40
Gas Chromatography

40.1 Introduction

Gas chromatography, GC, is probably the most utilized of all the chromatographic techniques. Since its theory was developed and amino acids were separated in the early 1950's, literally thousands of applications have been reported in organic, general, and biochemistry. It has been applied to a wide variety of theoretical and practical problems in the separation and identification of components of the atmosphere, gases liquids, drugs, and commercial products. The primary limitation is that the sample must be capable of being volatilized without undergoing decomposition, Because of this limitation it is now being replaced, to a large extent, by high performance liquid chromatography.

In **gas—liquid chromatography, GLC**, the mobile phase is a gas and the stationary phase is a thin layer of a non-volatile liquid bound to a solid support. A partition process occurs. On the other hand, **gas—solid chromatography, GSC**, utilizes a solid adsorbent as the stationary phase and an adsorption process takes place.

Gas chromatography is quite similar to column chromatography, except that a gas is used as the mobile phase instead of a liquid. Gas–solid chromatography (GSC) is based upon selective adsorption on a solid, whereas gas–liquid chromatography is base upon the partition between the gas and an immobile liquid phase. Gas solid chromatography was developed by O.D. Kohler and K. Thiele in 1943 followed by E. Cremer, T. Janak, H.W. Patton, J.S. Lewise and W.L. Kaye, C.S.G. Phillips contributed to frontal analysis whereas P. Schuftan, N.C. Turner, S. Claesson and N.M. Turkel gave displacement development or the elution technique. The gas–liquid chromatography is more popular than gas–solid chromatography as has many more applications. Gas–liquid chromatography was originated by A.J.P. Martin in 1951 together with A.T. James. It is noteworthy that Martin had theoretically predicted the feasibility of GLC almost a decade earlier while presenting his work on liquid–liquid chromatography (LLC).

In 1952 Martin and Synge were awarded the Nobel Prize in chemistry for their work on the development of partition chromatography.

The main advantages of gas chromatography are given below :

(i) The technique has strong separation power and even complex mixture can be resolved into constituents.

(ii) The sensitivity of the method is quite high. It is a micromethod and only a few mg of the sample is sufficient for analysis.

(iii) It gives good precision and accuracy.

(iv) The analysis is completed in a short time.

(v) The cost of instrument is relatively low and its life is generally long.

(vi) The technique is suitable for routine analysis because the operation of a gas chromatograph and related calculations do not require highly skilled persons.

Gas chromatography is at present one of the most widely used and powerful tools available for analysis. The major factors for this are the speed, resolving power and extreme sensitivity of the

technique. For example, using this technique, it is possible to separate the ten isomers of heptane in less than ten seconds. Detectors are available with detection limits as low as 10^{-12} to 10^{-14} g.

40.2 Principle of Gas Chromatographic Separations

When a gas or vapour comes in contact with an adsorbent, certain amount of it gets adsorbed on the solid surface. The phenomenon takes place according to the well known laws of Freundlich, *i.e.* $x/m=Kc^{1/n}$ or Langmuir, *i.e.*, $x/m=K_1 c/1+K_2 c$ where x is the mass of the gas or vapour sorbed in mass m of the sorbent and c is the vapour concentration in the gas phase and K, K_1 and K_2 are constants. Similarly if the vapour or gas comes in contact with a liquid, a fixed amount of it gas dissolved in the liquid. The phenomenon takes place according to Henry's law of partition, *i.e.*, $x/m=Kc$. Now both the phenomena are selective and there are different K-values for different vapour–sorbent pairs.

The principles of gas chromatography can be explained in terms of the following experiment. A gas that is flowing smoothly at the rate of 3 ft/min down an empty tube that is 6 ft long takes 6/3 = 2 min to flow from one end of the tube to the other (Fig. 40.1, top). If such a tube were filled with sand (Fig. 40.1, bottom), the gas would flow through it more slowly. If the rate at which the gas flows in the sand–filled tube is 2 ft/min, it will take the gas 6/2 = 3 min to traverse the tube. The sand-filled tube in this example has some properties of a gas chromatography column. The gas is the moving or *mobile phase*. The sand is the *stationary phase*. The gas that emerges after it has passed through the column is called the *effluent*. In practice the mobile phase should be relatively insoluble in the stationary phase; otherwise the stationary phase becomes overloaded.

To further our analogy, consider the following property of soluble gases. In a vessel containing non–volatile liquid A we place a soluble gas B (Fig. 40.2).

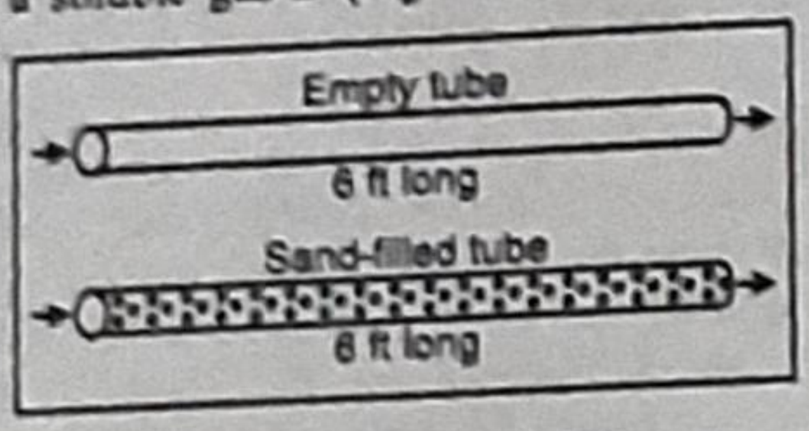


Fig. 40.1 : Flow of Gas.

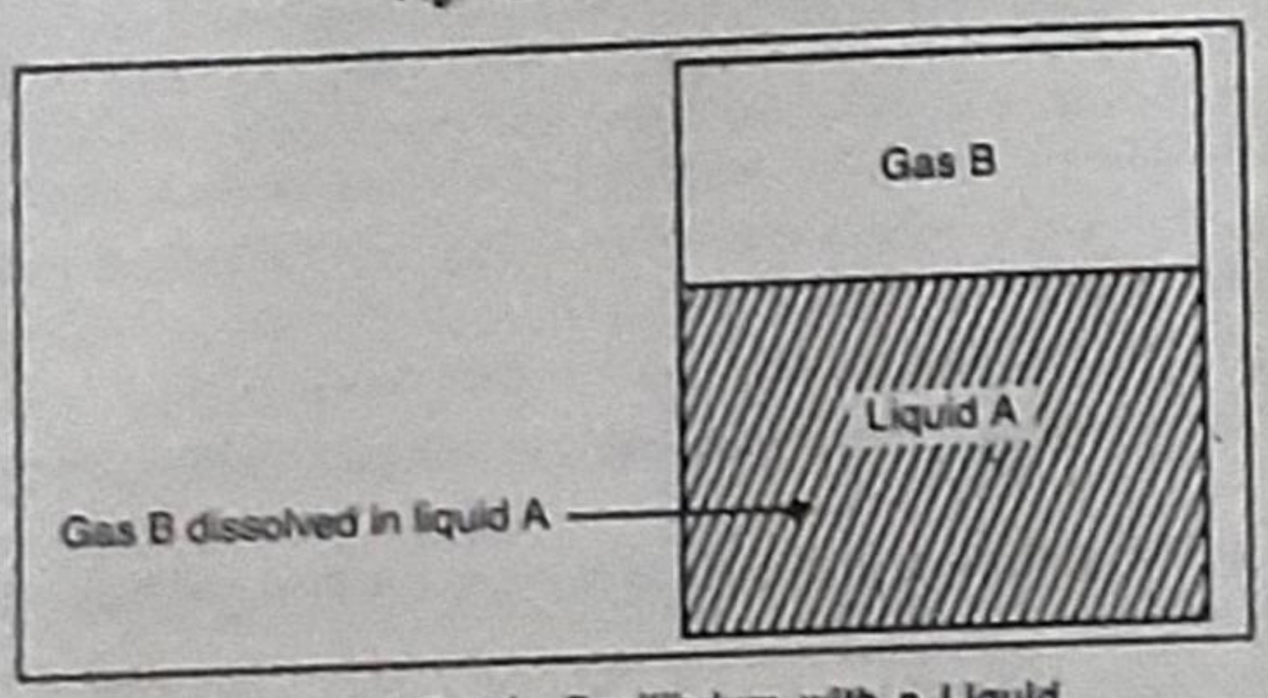


Fig. 40.2 : Gas in Equilibrium with a Liquid.

A short time after the vessel has been sealed, the gas B in the gas phase comes to equilibrium with the gas dissolved in the liquid phases, and the distribution of gas between the two phases remains constant. Although the total number of gas molecules above the liquid and in the liquid stays the same, a rapid interchange takes place between the molecules in the two states; that is, molecules from the gas pass into the liquid at the same rate that dissolved gas molecules leave the liquid and become gas. The molecules are said to be in *dynamic equilibrium*. This is constant if the temperature is kept constant.

It can be shown that, on the average, each molecule of B spends a constant fraction of the time in the liquid phase and the remainder of the time in the gas phase. For the purposes of our discussion, we hypothesize that gas B spends 40% of its time in liquid A and 60% of its time as a gas. Also, the equilibrium will be established more quickly if the thickness of the liquid is small—otherwise, dissolved molecules B wander from the interface and the equilibrium is disturbed.

To proceed with our illustration, we remove the sand from the tube shown in Fig. 40.2, coat it with a thin film of liquid A, and replace the coated sand in the tube. Liquid A is now known as the stationary phase, or substrate and gas B is known as the carrier gas. The sand is the support. Again, we allow gas B to flow down the packed tube. We noted earlier that it took 3 min for gas B to flow through the tube filled with uncoated sand; moreover, we have hypothesized that when this gas is in equilibrium with liquid A, it spends 40% of its time in the liquid. Therefore for 40% of the time gas B spends in the tube, it does not travel down the tube. During the remaining 60% of the time (*i.e.*, during the time in which it is in the gaseous state), gas B moves down the tube at the normal flow rate of 2 ft/min. On the average the gas flows at (60/100) × 2 ft/min = 1.2 ft/min. The time taken to pass through the column at this rate is

$$\frac{6\text{ft}}{1.2\text{ft}/\text{min}} = 5\text{min}$$

Alternatively, we can say that if gas B were flowing all the time, it would take 3 min to flow through the tube. Therefore it must spend 3 min flowing to reach the end of the tube. But it spends only 60% of its time in the gas phase, and 3 min is 60% of the total time in the column. Therefore the total time in the tube is 3 × (100/60) = 5 min.

The distribution coefficient for the gas in Fig. 40.2 is given by

$$K = \frac{\text{concentration in liquid}}{\text{concentration in gas}} = \frac{40}{60}$$

and this is reflected directly in the time it takes for the gas to elute from the chromatography column.

Based on this relationship, we can state that the time spent in the gas phase, or mobile phase, is the same as the time it takes for the carrier gas to pass through the column, that is, t_0. The time spent in the solvent phase, or stationary phase, is the *extra* time it takes for the sample to pass through the column, that is, $t_r - t_0$, where t_r is the total time for the sample to pass through the column, or the retention time. We can therefore present K in terms of the chromatographic experiment as

$$K = \frac{t_r - t_0}{t_0}$$

or

$$K = \frac{t_r}{t_0} - 1$$

If we substitute a second gas C for gas B in Fig. 40.2 and it is found that gas C spends only 20% of its time in liquid A and 80% of its time as a gas, then when C is permitted to flow through the tube packed with coated sand, it moves at the rate of 2 ft/min for 80% of the time and is at rest for 20% of the time. The average flow rate should be 2 × (80/100) = 1.6 ft/min. The total time needed for gas C to flow through the tube is 6/1.6 = 3.75 min.

If we were to pass a mixture of gases B and C down the tube, gas C would emerge after 3.75 min and gas B after 5 min. Passage of a mixture of the two gases down the tube results in separation of the mixture.

Inert gases, such as helium, flow through chromatographic columns at a constant velocity; that is, such gases do not interact with and thus spend no time in the stationary phase. For this reason inert gases are often used as carrier gases. In practice, the carrier gas flows steadily through the column. The sample

is injected into the carrier gas and is swept into the chromatographic column. The sample then distributes between the stationary phase and the moving carrier gas.

We have seen with a liquid substrate, the process can be used to separate gases from each other (gas liquid chromatography) or using solid substrates, we can separate liquids from each other (liquid solid chromatography). Different species of molecules spend different periods of time in the stationary phase (or substrate) and therefore different percentages of time in the mobile (or moving) phase. The variation in time spent in the moving phase, in turn, affects the length of time a gas takes to pass through and emerge from a chromatographic column. A record of the emergence of various components plotted against time is called a *chromatogram*.

This process of separation is the basis of all forms of chromatography. The factors that affect the zone distribution between the mobile phase and the stationary phase differ from one branch of chromatography to another. The most important branch is gas liquid chromatography, which will be discussed later. The next three most important branches are gas solid, liquid liquid, and liquid solid chromatography, where the first phase is the mobile phase and the second is the stationary phase. In the following paragraphs, we consider some properties of the substances involved in chromatography, as well as some hypothetical data illustrating chromatographic analysis.

In the example described previously, compound B distributed itself between the liquid phase and the gas phase in the ratio of 40% (liquid) to 60% (gas). If we add a second gas such as helium that can be used as a carrier gas, the distribution of B between the gas phase and the liquid phase will not be disturbed. The distribution can then be described by

$$K_B = \frac{\text{concentration of B in liquid (stationary) phase}}{\text{concentration B in gas (mobile) phase}} = \frac{X_L}{X_G} = \frac{40}{60} \qquad (40.1)$$

In this case K is the partition coefficient. Frequently it is expressed in terms of moles per litre, in which case

$$k'_B = \frac{\text{moles in stationary phase}}{\text{moles in mobile phase}} = K_B \frac{V_L}{V_m} \qquad (40.2)$$

where V_m and V_L are the volumes of the mobile (gas) and stationary (liquid) phases, respectively, and k'_B is the solute partition ratio. Also, more correctly,

$$k' = (t_r - t_0)/t_0.$$

Suppose that we have a second gas C which spends 80% of its time in the gas phase and 20% in the liquid phase. The partition coefficient for gas C is given by

$$k'_{(C)} = \frac{\text{moles of C in stationary phase}}{\text{moles of C in mobile phase}} = \frac{20}{80}$$

When the partition coefficients are widely different, we can expect separation. On the other hand, if the partition coefficients are similar, it is less likely that separation will be achieved. The separation factor or *selectivity factor* α can be given as

$$\alpha = \frac{K_B}{K_C} \text{ or } \frac{k'_B}{k'_C} \qquad (40.3)$$

Unfortunately, selectivity factors are only a guide to the actual ability of a column to separate components. Other factors influence the final result. For example, the two gases are in dynamic equilibrium between the liquid phase and the mobile phase. If the mobile phase is flowing continuously, then the dynamic equilibrium is approached at all times but never quite achieved. This results in a spreading of the components B and C along the column. Sometimes this results in a lack of separation, even though

the selectivity factors, indicate that the two components should be separable. Here the capacity fraction K is deduced in terms of the retention time of the sample and the flow rate of the solvent.

40.3 Gas–Liquid Chromatography

Gas–liquid chromatography consists of a mobile gas phase and a stationary liquid phase that is coated on to either a solid matrix (*e.g.*, diatomaceous earth) or the wall of a capillary tube. Typically the stationary phase has a sufficiently low vapor pressure (mm) at the column temperature so that it can be considered as non–volatile. The sample mixture in gaseous form is run through the column with a carrier gas. Separation can be achieved by the differences in the distribution ratios of the components of the sample between the mobile (gaseous) and stationary (liquid) phases causing them to move through the column at different rates and with different retention times. After elution, the sample components can be detected by a suitable detector at the exit.

40.4 Instrumentation

Although many commercial variations are available, basically all gas chromatographs, whether GLC or GSC, consist of six basic components:

1. a carrier gas which is maintained at a high pressure and is delivered to the instrument at a rapid and reproducible rate
2. a sample injection system
3. the separation column
4. one or more detectors
5. thermostated chambers for the temperature regulation of the column and detectors
6. an amplification and recorder system.

A schematic diagram of a gas chromatographic system is shown in (Fig. 40.3).

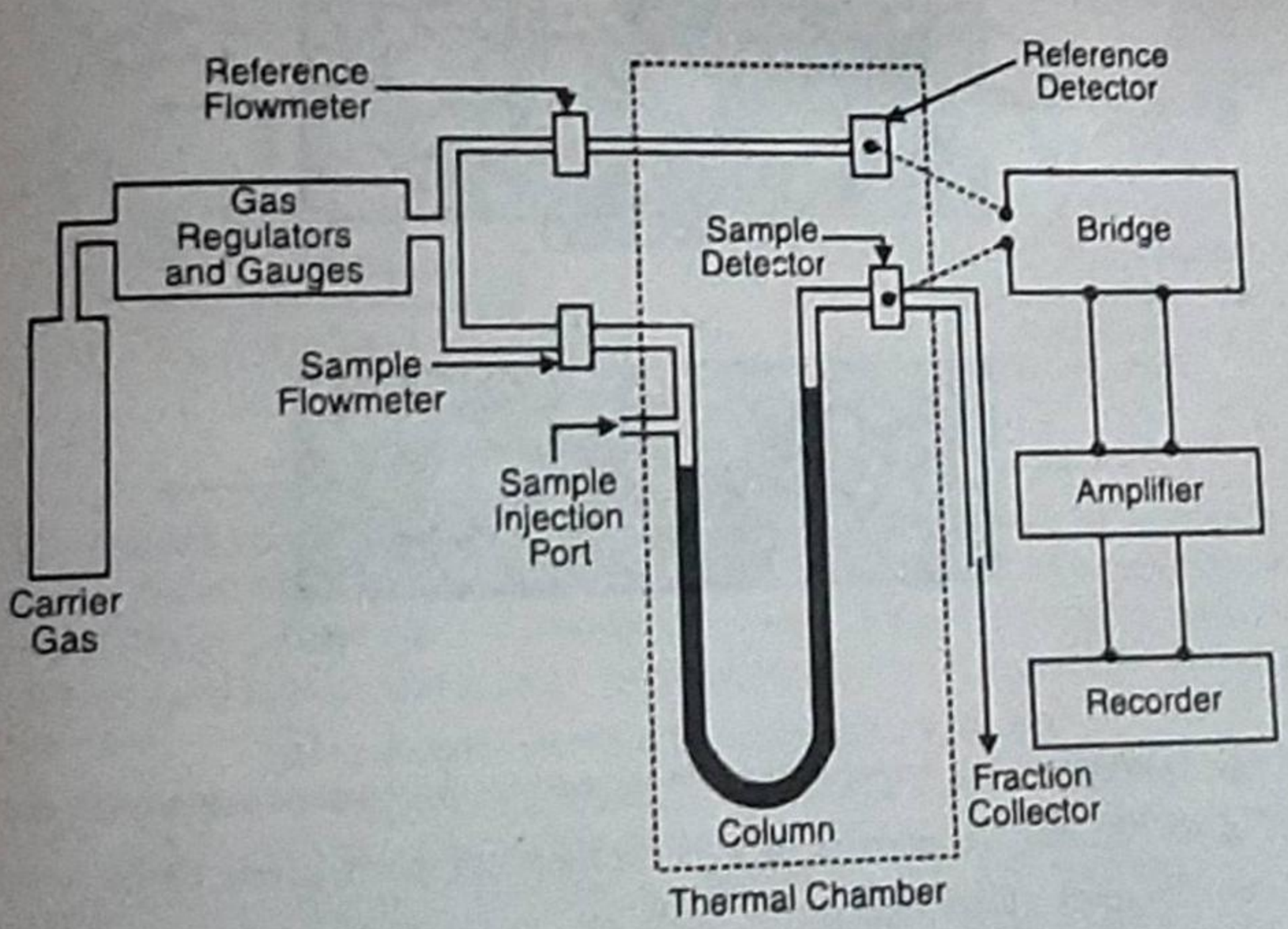


Fig. 40.3 : Gas Chromatograph (Schematic)

The gas chromatographic separation is carried out in a tubular column made of glass, metal or teflon, In this column a sorbent is filled as the stationary phase. The adsorbents are packed in the form of fine size graded powder, whereas the liquids are either coated as fine film on the column wall or first

III. Sample overload recovery is rapid and without tailing. Thus it is quite useful in preparative and trace analysis work.

IV. Porus polymer beads are mechanically strong and can be easily packed on columns.

V. Retention data are highly reproducible.

VI. Some of the separations provided are unique.

(*ii*) ***Open Tubular Columns.*** These columns are also referred to capillary or Golay columns and are made of long capillary tubing (30–90 meters) having uniform and narrow internal diameter (0.025–0.075 cm). They are made of stainless steel, copper, nylon, or glass etc., the stainless steel being the most popular. The inside wall of the capillary tubing is coated with the liquid phase in the form of a thin (0.5–1 micron) and uniform film. The carrier gas flow faces less resistance because there is no packing in the column.

(*iii*) ***Support Coated Open Tubular Columns.*** These columns are made by depositing a micron size porous layer of support material on the inside wall of a capillary column and then coating with a thin film of liquid phase. These columns have more sample capacity and an inlet splitter may not be required. SCOT columns are preferred for trace analysis.

A number of nonvolatile liquids are used as stationary phases. Some of them are listed in Table 40.1.

Table 40.1 : Some GC Stationary Phases

Abbreviation	*Identification*
Apiezon L and M	
Carbowax	polyethylene glycols
CDMS	cyclohexane dimethanol succinate
DEGS	diethylene glycol succinate
Dexsil—300	carborane–silicone
Dexsil—400	carborane–silicone
FFAP	Free fatty acid phase
Igepal CO–880	nonylphenoxypoly (ethuleneoxy) ethanol
NPGS	neopentylglycol succinate
OV–1	methyl silicone
OV–7	20% phenyl, methyl silicone
OV–17	50% phenyl, methyl silicone
OV–25	75% phenyl, methyl silicone
OV–61	33% phenyl, methyl silicone
OV–101	liquid methyl silicone
OV–210	50% trifluoropropyl, methyl silicone
OV–225	25% phenyl, 25% cyanopropyl methyl silicone
PEC–A	di–n–decyl phthalate
QF–1	50% trifluoropropyl, methyl silicone
SE–30	methyl silicone
SE–52	5% phenyl, methyl silicone
SF–96	methyl silicone
SP–525	aromatic polymer
SP–2250–BD	50% phenyl, methyl silicone
Thermol–3	phenetidine derivative
UCW–98	vinyl, methyl silicone
XE–60	25% cyanothyl, methyl silicone
Ucon	polypropylene glycols

The above table only provides information concerning the types of chemical compounds in a separation. The actual separation problem may be more complex. Detailed information for separations may be obtained from monographs on gas chromatography and technical bulletins manufacturers.

(d) **Detectors.** Almost all the detectors monitor the GLC column effluent by measuring the change the composition arising from the variations in the eluted components. When the carrier gas is passing they give a zero signal. When a component is eluted it is detected and a signal proportional to the concentration of that component is produced. Integrated detectors which give signals proportional to the amount of the eluted component are also available. Some commercially available detectors will be described now.

(i) ***Differential Thermal Conductivity Detector.*** One of the first detectors used was the differential thermal conductivity detector. The principle of the detector is that the temperature and thus the resistance of a wire through which a current is flowing is dependent upon the thermal conductivity of the gas in which it is immersed. The thermal conductivity of a gas is a function of composition.

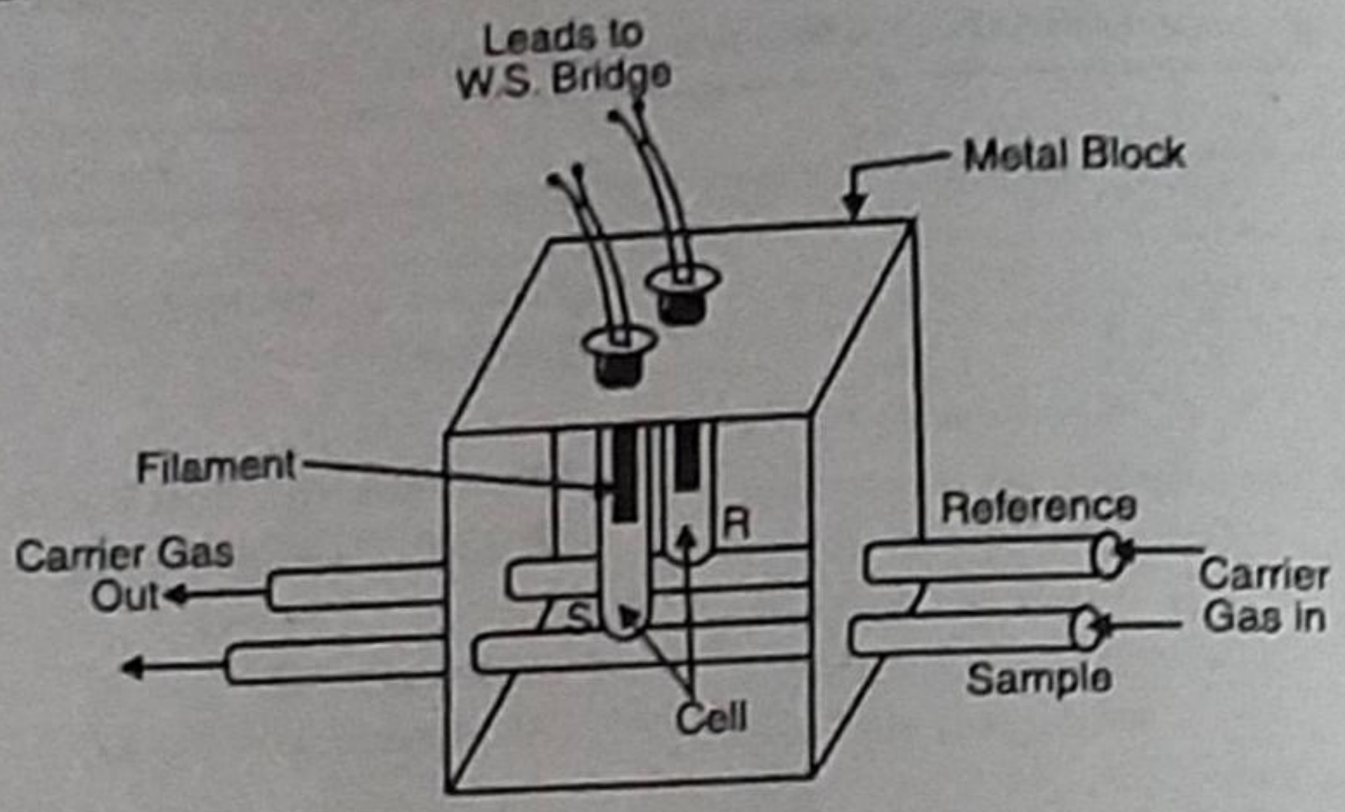


Fig. 40.7 : Typical Thermal Conductivity Detector.

A typical DTCD cell is shown in Fig. 40.7.

It consists of two chambers of small volumes, made within a metal block, each containing resistance wire of thermister which has a high temperature coefficient of resistance *i.e.*, resistance varies greatly with temperature. These resistances constitute the reference (R) and sensing (S) elements respectively and are included in two arms of a Wheatstone bridge circuit as shown in Fig. 40.8.

The carrier gas passes in both the cells and the arrangement is such that the column effluents are moved into the sensing side only. When a sample component enters the sensing cell the temperature of the filament S changes due to widely different thermal conductivity of the sample component than of the carrier gas. As a result the resistance of S also varies and the bridge becomes unbalanced. This off–balance current is signalled to the recorder which draws the elution curve for the chromatographic separation.

A differential thermal conductivity detector generally responds to all substances except the carrier gas. The sensitivity depends on the type of carrier gas, filament current detector block temperature and flow rate of carrier gas.

The important precaution with DTCD is that first turn the carrier gas 'ON' and then only switch on filament current/detector block heater. Similarly do not turn off the carrier gas before switching off the detector current or before the detector block has cooled down. This avoids damage to the filaments and gives long life to the detector.

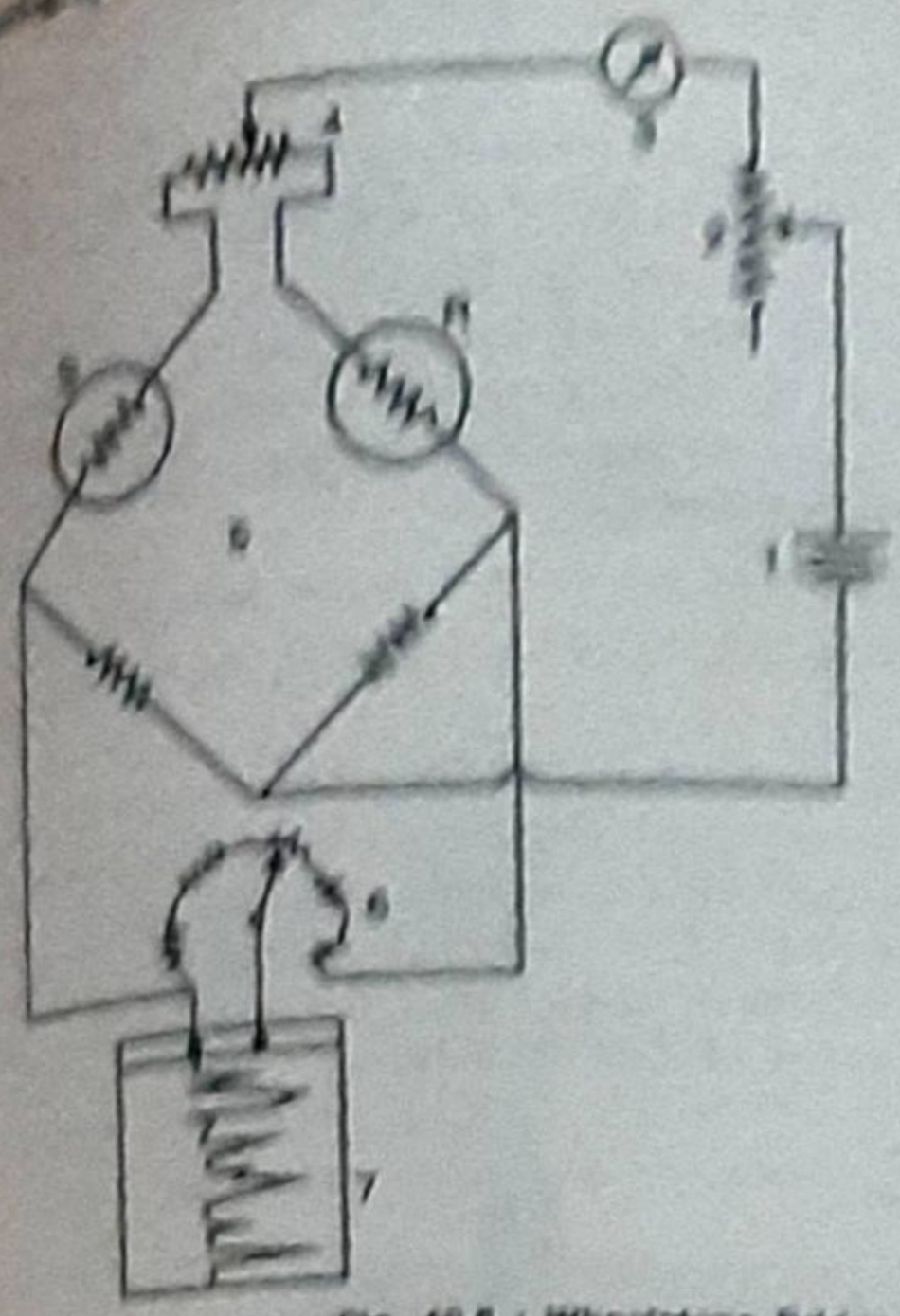


Fig. 40.8 : Wheatstone Bridge Circuit for TCD.

The TCD responds to all types of organic and inorganic compounds including those not detected by FID. Further, it does not destroy the eluted components and therefore is useful for preparative work. It is however less sensitive than the FID with a minimum detection limit of 10^{-9} g.

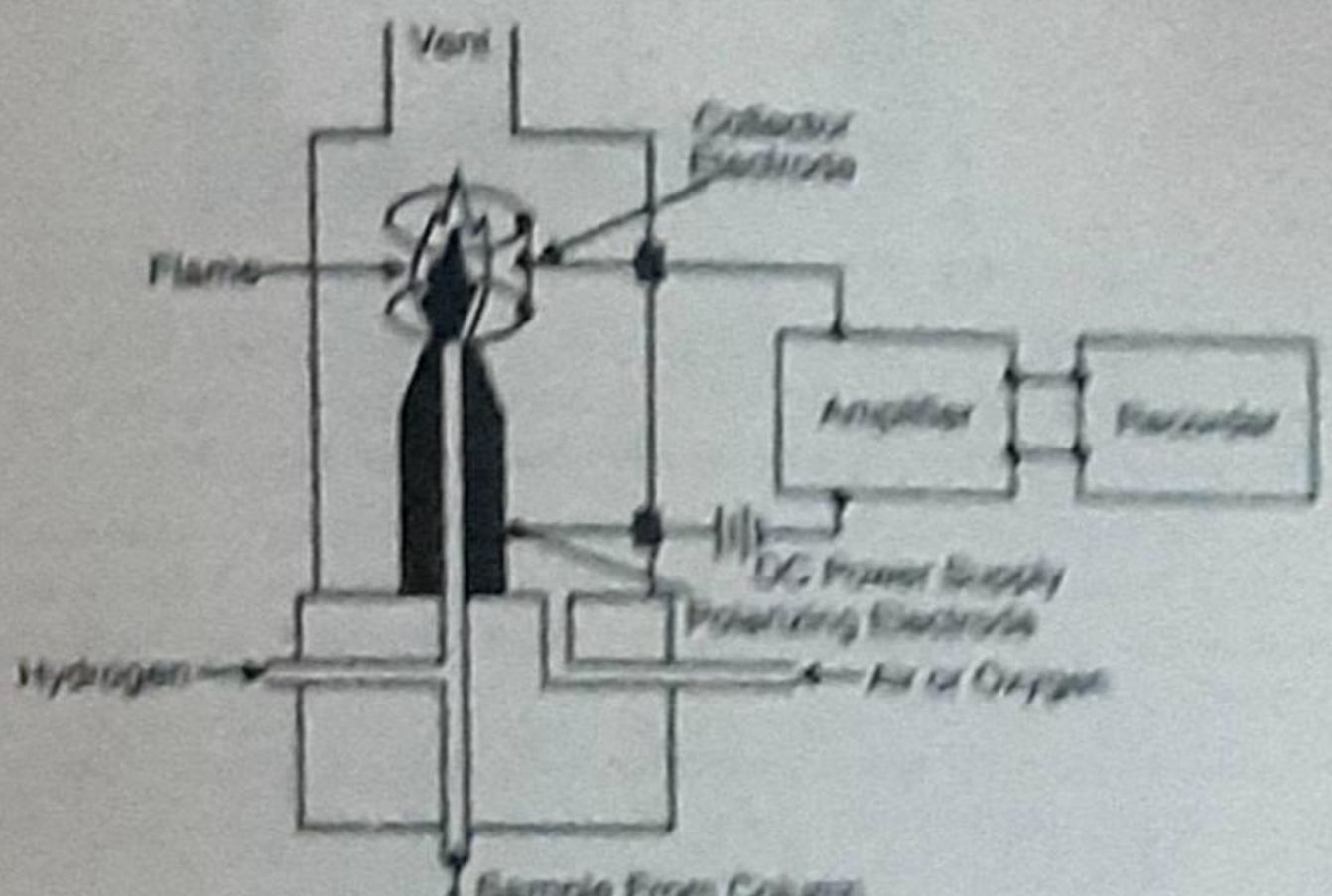


Fig. 40.9 : Flame Ionization Detector (schematic)

(ii) ***Flame Ionization Detector.*** The ionization detectors are based on the electrical conductivity of gases. At normal temperatures and pressures gases act as insulators but will become conductors of ions if electrons are present. If the conditions are such that the gas molecules themselves do not ionize, the change in conductivity due to the presence of a very small number of ions can be detected.

Table 40.2 gives the typical properties and characteristics of various GLC chromatographic columns.

Table 40.2 : Typical Properties and Characteristics of GLC Chromatographic Columns

	WCOT	*SCOT*	*Micropacked*	*Packed*
Typical inside diameter	0.25 mm 0.50 mm	0.50 mm	1 mm	2 mm 4 mm
Typical length	10–100 m	10–100 m	1–6 m	1–4 m
Typical efficiency	1000–3000 plates/metre	600–1200 plates/metre	1000–3000 plates/metre	500–1000 plates/metre
Sample size	10–100 ng	10 ng–1 μg	10 ng–10 μg	10 ng–1 mg
Pressure required	Low	Low	Very high	High

Table 40.3 gives some of the stationary phases with which bulk of the separations can be achieved.

Table 40.3 : Column Packings for GC Separations

Compound	*Packing*
Acids	
C_1–C_6	Pora Pak Q, SP–1200/H_3PO_4, Chromosorb 102
C_6+	DEGS–PS
Alcohols	
C_1–C_5	Chromosorb 102, Pora Pak Q, Carbowax 1500
C_6+	SP–1200, OV–101, SE–30, OV–1
Amines	
C_1–C_5	Chromosorb 103
C_6+	10% Apiezon L + 2% KOH
Gases	
O_2–N_2	Molecular Sieve 5A, Carbosieve B
CO, CO_2, N_2, CH_4	Pora Pak Q, Chromosorb 102
Hydrocarbons	
C_1–C_3	Carbosieve B
Xylenes	5% SP–1200/5% Bentone 34
Aliphatics from Aromatics (medium boiling)	10% SP–1200, OV–101, SE–30, OV–1
Aliphatics from Aromatics (high boiling)	3% SP–2100, OV–101, SE–30, OV–1
Aliphatics from Aromatics (very high boiling)	1% Dexsil 300
Pesticides	1.5% SP.2250/1.95% SP–2401 1.5% OV–17/1.95% OV–210
Phenols	10% SP–2100, OV–1, SE–30, OV–101
Steroids	3% SP–2100, 2250, 2401, or, 2340, OV–1, OV–101, SE–30, OV–17, OV–210, Silar–10C

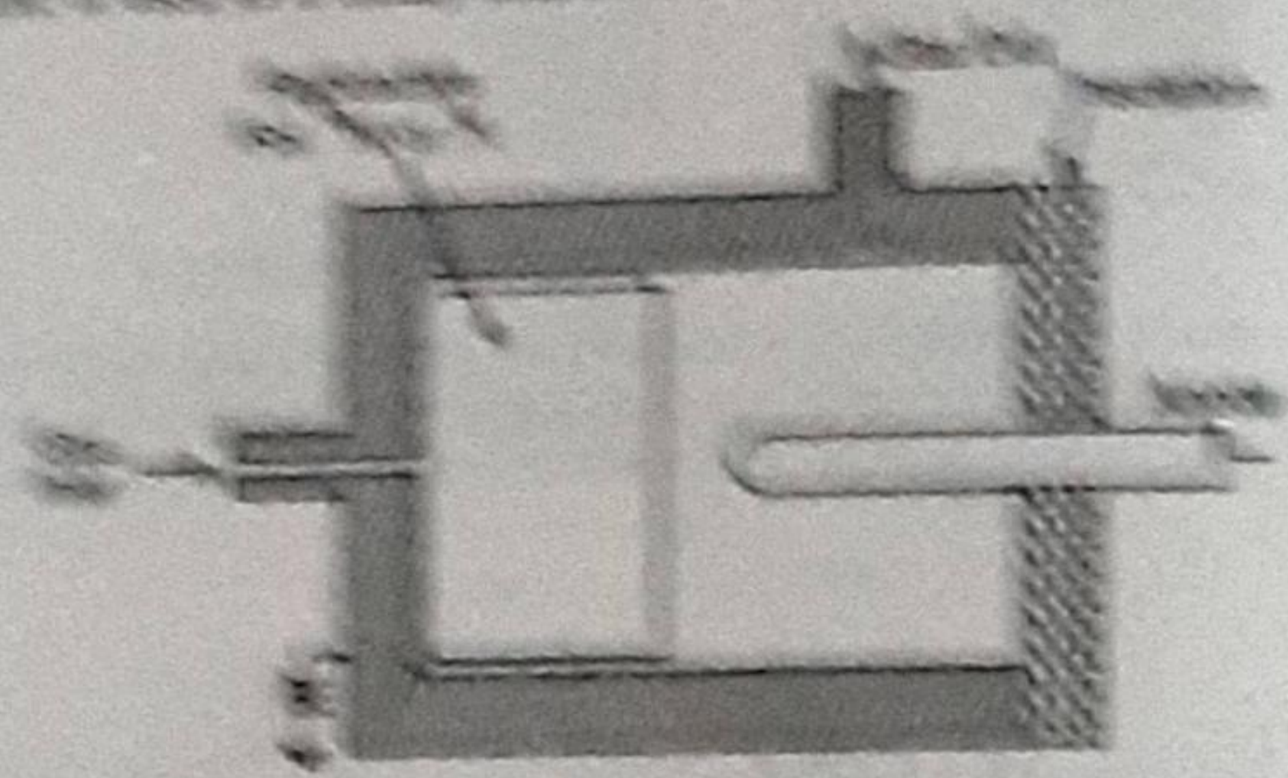


Fig. 6.5 Thermo Electric Device

Table 40.4 : Sensitivity of GC Detectors

Detector	*Sensitivity (g)*	*Linear range*	*Comments*
Thermal conductivity	10^{-8}	10^{4}	Universal sensitivity, non-destructive
Flame ionization	10^{-11}	10^{6}	Detects all organic compounds, the most widely used GC detector, destructive
Electron capture	10^{-13}	10^{3}	Detects halo-, nitro-, and phosphorus compounds, response varies significantly, nondestructive
Flame emission	10^{-6}	10^{3}	Sulfur and phosphorus compounds, response varies widely with compound, destructive
Gas density balance	10^{-6}	10^{5}	Universal; low sensitivity; nondestructive
Argon ionization	10^{-12}	10^{5}	Universal; argon carrier gas necessary non-destructive
Cross section	10^{-6}	10^{5}	Universal; detects major components

(e) **Substrates.** The solid support is generally coated with a high boiling liquid known as the substrate which acts as the immobile phase in GLC. The general requirements for the liquid phase are given below :

(*i*) Good solvent property of the component.

(*ii*) Differential partitioning of sample components.

(*iii*) Low vapour pressure at the column temperature.

(*iv*) High thermal stability.

Some of the typical substrates are given in Table. 40.5.

Table 40.5 : Some Typical Substrates

Substrates	*Solute type*	*Temperature (°C)*
(*i*) Polyglycols	Amines, ethers, alcohols, ketones, esters, aromatics	100–200
(*ii*) Paraffin oil (Nujol)	Paraffins, olefins, and halides	150
(*iii*) Silicone oils	Paraffins, olefins, esters and ethers	200
(*iv*) Didecyl phthalate	Polar Compounds	170

(f) **Temperature Control.** A temperature programming facilitates controlled increase of even temperature during an analysis. Thus, the latter peaks also become sharp and emerge quickly. Thus in temperature programming the components of a wide boiling range mixture may be resolved efficiently.

The temperature programming may be carried out in three different modes. These are :

(*i*) Natural or ballastic

(*ii*) Linear

(*iii*) Matrix or multilinear.

In general the operation with linear temperature programme is more common. The requirements for good temperature programming are discussed below :

(*i*) Dual column system. This compensates for bleeding of liquid phase from columns during increase of temperature.

(*ii*) Separate heaters for injector, column, oven and detector system.

(*iii*) Differential flow controllers.

(*iv*) Low mass column oven for rapid heat transfer.

(*v*) Thin walled columns.

(*vi*) Low liquid phase loading.

(*vii*) Pure dry carrier gas.

(*viii*) Stable and non–bleeding injection septums.

40.5 Evaluation

The efficiency of a column is expressed by the number (N) of theoretical plates in the colum by the height equivalent of a theoretical plate (HETP). The larger the number of theoretical plates or the smaller the HETP, the more efficient the column is for separations. A theoretical plate is that distance on the column in which equilibrium is attained between the solute in the gas phase and the solute in the liquid phase. It is equivalent to one equilibrium stage in a distillation.

From the elution curve, the number of the theoretical plates in any column can be calculated (Fig. 40.11). The distance *d* from the point of injection to peak maximum can be measured in units of length, time or volume and is generally called the retention volume V_R. In Fig. 40.11, H is the height of the peak maximum, W is the width of the peak measured as the distance between the intersection of the tangents to the inflection points with the base line; B is the width of the peak at a height of H/e; t is the time lapse between the injection and the initial rise of the peak and return of the peak to the base line respectively, and the area is calculated as the height multiplied by the width of the peak at one–half the height.

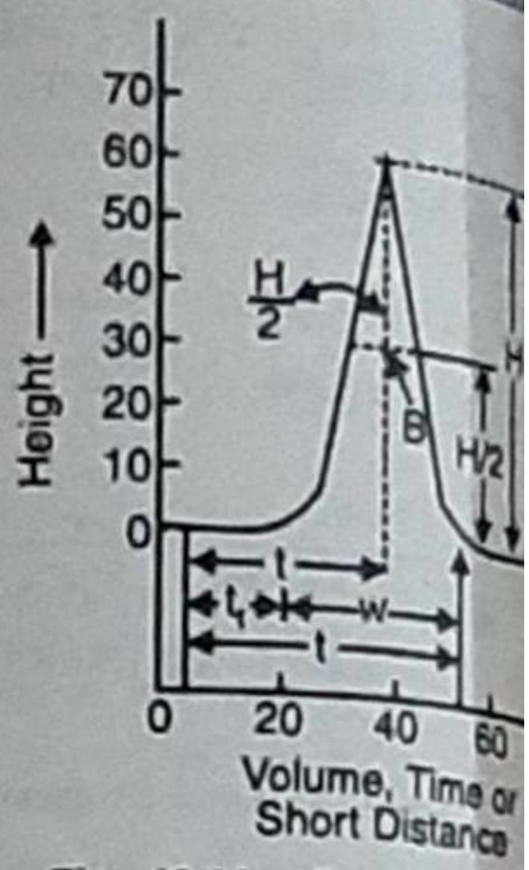


Fig. 40.11 : Calculation o Number of Theoretical Plat

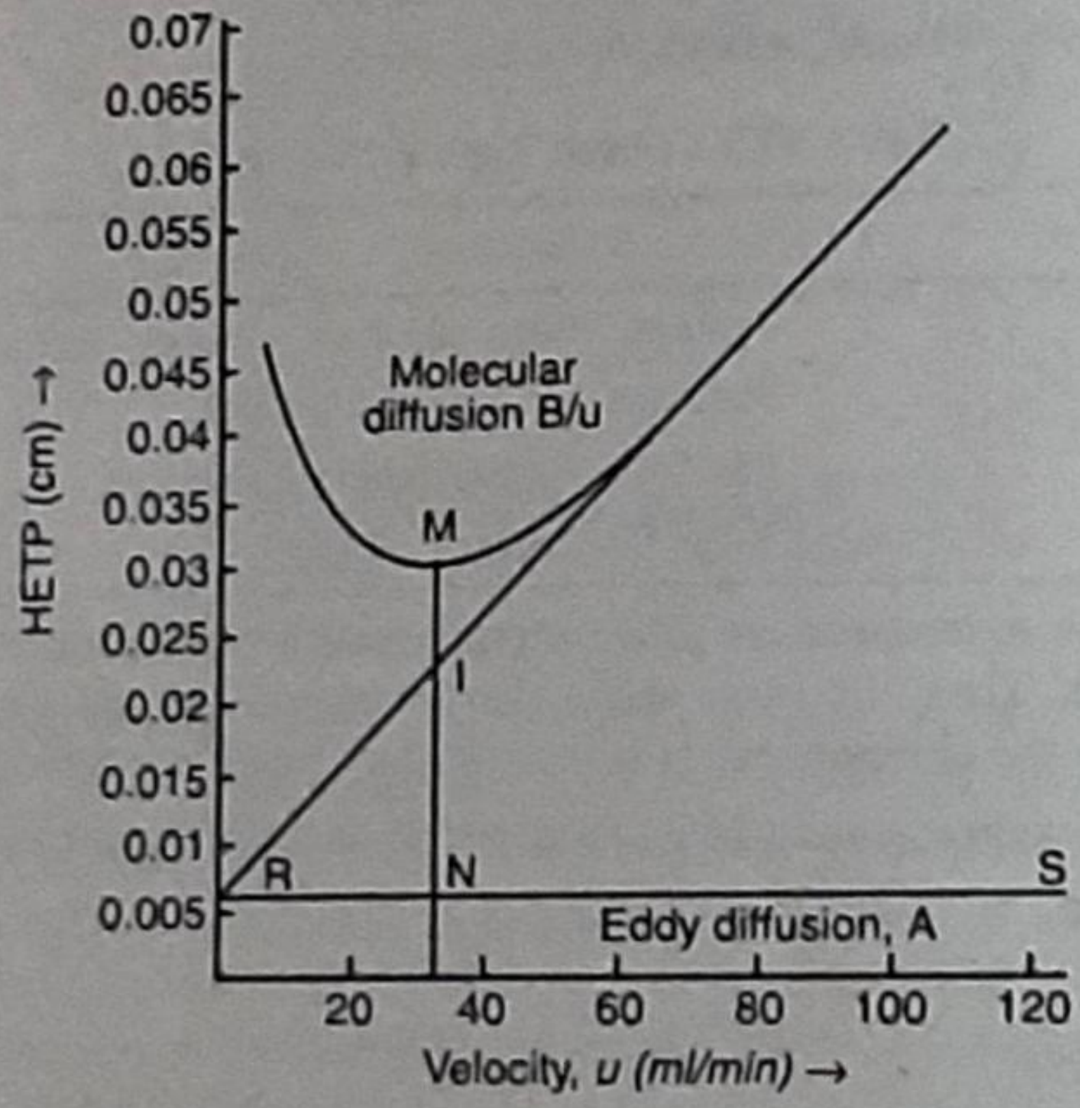


Fig. 40.12 : Typical Van Deemter Graph.

The number of theoretical plates gives the efficiency of a column but does not give any informat concerning the effect of various parameters upon the efficiency. Taking into account the different m transfer processes contributing to peak broadening in the column. Van Deemter derived a theoret equation equating H to the sum of three terms as a function of linear gas velocity *u*.

$$H = 2\lambda dp + \frac{2\lambda Dg}{u} + \frac{8}{n^2}\frac{k}{(1+k)^2}\frac{d^2 f}{D_l}u$$

$$= A + \frac{B}{u} + Cu$$

where u is the gas velocity or flow rate; A is a constant that involves the packing effect in the column and the partice diameter and is called the eddy diffusion term, B is a constant that includes the effect of diffusion in the gas phase and the correction for the tortuosity of the path and is called the molecular diffusion term. C is a constant that reflects the resistance to mass transfer between the gas and the liquid.

The curve of a typical Van Deemter study is given in Fig. 40.12.

It shows the effect of each term of the equation on the relation ship between the flow rate and the HETP

40.6 Retention Volume

The uncorrected or experimental retention volume for a chromatogram is given by :

$$V_R = t_r F_c \tag{1}$$

where t_r is the time in minutes on the time axis from the point of injection to the peak maximum and F_c is the volumetric flow rate in millilitres perminute. As already discussed the retention volume is also related to the number of theoretical plates by the relation

$$V_R = NV_h \tag{2}$$

where V_h is the effective plate volume. The relation of the effective plate volume to the partition coefficient is given by

$$V_h = h\,(A_m + PA_s) \tag{3}$$

where h is height equivalent of theoretical plate, A_m is the cross sectional area of the stationary phase and P is the partition coefficient. From equations (2) and (3) we get

$$\begin{aligned} V_R &= Nh\,(A_m + PA_s) \\ &= V_m + PV_s \end{aligned} \tag{4}$$

Because Nh A equals height times area, which equals volume. V_m is the volume of the mobile phase and V_s is the volume of the substance. P is defined as

$$P = \frac{\text{Wt of solute per ml substrate}}{\text{Wt of solute per ml gas}}$$

40.7 Resolution

The efficiency of the separation of the components of a mixture is generally expressed as separation factor of the resolution between the peaks. It is also expressed in terms of the distance of separation of the peak maximum and width of the phase using the relation.

$$R = \frac{2(V_{R_2} - V_{R_1})}{w_2 + w_1} = \frac{2(t_{R_2} - t_{R_1})}{w_2 + w_1}$$

where V_R and t_R represent the retention volume and retention time as shown in Fig. 40.13.

The value of R in the figure is about 1.14. It should be kept in mind that values of R equal to or greater than 1.5 indicate baseline or essentially complete resolution.

40.8 Branches of Gas Chromatography

There are a number of different analytical requirements that elicit different aspects of gas chromatography. For this reason, modification of the equipment has led to the development of the distinct branches described in the following sections.

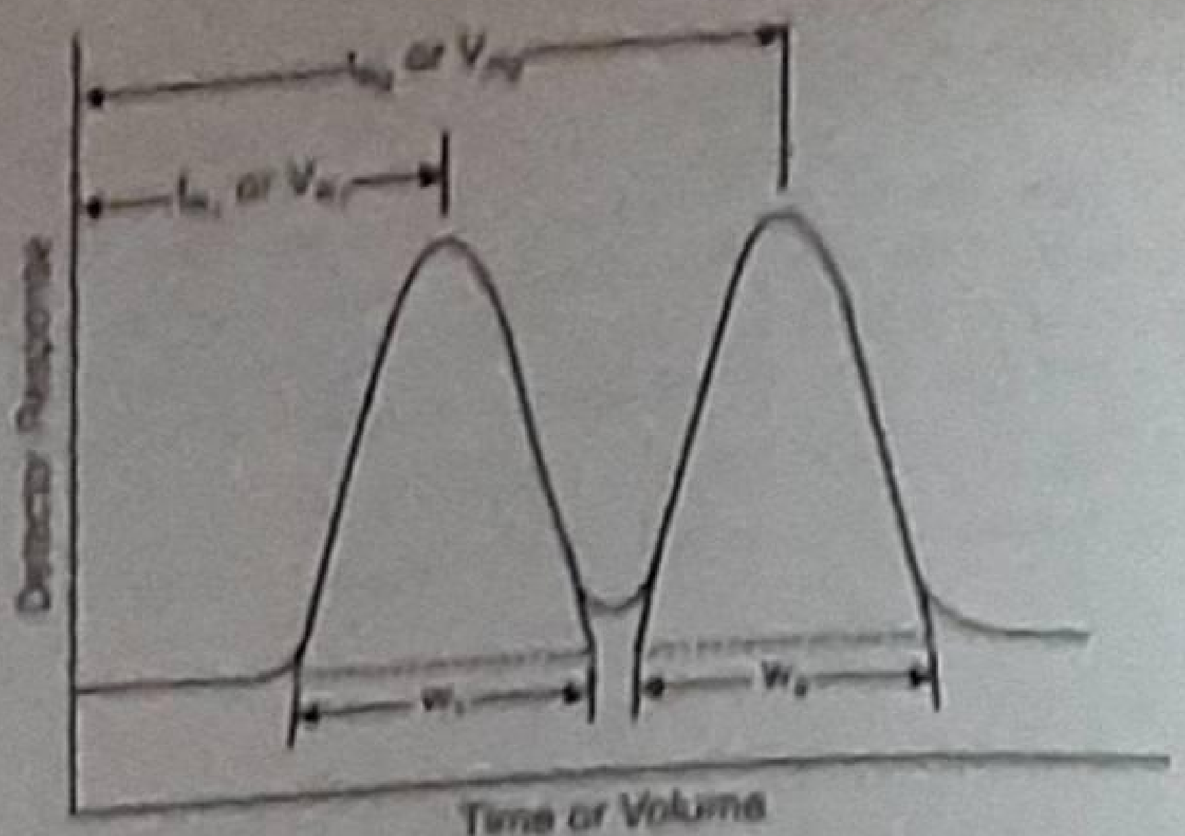


Fig. 40.13 : Measurements Used in Calculation of Peak Resolution.

1. Packed-Column Gas Chromatography

Packed-Column gas chromatography, as the name implies, involves the use of packed columns having internal diameters of about 0.25 in. The tube may vary in length from 3 to 20 ft. This method is the workhorse of gas–liquid chromatography, is used for all forms of conventional organic molecular analysis including both qualitative and quantitative analysis. It is most satisfactory for quantitative analysis because the reasonably large sample size allows accurate measurement. Samples of the order of 0.01 ml are usually analyzed. The resolution is reasonable, but not excellent. The injection of a large quantity of sample permits the trapping of components as they emerge from the column. The trapped fractions can be positively identified by other methods, such as IR absorption spectroscopy, UV absorption, NMR, or mass spectrometry. The large sample size allows the use of all conventional detectors. Highly sensitive detectors are not required and may be overloaded by these large quantities. To prevent overloading it may be necessary to split the sample into two streams before the components reaches the detector. A large portion is thrown away and a small portion goes through the detector. The splitting is performed by a sample splitter (Fig. 40.14).

The splitter separates off the bulk of the sample. In doing so, it allows a constant fraction of the samples to reach the detector. It is important that the fraction that reaches the detector be a constant fraction of the sample; otherwise quantitative interpretation is impossible. Detectors that need the protection of sample splitters in packed-column gas chromatography include flame and electron capture detectors.

The use of packed columns permits many variations of substrate and support. The substrates can therefore be selected for use at high or low temperatures or for programmed temperature work. This type of column is therefore widely used for routine analysis; however, it has neither the high resolution of capillary columns nor the high sample capacity of preparative-scale columns, each of which is described in the following sections.

2. Capillary Column Gas Chromatography

Very narrow columns (less than 0.1 in. in diameter) are used in capillary column gas chromatography. The capillary columns, which may be several hundred feet long, are too thin and too long to pack with a support coated with a substrate. Usually, therefore, they are not packed; instead, a liquid substrate coats the inside walls of the column. The smooth unpacked interior considerably reduces the *A* (geometry) factor in the Van Deemter relationship and contributes to the high resolving power of the technique. In addition, the low pressure drop per unit length allows very long columns to be used.

The inside walls of the capillary are coated with substrate by forcing the latter through the column under high pressure, a difficult operation that requires special equipment. In general, capillary columns are prepared by the manufacturer and are sold ready to use. Samples of the order of 1 μg are analyzed.

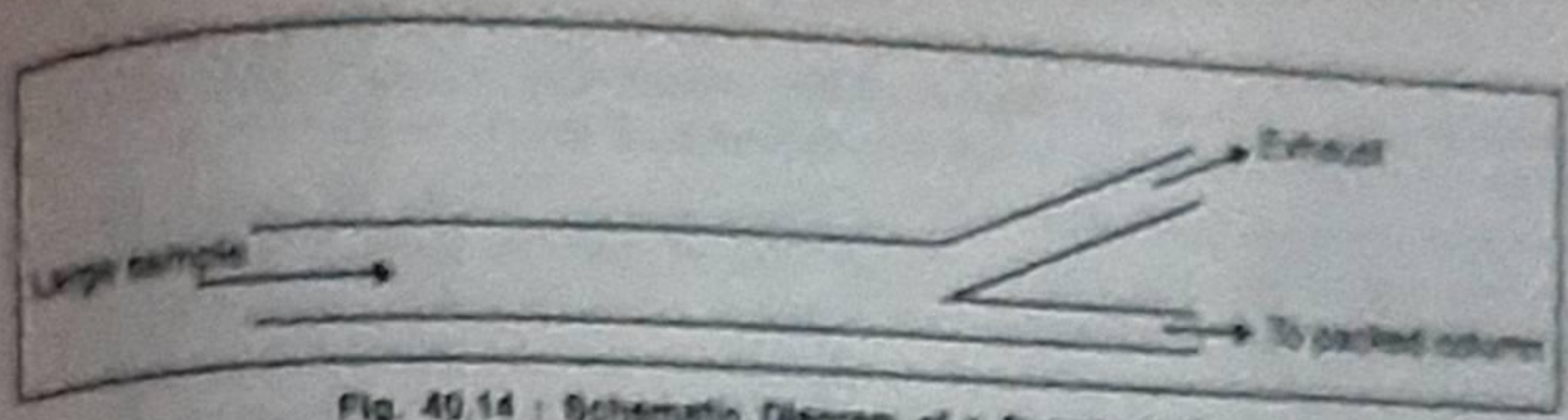


Fig. 40.14 : Schematic Diagram of a Sample Splitter

for runs these may require size reduction with a sample splitter to avoid swamping the detector. Because of the small sample size, highly sensitive detectors (such as flame ionization or electron capture detectors) must be used for this branch of gas chromatography. The method is capable of very high resolution. 10⁵ theoretical plates on a single column is not uncommon. It is therefore excellent for multicomponent mixtures, particularly if these are present in small concentrations that might not be easily resolved in the presence of the large chromatographic peaks generated by major components in packed-column chromatography. An example is the trace developed by Miles Novotny on the volatile components of a body fluid (Fig. 40.15). The sample cannot be recovered after injection into these columns, particularly if destructive flame ionization detectors are used. The column is therefore unsuitable for isolating pure compounds.

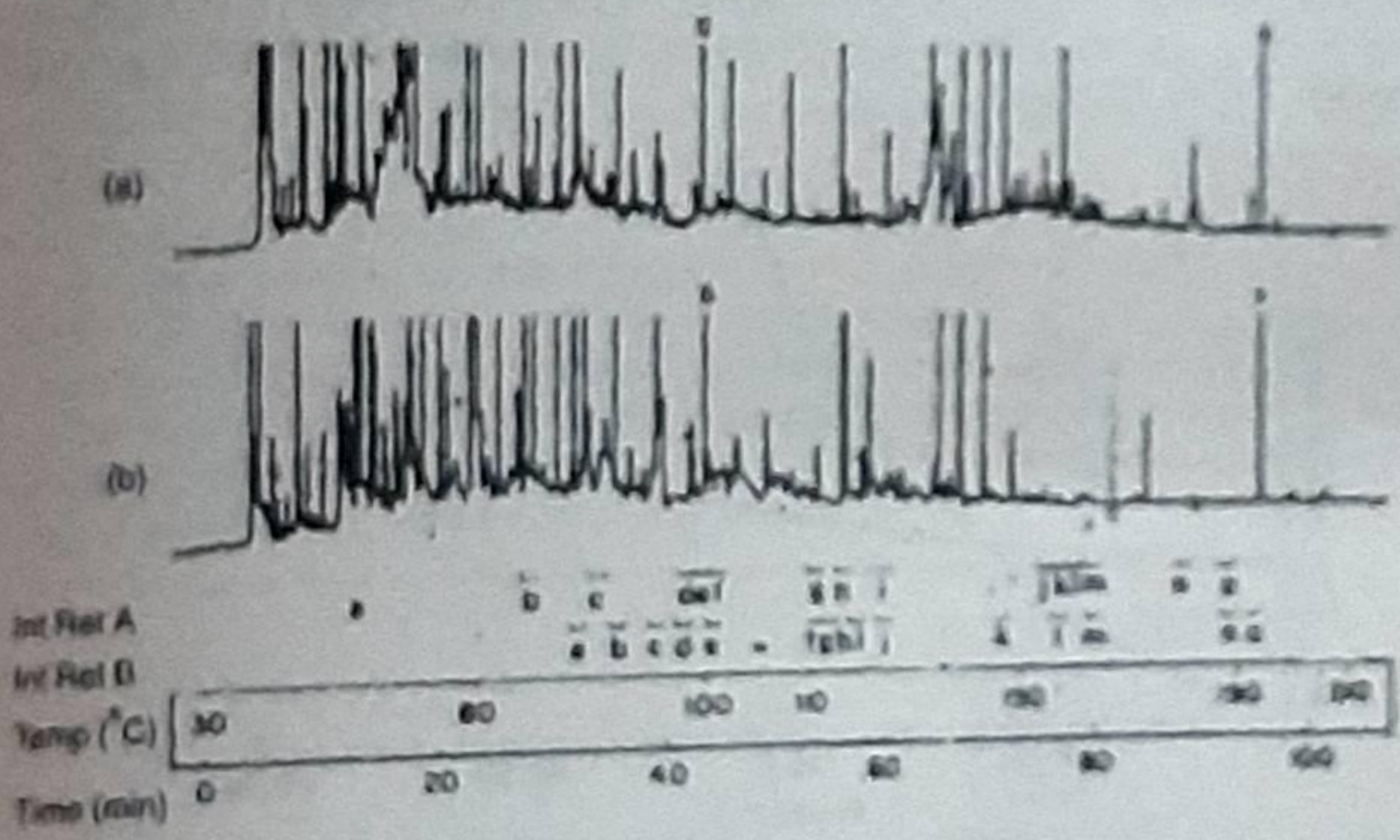


Fig. 40.15 : (a) Chromatogram of the urinary volatiles of a diabetic male.
(b) Chromatogram of the urinary volatiles of a normal female.

3. *Preparative-Scale Gas Chromatography*

Preparative-scale gas chromatography is more important to the organic chemist than to the analytical chemist. Large-bore (1/5–1 in.) packed columns are used with any suitable nondestructive (thermal conductivity) detector. This system lacks high resolution, but mixtures of components that are not difficult to separate can be separated on such columns. Relatively large samples (up to 100 ml) can be handled with comparative ease. The components are usually trapped as they leave the column, at which stage they are very pure. The analytical chemist uses the trapped fractions for identification when the mixture's components are unknown.

One of the most important uses of preparative-scale gas chromatography is in the preparation of pure organic compounds. By trapping the required fraction but not the other fractions, it is possible to obtain a compound in a very pure form. Thus chemists need not go through the tedious steps of

multidistillation or multicrystallization. Conversely, the other components, which include the impurities, may also be trapped and identified. Identification of side products in an organic reaction provides important information to the organic chemist regarding the mechanism of the reaction.

In analytical chemistry, preparative-scale gas chromatography is used for trapping the components of a mixture of organic materials and identifying each of them by methods such as IR absorption spectroscopy, NMR, and mass spectrometry. This analytical information is of great service to the chemist, chemical engineer, and pharmacist, and to all concerned with the manufacture or research synthesis of organic and some inorganic compounds. Also, preparative-scale gas chromatography can be used to reveal the source of unwanted impurities. It has been used to collect and purify pollutants in air or water. It has also disclosed impurities in many forms of samples.

Commercial operations based on preparative-scale gas chromatography have been developed as a means of making large quantities of highly pure compounds. Large columns up to several feet wide have been investigated, with encouraging results indicating that gross samples of many litres can be purified this way. This technique can be used to provide a commercial source of very pure compounds that hitherto have had to be prepared individually by the bench chemist. Compounds such as pharmaceuticals, drugs and fine chemicals produced by preparative-scale gas chromatography are purer, cheaper, and more readily available than those purified by other means, such as distillation.

As a commercial means of purification, preparative-scale gas chromatography competes with distillation, the cost of which increases rapidly as increased purity is required. Fig. 40.16, indicates the relative cost per pound of a given compound, at various degrees of purity, produced by preparative-scale gas chromatography and by distillation.

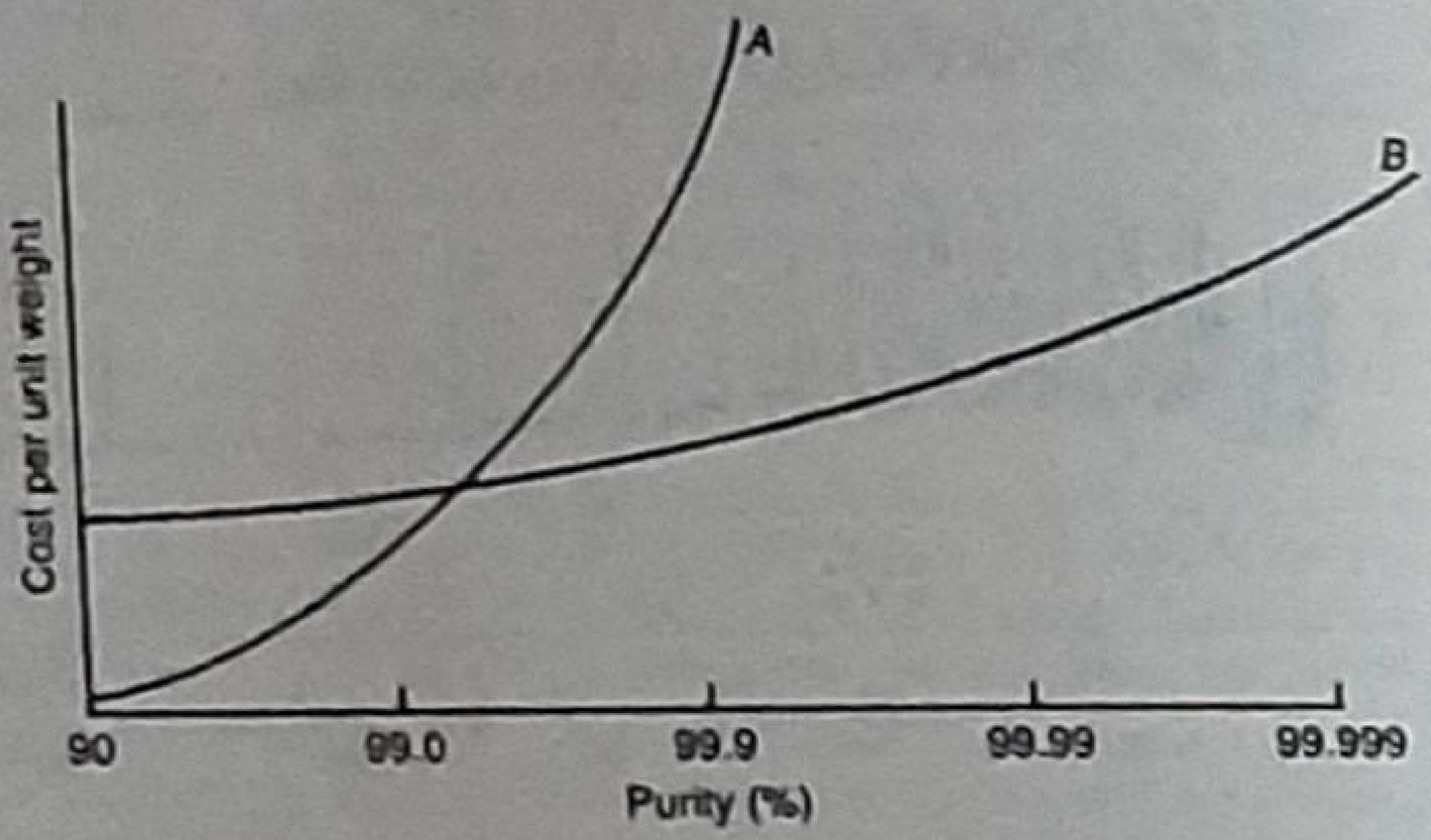


Fig. 40.16 : Relative cost per pound of a given compound at various degrees of purity: (A) distillation and (B) preparative-scale gas chromatography.

Specific commercial applications of preparative-scale gas chromatography include (1) the isolation of flavors from coffee, tea, wines, beer, whiskey, and citrus fruits; (2) the production of fine chemicals; (3) the reduction of the toxicity of pharmaceuticals along with increased purity; and (4) preparation of pure drugs for medical research.

4. Programmed-Temperature Gas Chromatography

In order to obtain reproducible analytical results, it is necessary to control the temperature of the column very carefully. The temperature directly affects the tendency of organic compounds to enter the gas phase and therefore affects K, the distribution coefficient. At low temperatures, if the boiling point is high, the compound will spend most of its time in the stationary phase and emerge from the column only after a prolonged time period. Under these circumstances, the GC peak is very much broadened

is also very useful. However, the temperature may be controlled such that the vapor pressure of each component is reasonably high. It should not be so high that the components are volatile and boil. If compounds boil, they spend their time in the gas phase and pass through the column without separating. In general, however, a suitable temperature can be found at which all compounds spend appreciable but different times in the gas phase. If the sample contains compounds with widely different boiling points, however, a suitable single temperature cannot be found at which each component spends a reasonable fraction of time in the gas phase.

This problem was overcome by using a programmed-temperature procedure, which was developed by Hewlett-Packard Company of du Pont. In this method, the sample is injected into the column in the normal manner. The temperature of the column is maintained at some suitable low temperature, such as 50°C, during injection. The column temperature is then increased at a controlled rate (e.g., 20°C/min) up to a maximum temperature as high as 300°C. Higher temperatures are not used because at such temperatures the substrate may be lost by vaporization, and in the process it may coat and destroy the detector. It is also necessary to check that the sample components are stable at these temperatures. If the components decompose or react with the column and not the sample, the analyses will be unreliable because any such fractions collected will have originated in the column and not the sample. It is also important that the column be heated uniformly during the analysis; otherwise the sample components will be diffused and column efficiency decreased. It should be noted that although the column may be at a low temperature initially, the inlet port must be maintained at a high temperature (350°C) to ensure rapid vaporization of the sample after injection.

At the beginning of the chromatogram, the temperature of the column is low. The low-boiling components emerge in an orderly fashion and can be resolved. As the temperature increases, the vapor pressure of the middle- and higher-boiling components increases and they in turn emerge from the column and are resolved and analyzed.

This technique has extended the use of gas chromatography to the analysis of mixtures containing components with a wide range of molecular weights. Typical examples include (1) alcohols from CH_3OH to $C_{30}H_{61}OH$, (2) paraffins from CH_4 to $C_{40}H_{82}$, (3) olefins from C_2H_4 to $C_{40}H_{80}$, and (4) natural fatty acids (up to $C_{17}H_{31}COOH$). There are numerous examples in other molecular species, such as amines and halides. Also, mixtures of these compounds can be successfully separated and analyzed via this technique. A typical chromatogram is shown in Fig. 40.17. It is not difficult to imagine that the separation of such a mixture by any other means would be virtually impossible.

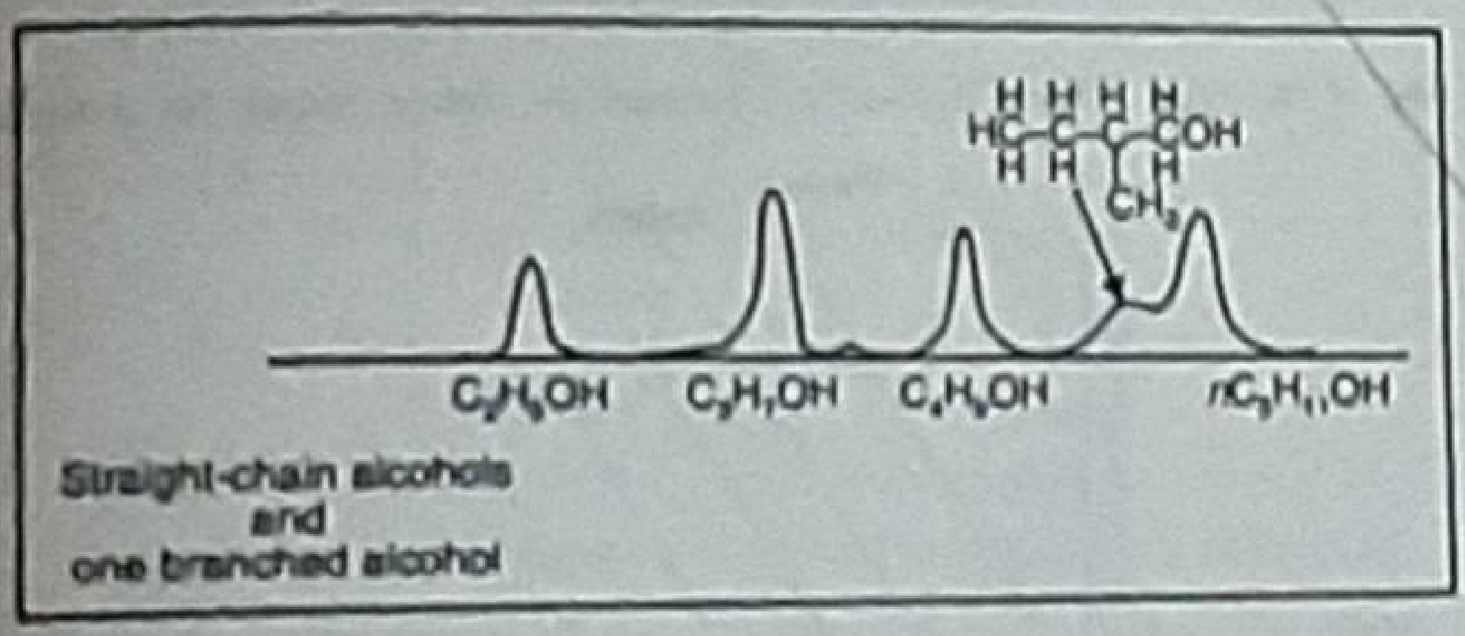


Fig. 40.17 : Typical chromatogram of straight-chain alcohols.

40.9 Applications

The principal applications of gas chromatography are the qualitative and quantitative analysis of liquids, gases, and vapors, particularly of organic compounds. Any stable compound that can be vaporized below 300°C can be determined by this method. It should be noted that the compound must be stable with respect to isomerization and decomposition at these temperatures, or the method gives erroneous results. Compounds that are unstable at these temperature or that are not volatile can be analyzed by liquid chromatography.

1. Qualitative Analysis

Qualitative analysis of the individual components of a mixture may be obtained by either of t following:

a. By comparing the retention times or volumes of the unknown to the retention times or volumes a series of standards, or

b. By collecting the individual components as they emerge from the chromatograph and subsequen identifying these components by other methods.

When retention times or volumes are used, all of the experimental parameters, especially the fl rate and temperature, of all the standards and unknowns must be carefully controlled and duplicat Since many compounds may have the same retention time, the data from a single set of conditions often inadequate for positive identification. Confirmation should be assumed only after several differ substrate–solvent combinations have been used.

At times it may be desirable to spike the sample mixture with a known compound. If an incre in area of a formerly present band occurs and if no extra elution band is obtained under several sets conditions, positive identification is practically assured.

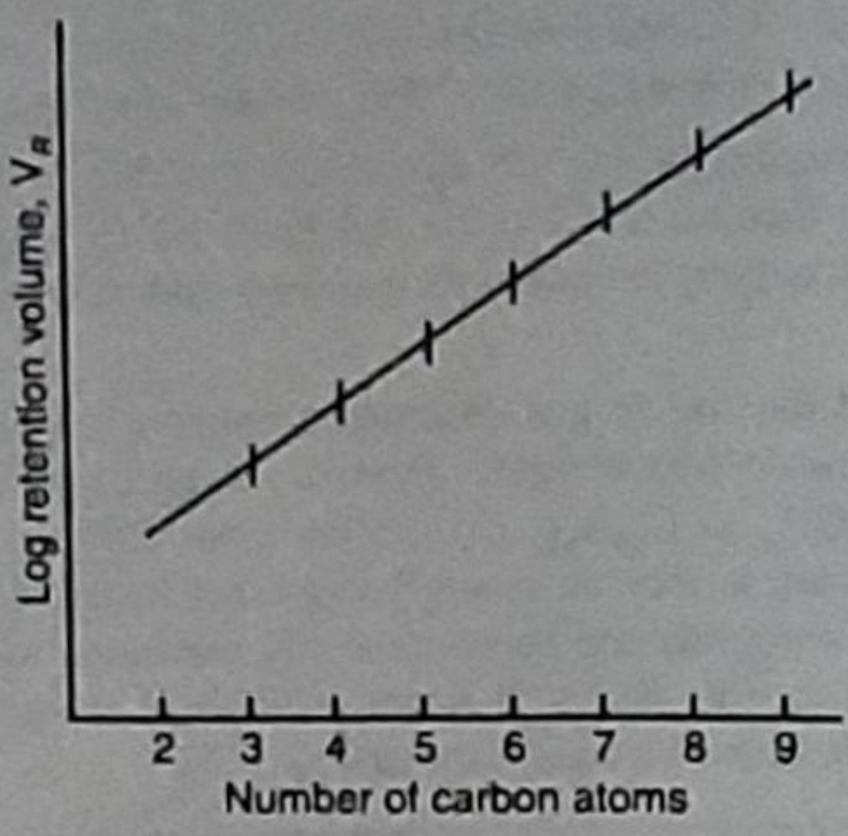


Fig. 40.18 : Plot of log of retention volume against the number of carbon atoms for a homologous se

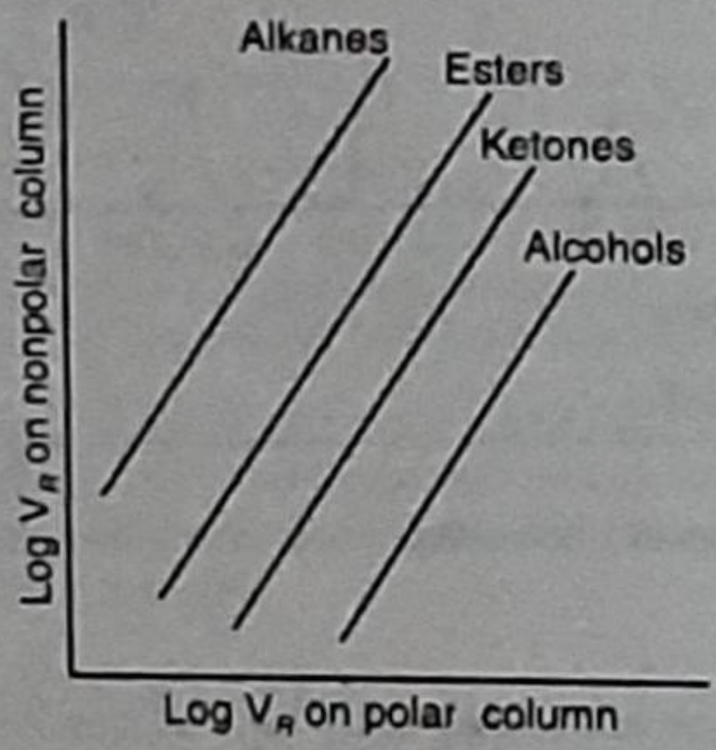


Fig. 40.19 : Plot of log retention volume on a polar column against the log of retention volume on non-polar column.

It has been found that under a given set of experimental conditions the log of the retention vol is a linear function of the number of carbon atoms in each homologous series. A different line is obta

for each different homologous series. (Fig. 40.18). From these graphs the number of carbon atoms may be determined if the series is known. A plot of the log of the retention volume on a polar column against the log of retention volume on non-polar columns may be used to determine the series to which a compound belongs. (Fig. 40.19)

When the samples are collected, final identification is usually made by mass spectroscopy, nuclear magnetic resonance, or infrared absorption techniques. In most cases interfacing devices are required to compensate for sample size, sample composition, and/or the pressure requirements of the different instruments.

1. Quantitative Analysis

Quantitative analysis of a chromatogram depends upon the fact that *the area under a single component elution peak is proportional to the quantity of the detected component.* In order for any peak area measurements to be meaningful, the output at the recorder must be linear with concentration and the time response of the recorder must either match the time response of the detector or, as an alternate, an automatic integrator must be coupled directly to the detector. In addition, the flow rate of the carrier gas must be reproducibly constant in order to allow a conversion between flow rate and time.

Because of the speed, accuracy, and ease of interpretation of the results, the automatic integrators are preferred. If no integrator is available, the area may be determined by the **cut and weigh** procedure. In this, a known weight of the sample is injected. The paper, which is of constant thickness and moisture content, is cut and a weight component per weight of paper factor is determined.

The peak area may also be determined by triangulation procedures. In this method tangents are drawn (Fig. 40.20) on the two sides of the curve. The intercept of the two tangents with each other at the top is the height, H. The distance between the intercepts of the two tangents with the baseline is the width, W. The area, A, is then calculated from the formula

$$A = \tfrac{1}{2} WH$$

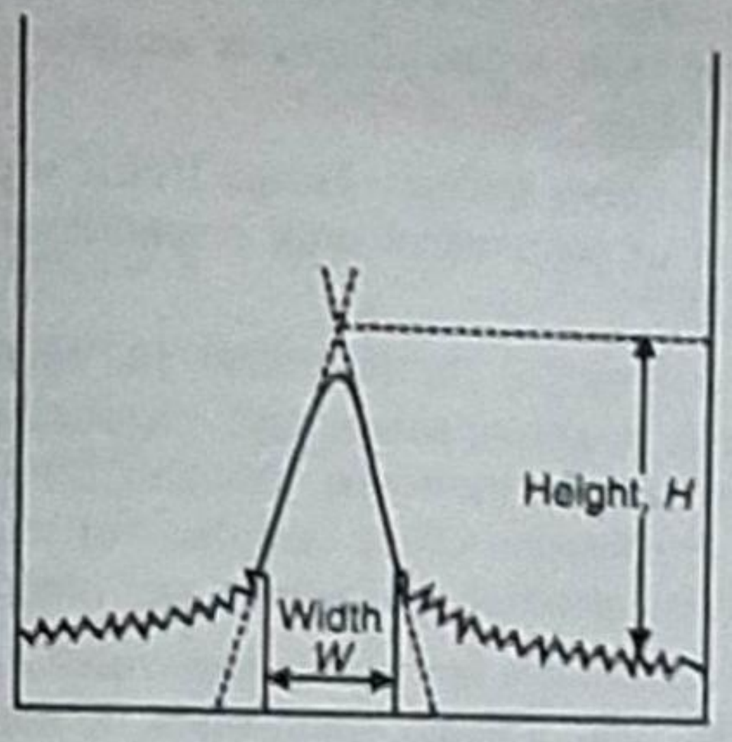


Fig. 40.20 : The triangulation method of determining peak area.

The Response factor. Since none of the detectors respond to the same degree for an equal mass of different compounds, a **response factor** must be determined for each pure compound under any given set of conditions. This response factor is used in subsequent calculations for the quantitative evaluation of an unknown in a mixture under the same set of conditions.

Graphs and Calibration. Rather than calculating the response factor, a number of exact known weights of the known pure component may be chromatogramed. The concentration of the unknown is then obtained from the standard plot of concentration against peak height or, more often, peak area, provided all experimental conditions are held constant.

Internal Standards. In this procedure a known weight of an internal standard is added to each of a series of known sample weights. The internal standard must be similar in structure to the sample

component and the retention times of the standard and sample component must be close to e
but must be resolved from each other as well as from other sample components. A linear calibrat
is prepared by plotting the ratio of the peak areas which result from the spiked samples to th
the unknown against the weight ratio of the added standards to the unknowns. The weight of the
is then determined from the graph.

3. Miscellaneous Applications

A large number of applications have been achieved by GLC. The applications happen
varied that it is just not possible to cover them completely. During the last few decades we h
the rapidly changing complexities of the product (*e.g.*, drugs, foodstuffs, consumer products)
technology with the concomitant environmental problems. All this has necessitated development o
that can separate and identify numerous species (known and unknown) with increased sensitivi
plays a vital role in this task. A few typical applications will be discussed as follows :

(*i*) The detections of steroid drugs used by athletes in international sports competitions and the
administered to animals in traces are being carried out by GLC. Hazardous pollutants
formaldehyde, carbon monoxide, trichloroethylene, benzene and acrylonitrile can be moni
GLC. Analysis of volatile fatty acids produced by bacteria, particularly anaerodic bacteria,
fingerprinting of the particular micro-organisms giving rise to the identification of the ba

(*ii*) In analysis of foods, the separation and identification of lipids, proteins, carbohydrates, preser
flavors, colorants and texture modifiers, as well as vitamins, steroids, drug and pesticide
and trace elements are involved. As most of the compounds are non-volatile, HPLC is ne
for food analysis but GLC is also frequently used by converting them into a volatile form
for separation. This is termed as '*derivatization*'. Some examples include, conversion of
fatty acid methyl esters, of proteins by acid hydrolysis followed by esterification, and silyl
carbohydrates.

(*iii*) It is possible to analyse the dairy products by GLC for aldehydes and ketones (for rancidity
acids (by derivatization), and milk sugars. Butter is analyzed for the butter fat content,
added colors and flavours.

(*iv*) GLC finds valid applications in drug analysis, though HPLC is playing increasingly promine
Some examples are analyses of commercial drug preparations, illicit drug samples, blood
samples and stomach contents.

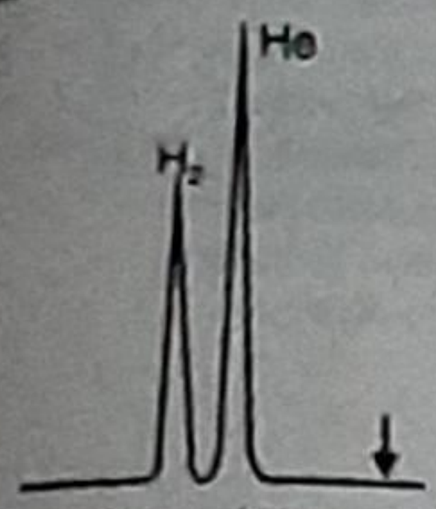
(*v*) It is possible to use the Pyrolysis GC for separation and identification of volatile materia
plastics, natural and synthetic polymers, paints, and microbiological samples. The chromato
provide valuable information on the molecular structure from the identification of the py
products and the chemical composition of the samples. For example, information can be ob
on the nature of the polymeric material, plasticizers and other additives in a plastic sample

(*vi*) It is known that inorganic compounds are mostly non-volatile. However they can be subjec
GC studies by derivatization into volatile compounds. Some metal chlorides and hydrid
inherently volatile. Some organometallics such as boranes, silanes, germanes, organotin and o
lead compounds are amenable for GLC separations. A number of metal chelates have been sep
by GLC. A typical example involves the separation of the β-diketonates of chromium (III
other di-, tri-, and tetravalent metals which are thermally stable, soluble in organic solvent
volatile. The most frequently used ligands for derivatization of metal ions include acetyl ac
trifluoroacetyl acetone, and hexafluoroacetone. Increasing fluorination of the ligands gives
a more volatile metal chelate.

(*vii*) Some examples of application for GC for environmental studies include separation and identific
of polycyclic aromatic hydrocarbons, chlorinated pesticides (*e.g.*, DDT, BHC), organophosp
and sulphur compounds, phenols, amines and organic acids, organotin biocides, organolead
organomercury compounds.

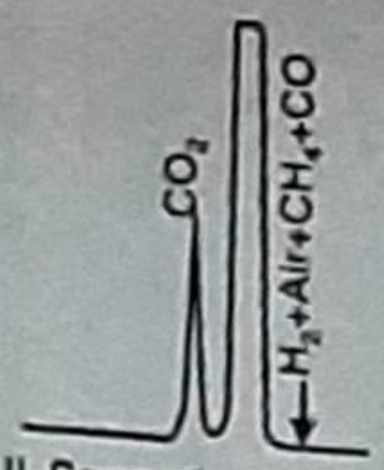
Some chromatograms are given below to illustrate typical applications of gas chromatography.

Conditions :
Column : 18'x 1/4" OD, Molecular Sieves 5A
Temp. : 40°C



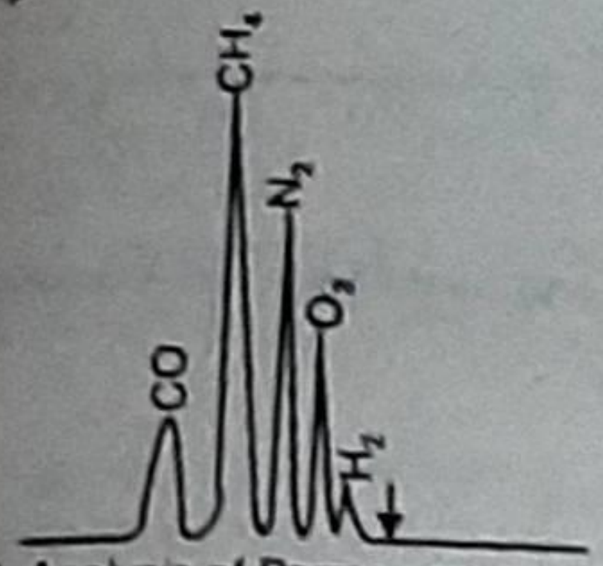
I. Separation of He and H_2

Conditions
Column : 2'x 1/4" OD, Silica Gel
Temp : 100°C



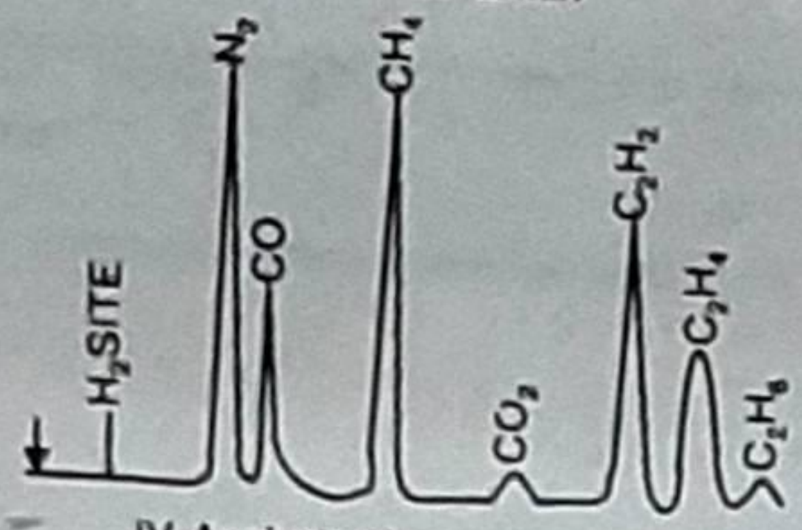
II. Separation of CO_2

Conditions :
Column : 6'x 1/4" OD, Molecular Sieves 5A
Temp. : 50°C



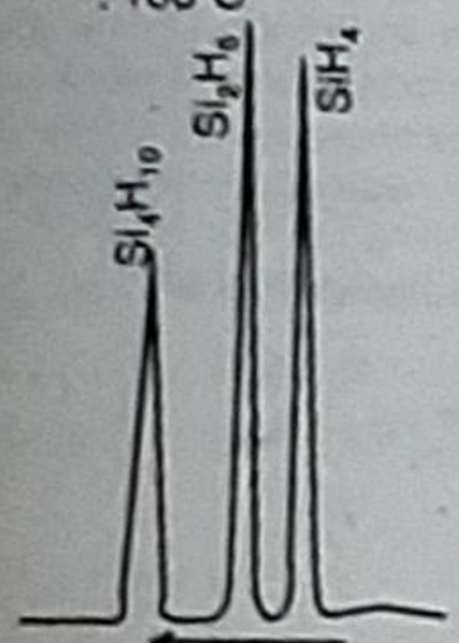
III. Analysis of Permanent gases.

Conditions :
Column : 9'x 1/4" OD, Active Charcoal
Temp. : 50–250°C @ 30°C/min



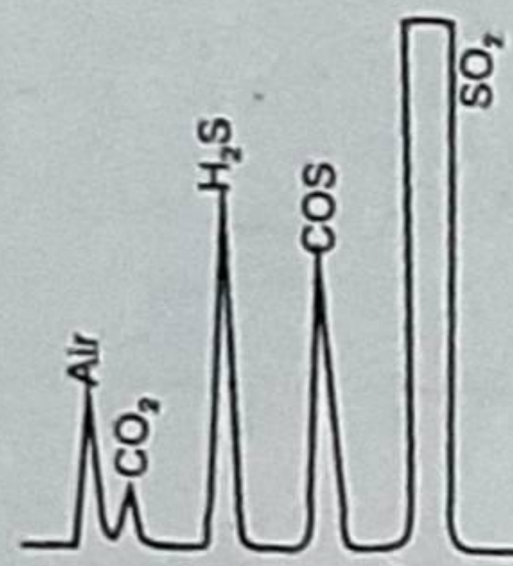
IV. Analysis of gaseous mixture.

Conditions :
Column : 4' 1/4" OD, 25% Silicone oil 702 on Chromosorb P
Temp. : 100°C

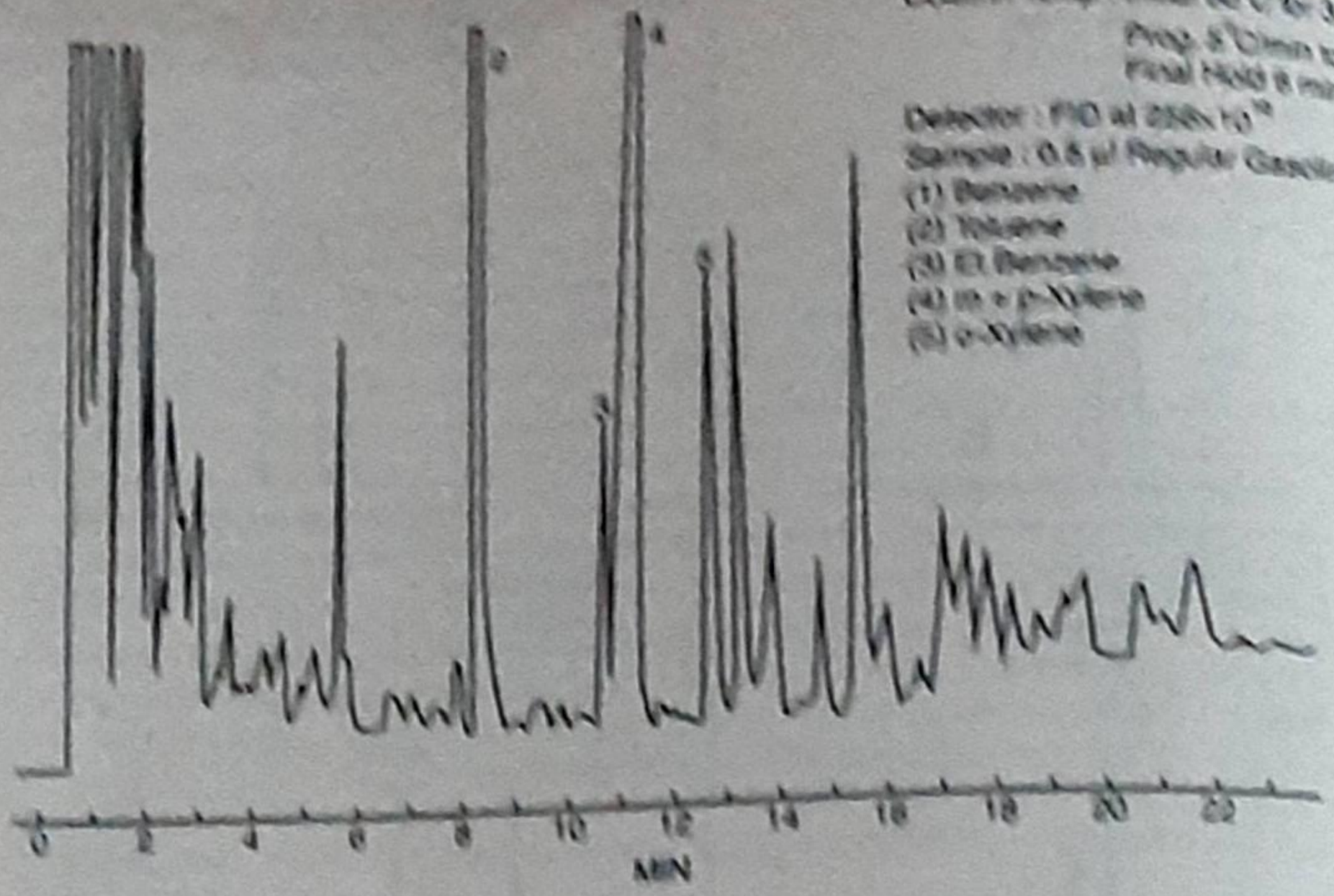


V. Analysis of Silanes.

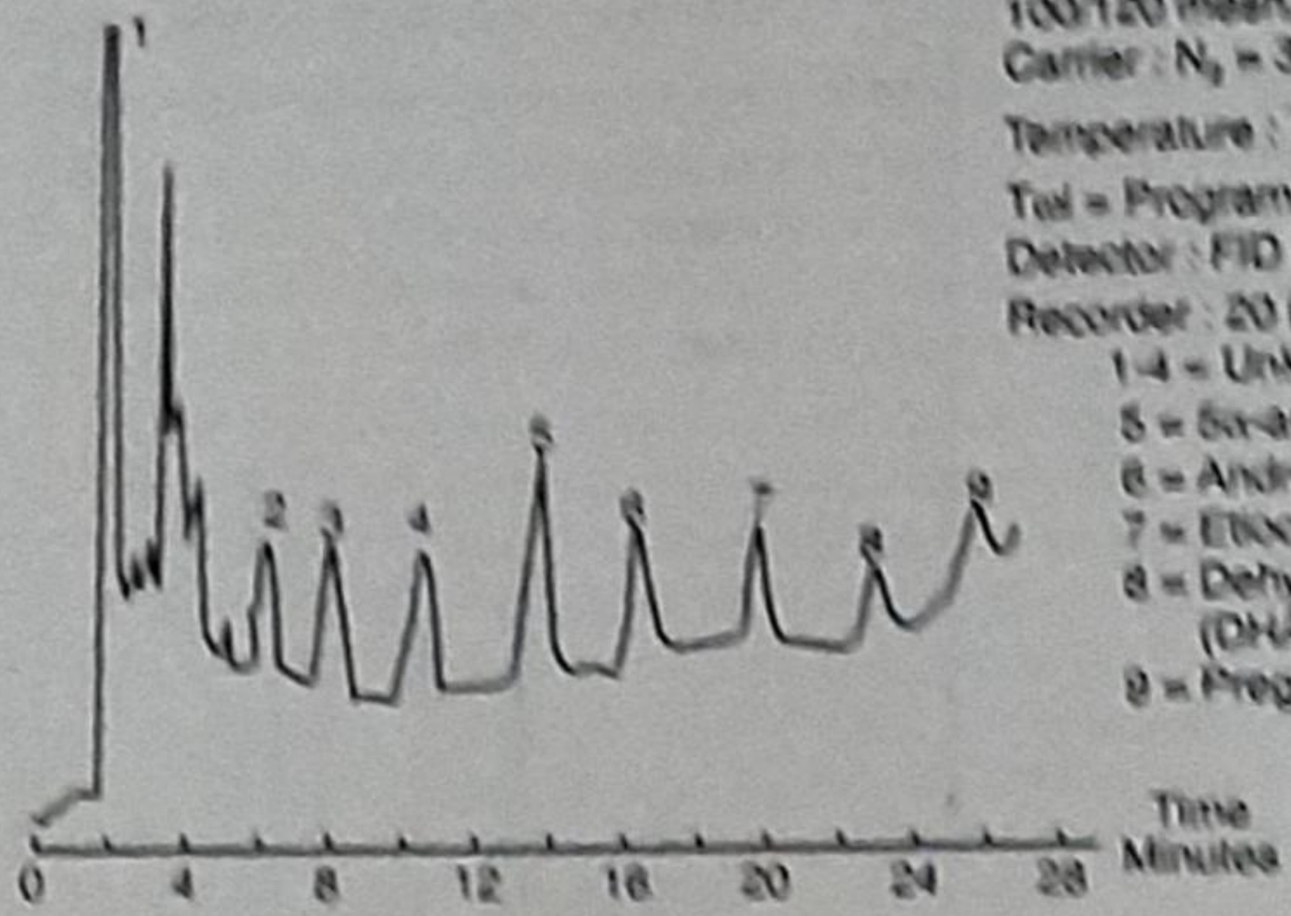
Conditions :
Column : 6' x 1/8" pora pak OD,
Temp. : 70°C



VI. Analysis of Sulphur gases.



Gas chromatographic separation of benzene and homologues in gasoline.



Gas chromatographic separation of steriod androgens by temperature programming.