

# Gas Chromatography

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In gas chromatography, the sample is vaporized on to head of chromatographic column possessing stationary phase and then eluted by the flow of an inert gaseous mobile phase. In contrast to most other types of chromatography, the mobile phase does not interact with molecules of analyte it only transport the analyte molecule through the column. Two types of gas chromatography are encountered: gas solid chromatography (GSC) and gas liquid chromatography (GLC). Gas solid chromatography uses a solid stationary phase on which retention of analytes is the consequence of physical adsorption. Gas liquid chromatography is based upon the partition of the analyte between a gaseous mobile phase and liquid phase immobilized on the surface of an inter solid.

Gas liquid chromatography is most common; in which mobile phase is inert gas commonly called carrier gas. The stationary phase is high boiling liquid present as a coating on granular particles (support) packed in a column. Here, a component of the sample volatilize and carried to the column by the carrier gas and transported through down the column at different rates depending upon the physicochemical properties of the substance to be gas chromatographed, liquid substrate solid support and the operating conditions of GC apparatus. When a component of the sample finally reaches the end of column, it passes through a differential detector and so recorded as peak on a chart paper. The position

of the peak on the chromatogram is characteristic of the component and is measured in terms of its **retention** on the column. The term most frequently used to describe retention are **retention time** and **retention volume**.

Retention time ( $t_R$ ) is defined as the time required by an average molecule of component to pass from the injection point through the column to the detector. It may be determined from a chromatogram by measuring the distance from the point of injection A to peak maximum C (Fig 20.1) and multiplying this value by reciprocal of the records speed.

Retention volume ( $v_R$ ) is defined as the volume of carrier gas necessary to carry an average molecule of the component from the point of the retention time by the gas flow rate of carrier.

Due to prevalence of many operating variables of GC apparatus which influences the retention data, most substances are best identified by determining relative retention ( $\alpha$ ) which is defined by the equation.

$$\alpha = \frac{x_2 - x_a}{x_1 - x_2}$$

$x_1$ ,  $x_2$  and  $x_a$  represent the retention from the point of injection measured in distance on the chromatogram to the peak maximums

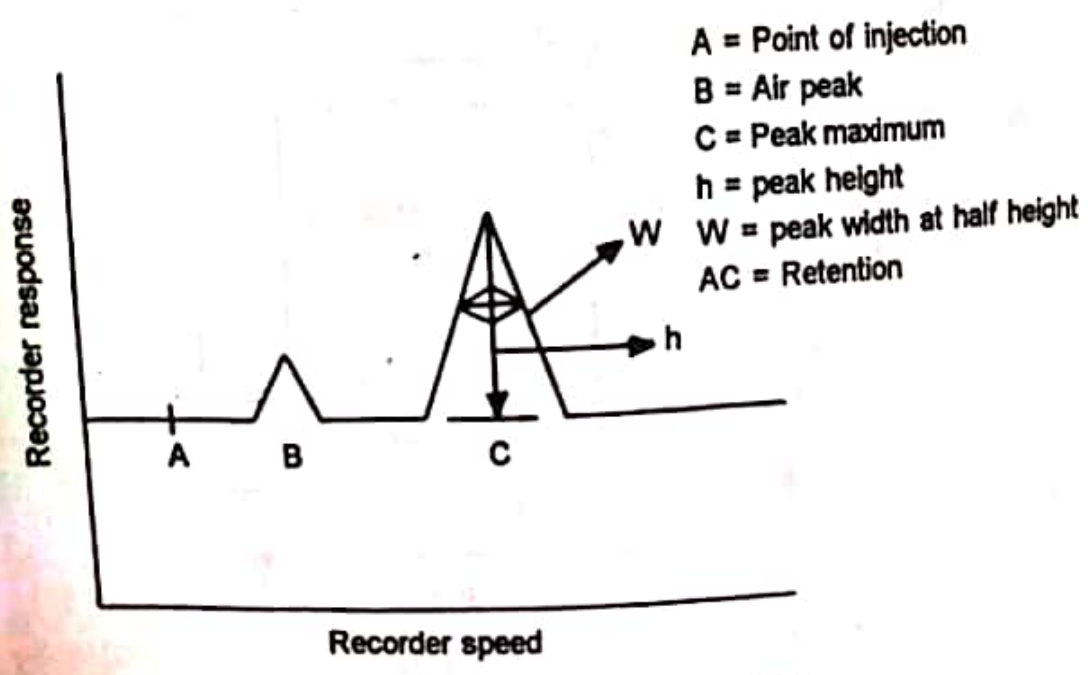


Fig. 20.1 Gas chromatogram

of the test drug standard such as n-pentane and inert compound such as air respectively

## Instrumentation

It consist of following components :

### 1. Carrier Gas Supply

Carrier gases must be chemically inert. These include helium, argon, nitrogen, carbon dioxide and hydrogen gases. The choice of carrier gas is often dependent upon the type of detector which is used. Associated with the gas supply are pressure regulators, gauzes and flow meters. In addition, the carrier gas system often contains a molecular sieve to remove water or other impurities. The flow rate is generally controlled by a two stages pressure regulators fixed at the gas cylinder. Inlet pressures of gas of usually ranges from 10 to 50 psi which give the flow rate of 25 to 150 ml/min with packed columns and 1 to 25ml/min for open tubular capillary columns. Flow rate can be controlled by a rotameter or soap bubble meter at column head.

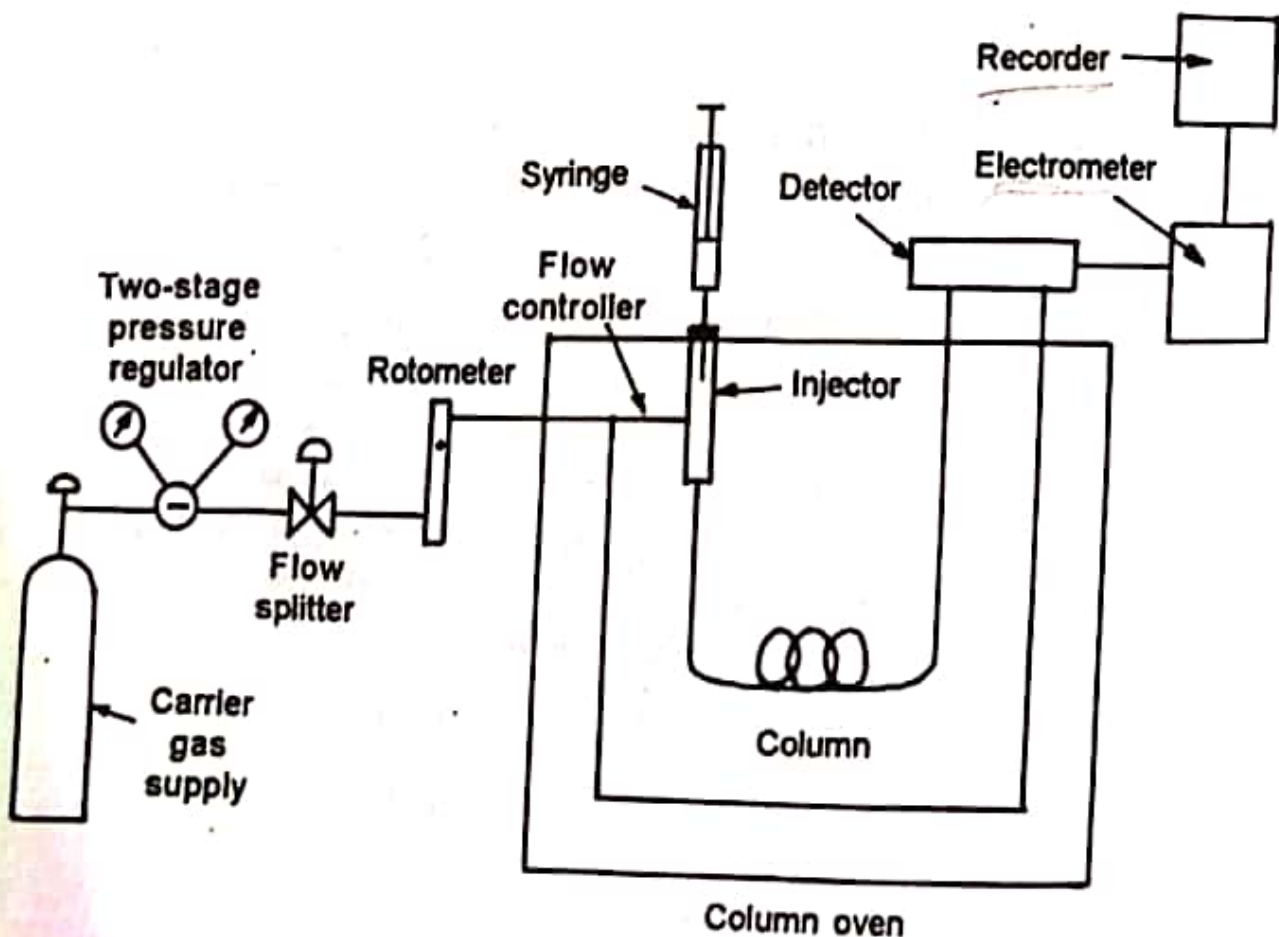


Fig. 20.2. Schematic diagram of instrumentation for gas chromatography.

## 2. Sample injection system

To increase the column efficiency, suitable size of sample should be selected and introduced as a 'plug' of vapour. The slow injection of over sized samples causes band broadening and poor resolution of various components of a mixture. The most common method of sample injection involves the use of a micro syringe to inject a liquid or gaseous sample through a silicone rubber diaphragm or septum into a flash vaporizer port located at the head of column (Fig. 20.3) the sample port is ordinarily about 50°C above the boiling point of least volatile component of the sample for ordinary analytical column. The sample size vary from a few tenth of microliter to 20 mL in packed column. Capillary column require much smaller samples (~ 10<sup>-3</sup> µL).

## 3. Analytical Column

Two general types of column are encountered in gas chromatography.

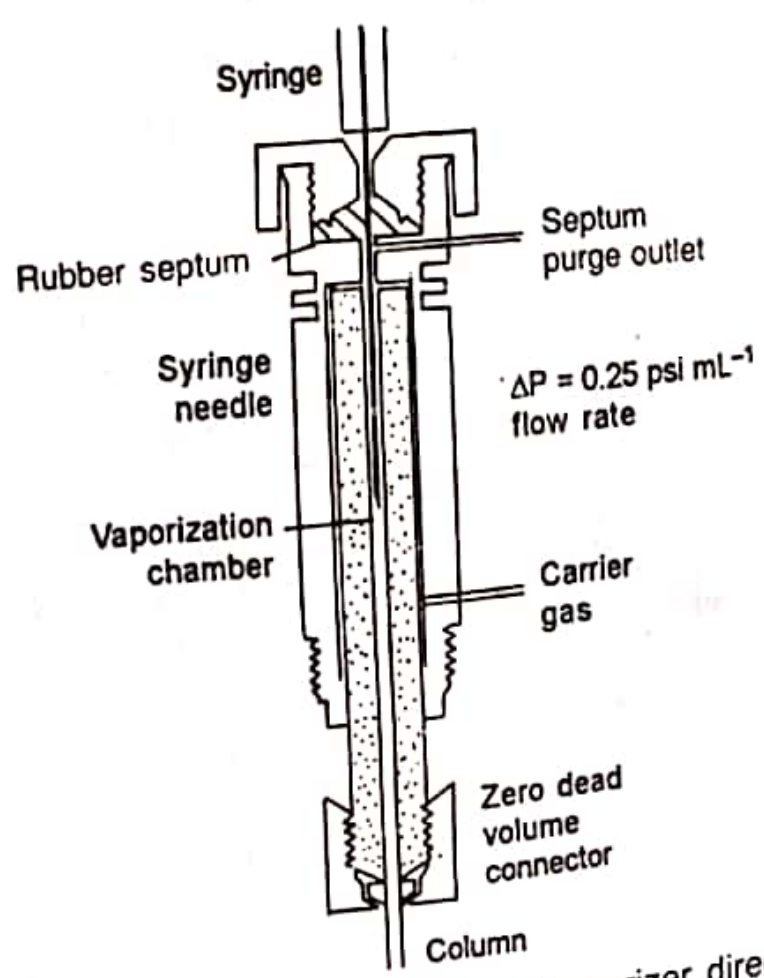


Diagram of vaporizer direct injector.

- (i) Packed column
- (ii) Open tubular or capillary column

To date, the vast majority of gas chromatographic analysis has been carried out on the packed column currently. However, this situation is changing rapidly, and suggesting the replacement of packed column by the more efficient open tubular column.

Chromatography columns vary in length from less than 2 m to 50 m or more. They are constructed of stainless steel, glass, fused silica or Teflon. In order to fit into an oven for thermostating, they are usually framed as coils having diameters of 2–4 mm.

### **Column temperature**

Column temperature is important variable that must be controlled to within tenth of a degree for precise work. Hence, the column is ordinarily housed in a thermostated oven. The optimum column temperature depends upon the boiling point of sample and degree of separation required. Roughly, a temperature equal to or slightly above the average boiling point of a sample results in a reasonable elution time (2 to 30 min). For samples with a broad boiling range, it is often desirable to use **temperature programming** in which the column temperature is increased continuously in regular fashion or in stepwise as the separation proceeds (Fig. 20.4)

In general, optimum resolution is achieved with the minimum temperature but there is an increase in elution time and therefore the time required to complete an analysis at the low temperature.

### **Packed columns**

Column tube, made of stainless steel, copper, aluminium or teflon are densely packed with a uniform, finely divided packing material (usually diatomaceous earth) or solid material (fire bricks). These materials are coated with a thin layer of (0.05 to 1  $\mu\text{m}$ ) of the stationary phase. The efficiency of a gas chromatographic column increases rapidly with decreasing particle diameter of the packing. The pressure difference required to maintain a given flow rate of carrier gas however varies inversely as the square of the particle diameter. This relationship has restricted the use of small sized

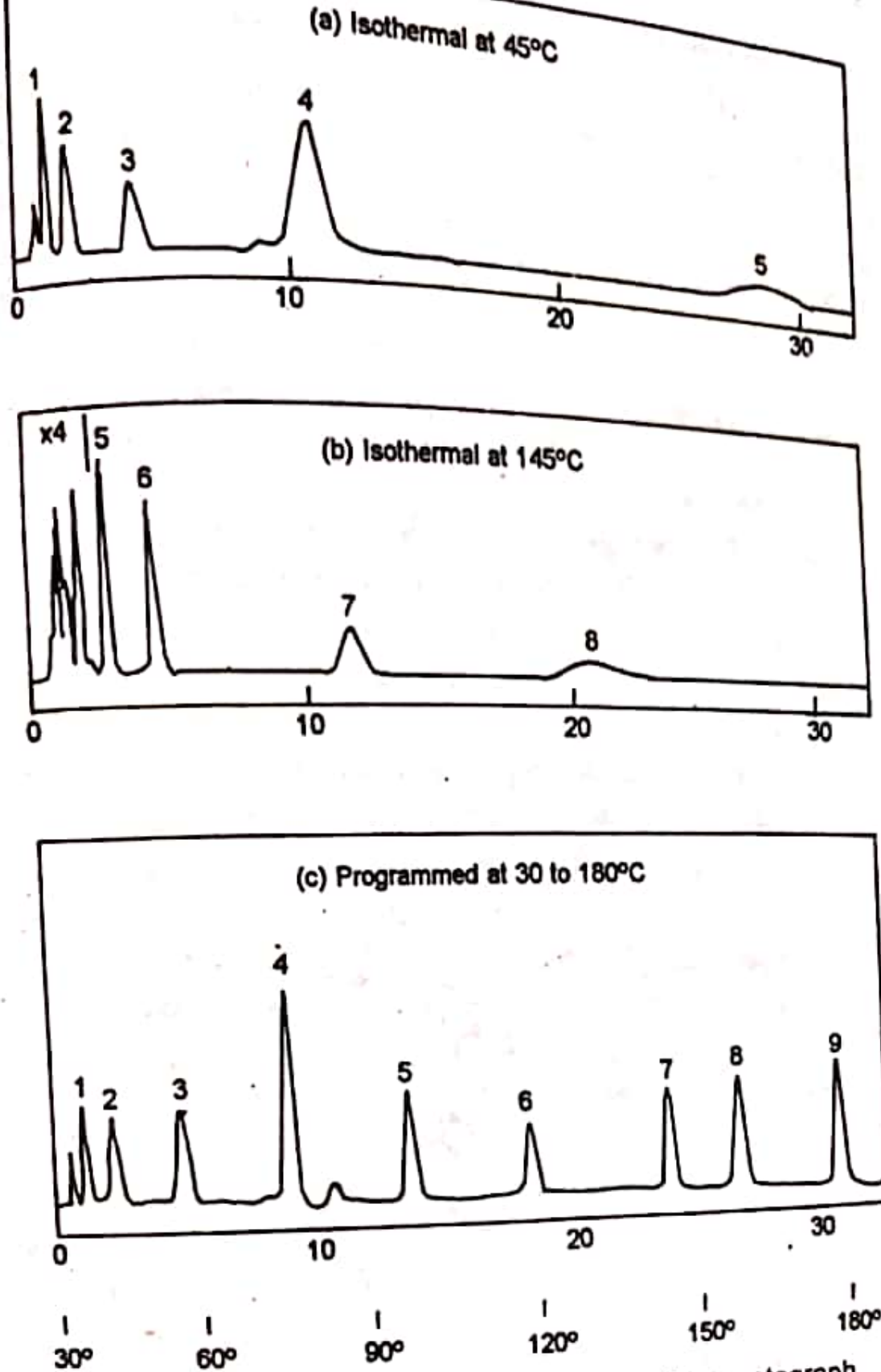


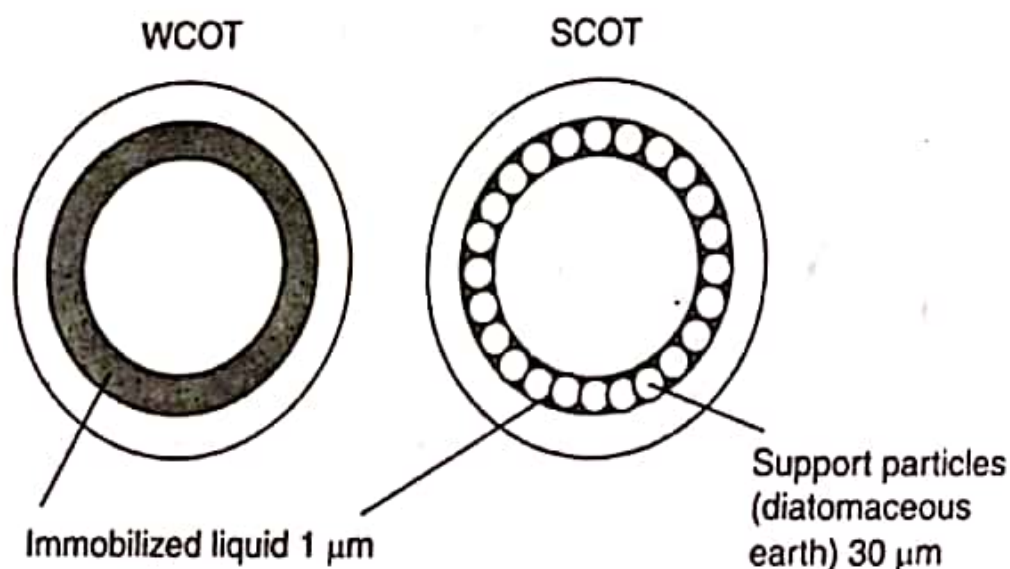
Fig. 20.4. Effect of temperature on Gas Chromatograph.

particles employed in gas chromatography because it is not convenient to use pressure difference that are greater than about 50 psi. As a result, the usual support particles are 60 to 80 mesh (250 to 170  $\mu\text{m}$ ) or 80 to 100 mesh (170 to 149  $\mu\text{m}$ ). The packed column is best for large scale but slow and inefficient.

### Open tubular column

Due to relatively low viscosity of the mobile phase in GC the contribution of solute diffusion to band broadening can be substantial. In an effort to reduce the volume with in which solvent can diffuse, narrow diameter column are used frequently. A small increase in efficiency has been achieved by using a tube of 1 mm diameter: Greater increase in efficiency can be increased with the capillary column. These are tubes of glass or highly purified fused silica with internal diameter of 0.2 to 0.75 mm. The liquid phase is kept within this column in either two ways. Accordingly, these are classified as:

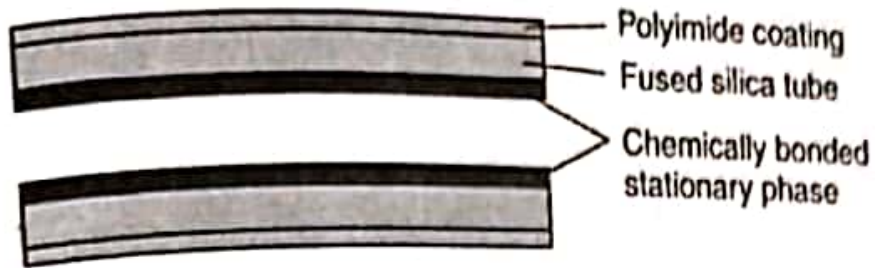
- (i) *Wall coated open tubular (WCOT) columns* : In this, the stationary phase is deposited as an extremely thin layer directly on the inner surface of the tube. This may be either as a film or more frequently by bonding chemically to the wall of the capillary column. The latter method is advantageous since it prevents bleeding or loss of the liquid phase due to its volatility at elevated temperature.



- (ii) *Support coated open tubular (SCOT) columns* : In this inner surface of the tube is lined with a layer of inert support such as diatomaceous earth in which stationary phase is coated. Because of irregularity of the support particles, the surface area of the SCOT column is larger and more stationary phase, therefore is available to interact with the analyte. However, the mechanics of packing, this column

are difficult and they are not used as often as WCOT type due to less efficiency.

Recently, a new type of WCOT column was devised - the *Fused Silica Open Tubular (FSOT)* column.



Cross-section of a fused silica open tubular column

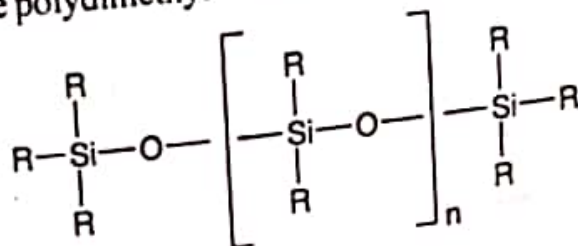
These have much thinner walls than the glass capillary columns, and are given strength by the polyimide coating. These columns are flexible and can be wound into coils. They have the advantages of physical strength, flexibility and low reactivity.

#### 4. Stationary phase

The desirable properties for the immobilized liquid phase in gas liquid chromatography column include.

- (i) Low volatility (ideally boiling point of the liquid should be at least 100°C higher than the maximum operating temperature for the column).
- (ii) Thermal stability
- (iii) Chemical inertness
- (iv) Solvent characteristics (suitable for partition coefficient for separation)

Table 20.1 lists the most widely used stationary phases for both packed and open tubular gas chromatography column in order of increasing polarity. Five of the stationary phases mentioned in Table 20.1 are polydimethyl siloxanes having the general structure.



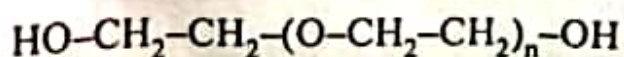


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Table 20.1. Some common stationary phases for gas – liquid chromatography

Stationary phase	Common trade name	Maximum temperature °C	Common application
Polydimethyl siloxane	OV-1, SE-30	350	General-purpose nonpolar phase; hydrocarbons; polynuclear aromatics; drugs: steroids; PCBs
Poly (Phenylmethyl-dimethyl) siloxane (10% phenyl)	OV-3, SE-52	350	Fatty acid methyl esters; alkaloids; drugs; halogenated compounds
Poly (phenylmethyl) siloxane (5% phenyl)	OV-17	250	Drugs; steroids; pesticides; glycols
Poly (Trifluoropropyl-dimethyl) siloxane	OV-210	200	Chlorinated; aromatics; nitroaromatics; alkyl-substituted benzenes
Polyethylene glycol	Carbowax 20M	250	Free acids; alcohols; ether, essential oils; glycols
Poly (dicyanoallyl dimethyl) siloxane	OV-275	240	Polyunsaturated fatty acids; rosin acids; free acids; alcohols

In the first of these polydimethyl siloxane, the methyl groups are present in place of R groups giving a liquid that is relatively non polar. In other polysiloxanes, shown in Table 20.1, fraction of methyl groups is replaced by functional groups such as phenyl ( $-C_6H_5$ ), cyano propyl ( $-C_6H_5CN$ ) and trifluoropropyl ( $-C_3H_6CF_3$ ). These substitution increase the polarity of the liquids to various degrees. The fifth entry in Table 20.1 is a polyethylene glycol having the structure.

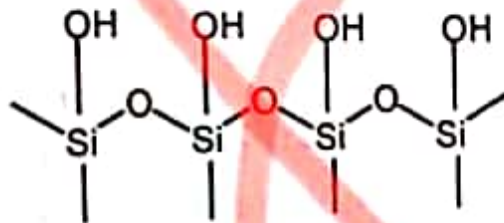


It is widely used for separating polar species.

Non-polar stationary phases are for non-polar analytes. They are used preferentially. Polar stationary phase are best for polar analytes, they retain preferentially in the column.

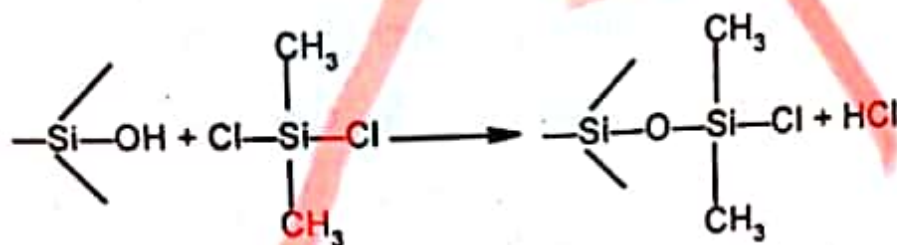
### Adsorption on column packings or capillary walls

A problem that has decreased the efficiency of gas chromatography from its inception is the physical adsorption of polar or polarizable analyte species, such as alcohols or aromatic hydrocarbons, on the silicate surfaces of column packings or capillary walls. Adsorption results in distorted peaks, which are broadened and often exhibit a tail. It has been established that adsorption is the consequence of silanol groups that form on the surface of silicates by reaction with moisture on the surface of silicates. Thus a fully hydrolyzed silicate surface has the structure.

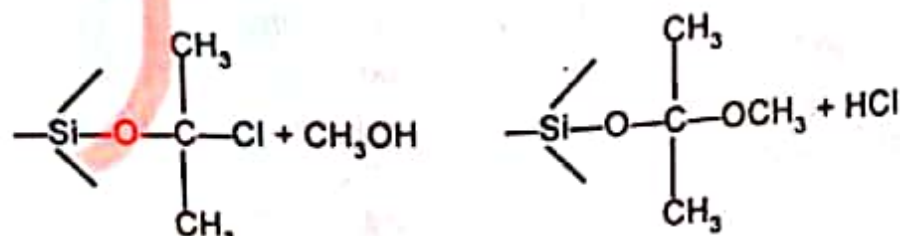


The SiOH groups on the support surface possess a strong affinity for polar organic molecules and tend to retain them by adsorption.

Support materials can be deactivated by silylation with dimethylchlorosilane (DMCS). The reaction is represented as:



Upon washing with alcohol, the second chloride is replaced by a methoxy group that is :



Salinized surfaces of column packings may still show a residual adsorption, which apparently arises from metal oxide impurities in the diatomaceous earth. Acid washing prior to salinization removes these impurities. Fused silica that is used for manufacturing open tubular columns is largely free of this type of impurity. As a consequence, fewer problems with adsorption are encountered with fused silica columns.

### ***The use of solvent as stationary phase***

A number of solvents have been proposed as stationary phases in the course of the development of gas liquid chromatography but only a dozen or less are sufficient for most of the applications. The proper choice among these solvents is often critical to the success of a separation. Qualitative guidelines exist for making this choice, but in the end, the best stationary phase can only be determined in the laboratory.

The retention time for a solute on a column depends upon its partition ratio which in turn is related to the chemical nature of the stationary phase. Clearly, to be useful in gas-liquid chromatography, the selection of immobilized liquid to be used as stationary phase depends on its ability to generate different partition coefficients for different solutes. In addition, however, these ratios must not be extremely large or extremely small, because the former leads to prohibitively long retention times and the latter results in short retention causing incomplete separation.

### ***Bonded and crossed linked stationary phases***

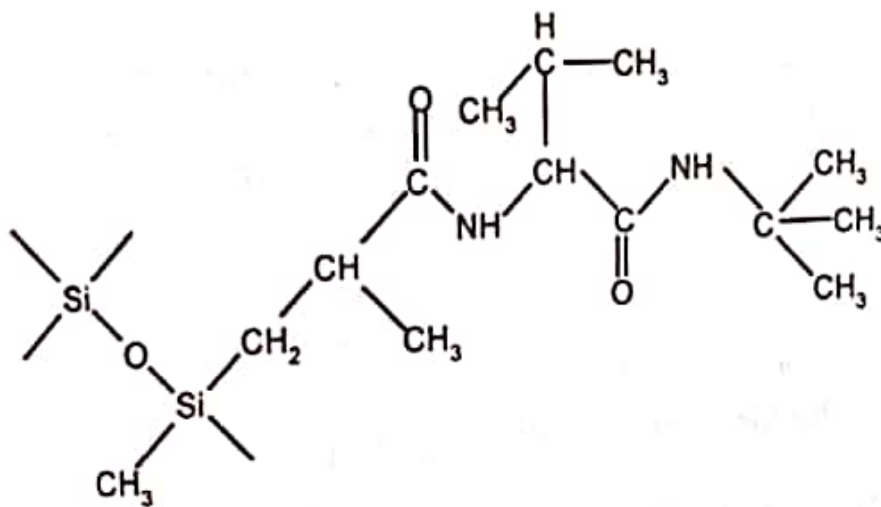
Bonded involves attaching a monomolecular layer of stationary phase to the silica surface of column by a chemical reaction. For commercial column, the nature of reaction is ordinarily proprietary.

Cross linking is carried out in situ after a column is coated with one of the polymer listed in Table 20.1. One way of cross linking is to incorporate a peroxide into original liquid. When a film is heated, reaction between the methyl group in polymer chain is initiated by a free radical mechanism. The polymer molecules are then cross linked through carbon to carbon bond. The resulting films are less

extractable and have considerably greater chemical stability than do untreated films. Cross linking has also been initiated by enclosing the coating film to gamma radiation.

### Chiral stationary phases

Enantiomers can be separated by making two approaches. In the first, derivatives of analyte with an optically active reagent are formed. It results in production of a pair of diester isomers that can be separated on a chiral column. The alternative (second) method is to use a chiral liquid as the stationary phase. A number of amino acid derived chiral phases have been developed for the purpose. The structure of one of these liquid that has been used for separation of optically active amino acids is presented here.



### Film thickness

Commercial columns are available having stationary phases that vary from 0.1 to 5  $\mu\text{m}$ . Film thickness primarily affect the retentive character and the capacity of column.

### 5. Detectors

There are number of detectors used during the development of gas chromatography but here, the most widely used are described here. Sometimes, gas chromatographs are coupled with either mass spectrometers or infrared spectrophotometers. Here, the spectral device serves not only to detect the appearance of analytes at the end of the column but also provides information about their identity.

**Characteristics of the ideal detector**

The ideal detector for gas chromatography has the following characteristics:

- (1) **Adequate sensitivity:** The sensitivities of the detectors we are about to describe differ by a factor of  $10^7$ . Yet all are widely used and are clearly adequate for certain tasks; the least sensitive are not, however, satisfactory for certain applications. In general, the sensitivities of modern detectors lie in the range of  $10^{-8}$  to  $10^{-15}$  g analyte.
- (2) **Good stability and reproducibility.**
- (3) A linear response to analytes that extends over several orders of magnitude.
- (4) A temperature range from room temperature to at least  $400^\circ\text{C}$ .
- (5) A short response time that is independent of flow rate.
- (6) **High reliability and ease to use :** To the extent possible, the detector should be foolproof in the hands of inexperienced operators.
- (7) Similarly in response towards all analytes or alternatively a highly predictable and selective response towards one or more classes of analytes.
- (8) Nondestruction of the sample.

**Flame ionization detector**

The flame ionization detector (FID) is one of the most widely used detectors in gas chromatography. It is used with a burner such as that shown in Fig. 20.5. The effluent from the column is mixed with hydrogen and air and then ignited electrically. Most organic compounds, when pyrolyzed at the temperature of a hydrogen/air flame, produce ions and electrons that can conduct electricity through the flame. A potential of a few hundred volts is applied across the burner tip and a collector electrode located above the flame. The resulting current ( $\sim 10^{-12}\text{A}$ ) is amplified for the measurement.

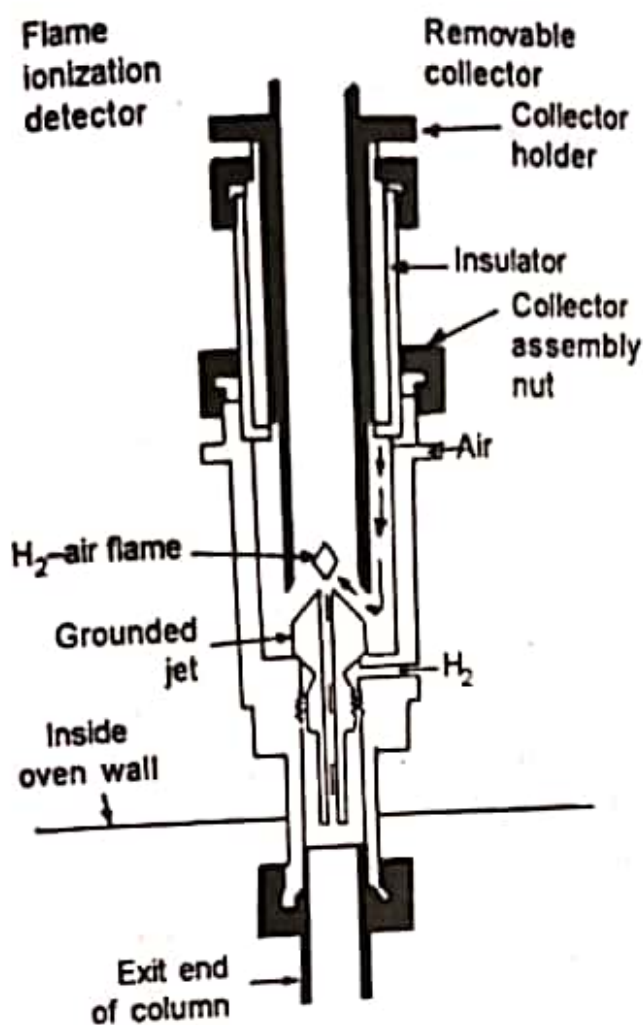


Fig. 20.5. Diagram of flame ionization detector.

understood process, although it is observed that the number of ions produced is roughly proportional to the number of reduced carbon atoms in the flame. Because the flame ionization detector responds to the number of carbon atoms entering the detector per unit of time, it is a mass sensitive, rather than a concentration sensitive device. As a consequence, this detector has the advantage that changes in flow rate of the mobile phase have little effect on detector response.

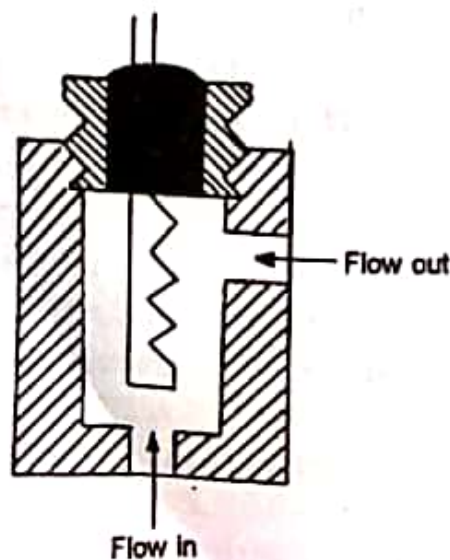
Functional groups, such as carbonyl, alcohol, halogen, and amine, yield fewer ions or none at all in a flame. This makes the FID unsuitable. In addition, the detector is insensitive toward noncombustible gases such as  $H_2O$ ,  $CO_2$ ,  $SO_2$  and  $NO_2$ . These properties make the flame ionization detector a most useful general detector for the analysis of most organic samples, including those that are contaminated with water and the oxides of nitrogen and

The flame ionization detector exhibits a high sensitivity ( $\sim 10^{-13}$  g/s), large linear response range ( $\sim 10^7$ ), and low noise. It is generally rugged and easy to use. It causes destruction of sample which is main drawbacks.

### ***Thermal conductivity detector (TCD)***

Its application is based upon changes in the thermal conductivity of the gas stream due to the presence of analyte molecules. This device is sometimes called a katharometer. The sensing element of a katharometer is an electrically heated element whose temperature at constant electrical power depends upon the thermal conductivity of the surrounding gas. The heated element may be a fine platinum, gold, or tungsten wire or, alternatively, a semiconducting thermistor. The resistance of the wire or thermistor gives a measure of the thermal conductivity of the gas; in contrast to the wire detector, the thermistor has a negative temperature coefficient. Fig. 20.6 is a cross sectional view of the temperature sensitive elements in a thermal conductivity detector system.

The Fig. 20.6 shows the arrangement of detector elements in a typical detector unit. Two pairs of elements are employed, one pair being located in the flow of the effluent from the column and the other in the gas stream ahead of the sample injection chamber. (These elements are labeled "Sample" and "Reference" in Fig.



**Fig 20.6.** Thermoconductivity detector cell.

### ***Thermionic detector or nitrogen phosphorus detector***

The thermionic detector (TID) is selective towards organic compounds containing phosphorus and nitrogen atoms. Its response to a phosphorus atom is approximately 10 times greater than to a nitrogen atom and  $10^4$  and  $10^6$  larger than a carbon atom. Compared with the flame ionization detector, the thermionic detector is approximately 500 times more sensitive for compounds containing phosphorus and 50 times more sensitive for nitrogen atom bearing species in comparison to FID. Due to this fact, the thermionic detector is particularly useful for detecting and determining the many pesticides that contain phosphorus atom.

A thermionic detector is similar in structure to the flame detector shown in Fig. 20.5. The column effluent is mixed with hydrogen, passes through the flame tip assembly, and is ignited. The hot gas then flows around an electrically heated rubidium silicate bead, which is maintained at about 180 V with respect to the collector. The heated head forms plasma having a temperature of 600 to 800°C. It produces usually large numbers of ions from phosphorus – or nitrogen resulting in generation of large ion currents results, which are useful for determining compounds containing these two elements.

### ***Electron Capture Detector***

Electron capture Detector (ECD) operates in much the same way as a proportional counter for measurement of X-radiation. Here the effluent from the column passes over a  $\beta$ -emitter, such as nickel-63 or tritium (absorbed on platinum or titanium foil). An electron from the emitter causes ionization of the carrier gas (often nitrogen) and the production of a burst of electrons. In the absence of organic species, a constant standing current between a pair of electrodes results from this ionization process. The current decreases, however, in the presence of those organic molecules that tend to capture electrons. The response is nonlinear unless the potential across the detector is pulsed.

The electron capture detector is selective in its response, being highly sensitive toward molecules that contain electronegative



functional groups such as halogens, peroxides, quinones and nitro groups. It is insensitive towards functional groups such as amines, alcohols, and hydrocarbons. An important application of the electron-capture detector has been for the detection and determination of chlorinated insecticides.

Electrons-capture detectors are highly sensitive and possess the advantage of not alluring the sample significantly (in contrast to the flame detector). On the other hand, their linear response range is usually limited to about two orders of magnitude.

### **Atomic emission detector (AED)**

The newest commercially available gas chromatographic detector is based upon atomic emission. In this device, the eluent is introduced into microwave-energized helium plasma that is coupled to a diode-array optical emission spectrometer. The plasma is sufficiently energetic to atomize all of the elements in a sample and to excite their characteristic atomic emission spectra. These spectra are then observed with a spectrometer that employs a movable, flat diode array capable of detecting emitted radiation from about 170 to 780 nm.

### **Other types of detectors**

The flame photometric detector (FPD) has been widely applied to the analysis of air and water pollutants, pesticides and coal hydrogenation products. It is a selective detector that is primarily responsive to compounds containing sulphur and phosphorus. In this detector, the eluent pressure regulator or flow regulator mounted in the chromatograph.

## **6. Gas chromatograph-mass spectrometer (GC-MS)**

The GC-MS is composed of two major building blocks: the gas chromatograph and the mass spectrometer (Fig. 20.8). The gas chromatograph utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase properties (e.g. 5% phenyl polysiloxane). The difference in the chemical properties between different molecules in a mixture

will separate the molecules as the sample travels the length of the column. The molecules take different period of time (called the retention time) to come out of (elute from) the gas chromatograph, and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass to charge ratio.

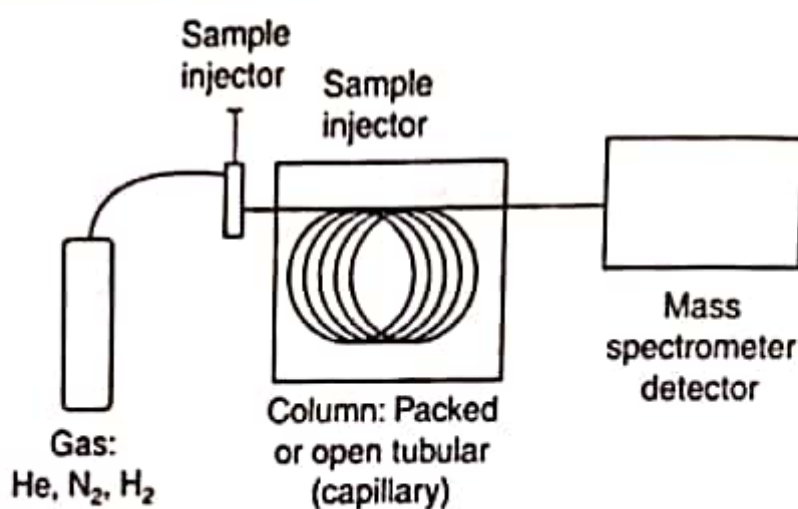


Fig 20.8. Schematic diagram of GC-MS.

These two components, used together, allow a much finer degree of substance identification than either unit used separately. It is not possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone. The mass spectrometry process normally requires a very pure sample while gas chromatography using a traditional detector (e.g. Flame Ionization Detector) detects multiple molecules that happen to take the same amount of time to travel through the column (i.e. have the same retention time) which results in two or more molecules to co-elute. Sometimes two different molecules can also have a similar pattern of ionized fragments in a mass spectrometer (mass spectrum). Combining the two processes makes it extremely unlikely that two different molecules will behave in the same way in both a gas chromatograph and a mass spectrometer. Therefore when an identifying mass spectrum appears at a characteristic retention time in a GC-MS analysis, it typically lends to increased certainty that the analyte of interest is in the sample.

Table 20.2. Summary of common GC detectors

Detector	Type	Support gases	Selectivity	Detectability	Dynamic range
Flame ionization (FID)	Mass flow	Hydrogen and air	Most organic compounds	100 pg	$10^7$
Thermal conductivity (TCD)	Concentration	Reference	Universal	1 ng	$10^7$
Electron capture (ECD)	Concentration	Make-up	Halides, nitrates, nitriles, peroxides, anhydrides, organometallics	50 pg	$10^5$
Nitrogen-phosphorus	Mass flow	Hydrogen and air	Nitrogen, phosphorus	10 pg	$10^6$
Flame photometric (FPD)	Mass flow	Hydrogen and air possibly oxygen	Sulphur, phosphorus, tin, boron, arsenic, germanium, selenium, chromium	100 pg	$10^3$
Photo-ionization (PID)	Concentration	Make-up	Aliphatics, aromatics, ketones, esters, aldehydes, amines, heterocyclics, organo-sulphurs, some organometallics	2 pg	$10^7$
Electrolytic conductivity	Mass flow	Hydrogen, oxygen	Halide, nitrogen, nitrosamine, sulphur		

## 7. Applications of Gas Chromatography

It is a process, used for: (i) Separation, (ii) Qualitative analysis, (iii) Quantitative analysis.

The separation and identification of compound are possible when quantity of sample is very small.

### *Qualitative analysis*

The retention volume/retention time is characteristics of particular component in a mixture under specified condition. For example, various components of volatile oil can be identified and compared with the standard sample of individual component.

### *Purity of sample*

The standard sample and test sample are compared in gas chromatograph. The additional peak in gas chromatogram will indicate the presence of impurity in the test sample.

### *Quantitative analysis*

With the help of peak height or peak area, it is possible to perform the quantitative analysis of particular component in the sample. It is generally used for the quantitative analysis of steroids, bile acid, cholesterol, some antibiotics e.g. penicillins, tetracyclines, erythromycin, etc.

It is based on a UV radiation emitted by sulphur compounds, during the burning process in a hydrogen rich flame in the region of 394 nm while phosphorus containing molecules at 526 nm. Photoionization detector can also be used. It is based on ionization of organic and inorganic molecules in UV radiation of sufficient energy. Thus, if the effluent from GC column is driven to an ionization cell with two polarized electrodes (the polarization potential is approximately 200 V) placed under the light of UV lamp. Those substances having an ionization potential lower than UV source energy will undergo ionization and produce an ion current which is detector response. If the UV energy is lower than 10.17 eV but higher than 4.2 eV which is the ionization potential of benzene and the alicyclic and aromatic hydrocarbons, only these compounds will be ionized and detected.