CHROMATOGRAHPY

(CHROMO=COLOUR,GRAPHY=GRAPHICAL REPRESENTATION)

DEFINITION OF CHROMOTOGRAPHY

- Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is **stationary phase**, while the other is **mobile phase** which moves in a definite direction. The stationary phase may be a solid, or a liquid supported on a solid or gel, the mobile phase may be either a gas or a liquid.

- The interaction of components to be separated with mobile phase and stationary phase result in the separation of components from the mixture.

Gas chromatography

- Gas chromatography is the chromatographic technique used for the separation of volatile components ,Volatiles compounds are the components that can easily vaporized at room temperature.

INSTRUMENTATION;

1. Column 2.Stationary phase 3.Mobile phase 4.Detector 5.Amplifier 6.Recorder

1.COLUMN;

Column used in GC is very long and arrange it as coil ,Column used in are of two types

one is packed and another one is stationary column;

1.Packed column is made up of glass or steel stainless steel(Length of the column 1 to 3m and Internal diameter of the column is 2 to 4 mm)

2. Capillary column is made up of fused Quartz (length of the column is 10 to 100m, Internal diameter of the column is 0.1 to 1mm)

3. Coulmn is placed in the champers, so that uniform temperature can be maintained.

2. Stationary phase;

- It is packed with the inner walls of the column
- It is made of Silicon grease or wax which can withstand high temperature
- **Mobile phase;**
- Inert gas helium or unreactive nitrogen gas is used as mobile phase. It is taken in the cylinder and connected to the column via molecular sieve. The molecular sieve removes unwanted hydro carbons and water vapor and oxygen that may interfere with the test sample during analysis .

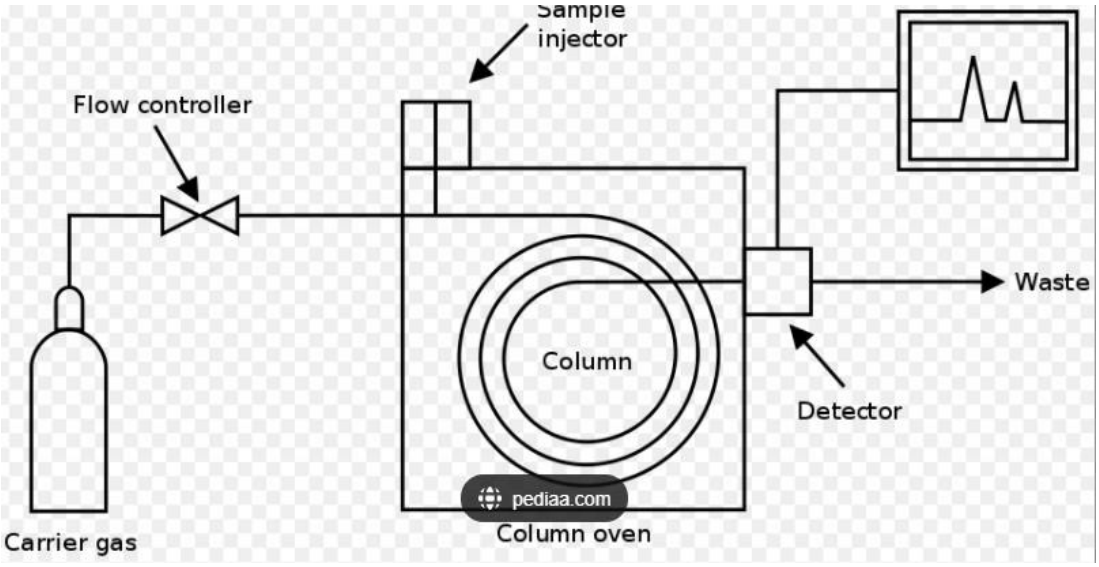
4.Detector;

- It is used to detect the sample which is placed at the end of the column; most commonly used detector is flame ionization detector.

5.Recorder;

- It records the signal received from detector

<u>WORKING</u>;



- Sample which is to be separated is mixed with appropriate volatile solent such as heptane,acetone or methanol,just before the column there is a septum(injector) which inject the sample to the column,Temperature of the injection tegion is kept 20 to 50 degree celcius higher than the column,this allows the rapid volatilization of the sample. once the sample volatilized ,it persist on the column where the separation occurs.

Separation of the molecule

- During analysis the temperature of the column maintained between 150 to 300 degree Celsius ,separation occurs based on the interaction of molecule between mobile phase and stationary phase.
- Less volatile molecule interacts more with stationary phase and moves slowly and more volatile molecule interacts more with mobile phase and moves fast down the column

  Once the separation is completed, the sample is detected by the detector which is attached at the end of the column. The detected sample recorded by the recorder it shows the graph drawn between current and time. Based on the time,the elements present in the sample is identified.

USES;

- The chromatographic technique is used for the separation of amino acids, proteins & carbohydrates.
- —It is also used for the analysis of drugs, hormones, vitamins
- —Helpful for the qualitative & quantitative analysis of complex mixtures.
- —The technique is also useful for the determination of molecular weight of proteins.