principle of chromatography. Unit - II

1. chromatography is a method of physical separation in which components of mixture gets separated on two phase.

2. one of the phase is the immobile porous bod bulk liquid ~~with~~ which is called stationary phase and the other phase is the mobile fluid ~~that~~ flows over the stationary phase under gravity.

3. During the movement of the sample a separated result is formed by the repeated desorption an sorption in the direction of the mobile phase migration.

4. several key factors are responsible on the separation process like partition between liquid – liquid, affinity between molecular weight and characteristics related to ~~the~~ ~~stationary components~~. liquid solid ad sorption.

5. An interaction between the molecules are physical and involves weak chemical bonds like dipole-dipole interaction and hydrogen bond formation and adhere to the stationary components.

6. Components that adhere strongly to the stationary phase move slowly than those who adhere weakly.

Application of Chromatography.

1. In molecular Biology: Protein separation, insulin and enzyme purification and some biochemical processes. HPLC is used. It is also applied in the study of proteomics and metabolomics.

2. In chemical Industries: HPLC and GC is used for the water and air purification and in Pesticides and oil testing.

3. In pharmaceutical Industries, chemicals of different weight and size are separated and identified and also detects the unknown compounds.

4. In food Industries. toxins and food additives are determined.

HPLC Chromatography.

High Performance liquid chromatography is a form of column chromatography that pumps a sample mixture or analyte in a solvent at high pressure through a column with chromatographic packing material.

Principle of HPLC

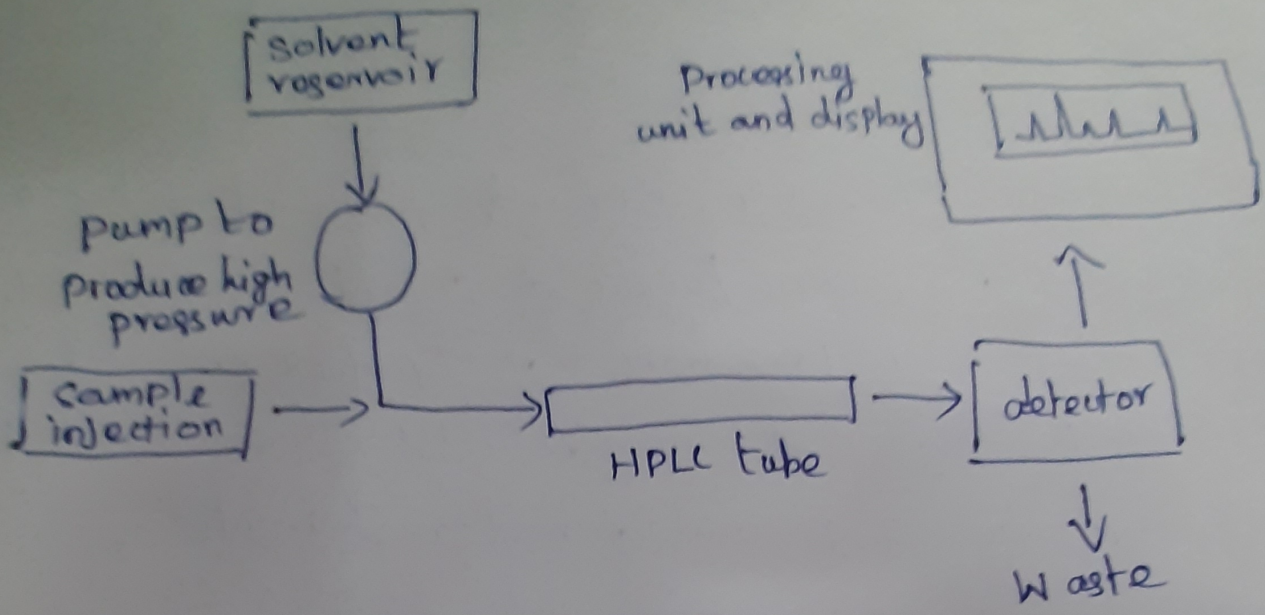
The specific intermolecular interactions between the molecules of a sample and the packing material define their time on column. Hence different constituents of a sample are eluted at different times.

Thereby, the separation of the sample ingredients is achieved.

HPLC is a highly improved form of column chromatography. A pump forces a solvent through a column under high pressures of up to 400 atmospheres. The column packing material or adsorbent (or) stationary phase is typically a granular material made of solid particles such as silicaa (or) polymers.

HPLC uses :

1. Analysis of drugs
2. Analysis of synthetic polymers
3. Analysis of pollutants in environmental analytics
4. Determination of drugs in biological matrices.
5. product purity and quality control of industrial products and fine chemicals.
6. water purification.
7. Pre-concentration of trace components
8. ~~Ligand - Ion - exchan~~
8. crime scene testing
9. ~~Forensic~~



High performance liquid chromatography.

1. Paper chromatography

Paper chromatography is an analytical method used to separate colored chemicals or substances. It is primarily used as a teaching tool, having been replaced by other chromatography methods, such as thin layer chromatography.

Principle of paper Chromatography.

Principle of paper chromatography is based on liquid - Liquid partition chromatography. In paper chromatography special quality paper known as chromatography paper containing water trapped in it which acts as the stationary phase. The solvent rises up the paper by capillary action and flow over the spot

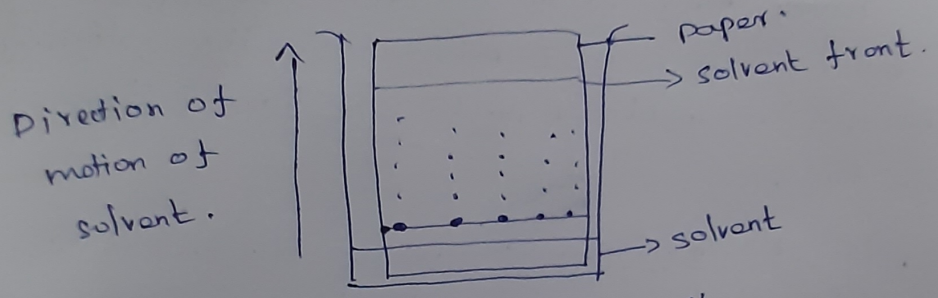
paper chromatography can be used to separate mixtures of coloured compounds. As the solvent soaks up the paper, it carries the mixtures with it. Different components of the mixture will move at different rates.

1. Ascending type or radial paper chromatography is used as they are easy to perform. Selection of
2. selection of filter paper is done based on the size of the pores and the sample quality.
3. preparation includes the dissolution of the the sample in a suitable solvent used in making the mobile phase.
4. Sample should be spotted at a proper position on the paper by using a capillary tube.
5. chromatogram development is spotted by immersing the paper in the mobile phase. Due to the capillary action of paper, the mobile phase moves over the sample on the paper.

once the chromatogram is developed the paper is dried detecting solution can sprayed chromatogram developed paper

Applications of paper chromatography :-

1. Study the process of fermentation and riponing
2. check the purity of Pharmaceuticles.
3. Inspect cosmetics.
4. detect the adulterants.
5. detect the contaminants in drinks and foods.
6. Examine the reaction mixtures in biochomical Laboratories.



paper chromatography.

2. Thin Layer chromatography.

Thin Layer chromatography technique used to separate non volatile mixtures. Thin layer chromatography is performed on a sheet of an inert substrate such as glass, plastic or aluminium foil which is coated with a thin layer of adsorbant material, usually silicagel, aluminium oxide, or cellulose.

Principle :-

Chromatography works on the principle that different compounds will have different solubilities and adsorption to the two phases between which they are

to be partitioned. TLC is a solid-liquid technique in which the two phases are a solid and a liquid.

For Thin layer chromatography a sample of the mixture to be separated is deposited at a spot near one end of the plate and a suitable solvent is allower to rise up the plate by capillary action. The components of the sample become separated from one another because of their different degrees of attachment to the coating material on the plate or sheet. The solvent is then allowed to evaporate, and the location of the separated components is identified, usually by application of reagents that form coloured compounds with the substance.

* purpose of thin layer chromatography:.

* Thinlayerchromatography is a very commonly used. technique in syothetic chemistry for identifying compounds, determining their purity and following the progress of a reaction.

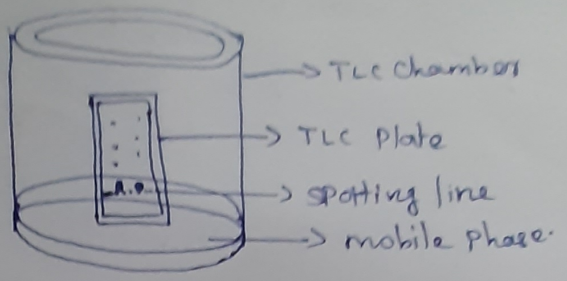
* TLC is extremly useful in Biochemical analysis

* It's used widely in separating multicomponent pharmaceutical formulations.

* It is used in the cosmetic industry.

* It is used in the food industry, to separate and identify colours.

Thin layer chromatography (TLC)



Gas - Liquid Chromatography.

Gas Liquid Chromatography called gas chromatography. It is a powerful tool in analysis. It has all sorts of variations in the way it is done - ~~it you must~~ ~~exceed~~, In gas Liquid chromatography the mobile phase is a gas such as helium and the stationary phase is a high boiling point liquid adsorbed onto a solid.

How fast a particular compound travels through the machine will depend on how much of its time is spent moving with the gas as opposed to being attached to the liquid in some way.

Injection of the sample.

very small quantities of the sample injected into the machine using a small syringe.

the injector is contained in an oven whose temperature can be controlled. It is hot enough so that all the sample boils and is carried into the column

as a gas by the helium.

The column packing materials:

Column in gas liquid chromatography is a long ~~thin~~ tube packed with the satationary phase. the other is even thinner and has the satationary phase bonded to its inner surface.

The column made of stainless steel, 1 to 4 meters long 4mm in diameter. It is coiled, fit into thermostatically controlled oven.

The column packed with finely ground diatomaceous earth. This is coated with a high boiling liquid – waxy polymer.

The colum temperature:

varied from 50°c to 250°c.

molecule in the mixture injected into the colum.

1. it may condence on the stationary phase
2. it may dissolve in the liquid on the surface of the stationary phase
3. It may remain in the gas phase.

A compound with a boiling point higher than the temperature of the column will obviously tend to condense at the start of the column.

Some molecules may dissolve in the liquid stationary phase. Some compounds will be more soluble in the Liquid than others.

Any moleale in the substance spends some of its time dissolved in the liquid and some of its time carried along with the gas.

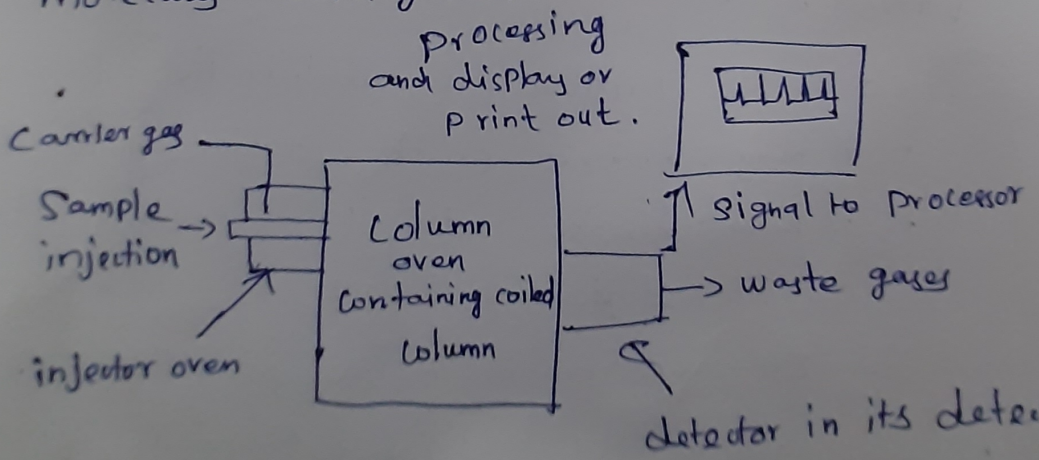
* Detectors sense the arrival of the separated components and provide a signal.

* These are either concentration dependent (or) mass dependant.

* The detector should be close to the column exit and the correct temperature to prevent decomposition.

Uses :-

Gas chromatography is an analytical technique used to separate chemical components of a organic molecules and gases.



Gas-Liquid chromatography.