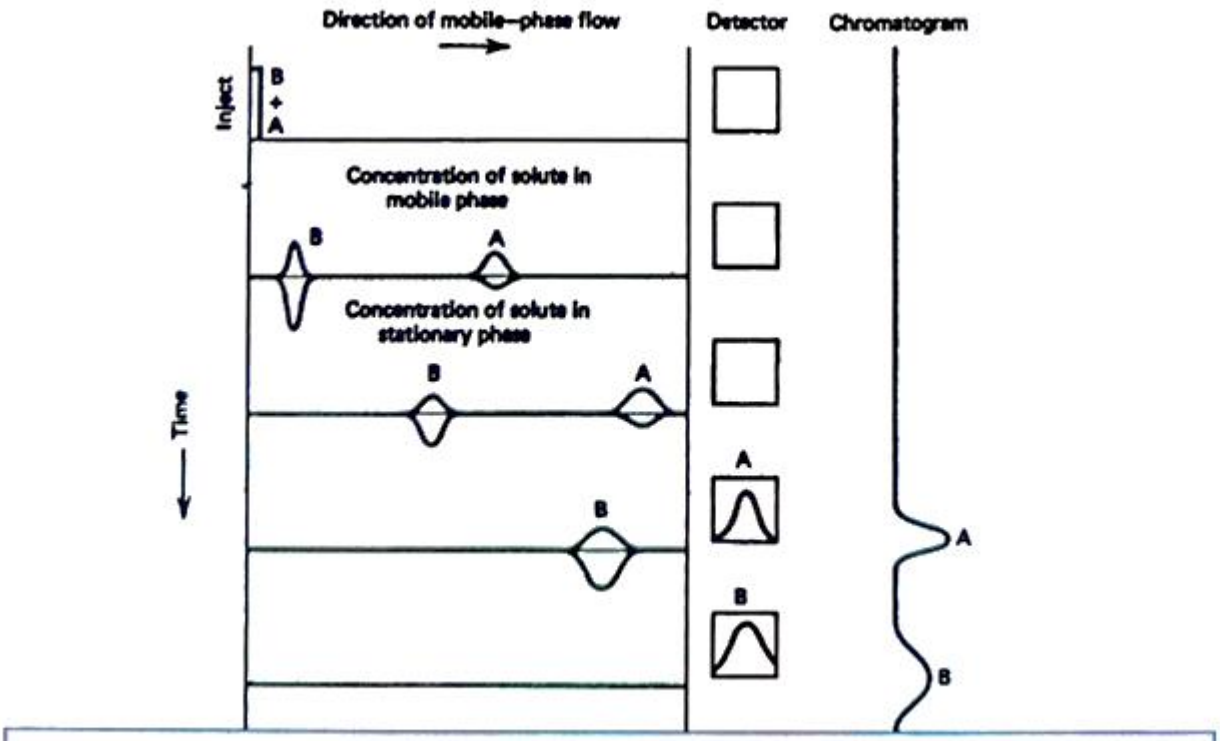
❖ PRINCIPLE

- In gas chromatography, the substance to be analysed is **partitioned between the mobile and stationary phases**.
- During separation, the **sample is vaporised** and **carried through the column** by the mobile gas phase (carrier gas).
- Different components separate **based on their vapour pressure and affinity** for the stationary phase.
- The affinity of a component for the stationary phase is expressed as the **distribution constant (Kc)** or **partition coefficient**:

$$\mathbf{Kc = [A]s / [A]m}$$

Where,

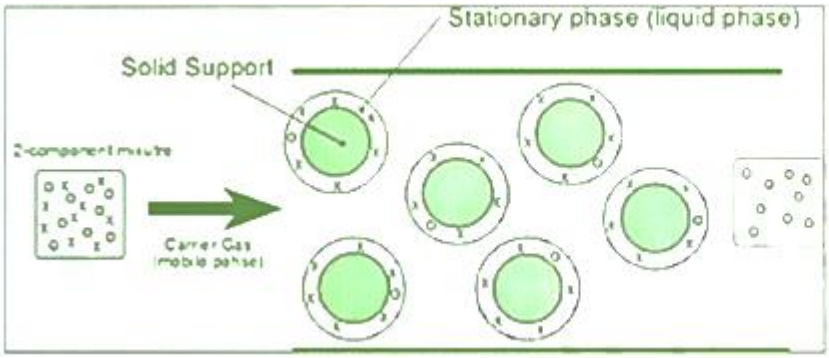
- **[A]s** = concentration of component A in stationary phase
- **[A]m** = concentration of component A in mobile phase
- Movement of components through the column is controlled by Kc, so separation occurs based on differences in distribution constants.

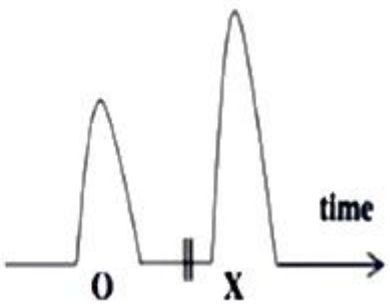


Schematic Representation of the Chromatographic Process

❖ THEORY

- Separation of compounds in a mixture is based on **different polarities**, either by direct interaction (solubility, stationary phase interaction) or indirect (boiling point).
- GC column contains **solid support coated with high-boiling liquid** in a capillary tube.
- In the example, **compound X has a higher affinity** toward stationary phase compared to **compound O**.
- **Compound O elutes first** because of lower boiling point and weaker interaction.





❖ Factors that influence GC results

➤ Vapor Pressure of the Compound

- Higher boiling point ⟶ lower vapor pressure.
- Higher boiling compounds **migrate slower**, giving longer retention time.
- Low boiling solvents such as **diethyl ether, dichloromethane, ethyl acetate,** methanol are commonly used because they elute early.

➤ Polarity of Compound and Column

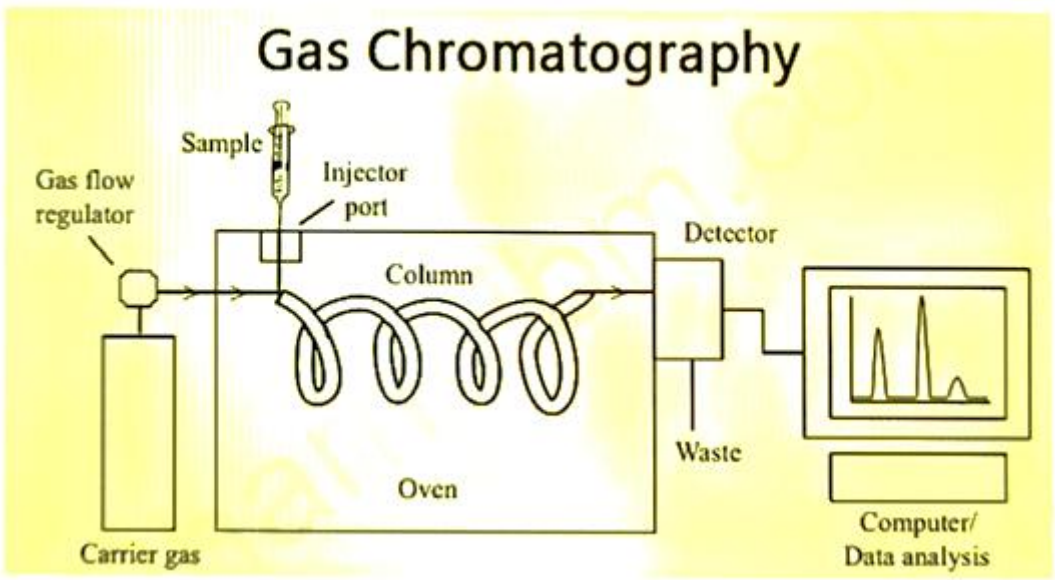
- Stronger interaction with stationary phase ⟶ longer retention time and **peak broadening**.
- Polar compounds (alcohols, amines) cause tailing on polar columns.
- Many column polarities are available for GC separations.

✤ 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.**

Gas Chromatography



❒ 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 **special gas sampling valves**.

❒ Separation Columns

• The columns used are made of **glass or metal tubing**, and have a diameter of **4.8 mm**.

• 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:

➤ 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 **(30–90 m) and have uniform and** narrow internal diameter **(0.025–0.075 cm)**.

• 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 **(0.5–1 μ) 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.

• 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:

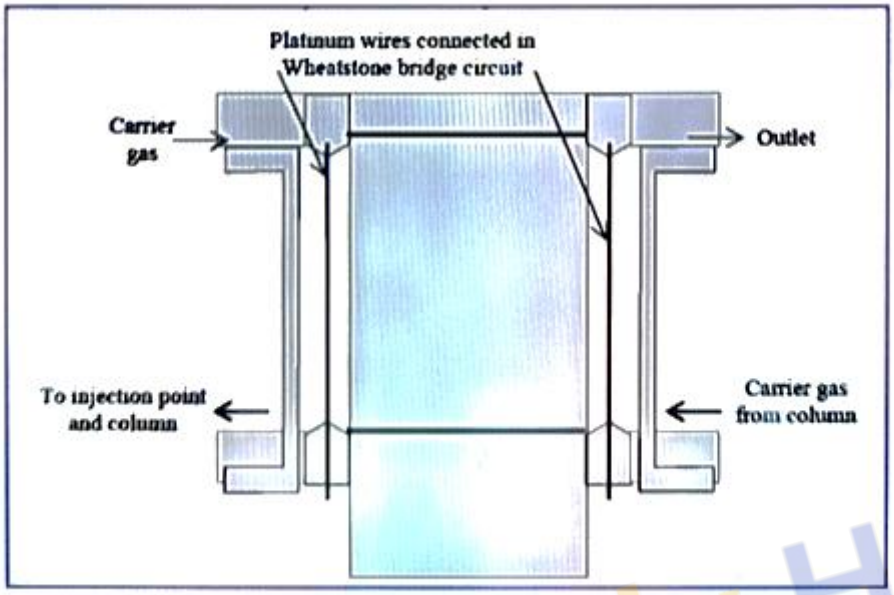
➤ **Katharometer:**

• 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.



Diagrammatic Representation of Katharometer

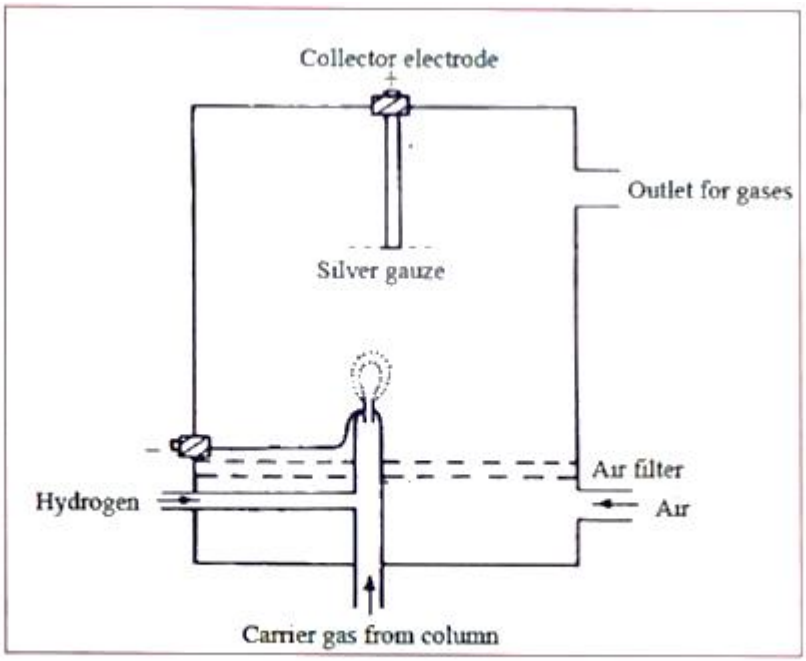
➤ **Flame Ionisation Detector (FID):**

• 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

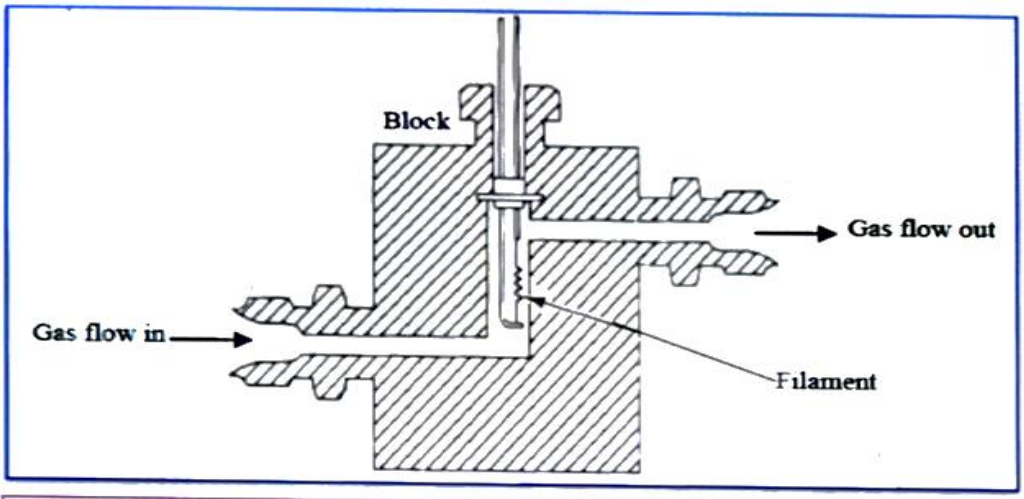
➤ Thermal Conductivity Detector (TCD):

• 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.

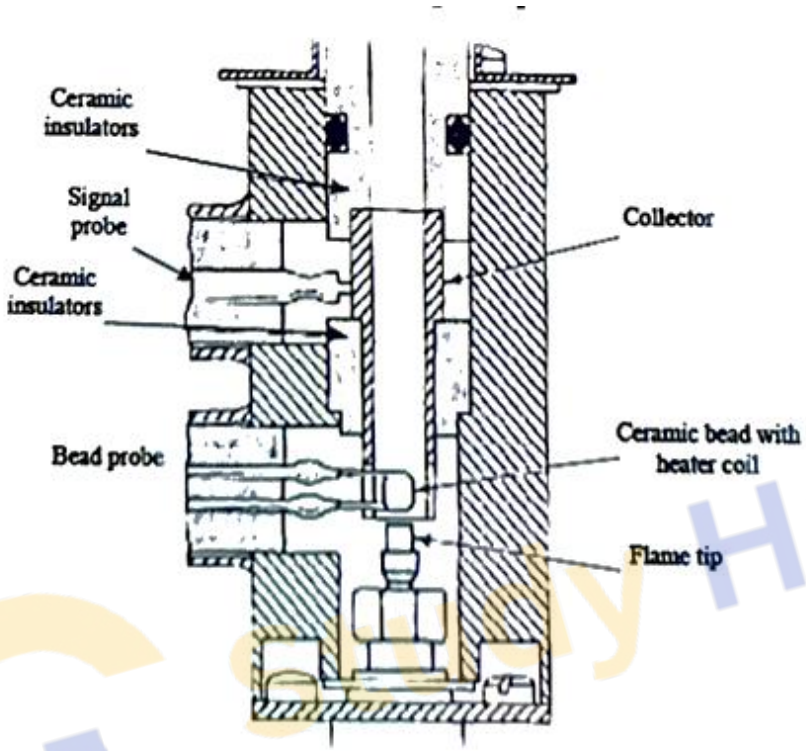


Cross-Sectional View of a Thermal Conductivity Detector

➤ 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

➤ Electron Capture Detector (ECD):

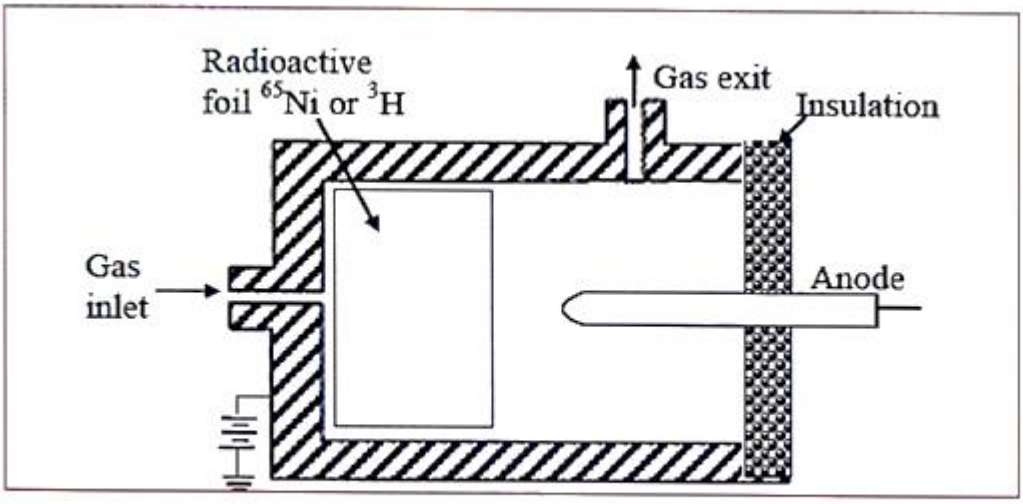
• This detector relies on the **electron affinity of different substances**.

• 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**.

✤ 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 then be 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**.

➤ 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**

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

➤ **Isolation and identification of mixture**

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

UNIT–IV HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (PART - 2)

Points to be covered in this topic

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

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.

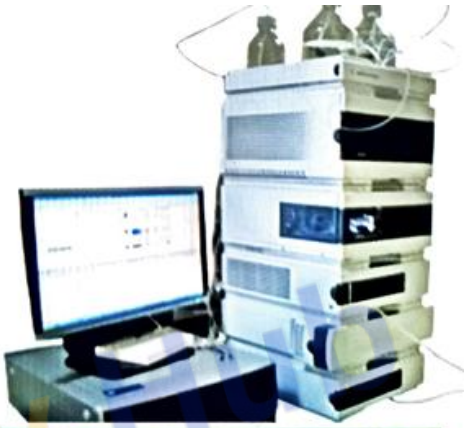
• Following are the points showing the **advantages of HPLC over gravity-fed (classical)**

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

❒ 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.

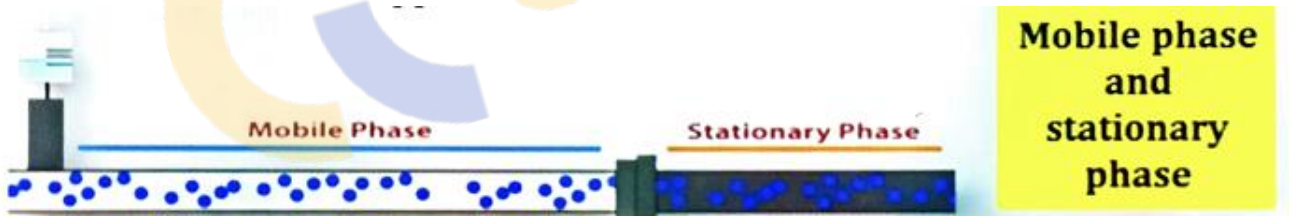
• Selection of method for the separation process depends on the stationary phase nature.

• 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.



❖ 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 **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.

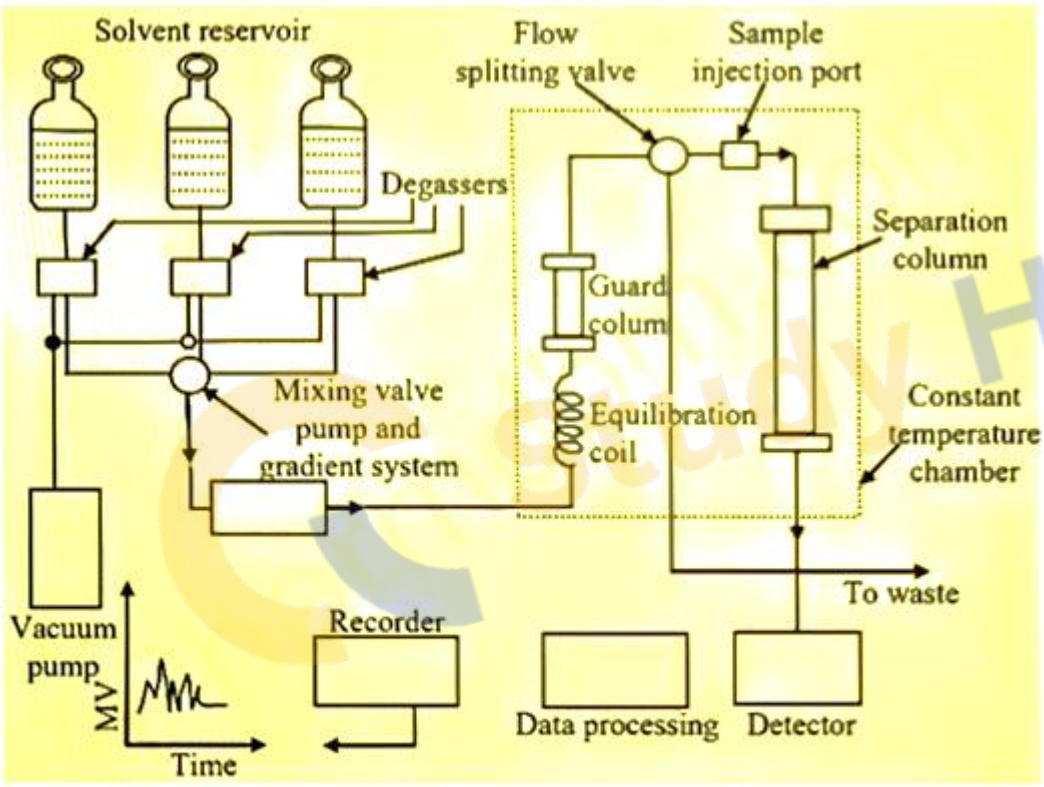
• In the HPLC system, a **UV absorption detector** or an **NMR detector** is preferred.

❖ 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 **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**.

❒ Pumping System

The types of pumps that are used in HPLC are:

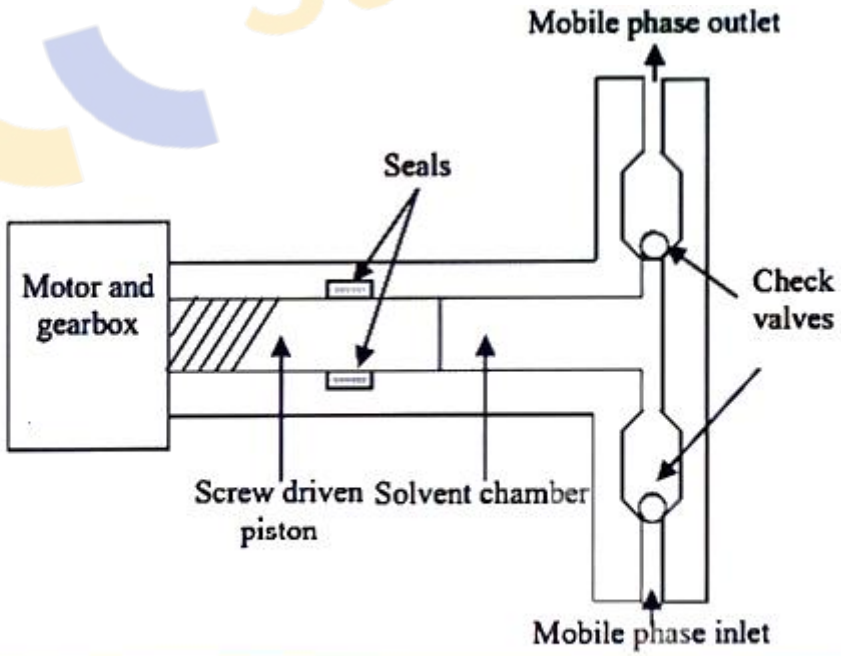
1. **Screw-driven syringe pump**,
2. **Reciprocating pump**, and
3. **Pneumatic or constant-pressure pump**.

➤ 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 200–500 cm^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 a **gear drives the piston in and** out of a solvent chamber.

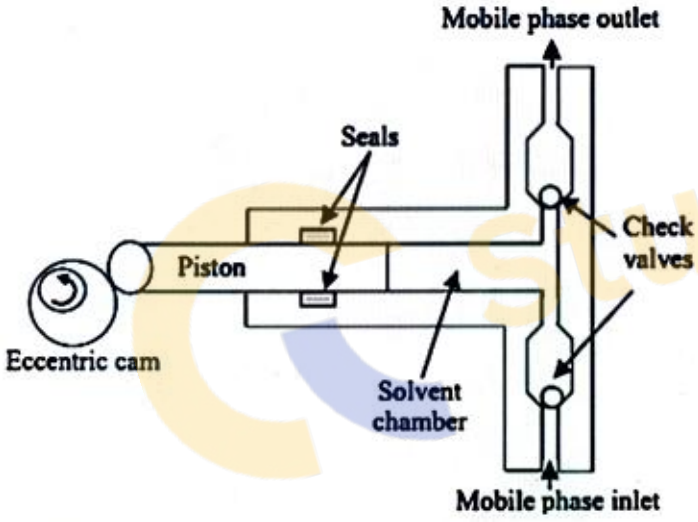
• Upon moving forward, the inlet check valve closes, the outlet check valve opens, thus pumping the mobile phase into the column.

• 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 10–100 µl.

• 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

➤ 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:

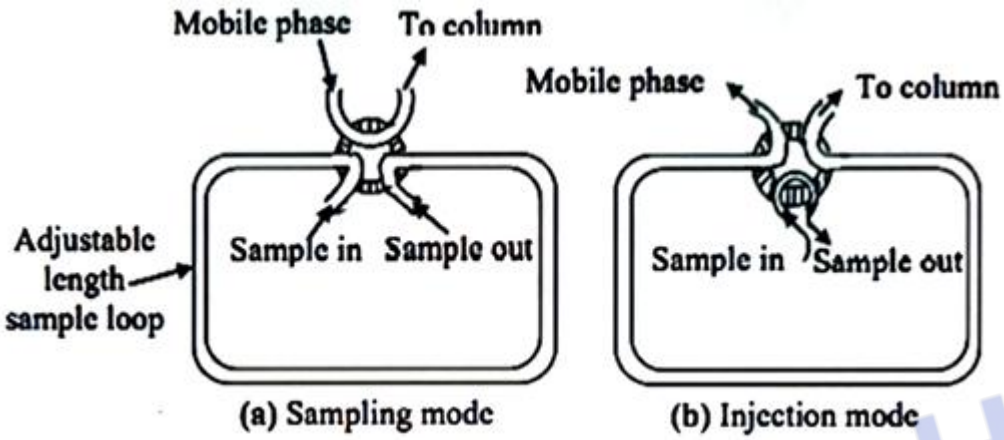
• In highly sophisticated modern HPLC apparatus, **micro-volume sampling valves**, having **good precision** and adaptable for automatic injection are used.

• 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**.



❒ Columns

The following two types of columns are used in HPLC:

1. **Guard columns**,
2. **Column thermostats**.

➤ Guard Columns

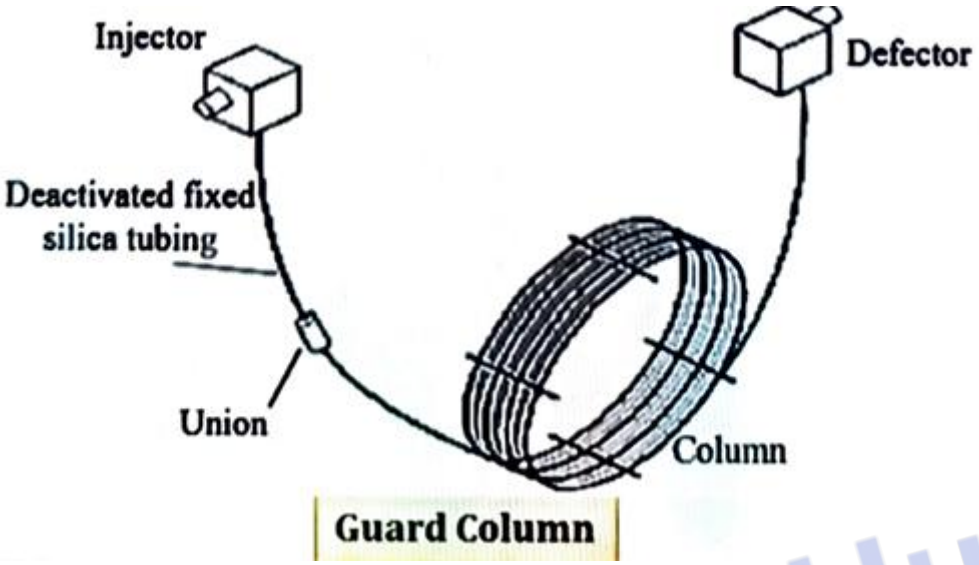
• It is a short column **present between the injector and** **analytical column**.

• 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.



➤ 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

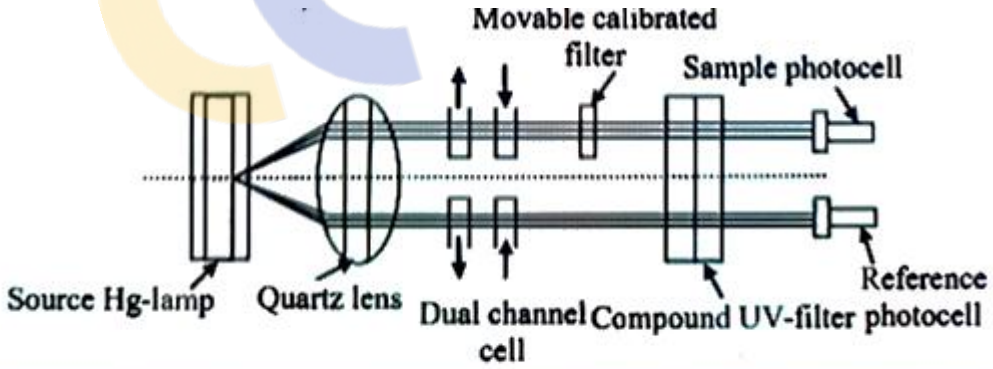
• In HPLC, the **detector monitors the mobile phase passing out of the column**, which further **releases electrical signals directly proportional to the characteristics of the solute or the mobile phase**.

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

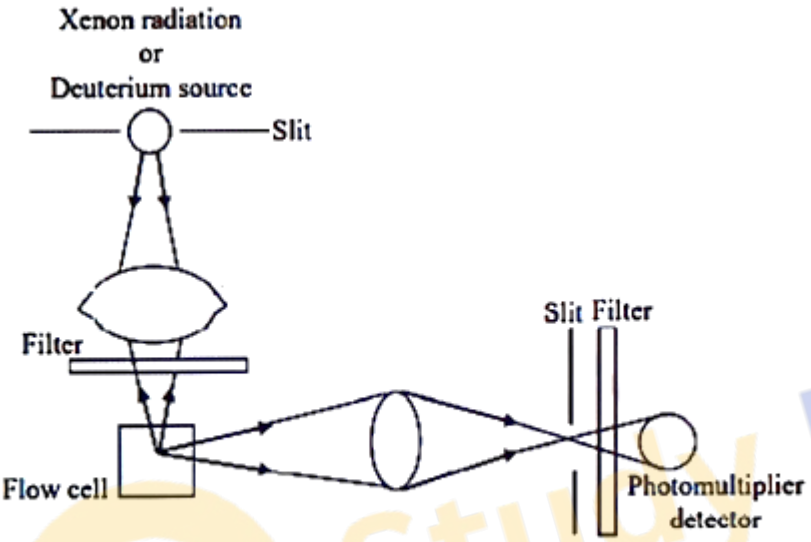
➤ Fluorescence Detector

• 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'**.



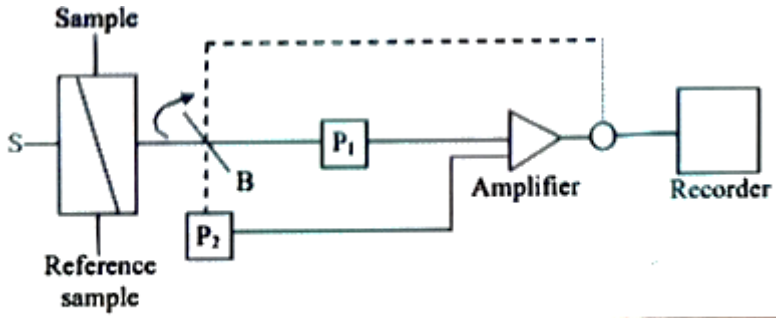
Schematic Representation of a Fluorescence Detector

➤ 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

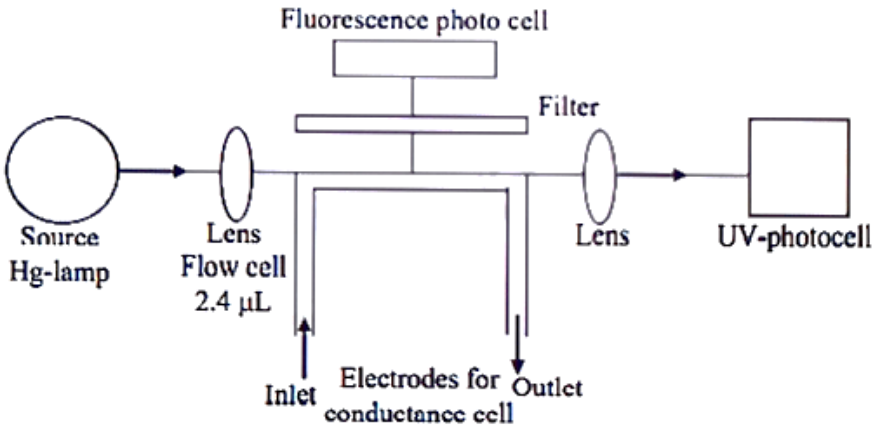
➤ Multipurpose Detector

• 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 280 nm, based on excitation at 254 nm.
2. **UV-Function**: It is a fixed wavelength (254 nm) 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

➤ 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 means 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 **1–10 mV 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 5–5 mm/min**.

• A feedback signal arrangement (device) using a servomechanism is used to balance the input signal of a potentiometric-recorder continuously.

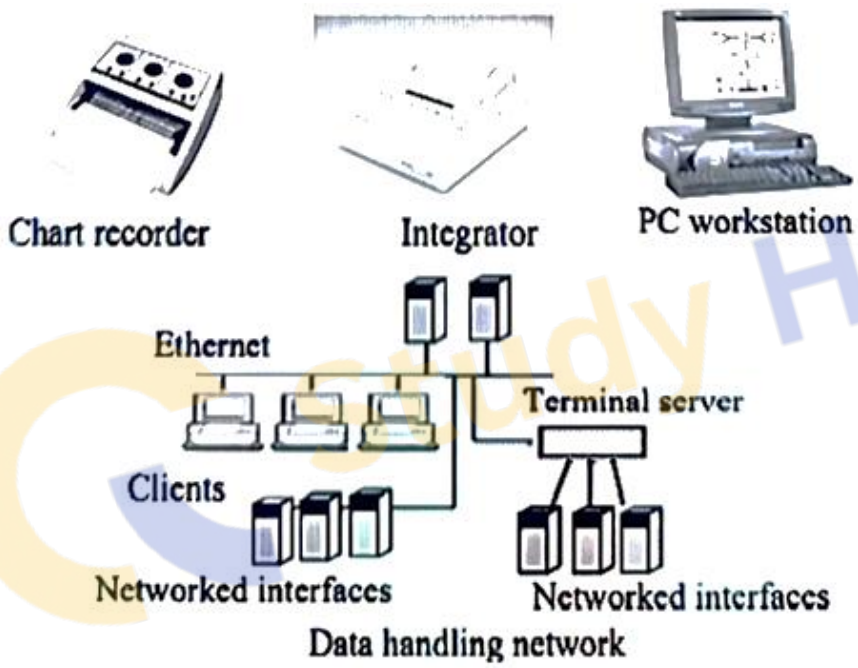
• With pre-adjusted attenuation **a pen is attached with this device**, which moves

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.

❖ 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

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.