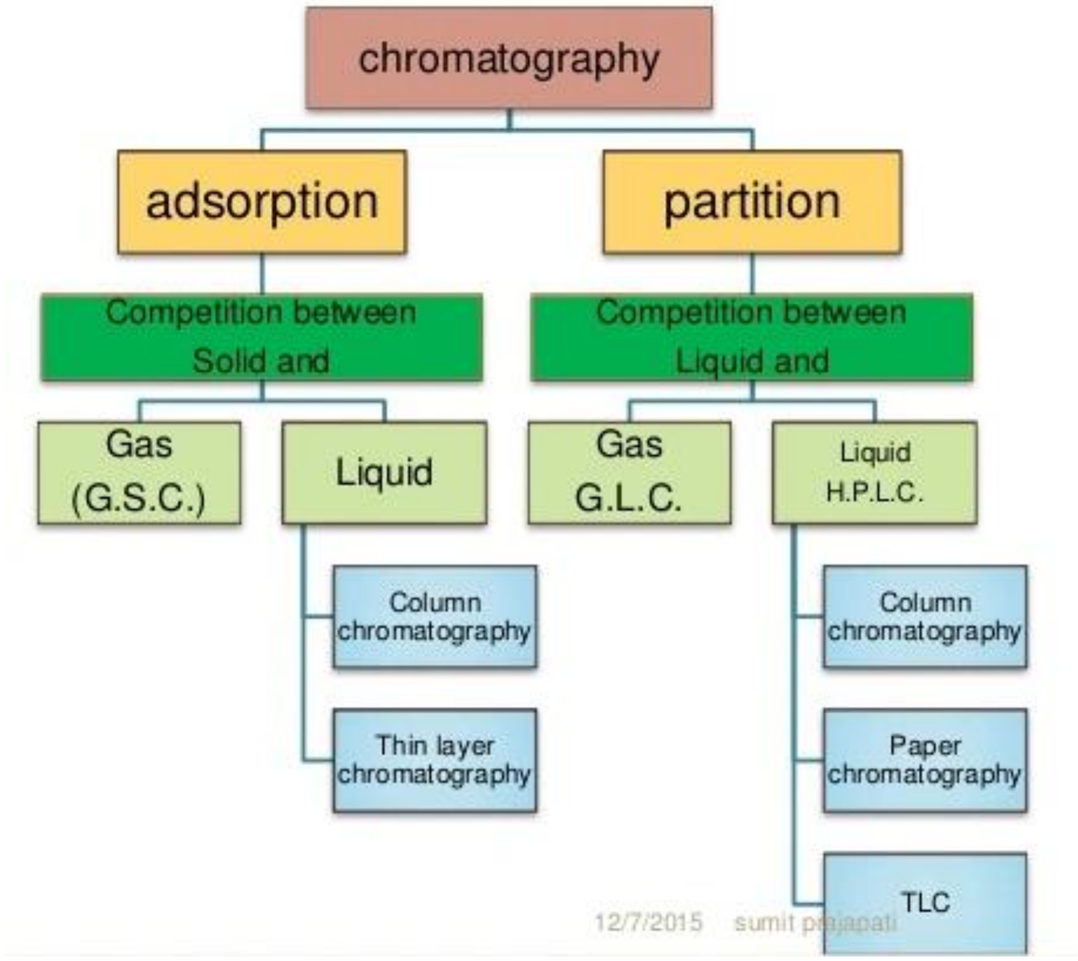
Chromatography, Gas chromatographic analysis

Chromatography Definition:

Chromatography is defined as physical method of separation, in which the method of separation, in which the mixture of Analytes is separated using two mixture of Analytes is separated using two phases, one is stationary phase and other is mobile phase which percolates through is mobile phase which percolates through the stationary phase. The separation occurs because of difference in affinity between Analytes and stationary phase.

Branches of Chromatography:

There are two main types of chromatography: liquid chromatography (LC) and gas chromatography (GC). Both LC and GC can be used for either preparative or analytical applications.



Comparison between LC and GC Techniques:

Gas chromatography(GC)	Liquid chromatography(LC)
1. Stationary phase: Solid/liquid 2. Mobile phase: GAS 3. Mobile phase does not take part in separation 4. Volatile Organic/inorganic compounds only. 5. Works at comparatively low pressure. 6. Works on both packed as well as capillary columns capillary columns 7. Fast and better efficiency obtained 8. Selective columns for applications 9. Range of selective detectors available for application available for application 10. Environmental friendly technique	1- Stationary phase: Solid/Liquid 2- Mobile phase: LIQUID 3- Mobile phase takes active part in separation. 4- Volatile as well as nonvolatile compounds can be separated compounds 5- Works at high pressure 6- Only packed columns for analysis 7- Slow and poor efficiency . 8- Very few selective columns available 9- Few selective detectors available 10- Solvents eluted after separation needs to be disposed off properly hence non environmental friendly technique

Gas chromatography

- Gas chromatography (GC) is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition.
- Gas chromatography is a term used to describe the group of analytical separation techniques used to analyze volatile substances in the gas phase.
- Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture. GC can be used to prepare pure compounds from a mixture.
- Gas chromatography is also sometimes known as vapor-phase chromatography (VPC), or gas–liquid partition chromatography (GLPC).
- In gas chromatography, the components of a sample are dissolved in a solvent and vaporized in order to separate the analytes by distributing the sample between two phases: a stationary phase and a mobile phase.
- The mobile phase is a chemically inert gas or an unreactive gas such as helium, argon, nitrogen or hydrogen. that serves to carry the molecules of the analyte through the heated

column. Gas chromatography is one of the sole forms of chromatography that does not utilize the mobile phase for interacting with the analyte.

- The stationary phase is a microscopic layer of viscous liquid on a surface of solid particles on an inert solid support inside a piece of glass or metal tubing called a column.
- The stationary phase is either a solid adsorbant, termed gas-solid chromatography (GSC), or a liquid on an inert support, termed gas-liquid chromatography (GLC)

Why use Gas Chromatograph (GC)?

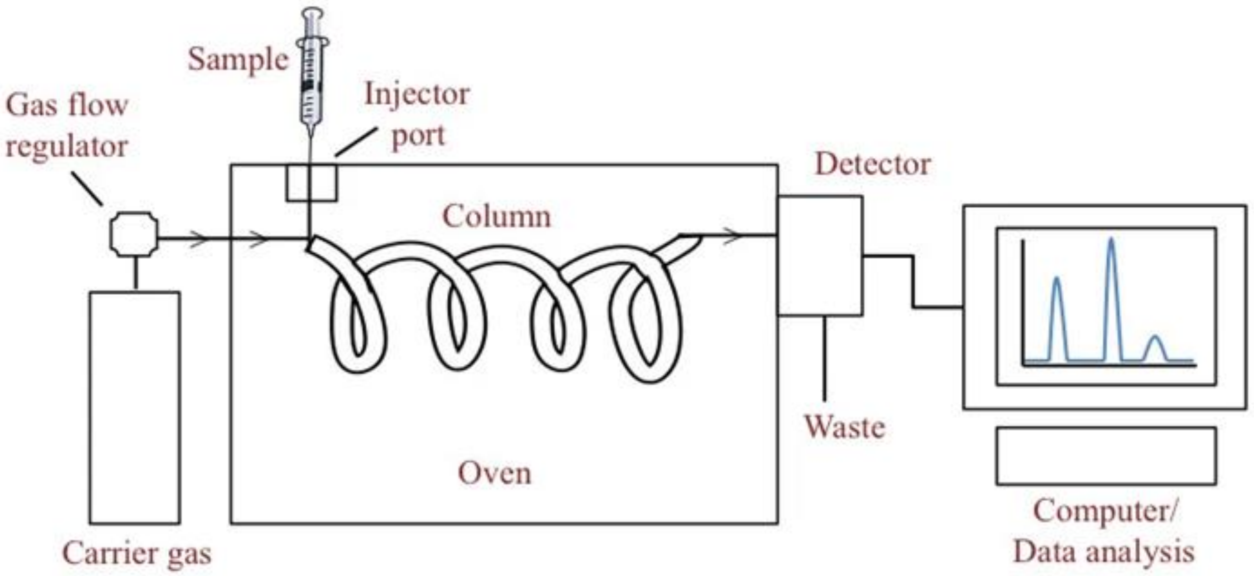
1- Short Analysis Time
2- Wide Choice of Stationary Phase
3- Wide Choice of Detectors
4- Ease of Operation

What are the types of gas chromatography?

Two types of gas chromatography are encountered.

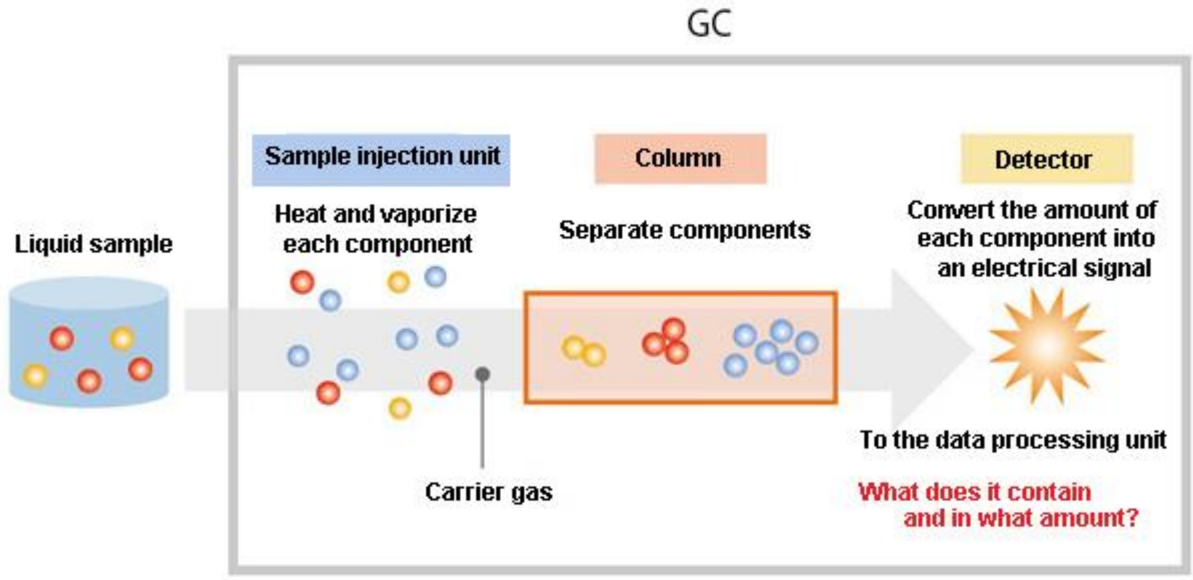
1. **Gas-solid chromatography (GSC):**It based upon a stationary phase on witch retention of analysis consequence of physical adsorption
2. **Gas-liquid chromatography (GLC):**Is useful for separating ions or molecules that are dissolved at absolvent.

Gas Chromatograph Main Components :



Separation of compounds:

- When analytes are introduced into the column, the molecules distribute between the stationary and mobile phases .
- Those in the stationary phase are temporarily immobile and do not move down the the phase .
- All molecules of the same compound travel through the column at nearly the same rate and appear as a band of molecules (called sample band)
- Sample band of compound which is less ‘soluble’ in the stationary phase moves faster, because more of the molecules spend time in the mobile phase (carrier gas).



Principle:

1- The analyte is loaded over the silica bed (packed in the column) and allowed to adhere to the silica. Here, silica acts as the stationary phase.

2- Solvent (mobile phase) is then made to flow through the silica bed(under gravity or pressure).

3- The different components of the analyte exhibit varying degrees of adhesion to the silica and as a result they travel at different speeds through the stationary phase as the solvent flows through it, indicated by the separation of the different bands.

4- The components that adhere more strongly to the stationary phase travel more slowly compared to those with a weaker adhesion.

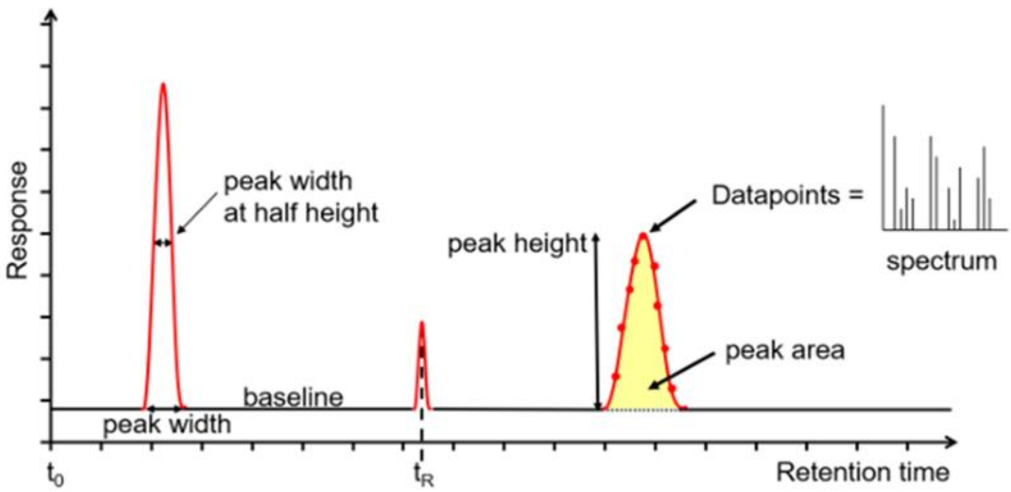
5- Analytical chromatography can be used to purify compounds ranging from milligram to gram scale.

principle of separation of different components:

1- Higher the adsorption to the stationary phase, the slower the molecule will move through the column.
2- Higher the solubility in the mobile phase, the faster the molecule will move through the column.
3- So, the interplay between the above two factors determines the differential rates at which the different components of the analyte will move through the column.
4- Adsorption and solubility of a molecule can be manipulated bychoosing the appropriate stationary phase and mobile phase.

How do you read a chromatogram and what does it tell you?

1. The x-axis is the retention time, taken from the time the sample was injected into the GC (t_0) to the end of the GC run.
2. Each analyte peak has a retention time measured from the apex of the peak, for example t_R .
3. The y-axis is the measured response of the analyte peak in the detector. The baseline shows the signal from the detector when no analyte is eluting from the column, or it is below the detection limit .
4. The baseline response is a mix of electrical noise (usually low) and chemical noise, such as impurities in the carrier gas, column stationary phase bleed and system contamination .
5. Hence, if the baseline is higher than it should be, it is an indication of a problem or that maintenance is required .
6. Various measurements can be taken from the peak, such as width at the baseline, width at half height, total height and area.



Narrower, sharper peaks give better sensitivity (signal to noise ratio) and better resolution (peak separation).

Migration rates of compounds in column :

Different migration rates of compounds can be achieved if these compounds have different interaction strengths with the stationary phase .

Migration rate of compounds in column depend on:

1. Compound chemical structure
2. Stationary phase chemical structure
3. Column temperature

Retention Time (t_R)

- ✓ The time an analyte takes to travel through the column
- ✓ A measure of the amount of time an analyte spends in the column
- ✓ Sum of the time spent in the stationary phase and the mobile phase

Retention Time = time spent by a compound inside the column

Retention time of an unretained compound ((t_M)

- ✓ The time an unretained compound takes to travel through the column
- ✓ Unretained compound travels down the column at the same rate as the mobile phase (carrier at the same rate as the mobile phase (carrier gas)
- ✓ Equivalent to the time a compound spends in the mobile phase .

GC Applications:

1. **Food Analysis:** Analysis of foods is concerned with confirming the presence and determination the quantities of the analytes (lipids, proteins, carbohydrates, preservatives, flavours, colorants, and vitamins, steroids, and pesticide residues).
2. **Drug Analysis:** GC is widely applied to identification of the active components, possible impurities as well as the metabolites.
3. **Environmental Analysis:** The environmental contaminants; e.g. (DDT) is present in the environment at very low concentrations and are found amongmany of other compounds. GC, with its high sensitivity and high separating power, is mostly used in the analysis of environmental samples.
4. **Forensic Analysis:** In forensic cases, very little sample is available, and the concentration of the sample components may be very low. GC is a useful due to its high sensitivity and separation efficiency.