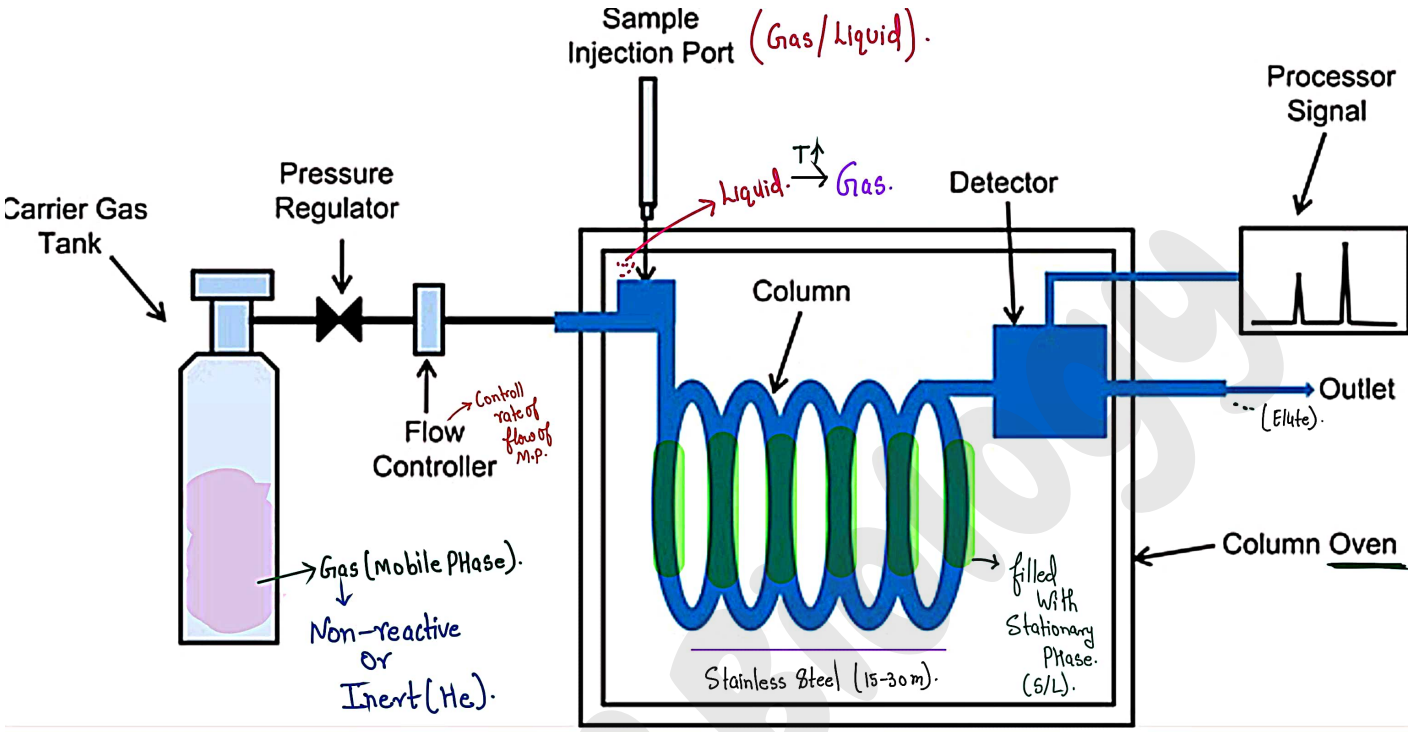
UNIT –IV

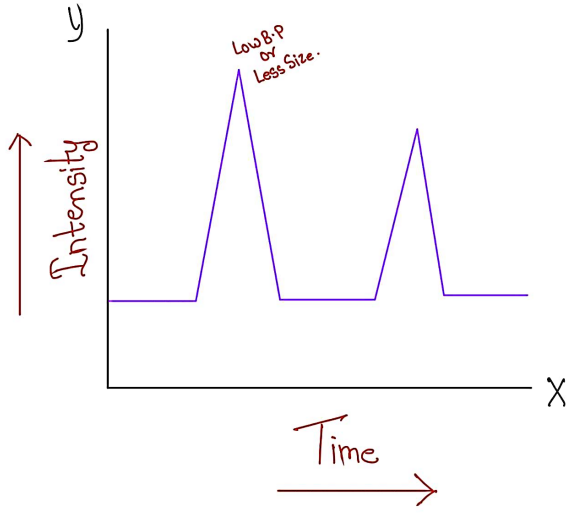
Gas chromatography - Introduction, theory, instrumentation, derivatization, temperature programming, advantages, disadvantages and applications

High performance liquid chromatography (HPLC)-Introduction, theory, instrumentation, advantages and applications.



S.P → Solid → GSC
S.P → Liq. → GLC.
Generally Liquid is Used (High B.P).

Separation of Basis
of B.P → Low B.P 1st Peak.
If B.P Same → Size decide.



Gas Chromatography

1.

Chromatography → Separation Technique.
↳ Isolate a Single Component from Mixture of Component.

- In this Chromatography Gas act as Mobile Phase.
& Stationary Phase is Solid or Liquid.

- Gas Chromatography also known as Vapour Phase Chromatography
& Gas-Liquid partition Chromatography.

★ The Gas Used in Gas Chromatography must be Non-reactive or Inert (eg → He gas).

- Sample generally Used in Gas form.
- But if sample is in Liquid format then firstly Convert it into Gaseous form without Altering the properties.
- Flow Controller ⟶ decide the flow rate of gas.
- Sample is Injected by Injector.
- If we Inlet Sample (at liquid form) then liquid will Convert into gas due to High Temp. of Inlet Point.

★ Mobile Phase (Gas) & Sample will be Mixed & then run in 2 Column (Tube like Structure)

- Column is made up of Stainless Steel or Glass.
- Column lenght 15 – 30 Meter
- Stationary Phase filled into Column. (Solid / Liquid).
- If Stationary Phase is Solid then it is Called Gas-Solid Chromatography
- If Stationary Phase is Liquid then it is Called Gas-Liq. Chromatog.

★ Generally Liq. is Used in Column as a Stationary Phase. & this Liquid must have High B.P (eg→ Silicon Grease).

★ The Column is placed Inside Oven. (Temp. of Oven = 300°c)

★ Sample Contain Molecule of High & Low B.P.

→ Molecule who Have High B.P moves Slower.

→ Molecule who have low B.P moves faster.

★ So, here purification is done on the basis of Boiling Point.

★ First Peak in Graph is shown by low B.P molecule.

★ Second peak in Graph is shown by High B.P molecule.

⇒ Elute (particle (molecule) which is obtained after Gas Chromatography

3.

⇒ Temprature Programming → Means we change the oven temprature to elute those molecule who have High Boiling Point.

✡ If Molecules Have Same Boiling Point then Separation is depends on Size.

→ Small Size molecules easily pass. (first peak /\)

→ Large Size molecule moves slowly in the column. (second peak)

✡ On the basis of Graph (b/w Intensity & Retention Time). Compare the Retention time with standard & then we can easily Identify the Molecule.

✡ Here detector is Used Mass spectroscopy So, this is also known as Gas Chromatography Mass Spectroscopy.

What is derivatization?

- What is GC Derivatization?
- Derivatization is the process of chemically modifying a compound to produce a new compound which has properties that are suitable for analysis using a GC.

What Does Derivatization Accomplish?

✓ Increases volatility (i.e. sugars):
✓ Eliminates the presence of polar OH, NH, & SH groups
✓ Derivatization targets O,S, N and P functional groups (with
hydrogens available.
✓ Increases detectability, I.e. steroids/ cholesterol
✓ Increases stability.
✓ Enhances sensitivity for ECD (Electron Capture Detection). The introduction of ECD detectable groups, such as halogenated acyl groups, allows detection of previously undetectable compounds.

Types of Derivatization

- pre-column derivatization
- post-column derivatization

❖ Precolumn derivatisation:

Components are converted to volatile & thermo stable derivative

Post column derivatisation

- Improve response shown by **detector**
- Components ionization / affinity towards electrons is increased

TECHNIQUES OF DERIVATISATION

- SILYLATION
- ACYLATION
- PERFLOURO-ACYLATION
- ALKYLATION
- ESTERIFICATION
- CONDENSATION
- CYCLISATION

COMPONENT OF GAS CHROMATOGRAPH

- ✓carrier gas
- ✓flow regulator
- ✓injector
- ✓column
- ✓stationary phase
- ✓oven
- ✓detectors
- ✓display device

ADVANTAGES AND DISADVANTAGES OF GC

➢**ADVANTAGES:-**
✓High Resolution
✓Very high sensitivity, detect down to 100 ppm.
✓Very good precision and accuracy.
✓Very good separation
✓Time(analysis is short), fast analysis is possible.
✓Small sample is needed-ml
✓Good detection system
✓Quantitatively analysis
➢**DISADVANTAGES :-**
✓Sample must be volatile
✓Dirty sample choke the capillary

Applications of Gas Chromatography

- Miscellaneous-analysis of foods like carbohydrates, proteins, lipids, vitamins, steroids, drug and pesticides residues, trace elements
- Pollutants like formaldehyde, carbon monoxide, benzen, DDT etc
- Dairy product analysis- rancidity
- Separation and identification of **volatile materials**, plastics, natural and synthetic polymers, paints, and microbiological samples
- Inorganic compound analysis