

INTRODUCTION

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. Therefore **all the discussions in this chapter** refers to **GLC** technique **only**.

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 has 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

Two important criteria are

1. **Volatility**: Unless a compound is volatile, it cannot be mixed with mobile phase. Hence volatility is important.
2. **Thermostability**: 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 a higher temperature. At that temperature, the compounds have to be thermostable. 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

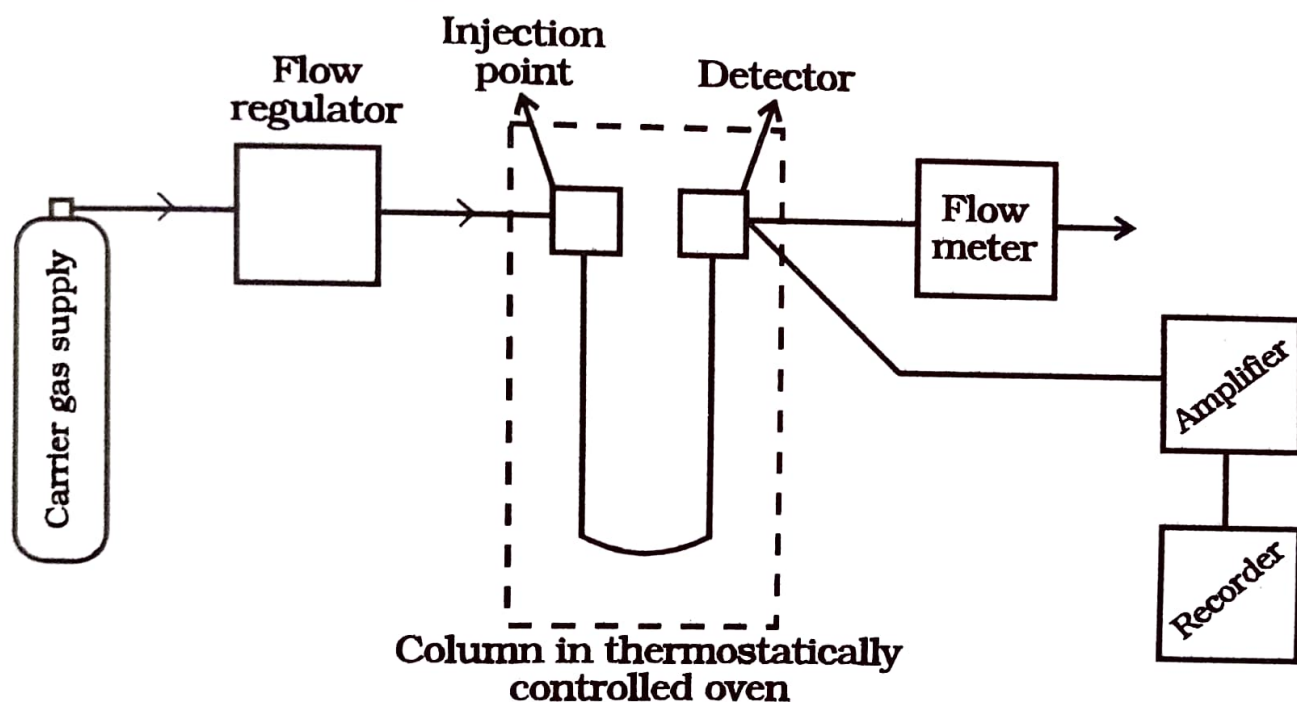


Fig 17.1. Gas chromatographic apparatus

1. 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.

3. INJECTION DEVICES

Samples for introducing into the column can be of any type i.e., either gas, liquid or solid in nature.

Gases can be introduced into the column by valve devices.

Liquids can be injected through loop or septum devices. Most GC instruments have a high quality rubber septum through which sample solution is injected. The rubber is made up of good quality silicone rubber, which can withstand high temperature of preheating device and withstand repeated injections over a period of time.

Solid samples are dissolved in a suitable solvent and then they are injected through a septum.

4. COLUMNS

Column is one of the important part 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 of fragility. But some samples react with them. Hence in such cases, glass columns are used. eg. Steroids. 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 as well as its use.

6. Detectors

Detectors are the most important part of gas chromatographic instruments. They are considered as heart of the apparatus. A detector

uses some property by which it can detect the difference between a pure carrier gas and a eluted component.

The requirements of an ideal detector are:

- i. Applicability to wide range of samples.
- ii. High Sensitivity to even small concentrations.
- iii. Rapidity of response.
- iv. Linearity: i.e. less response to low concentration and proportional response to high concentration.
- v. Response should be unaffected by temperature, flow rate or characteristics of carrier gases.
- vi. Non destructive to the sample in case of preparative work.
- vii. Simple and easy to maintain.
- viii. Inexpensive.

The different detectors used commonly are

- a. Katharometer or Thermal Conductivity Detector (TCD)
- b. Flame Ionisation Detector (FID)
- c. Argon Ionisation Detector (AID)
- d. Electron Capture Detector (ECD)

7. Recorders and Integrators

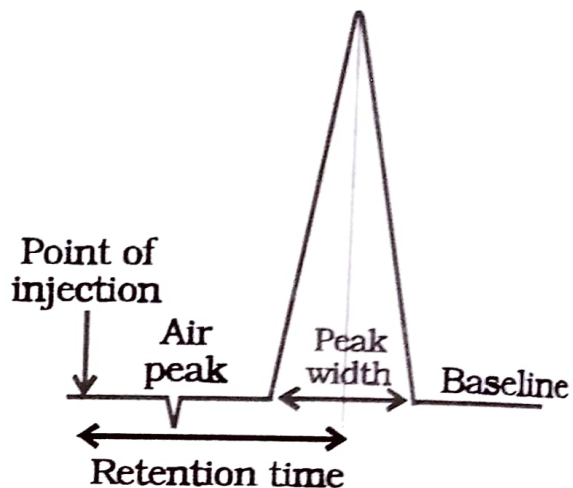
Recorders are used to record the responses obtained from detectors after amplification, if necessary. They record the baseline and all the peaks obtained, with respect to time. Retention time for all the peaks can be found out from such recordings, but the area of individual peaks cannot be known.

Integrators: Integrators are improved version of recorders with some data 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.

PARAMETERS USED IN GAS CHROMATOGRAPHY

Retention time (R_t)

Retention time is the difference in time between the **point of injection** and appearance of **peak maxima**. Retention time is the time required for 50% of a component to be eluted from a column. Retention time is measured in minutes or seconds. Retention time is also proportional to the distance moved on a chart paper, which can be measured in cm or mm.



Retention volume (V_r)

Retention volume is the volume of carrier gas required to elute 50% of the component from the column. It is the product of retention time and flow rate.

$$\text{Retention volume} = \text{Retention time} \times \text{flow rate}$$

APPLICATIONS OF GAS CHROMATOGRAPHY

1. **Qualitative analysis:** It is nothing but identification of a compound. This is done by **comparing** the **retention time** of the **sample** as well as the **standard**. Under identical conditions, the retention time of the standard and the sample are same. If there is a deviation, then they are not the same compound.
2. **Checking the purity of a compound:** By comparing the chromatogram of the standard and that of the sample, the purity of the compound can be reported. If additional peaks are obtained, impurities are present and hence the compound is not pure. From the percentage area of the peaks obtained, the percentage purity can also be reported.
3. **Presence of impurities:** This can be seen by the presence of additional peaks when compared with a standard or reference material. The percentage of impurities may also be calculated from peak areas.
4. **Quantitative analysis:** The quantity of a component can be determined by several methods like

a. Direct comparison method

5. Multicomponent analysis or Determination of mixture of drugs:

Similar to the quantification of a single drug, multicomponent analysis can also be done easily. The quantity of each component is determined by using any one of the above methods. Marketed formulations are available which contain several drugs and each component can be determined quantitatively.

6. Isolation and identification of drugs or metabolites in urine, plasma, serum etc can be carried out.

7. Isolation and identification of mixture of components like amino acids, plant extracts, volatile oils, etc.