UNIT-4 CHAPTER-1 GAS CHROMATOGRAPHY (GC)

Introduction

- Gas chromatography (GC) is a common type of chromatography used for separating and analyzing compounds in an unknown sample.
- In this method Gas is used as mobile phase and Solid or Liquid is used as stationary phase.
- In Gas chromatography, moving Gas phase is passed over a stationary phase to separate the mixture component.
- This method is first used by A.T. James and P. Martin in 1952 for separating long chain fatty acid.

Types of Gas chromatography

✓ Gas-Solid chromatography [GSC]
- M.P - Gas
- S.P - Solid

✓ Gas-Liquid chromatography [GLC]
- M.P - Gas
- S.P - Liquid.

Principle - The principle of separation in GC is :-

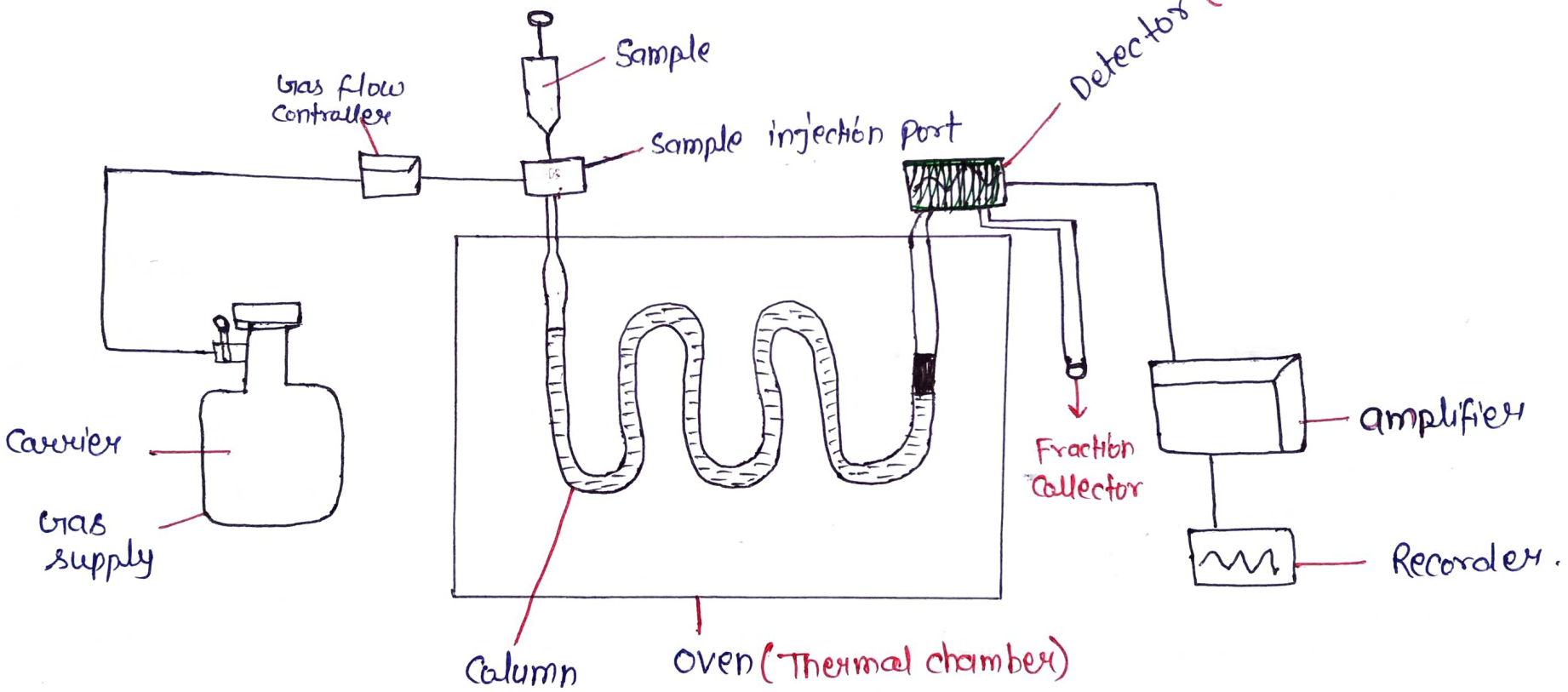
Partition – In Gas-Liquid chromatography
Adsorption – In Gas- Solid chromatography.

Theory

- The mixture of components to be separated is converted to vapour and mixed with gaseous mobile phase.
- The components which is more soluble in stationary phase travel slower and eluted later.
- The components which is less soluble in stationary phase travel faster and eluted out first.
- If components are used having same partition coefficient (PC), so they separated out according to the partition coefficient.//

Instrumentation of Gas chromatography

A, B



→ Carrier (Gas)
→ Sample Injection Port
→ Column
→ Detectors — Imp

① Carrier Gas

- The operating efficiency of a G.C. is dependent on the maintenance of a highly Constant carrier gas flow rate.
- Carrier gas from the cylinder tank passes through a flow meter, a few feet of metal capillary restrictors and a guage.
- The flow meter with a range of 0-20 ml/min, indicates flow rate in the reference side of the detector (thermal Conductivity Cell).
- Flow rate is adjustable and is controlled by the pressure regulator.

- The various types of carrier gas used in GC -

ⓐ H_2 - it has better thermal conductivity and lower density.
- Demerits are its reactivity with unsaturated compounds and hazardious explosive nature.

ⓑ He - it has excellent thermal conductivity, low density, inertness and it permits greater flow rates.
- It is highly expensive.

ⓒ N_2 - It offers reduced sentitivity and is expensive.

ⓓ Air - it is employed only when the atmospheric O_2 is beneficial to the detector separation.

② Sample Injection Port/System

- The sample injection system is very important and critical because GC makes use of very small amounts of the samples.
- A good and ideal sample injection system should be the one where the sample must not :-

  ⓐ Be decomposed at the point of injection.
  ⓑ Create pressure surges.
  ⓒ Undergo fractionation, condensation (gas to liquid) or adsorption of components during the course of transfer to the column.

ⓐ Liquid Samples

- They are usually injected by hypodermic syringes through a self-sealing silicon-rubber septum into a

preheated - metal - block flash evaporator.

(b) Solid Samples

- These are either dissolved into volatile liquids (solvents) or temporarily liquefied by exposure to infra-red heat.

(c) Gas Samples

- They are best handled and injected by gas-tight syringes or a gas-sampling valve, usually termed as a stream-splitter.

(3) Column unit

- Columns are of diff. shapes and sizes that includes U-type or coiled helix type.
- they are mainly made of copper, stainless steel, Aluminium, glass, nylon and other synthetic plastics.

Support medium :-

- its main function is to provide mechanical support to the liquid phase.
- An ideal support should have a large surface area, chemically inert, should get uniformly wet with liquid phase, should be thermostable.
- Commonly used **Solid phases** are, diatomaceous earth or kieselguhr glass beads, porous polymer, sand etc.
- **Liquid phase** :- It should have the following requirement -
  - It should be non-volatile,
  - chemically inert;
  - High decomposition temperature;

  e.g → Non polar - Paraffin oil, Silicon oil, Silicon rubber gum (used for high temp. of about 400°c)

  Polar - Polyglycols (Carbowaxes).

Column

- They are made up of glass or metal tubing have a diameter of 4.0 mm.
- They may be of any length ranging from few cm - 100 m.
- They may be coiled, bent or straight.

Types of Column

(a) **Packed Column** :- - In GLC, they are densly packed with finely divided, inert, solid support medium (diatomaceous earth) coated with liquid S.P;

- In GSC, the columns are packed with absorbents or porous polymers (Length 1.5-10 m, Internal diameter 2-4 mm).

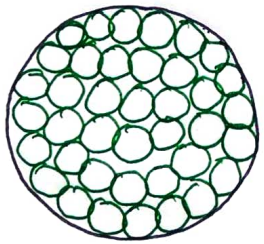
(b) **Capillary columns** :- - Length ranges from 10-100 m, inner diameter is usually 0.1-0.5 mm.
  - → wall coated columns
  - → Support coated columns.

(i) Wall Coated Columns :- Consist of a capillary tube whose walls are coated with liquid S.P.

(ii) Support Coated Column :- - The inner wall of the capillary is lined with a thin layer of support material such as diatomaceous earth.

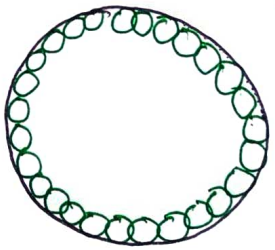
- It is also known as PLOT (Porous-Layer Open Tubular Column)

Packed

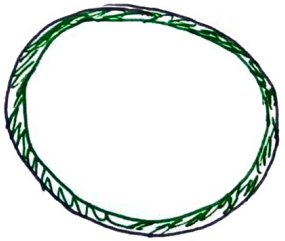


Packed Column.

open (capillary)



Porous layer open tube



wall coated open tube

④ Detectors

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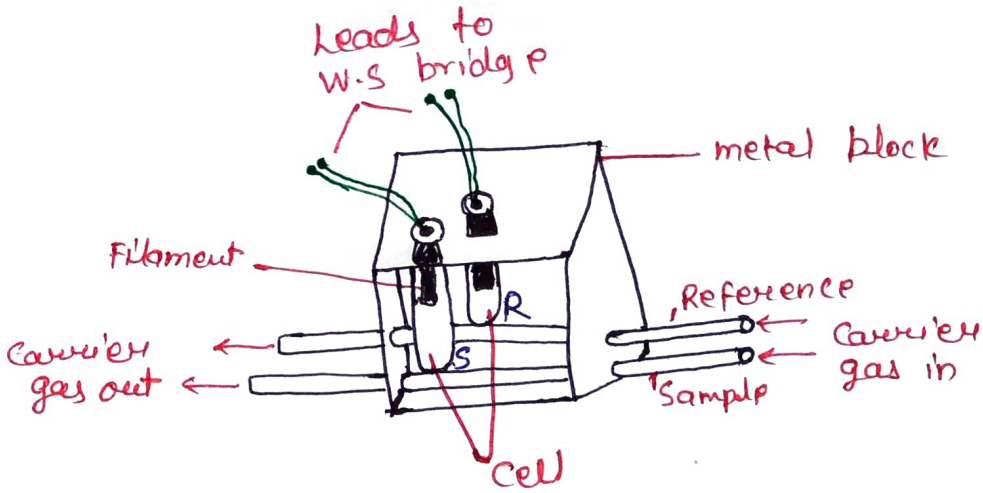
- Almost all the detectors monitor the GLC column effluent by measuring the changes in the composition arising from the variations in the eluted components.
- when the carrier gas alone is passing they give zero signal.
- when a component is eluted it is detected and a signal proportional to the concentration of that component is produced.

There are six different kinds of detectors used in 'Gas chromatography' –

1. Thermal Conductivity detector (TCD)
2. Flame Ionization detector (FID)
3. Electron capture detector (ECD)
4. Thermionic detector (NP-FID)
5. Flame photometric detector (FPD)
6. Photoionisation detector (PID)

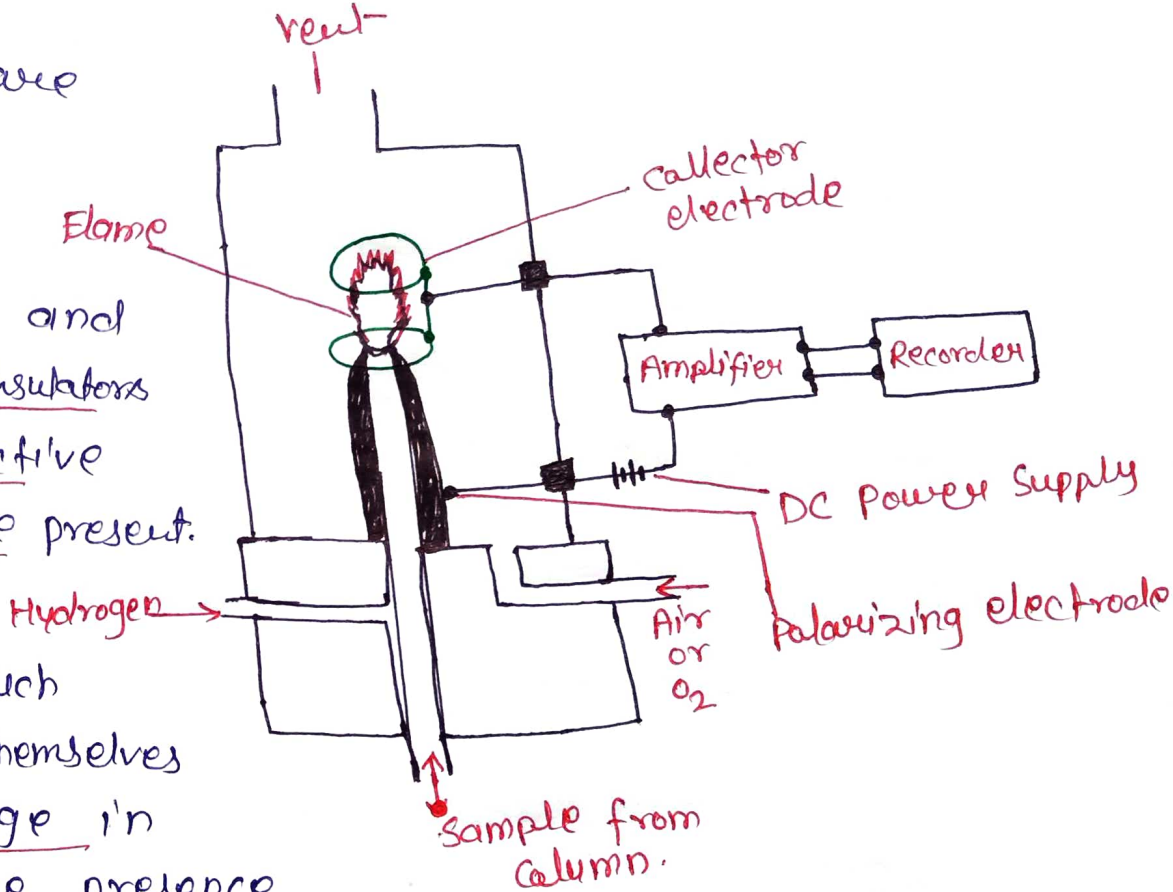
① Thermal Conductivity Detector

- One of the first detectors used was the "differential thermal conductivity detector".
- The principle of the detector is that the temp. and thus the resistance of a wire through which a current is flowing is dependent upon the thermal conductivity of the gas in which it is immersed. Time #
- The thermal conductivity of a gas is a function of its composition.
- The TCD respond to all types of organic and inorganic compounds including those not detected by FID.



② Flame Ionization Detector (FID)

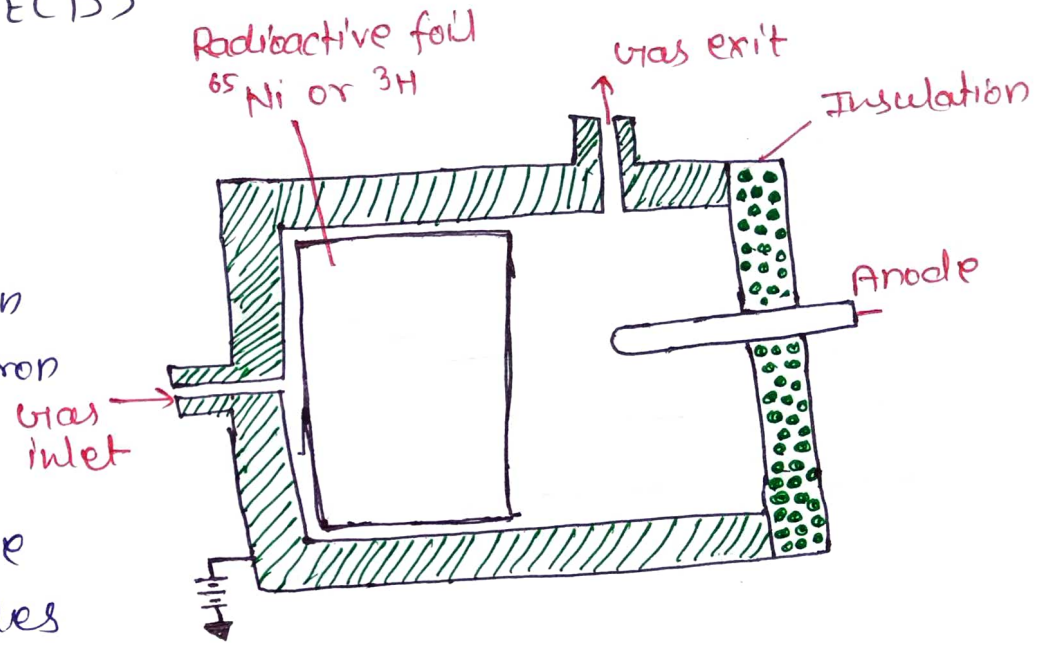
- The ionization detectors are based on the electrical conductivity of gases.
- At normal temprature and pressure gases act as insulators but will become conductive of ions if electrons are present.



- If the conditions are such that the gas molecules themselves do not ionize, the change in conductivity due to the presence of a very small number of ions can be detected.
- The detector responds to all organic compounds except formic acid. The following are some compounds that are not detected by FID -

H_2, O_2, N_2, $SiCl_4$, H_2O, SO_2.

③ Electron Capture Detector (ECD)



- The electron affinity of diff. substances can be used as the basis for an ionization detector known as the electron capture detector.
- it responds to only those compounds, whose molecules have an affinity for electrons, e.g.→ chlorinated compounds, alkyl lead etc.
- Electron capture detector in which a metal foil coated with a tritium-containing compound is used as a steady source of slow electrons.
- The ECD is most frequently used for detection and measurment of trace environmental pollutants.

④ Thermionic Detector (NP-FID)

- The thermionic detector functions on the principle of ion-current generated by the thermal production of ions.
- It may also be termed as a Nitrogen detector, a Sulphur detector, a phosphorous detector, and a halogen detector by virtue of the fact that its specificity in detecting organic compounds essentially containing these elements.
- it is also widely known as NP-FID because it is invariably employed for carrying out the analysis of N- or P- containing organic compounds.

⑤ Flame photometric Detector (FPD)

- it operates on the principle of photon emission.
- If P- or S- containing hydrocarbons are ignited in a hydrogen-rich flame, it gives rise to the chemiluminscent species spontaneously which may subsequently be detected by a suitably photomultiplier device.

- Hense, FPD is regarded as a specific detector for P- or S- containing compounds.
- The Flame photometric detector (FPD) which unfortunately could not get enough recognition in the field of gas chromatographic analysis due to the following reasons,

  (a) its selectivity,
  (b) Its poor commercial availability.

Derivatisation techniques in Gas chromatography

- Derivatisation is a technique of treatment of the sample to improve the process of separation by column or detection by detector.
- They are of two types :-

① Pre-column derivatisation :-

- This is done to improve some properties of the

sample for separation by column.

- By this derivatisation technique, the components are converted to more valatile and thermostable derivatives.
- moreover improved separation and less tailing will be seen after such treatment.
- In the following conditions, pre-column derivatisation is done :-
  - The component is less valatile;
  - The compounds are heat sensitive
  - To reduce tailing;
  - To improve separation factor;

examples :- Carboxylic acids, sugars, phenols, alcohols etc. can be converted to less palar compounds by using reagents like BSA reagent (Bi-trimethyl Silyl Acetamide).

They can also be converted to acetyl derivative or triflouro acetyl derivative.

② Post-Column derivatisation

- Post column derivatisation is done to improve the response shown by detector.
- The components may not be detected by detector unless derivatisation is done.
- 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.

Temperature programming in GC

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- it is a technique in which the column temperature is increased either continuosly or in steps as the separation proceeds.
- If column temp. increases — vapour pressure of Analyte ↑es
  ↓
  elute faster.

Advantages of GC

- High resolution power compared to other methods.
- High sensitivity.
- High accuracy and precision.
- Analysis of sample very quickly (minutes even seconds)
- Small sample needed.

Disadvantages of GC

- Limited to volatile sample.
- Not suitable for thermally labile samples.
- Samples be soluble and dont react with the column.
- During injection of the gaseous sample proper attention is required.

Applications

- miscellaneous analysis of foods like carbohydrates, proteins, lipids, vitamins, steroids, drugs and pesticides residues, trace elements.

- Pollutants like formaldehyde, carbon monoxide, benzene,

DDT etc.

- Dairy product analysis.
- Separation and identification of volatile materials, plastics, natural and synthetic polymeres, paints and microbiological samples.
- Inorganic compound analysis.

UNIT-4 CHAPTER-2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY [HPLC]

Introduction

- The HPLC is a method of Separation in which the stationary phase is contained in a Caloumn, one end of which is attached to a source of pressurized liquid eluent (mobile phase).
- HPLC is also called as High pressure liquid Chromatography.

Theory

- The main principle involved in high performance liquid chromatography is adsorption.
- when a mixture of component are introduced into a HPLC column, they travel according to their relative affinities towards the stationary phase.
- 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.
- It relies on pump to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material.

Types of HPLC :-

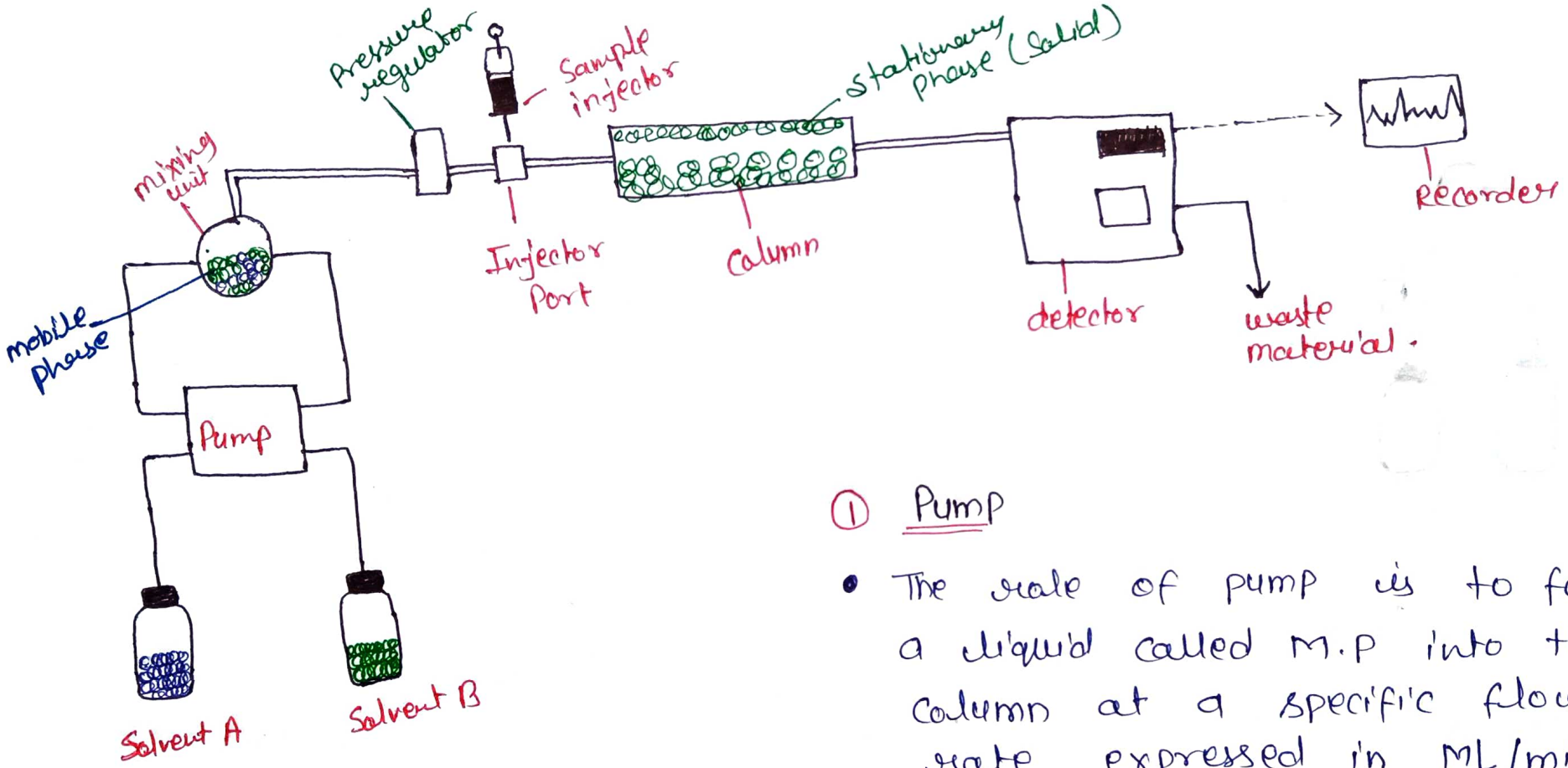
Normal phase mode - S.P is polar and M.P is non-polar

Reverse phase mode - S.P is non-polar and M.P is polar.

Principle :- Adsorption, Ion-Exchange, Size exclusion.

Instrumentation

1. Pump,
2. mixing unit,
3. Solvent degassing,
4. Injector,
5. Column,
6. Detector.



① Pump

- The role of pump is to force a liquid called M.P into the column at a specific flow rate expressed in ML/min.
- Normal flow rate in HPLC is 1-2 mL/min.

Types
- Constant flow reciprocating Pump.
- Syringe type pump.
- Pneumatic pump.

② Mixing Unit

Mixing unit is used to mix Solvents in different proportion and pass through the column.

- There are two types of mixing units –

(i) There are low pressure mixing chamber which uses helium for degassing Solvent.
  └ stop bubbles during High pressure Pump.

(ii) High pressure mixing chamber does not require helium for degassing ~~other~~ solvent.

- mixing of solvent is done either with static mixture ~~which~~ which is packed with beads and dynamic mixer which uses magnetic stirrer and operates under high pressure.

③ SDlvent Degassing

- Several gases are soluble in organic solvents.
- when solvents are pumped under high pressure gas

bubbles are formed which will interfare with separation process steady baseline and shape of peak.

- Hence degassing is necessory.

Techniques for Solvent degassing -

(i) Vaccum filteration :- which can remove air bubbles, but it is not always reliable and completed.

(ii) Helium purging :- By passing helium gas through the solvent.

- This is very effective but expensive.

(iii) Ultrasonification :- By Ultrasonificator which converts ultra high frequency to mechanical vibrations cause removal of air bubbles.

(4) Injector

- The injector serves to introduce the liquid sample into the flow M.P.
- Typical sample valumes are 5-20 microlitres.

- The injector must also be able to withstand the high pressure of the liquid system.

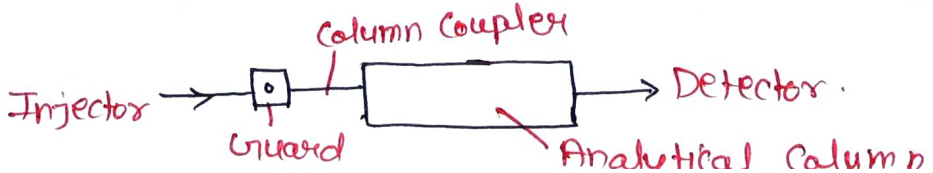
Types of injectors ⇒

(i) Septum injectors :- For injecting the sample through a rubber septum. This is not common, since the septum has to withstand high pressure.

(ii) Stop flow injector :- In which the flow of M.P is stopped for a while and the sample is injected through a value device.

(5) Column

- most important part of chromatography.
- Column length - varies from 5-30 cm.
- Column diameter - ranges from 2-5 cm.
- Particle size - 1-20 μm
- Particle nature - Spherical, uniform sized, porous material are used.



materials of construction for the tubing ⇒

- Stainless steel (most popular, gives high pressure capabilities)
- Glass
- PEEK (Poly ether - ether ketone)
- Polymers.

⑥ Detectors

- The detection of separated components in the elute from the column is based on -

  ⓐ Bulk property of elute.
  ⓑ Solute property of the individual

- Detector should respond to a particular property of the substance being separated and it should be sensitive to at least at 10^{-8} g/ml and give a linear response over a wide concentration range.

The most commonly used detectors for HPLC are -

① Photometric detectors
- Single wavelength detector
- multi wavelength detector
- Variable wavelength detector.
- Programmable detector.
- Diode array detector.

② Fluorescence detector
③ Refractive index detector
④ Electrochemical detectors..

Advantages of HPLC

① Simple, rapid, reproducible.
② High performance.
③ High sensitivity.
④ Rapid process, time saving.

⑤ High separation Capacity.
⑥ S.P and M.P are chemically inert.
⑦ wide varieties of S.P.
⑧ Important for qualitative and Quantitative Analysis.

Applications

① checking the purity of compounds.
② Isolation and purification of biologically active natural products.
③ Used for separation of antibiotic from broth mixture.
④ Bio-monitoring of pollutants.
⑤ Pharmaceutical Quality Control.
⑥ Identification of steroids in blood, wine etc.