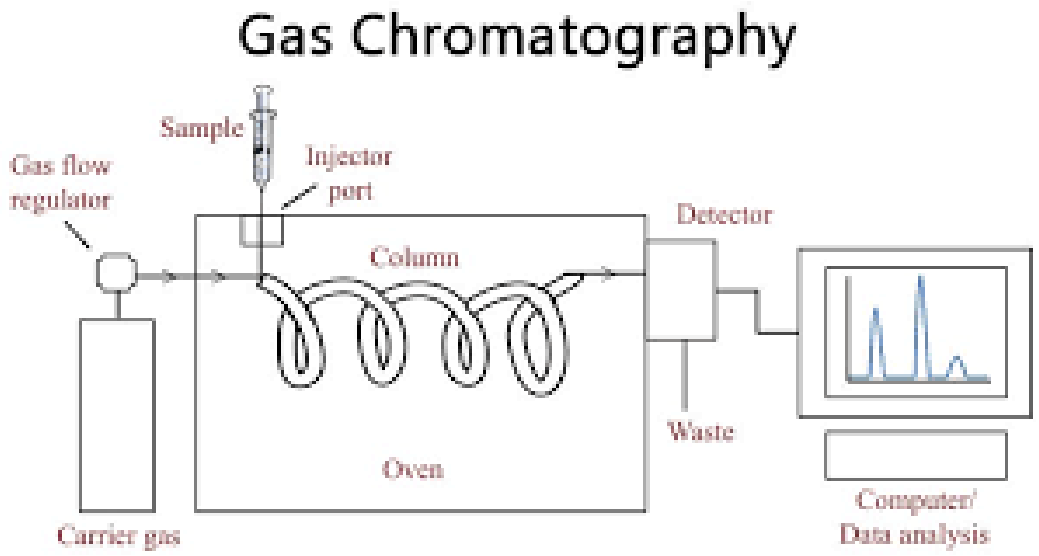
# 19CH103– ENGINEERING CHEMISTRY

## Unit-5 INSTRUMENTAL METHODS OF ANALYSIS

## Gas Chromatography

- Gas chromatography – "It is a process of separating component(s) from the given crude drug by using a gaseous mobile phase."
- It involves a sample being vaporized and injected onto the head of the chromatographic column.
- The sample is transported through the column by the flow of inert, gaseous mobile phase.
- The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid.




- **Two major types:**

- **Gas-solid chromatography:**

Here, the mobile phase is a gas while the stationary phase is a solid.
Used for separation of low molecular gases, e.g., air components, $H_2S$, $CS_2$,$CO_2$ ,rare gases, CO and oxides of nitrogen .

- **Gas-liquid chromatography**:

The mobile phase is a gas while the stationary phase is a liquid retained on the surface as an inert solid by adsorption or chemical bonding.

- The principle of separation in GC is "partition."
- The mixture of component to be separated is converted to vapour and mixed with gaseous mobile phase.
- The component which is more soluble in stationary phase travel slower and eluted later. The component which is less soluble in stationary phase travels faster and eluted out first.
- No two components has same partition coefficient conditions. So the components are separated according to their partition coefficient.
- Partition coefficient is "the ratio of solubility of a substance distributed between two immiscible liquids at a constant temperature.'

**INSTRUMENTATION**

1. Carrier gas - He (common), N2, H2, Argon
2. Sample injection port - micro syringe
3. Columns
4. Detectors

- Thermal conductivity (TCD)
- Electron capture detector(ECD)
- Flame Ionization detector (FID)
- Flame photometric (FPD)

- **CARRIER GAS** - The cylinder/ gas tank is fitted with a pressure controller to control the pressure of gas, a pressure gauge that indicates the pressure, a molecular sieve to transfer filtered dry gas and a flow regulator to ensure a constant rate of flow of mobile phase to the column.
- It should meet the following criteria:

✓ Should be chemically inert
✓ Should be cheap and readily available
✓ Should be of high quality and not cause any fire accidents
✓ Should give best possible results
✓ Hydrogen, helium, nitrogen and carbon dioxide are commonly used.
✓ Hydrogen has low density and better thermal conductivity. However, it reacts with unsaturated compounds and is inflammable and explosive in nature.
✓ Nitrogen is inexpensive but it gives
✓ reduced sensitivity.
✓ He is the most preferred gas.
✓ Inlet pressure ranges from: 10-50 psi
✓ -Flow rate : 25-150 mL/min for packed columns
✓ -Flow rate: 2-25 mL/min for open tubular columnSampling unit or injection port is attached to the column head.
✓ Since the sample should be in vapourized state, the injection port is provided with an oven that helps to maintain its temperature at about 20-50$^0$ C above the boiling point of the sample.
✓ Gaseous samples may be introduced by use of a gas tight hypodermic needle of 0.5-10 ml capacity.

- ✓ For Liquid samples , micro syringes of 0.1-100µL capacity may be used.
- ✓ Columns are of different shapes and sizes that includes: "U" tube type or coiled helix type.
- ✓ They are mainly made of copper, stainless steel, aluminium, Glass, nylon and other synthetic plastics.

## ✓ Support material:-

- ✓ Commonly used solid phases are: diatomaceous earth or kieselguhr, glass beads, porous polymers, sand,etc.

- ✓ *Types of columns:*- There are two general types of columns:

- ✓ ***1. Packed columns***:- In GLC, they are densely packed with finely divided, inert, solid support material ( *diatomaceous earth*) coated with liquid stationary
- ✓ phase. In GSC, the columns are packed with adsorbents or porous polymers.
- ✓ Length- 1.5 - 10m
- ✓ internal diameter- 2 - 4mm.
- ✓ ***1. Capillary columns***-
- ✓ length ranges from 10-100m
- ✓ inner diameter is usually 0.1-0.5mm.

**DETECTOR**

- ➢ The eluted solute particles along with the carrier gas exit from the column and enter the detector.
- ➢ The detector then produces electrical signals proportional to the concentration of the components of solute.
- ➢ The signals are amplified and recorded as peaks at intervals on the chromatograph.

## Properties of good detector

- ➢ Sensitive
- ➢ Operate at high T (0-400 °C)
- ➢ Stable and reproducible
- ➢ Wide dynamic range
- ➢ Fast response
- ➢ Simple (reliable)
- ➢ Nondestructive
- ➢ Uniform response to all analytes

- When only carrier gas flows heat loss to metal block is constant, filament T remains constant.
- When an analyte species flows past the filament generally thermal conductivity changes, thus resistance changes which is sensed by Wheatstone bridge arrangement.
- The imbalance between control and sample filament temperature is measured and a signal is recorded.

## WORKING

- This sample components now get distributed between the gas and stationary liquid phase depending upon their solubilizing tendencies.
- The components with minimal solubility move faster and those with maximum solubility travel slowly.
- The components leaving the column activate detector and recorder to give a plot.
- The components that slowly leave the column give a bell shaped curve of shorter peak while the one which travels faster gives a bluntly pointed curve of larger peak.
- In above graph, the component that first emerges out of the column is component 4 followed by 2,5,3 and 1.
- The area under the curve is determined in order to obtain the percentage composition of the mixture.

Retention Time: defined as the absolute time taken by a sample to show maximum peak after injecting

## HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

- HPLC stands for "High-performance liquid chromatography"(sometimes referred to as High-pressure liquid chromatography).
- High performance liquid chromatography is a powerful tool in analysis, it yields high performance and high speed compared to traditional columns chromatography because of the forcibly pumped mobile phase.
- HPLC is a chromatographic technique that can separate a mixture of compounds
- It is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of a mixture.

- **Chromatography** : physical method in which separation of components takes place between two phases-a stationary phase and a mobile phase
- **Stationary phase** : The substance on which adsorption of the **analyte** (the substance to be separated during chromatography) takes place . It can be a solid, a gel, or a solid liquid combination
- **Mobile phase** : solvent which carries the analyte (a liquid or a gas)

HPLC is a type of **liquid chromatography** where the sample is forced through a **column** that is packed with a stationary phase composed of irregularly or spherically shaped particles, a porous monolithic layer , or a porous membrane by a liquid (mobile phase) at high pressure.

## PRINCIPLE

To understand the principle of HPLC , we must first look at the principle behind liquid chromatography

Liquid chromatography is a separation technique that involves:

- the placement (injection) of a small volume of liquid sample
- into a tube packed with porous particles (stationary phase)
- where individual components of the sample are transported along the packed tube (column) by a liquid moved by gravity.

The main principle of separation is adsorption .

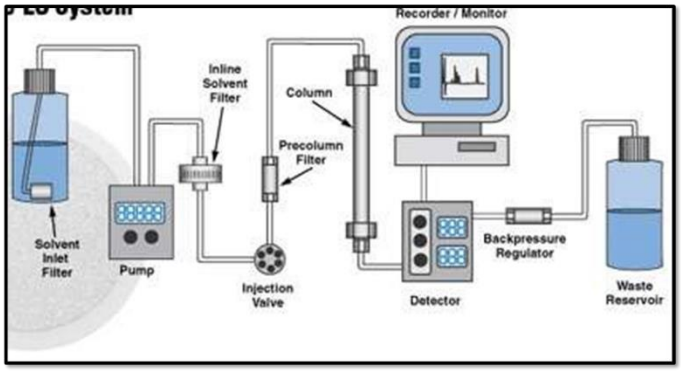
- When a mixture of components are introduced into the column . various chemical and/or physical interactions take place between the sample molecules and the particles of the column packing .

They travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent, travels slower.

The component which has less affinity towards the stationary phase travels faster.

- Since no two components have the same affinity towards the stationary phase, the components are separated
- **HPLC** is a separation technique that involves:
- the injection of a small volume of liquid sample into a tube packed with tiny particles (3 to 5 micron (μm) in diameter called the stationary phase)
- where individual components of the sample are moved down the packed tube (column) with a liquid (mobile phase) forced through the column **by high pressure delivered by a pump.**
- These components are separated from one another by the column packing that involves various chemical and/or physical interactions between their molecules and the packing particles.
- These separated components are detected at the exit of this tube (column) by a flow-through device (detector) that measures their amount.The output from the detector is called a liquid chromatogram

In principle, LC and HPLC work the same way except the speed , efficiency, sensitivity and ease of operation of HPLC is vastly superior .



## A . Solvent delivery system(mobile phase)

- The mobile phase in HPLC refers to the solvent being continuously applied to the column or stationary phase
- The mobile phase acts as a carrier to the sample solution
- A sample solution is injected into the mobile phase of an assay through the injector port
- As a sample solution flows through a column with the mobile phase,the components of that solution migrate according to the non-covalent interaction of the compound with the column

## B.Pumps

- The role of the pump is to force a liquid (called the mobile phase) through the liquid chromatograph at a specific flow rate, expressed in milliliters per min (mL/min).
- Normal flow rates in HPLC are in the 1-to 2-mL/min range.
- Typical pumps can reach pressures in the range of 6000-9000 psi (400- to 600-bar).
- During the chromatographic experiment, a pump can deliver a constant mobile phase composition (isocratic) or an increasing mobile phase composition (gradient).

## C.Column

- Considered the "heart of the chromatograph" the column's stationary phase separates the sample components of interest using various physical and chemical parameters.
- It is usually made of stainless steel to withstand high pressure caused by the pump to move the mobile phase through the column packing other material include PEEK and glass
- The small particles inside the column are called the "packing" what cause the high back pressure at normal flow rates.
- Column packing is usually silica gel because of its particle shape , surface properties , and pore structure give us a good separation

## D. Detector:

- The detector can detect the individual molecules that elute from the column and convert the data into an electrical signal
- A detector serves to measure the amount of those molecules
- The detector provides an output to a recorder or computer that results in the liquid chromatogram
- Detector is selected based on the analyte or the sample under detection

**Commonly used detectors in HPLC**

***Ultraviolet (UV)***

- This type of detector responds to substances that absorb light.
- The UV detector is mainly to separate and identify the principal active components of a mixture.
- UV detectors are the most versatile, having the best sensitivity and linearity.
- UV detectors cannot be used for testing substances that are low in chromophores (colorless or virtually colorless) as they cannot absorb light at low range.
- They are cost-effective and popular and are widely used in industry

***Fluorescence***

- This is a specific detector that senses only those substances that emit light. This detector is popular for trace analysis in environmental science.
- As it is very sensitive, its response is only linear over a relatively limited concentration range. As there are not many elements that fluoresce , samples must be syntesized to make them detectable.

***Mass Spectrometry***

- The mass spectrometry detector coupled with HPLC is called HPLC- MS. HPLC-MS is the most powerful detector,widely used in pharmaceutical laboratories and research and development.
- The principal benefit of HPLC-MS is that it is capable of analyzing and providing molecular identity of a wide range of components.

***Refractive Index (RI) Detection***

The refractive index (RI) detector uses a monochromator and is one of the least sensitive LC detectors.

- This detector is extremely useful for detecting those compounds that are non-ionic, do not absorb ultraviolet light and do not fluoresce.
- e.g. sugar, alcohol, fatty acid and polymers.

## E. Data processing unit (Computer)

- Frequently called the data system, the computer not only controls all the modules of the HPLC instrument but it takes the signal from the detector and uses it to determine the time of elution (retention time) of the sample components (qualitative analysis) and the amount of sample (quantitative analysis).
- The concentration of each detected component is calculated from the area or height of the corresponding peak and reported.
- **Retention time (RT)**
- In a chromatogram, different peaks correspond to different components of the separated mixture.

- Time elapsed between sample introduction and maximum of response, it is the characteristic time it takes for a particular analyte to pass through the system
- Time taken for the analyte to travel from the column inlet to the point of detection(maximum peak)

## APPLICATIONS

HPLC is one of the most widely appliedanalytical separation techniques**.**

**Pharmaceutical:**

- Tablet dissolution of pharmaceutical dosages.
- Shelf life determinations of pharmaceutical products.
- Identification of counterfeit drug products.
- Pharmaceutical quality control.
- Qualitative and quantitative analysis
- Trace metal identification ect.,

***ADVANTAGES OF HPLC:***

1. Separations fast and efficient (high resolution power)
2. Continuous monitoring of the column effluent
3. It can be applied to the separation and analysis of very complex mixtures
4. Accurate quantitative measurements.
5. Repetitive and reproducible analysis using the same column.
6. Adsorption, partition, ion exchange and exclusion column separations are excellently made.
7. HPLC is more versatile than GLC in some respects, because it has the advantage of not being restricted to volatile and thermally stable solute and the choice of mobile and stationary phases is much wider in HPLC
8. Both aqueous and non aqueous samples can be analyzed with little or no sample pre treatment
9. A variety of solvents and columnpacking are available, providing a high degree of selectivity for specific analyses.
10. It provides a means for determination of multiple components in a single analysis.