Gas chromatography

Gas chromatography consists of Gas solid chromatography (GSC) and Gas Liquid Chromatography (GLC). In both types, gas is used as mobile phase and either solid or liquid is used as stationary phase.

GSC is not widely used because of limited number of stationary phases available. In GSC, the principle of separation is adsorption. GSC is used only in case where there is less solubility of solutes in stationary phase, which is rare.

Principle of separation:-

The principle of separation in GLC is partition. Gas is used as mobile phase. Liquid which is coated on to a solid support is used as stationary phase. The mixture of components to be separated is converted to vapour and mixed with gaseous mobile phase. The component which is more soluble in the stationary phase travels slower and eluted later. The component which is less soluble in the stationary phase travels faster and eluted out first. No two components have the same partition co-efficient for a fixed combination of stationary phase, mobile phase and other conditions. Hence the components are separated according to their partition co-efficients. Partition co-efficient is the ratio of solubility of a substance distributed between two immiscible liquids at a constant temperature.

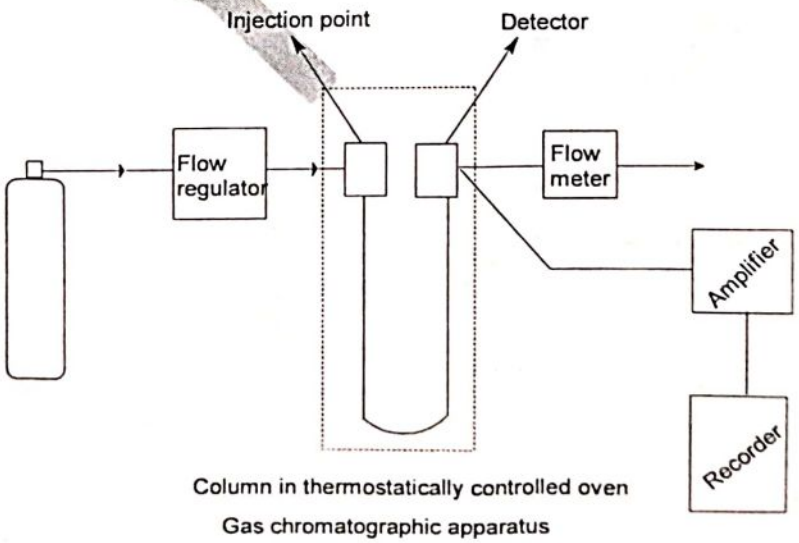
Criteria for compounds to be analysed by gas chromatography:-

1. Volatility: Unless a compound is volatile, it cannot be mixed with mobile phase. Hence volatility is important.

2. Thermo stability: All the compounds will not be in the form of vapour. There will be solid as well as liquid samples. Hence to convert them to a vapour form, they have to be heated to higher temperature. At that temperature, the compounds have to be thermo stable. If they are not thermostable, the compounds cannot be analysed by gas chromatography, since they will be decomposed.

Practical requirements:-

1. Carrier gas
2. Flow regulators and flow meters
3. Injection devices
4. Columns
5. Temperature control devices
6. Detectors
7. Recorders and integrators



Gas chromatographic apparatus

Carrier gas: The choice of carrier gas determines the efficiency of chromatographic separation. Most widely used carrier gases are Hydrogen, Helium, Nitrogen and Argon.

Hydrogen: It has better thermal conductivity, low density; it is useful in case of thermal conductivity detector and flame ionisation detector. The disadvantage is that it reacts with unsaturated compounds and it is inflammable.

Helium: It also has excellent thermal conductivity, but it is expensive. It is a good carrier gas when used with thermal conductivity detector.

Nitrogen: It is inexpensive but has reduced sensitivity.

Requirements of a carrier gas: 1. inertness, 2. Suitable to the detector used, 3. High purity, 4. easily available, 5. Cheap, 6. less risk of explosion or fire hazards, 7. should give best column performance consistent with the required speed of analysis.

As carrier gas is compressible, gases are stored under high pressure in cylinders and used when required.

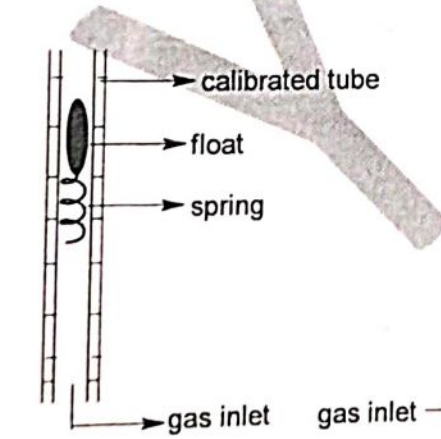
Flow regulators and flow meters:-

As carrier gases are stored under high pressure, flow regulators, are used to deliver the gas with uniform pressure or flow rate.

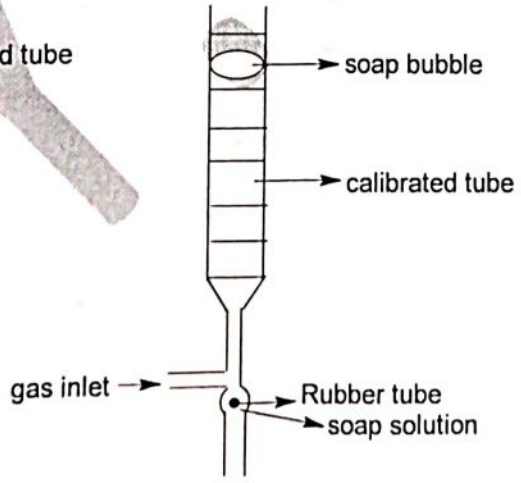
Flow meters are used to measure the flow rate of carrier gas. They are Rotameter and soap bubble flow meter.

Rota meter: It is placed conveniently before the column inlet. It has an ordinary glass tube (like burette) with a float held on a spring. The level of the float is determined by the flow rate of carrier gas and is precalibrated.

Soap bubble meter: It is similar to rotameter and instead of a float, soap bubble formed indicates the flow rate. It has a glass tube with a inlet tube at the bottom through which gas comes in. A rubber bulb is used to store soap solution. When the bulb is gently pressed, a drop of soap solution is converted into a bubble by the pressure of carrier gas and travels up. The distance travelled upwards is a measure of flow rate of carrier gas. The graduations are also precalibrated.



Rotameter



Soap bubbled meter

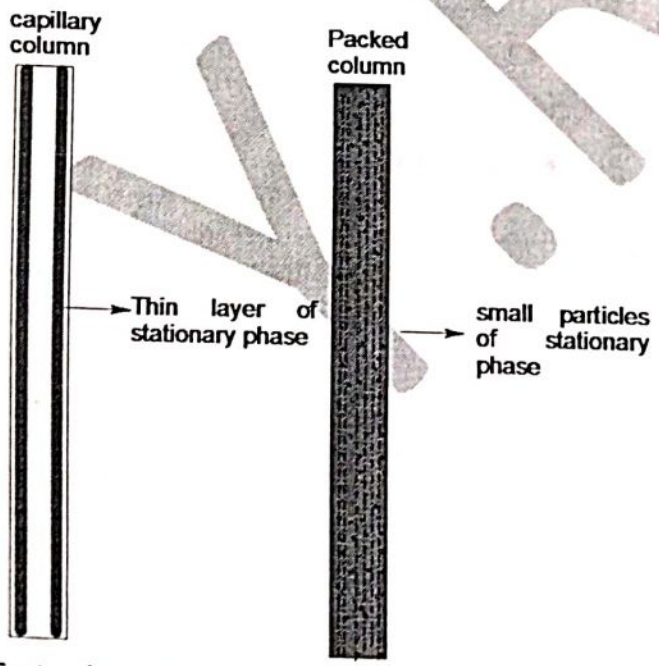
Injection devices:

Gas can be introduced into the column can be of any type either gas, liquid or solid in nature.
Liquids can be injected through loop or septum devices. Most GC instruments have a high quality rubber septum through which sample solution is injected.
Solid samples are dissolved in a suitable solvent and then they are injected through a septum.

Column: Column is one of the important parts of GC which decides the separation efficiency.
Columns are made up of glass or stainless steel. Stainless steel columns have the advantage of long life and can be easily handled without the fear or fragility. But some samples (steroids) react with them. Hence in such cases, glass columns are used. Glass columns have the advantage that they are inert and do not react with the any kind of sample. The great disadvantage is that they are highly fragile and are difficult to handle.
Columns can be classified according to the nature.

Packed column: Columns are available in packed manner commercially and hence are called as packed columns. Different columns ranging from low polar nature to high polar nature are available.

Open tubular column or capillary column or golay column: They are made up of long capillary tubing of 30-90 meters in length and have uniform and narrow internal diameter of 0.025-0.075cm. These are made up of stainless steel and are in the form of a coil. The inner wall of the capillary is coated with the stationary phase liquid in the form of a thin film (0.5 to 1μ). These columns offer least resistance to the flow of carrier gas and hence they are more efficient than packed columns which offer made resistance to the flow of carrier gas. But the disadvantage is that more sample cannot be loaded.



Scot column (support coated open tubular column): As golay or capillary columns have small sample capacity, they can be modified into scot columns. These columns are made by depositing a micron size porous layer of support material on the inner wall of the capillary column and then coated with a thin film of liquid phase. This column also has low resistance to the flow of carrier gas but offers the advantage of more sample load or capacity.

Temperature Control Devices:

Preheaters: Preheaters are used in Gas chromatography to convert the sample into its vapour form and mix them with the mobile phase or carrier gas. The preheaters are present along with injecting devices. As soon as liquid samples are injected, they are converted into vapour form.

Thermostatically controlled oven: The principle of separation in gas chromatography is partition. Since partition coefficient as well as solubility of a solute depends upon temperature, temperature maintenance in a column is highly essential for efficient separation. Hence the column as well as injecting devices should be maintained at a particular temperature.

Two type of operations are available they are

i) Isothermal programming: in which the same temperature is maintained throughout the process of separation.

Linear programming: In which the oven is heated linearly over a period of time eg. 150°c initially to 200°c at the end of separation with an increase in temperature at the rate of 5°c /minute this type of linear programming is required when a sample has a mixture of low boiling and high boiling point compounds. This method is efficient for separation of such complex mixtures.

Detector: A detector can detect the different between a pure carrier gas and a eluted component.

The requirements of the ideal detector are:

1. Applicability to wide range of samples.
2. High Sensitivity to even small concentrations.
3. Rapidity of response.
4. Linearity: i.e. less response to low concentration and proportional response to high concentration.
5. Response can be unaffected by temperature, flow rate or characteristics of carrier gases.
6. Non destructive to the sample in case of preparative work.
7. Simple and easy to maintain.
8. Inexpensive

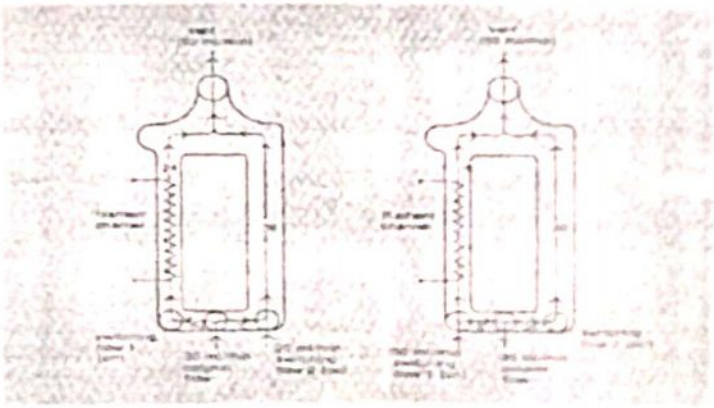
The different detectors used commonly are

Katharometer or Thermal conductivity Detector (TCD)

Flam ionisation detector (FID)

Katharometer or thermal conductivity detector:

The principle is based upon thermal conductivity difference between carrier gas and that of component. Katharometer has two platinum wires of uniform dimensions which form part of Wheatstone bridge. Through one of them, pure carrier gas always flows through and through the other, the effluents of the column passes. The two platinum wires are heated electrically and hence



Flow diagram of a commercially available TCD cell. In the left diagram, the switching flow causes the column effluent to pass through the filament channel; when the switching flow changes (right diagram), the column effluent will pass through the empty channel. During this time the filament channel fills with the switching gas, and reference measurements are made. Switching between the column effluent and reference gas occurs every 100 milliseconds.

assume equilibrium conditions of

temperature and electrical resistance. When pure carrier gas passes through both of them, there is no difference in temperature or resistance and hence a baseline is recorded. When a component emerges from the column, it alters the thermal conductivity and resistance of the wire. Hence this produces a difference in resistance and so conductivity between two wires, which is amplified and recorded as a signal.

Advantages of katharometer

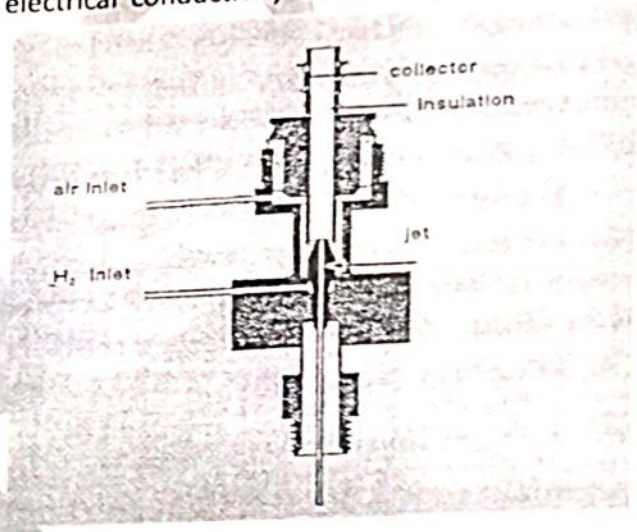
1. Applicable to most compounds
2. Linearity is good
3. The sample is not destroyed & hence used in preparative scale.
4. Simple, easy to maintain and inexpensive.

Disadvantages of Katharometer:

1. Low sensitivity
2. Affected by fluctuations in temperature and flow rate.
3. The response is only relative and not absolute.
4. Biological samples cannot be analysed.

Flam Ionisation detector (FID):

The ionisation detectors are based upon the electrical conductivity of carrier gases. At normal temperature and pressure, gases act as insulators, but become conductive if ions are present.



The carrier gas used with this type of detector can be hydrogen. If the carrier gas is either nitrogen or argon, it can be mixed with hydrogen and reach the burner tip made up of platinum capillary, which acts as one electrode (cathode). The anode is silver gauze placed little above the burner tip. When pure carrier gas alone passes, there is no ionisation and no current flows. When a component emerges from the column, number of ions is produced because a potential difference and causes a flow of current which is amplified and recorded as signal.

Advantages:

1. This detector is extremely sensitive and background noise is low. Hence µg quantities of the solute can be detected.
2. Stable and insensitive to small changes in the flow rate of carrier gas and water vapour.
3. Responds to most of the organic compounds.
4. Linearity is excellent.

Recorders and Integrations:

Recorders are used to record the responses obtained from detectors after amplification, if necessary. They record the baseline and all the peaks obtained, with respect to time. Retention time for all the peaks can be found out from such recordings, but the area of individual peaks cannot be known.

Integrators: Integrators are improved version of recorders with some date processing capabilities. They can record the individual peaks with retention time, height and width of peaks, peak area, percentage of area, etc. Integrators provide more information on peaks than recorders.

4. Size exclusion or gel permeation chromatography

In this type of chromatography, a mixture of components with different molecular sizes is separated by using gels. The gel used acts as molecular sieve and hence a mixture of substances with different molecular sizes is separated. Soft gels like dextran, agrarose or polyacrylamide are used. Semi rigid gels like polystyrene, alkyl dextran in non aqueous medium are also used. The mechanism of separation is by steric and diffusion effects.

5. Affinity chromatography

Affinity chromatography uses the affinity of the sample with specific stationary phases. This technique is used in the field of Biotechnology, Microbiology, Biochemistry, etc.

6. Chiral phase chromatography

Separation of optical isomers can be done by using chiral stationary phases. Different principles operate for different types of stationary phases and for different samples. The stationary phases used for this type of chromatography are mostly chemically bonded silica gel.

C. Based on elution technique

Isocratic separation: In this technique, a mobile phase combination of lower polarity or elution strength is used followed by gradually increasing the polarity or elution strength.

Gradient separation: In this technique, a mobile phase combination of lower polarity or elution is used followed by gradually increasing the polarity or elution strength.

D. Based on the scale of operation

Analytical HPLC: Where only analysis of the samples are done. Recovery of the samples for resulting is normally not done, since the sample used is very low. eg. µg quantities.

Preparative HPLC: Where the individual fractions of pure compounds can be collected using fraction collector. The collected samples are reused. eg. Separation of few grams of mixtures by HPLC.

E. Based on the type of analysis

Qualitative analysis: Which is used to identify the compound, detect the presence of impurities, to find out the number of components, etc. This is done by using retention time values.

Quantitative analysis: This is done to determine the quantity of the individual or several components in a mixture. This is done by comparing the peak area of the standard and sample.

Principle of separation in HPLC:-

The principle of separation in normal phase mode and reverse phase mode in adsorption. Where a mixture of components are introduced in to a HPLC column, they travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent, travels slower. 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.

INSTRUMENTATIONAL REQUIREMENTS

1. Pumps-solvent delivery system
2. Mixing unit, gradient controller and solvent degassing
3. Injector-Manual or auto injectors
4. Guard column
5. Analytical column
6. Detectors
7. Recorders and integrators.