Chromatography: principle and application of TLC ,HPLC ,and GC.

Chromatography:

In chemical analysis, chromatography is a laboratory technique for the separation of a mixture into its components. The mixture is dissolved in a fluid solvent called the Mobile phase , which carries it through a system on which a Material called the stationary phase is fixed.

Principle:

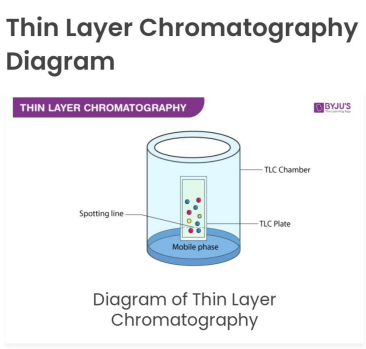
Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase.

Mobile phase flowing over the stationary phase is a gaseous or liquid phase. If mobile phase is liquid it is termed as liquid chromatography (LC) , and if it is gas then it is called gas chromatography (GC) . Gas chromatography is applied for gases , and mixtures of volitile liquid and solid material.

Stationary phase in chromatography is the one which does not move with the sample . Several physical and molecular changes take place during this stage that makes them intersting to explore. The characteristics proteins synthesized in the stationary phase are indispensable as they confer viability to the bacteria.

Chromatography plays a vital role in industries we interact with quite often. Pharmaceuticals, clinical trials , environmental and chemical safety . Food beverage , drug testing , forensic , petroleum creation , and molecular biology are some of molecular biology are some of the most common uses of chromatography.

TLC(Thin layer chromatography):



Thin layer chromatography is a technique used to isolate non - volatile mixtures. The experiment is conducted on a sheet of aluminium foil, plastic, or glass which is located with a thin layer of adsorbent material. The material usually used in aluminium oxide, cellulose , or silica gel .

On completion of the separation, each component appears as spots separated vertically. Each spots has rention factor (Rf) expressed as:

Rf= dist.travelled by sample / dist.trsvelled by solvent.

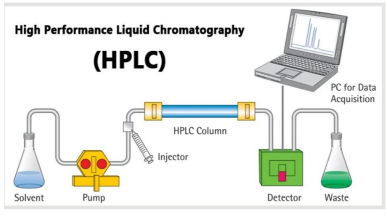
Principle:

TLC depends on the separation principle. The separation reliies on the relative affinity of compounds towards both the phases.

Application:

- The qualitative testing of various medicines such as sedatives , local anaesthetic, anticonvulsant tranquilisers ,analgesis, antihistamines , steroids ,hypnotics is done by TLC
- TLC is extremely useful in biochemical analysis such as separation or isolation of biochemical metabolites from its blood plasma , urine , body fluids , serum etc
- TLC can be used to identify natural products like essential oils or volatile oil ,fixed oil, glycosides, waxes ,alkaloids etc.
- It is widely used in separating multicomponent pharmaceutical formulations.
- It is used for the purification of samples and direct comparison is done between the sample and the authentic sample.
- It is used in the food industry , to separate and identify colours , sweeting agent, and preservatives

HPLC (High performance liquid chromatography) .



HPLC is an analytical technique used to separate , identify or quantify each component in a mixture.

The mixture is separated using the basic principles of column chromatography and then identified and quantified by spectroscopy .
In the 1960s, the column chromatography LC with its low- pressure suitable glass. Columns was further developed to the HPLC with its high - pressure adapted metal columns. HPLC is thus basically a high improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity , it is forced through under high pressures of up to 400 atmosphere.

Principle:

The purification takes place in a separation column between a stationary and a mobile phase. The stationary phase is a granular material with very small porous particles in a separation column.
The mobile phase , on the other hand , is a solvent or solvent mixture which is forced at high pressure through the separation column.

Applications:

Pharmaceutical Application

- To control drug stability
- Tablet dissolution study of pharmaceutical dosage form
- Pharmaceutical quality control

Environmental Application

- Detection of phenolic compounds in drinking water
- Bio- monitoring of pollutants

Application in forensics

- Quantification of drugs in biological samples.
- Identification of steroids in blood , urine etc
- Forensic analysis of textile dyes
- Determination of cocaine and other drugs of abuse in blood , urine etc.

GS(Gas chromatography)

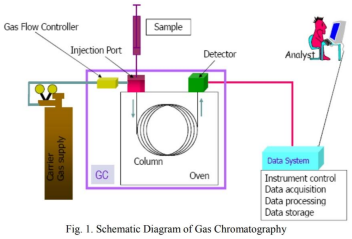


Fig. 1. Schematic Diagram of Gas Chromatography

Gas chromatography (GS) is a normally utilized analytic technique as a part of numerous research and industry research facilities for equality control and in addiction identification and quantitation of components in a mixture.

Principle:

The basis of the separation is a retardation of the individual components as they are moved through a long column by a carrier gas , usually helium or nitrogen . The column consists of a steel or glass tube filled with an inert packing material such as glass or ceramic beads.

Application:

- Food analysis
- Environmental monitoring
- Drug testing
- Forensics
- Manufacturing quality control
- Environmental research.