Talk on

GAS CHROMATOGRAPHY

Contents :

- **Introduction**
- **Instrumentation of GC**
- **Theory of gas chromatography**
- **Column efficiency parameters**
- **Derivatization methods used in GC**
- **Examples of GC applications in pharmaceutical analysis**

What is Gas Chromatography?

It is also known as…

Gas-Liquid Chromatography (GLC)

GAS CHROMATOGRAPHY

- Separation of gaseous & volatile substances
- Simple & efficient in regard to separation

GC consists of **GSC** (gas solid chromatography)

GLC (gas liquid chromatography

Gas → M.P

Solid / Liquid → S.P

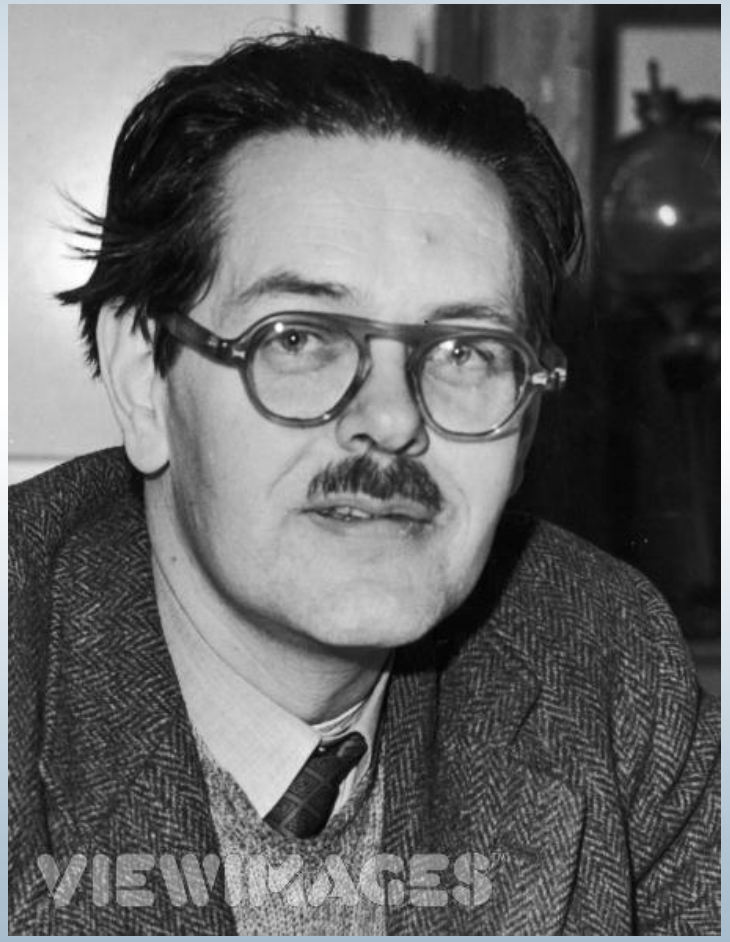
GSC not used because of limited no. of S.P

GSC principle is **ADSORPTION**

GLC principle is **PARTITION**

What is Gas Chromatography?

The father of modern
gas chromatography
is Nobel Prize winner
John Porter Martin,
who also developed the first
liquid-gas chromatograph. (1950)



Agilent Technologies

EVOLUTION of EXCELLENCE

First Electronic Pneumatics Control

First Capillary Flow Device (μ-dean switch)

First Fused Silica Capillary Column

First Bench Top GC/MS

First Microprocessor Controlled GC

1958

1973

1976

1979

1984

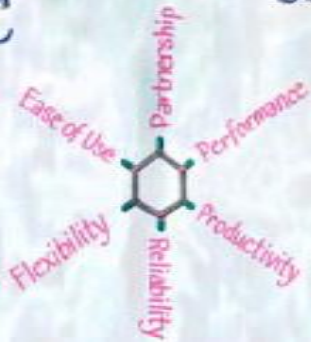
2004

5880GC

5890GC

6890GC

5830GC



GAS CHROMATOGRAPHY

- **Gas chromatography (GC) is a widely used technique for separation & analysis of gaseous & volatile substances which are difficult to separate & analyze.**
- **In performing gas chromatographic separation, the sample is vaporized & injected onto the head of a chromatographic column.**
- **Elution is brought about by the flow of an inert gaseous mobile phase.**
- **In GC gas as a moving phase is passed through a column containing solid adsorbent or liquid adsorbent. Thus adsorption or partition is possible.**
- **Based on stationary phase used in column, G.C is of 2 types :**

a. Gas solid chromatography (GSC)

b. Gas liquid chromatography (GLC).

a. GSC : **Mobile phase – gas**

Stationary phase – solid

In GSC, when a carrier gas containing analytes is passed through a column containing solid Stationary phase, the analytes get adsorbed on to the solid Stationary phase & the separation is due to differences in their adsorptive behavior.

b. GLC : **Mobile phase – gas**

Stationary phase – liquid

In GLC, when a carrier gas containing analytes is passed through a column containing liquid Stationary phase, the analytes get distributed themselves between the liquid Stationary phase & the carrier gas phase according to their partition coefficients.

- **In GLC, Stationary phase is liquid that is retained/coated on the surface of an inert solid by adsorption or chemical bonding.**

GLC finds much greater application in the analysis of most of the organic compounds, while GSC has limited application owing to :

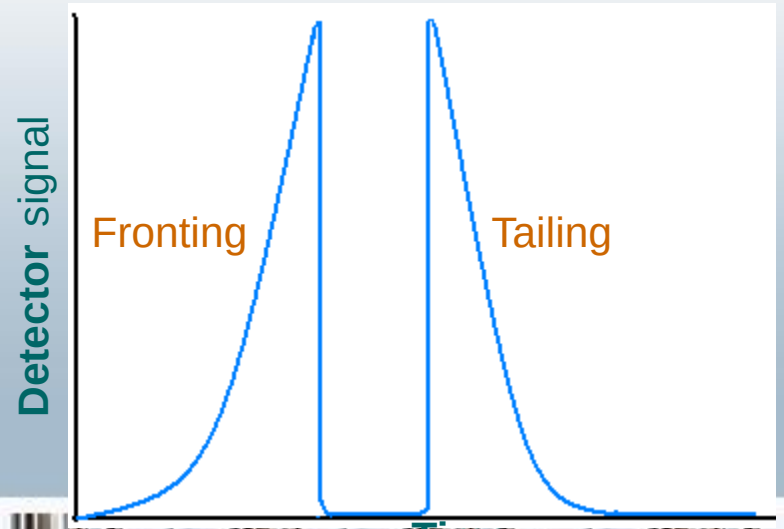
a. Semi permanent retention of active or polar molecules which reduces the available area

b. Severe tailing of elution peaks (a consequence of nonlinear character of adsorption process)

c. Difficulty in reproducing surface conditions

d. Surface catalyst also plays a restricting role

e. GSC finds application only in the separation & determination of low molecular mass gases such as air components, hydrogen sulfide, CO and nitrogen oxides.



Criteria for compounds to be analyzed by GC :

- Volatility
- Thermostability
- The analyte should have a measurable vapour pressure at the temperature employed.

Advantages of Gas chromatography :

a. It is a simple & inexpensive method, generally efficient with regard to separation.

b. The technique has a very high resolution power.

c. Small sample is needed – µLs.

d. Sensitivity of detection is very high (PPB or Picograms).

e. The speed of analysis is fast.

e. Qualitative & quantitative analysis at a time is possible. The area produced under each peak is proportional to that concentration.

How a Gas Chromatography Machine Works

- **First,** a vaporized sample is injected onto the *chromatographic column*.
- **Second,** the sample moves through the column through the flow of inert gas.
- **Third,** the components are recorded as a sequence of peaks as they leave the column.
- Deals with both the *stationary phase* and the *mobile phase*.
  - **Mobile** – inert gas used as carrier.
  - **Stationary** – liquid coated on a solid or a solid within a column

Chromatographic Separation

- In the mobile phase, components of the sample are uniquely drawn to the stationary phase and thus, enter this phase at different times.
- The parts of the sample are separated within the column.
- Compounds used at the stationary phase reach the detector at unique times and produce a series of peaks along a time sequence.
- The peaks can then be read and analyzed by a forensic scientist to determine the exact components of the mixture.
- Retention time is determined by each component reaching the detector at a characteristic time.

Instrumentation :

It consists of:

1. **Carrier gas tank**
2. **Flow regulators**
3. **Sample injection system**
4. **Column & Stationary phase**
5. **Detectors**

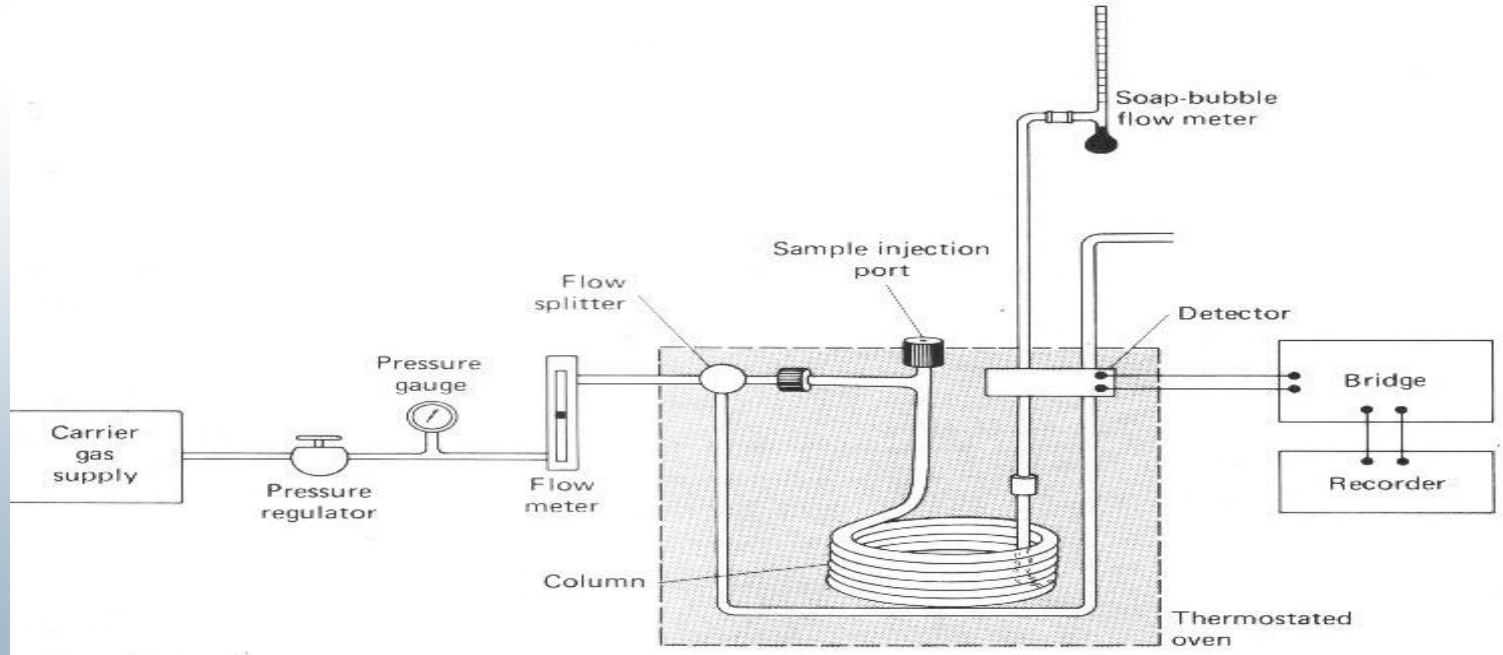


FIGURE 26–2 Schematic diagram of a gas chromatograph.

Carrier gas system :

The main purpose of carrier gas is to transport sample components through the column.

It determines the efficiency of the column, the time of analysis and the sensitivity of a given detector.

Factors are considered while selecting a carrier gas :

- **Chemically Inert**
- **Available in pure and stable form**
- **Free from moisture**
- **Should give best column performance consistent with desired speed of analysis**
- **Suitable for detector used**
- **Inexpensive, readily available**
- **Safe in handling**

Make up gas: Flow rate required for most detectors is 30 - 40 ml/min

Mobile phases or carrier gases used in GC :

GAS	APPLICATION	COMMENTS
Helium	General carrier gas or make up gas	Expensive
Nitrogen	General carrier gas or make up gas	Very cheap but not good for capillary columns as it gives a long run time
Oxygen	Combustion gas for FID	Not normally used
Argon	Carrier gas for TCD	----------
Hydrogen	Carrier gas for capillary column & combustion gas for FID	Cheap & explosive
Air	Combustion gas for FID, NPD, FPD	Cheap & readily available
Argon + methane	Make up gas & carrier gas for packed columns with ECD.	Better linearity & selectivity than N_2 but poor detection limits.

- **Pressure regulators, gauges & flow meters are required to control the flow rate of carrier gas.**
- **Inlet pressures usually range from 10 to 50 psi greater than the room pressure, which lead to flow rates of –**

25 to 150 ml/min for packed column

1 to 25 ml/min with open-tubular capillary columns.

Measurement of flow rate :

- **To maintain optimum operating conditions for analysis**
- **To reproduce these conditions when necessary.**

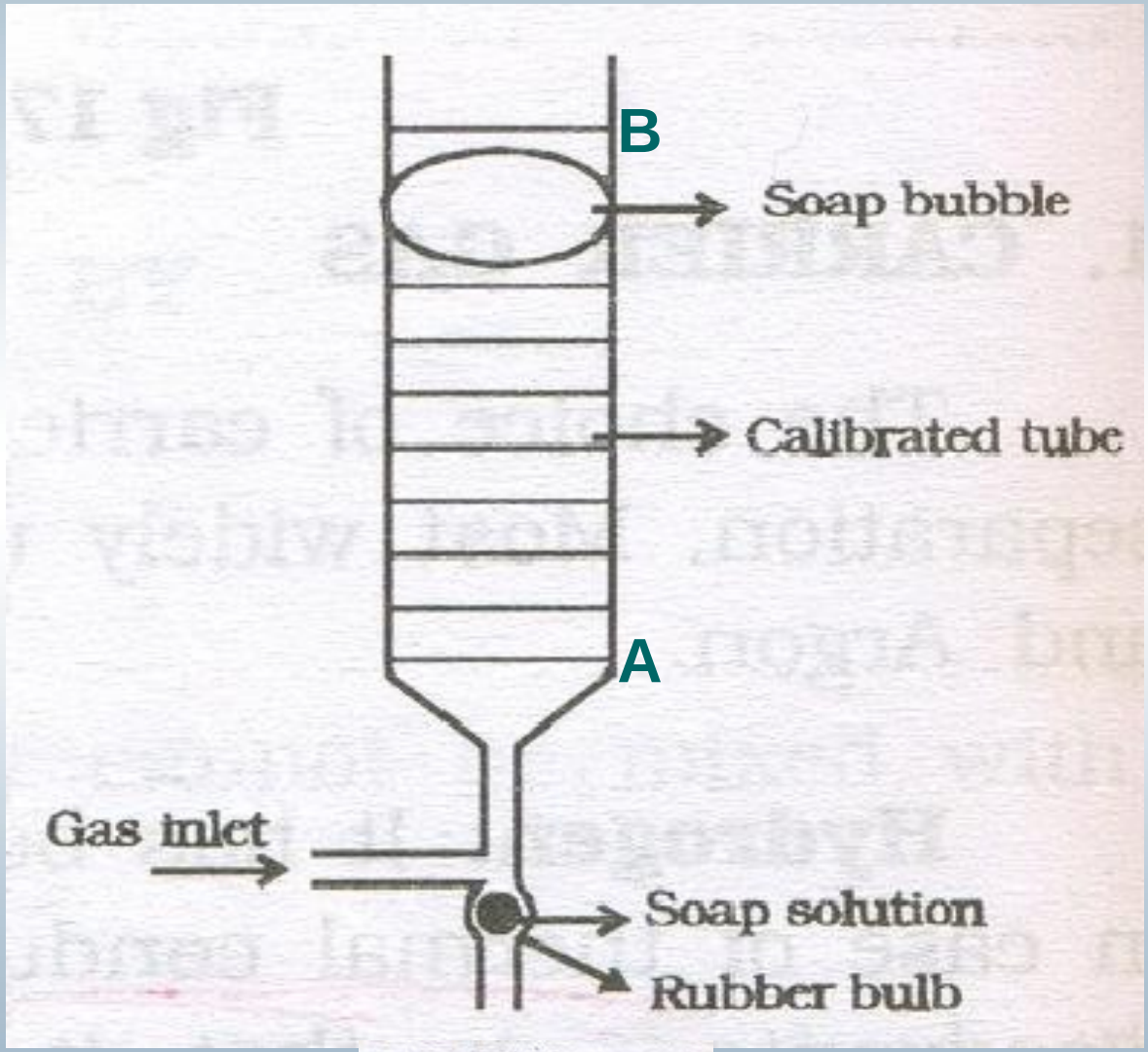
Devices commonly employed to measure flow rate :

a. Rotameter :

- **It is fixed in line with carrier gas, at the column head.**
- **This device is not as accurate as the simple soap bubble meter.**

b. soap bubble meter :

- It is located at the end of the column.



Soap bubble meter

The time is then converted into volumetric flow rate by the equation:

$$\text{Volumetric flow rate} = \frac{\text{Distance between graduations A \& B}}{\text{Time taken}}$$

Volumetric flow rate can be converted into linear flow velocities by the equations :

$F = u_o A = u_o X \P r^2$ ⟶ **for an open tubular column**

$F = \P r^2 u_o \varepsilon$ ⟶ **for packed column.**

Where, F - Volumetric flow rate (Cm^3/min)

u_o- Linear flow velocity

A – Area of the tube

ε – Fraction of total volume of column available for gas.

Sample injection system :

- Column efficiency requires that sample be of suitable size & be introduced as a plug of vapour.
- Slow injection or over sized samples cause band spreading & poor resolution.
- Calibrated micro syringes are used to inject liquid samples through a rubber or silicone diaphragm, or septum, into a heated sample port located at the head of the column.

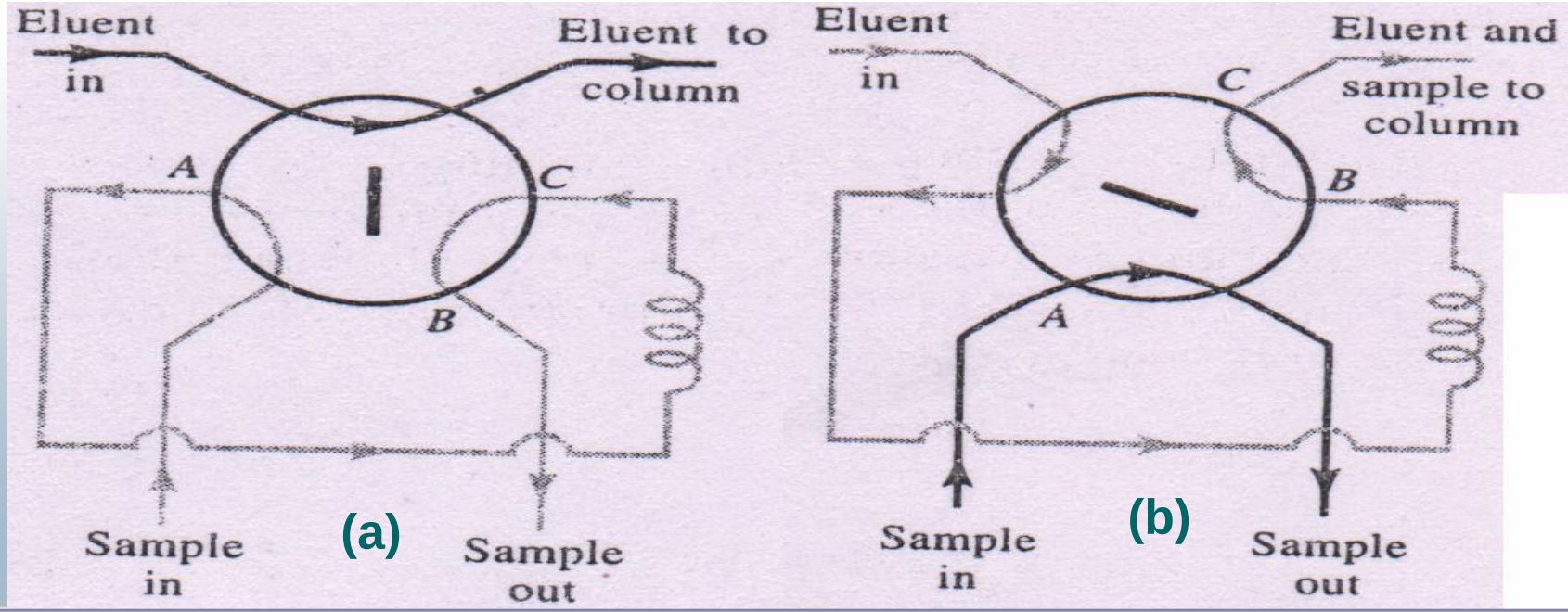
- **The sample port is ordinarily kept at about 50^0 C greater than the boiling point of the least volatile component of the sample.**

SAMPLE SIZE

Ordinary packed column ---- 0.1 uL to 20 uL

Capillary column ----- smaller by a fraction of 100 or more.

- **A sample splitter is always needed with capillary columns.**
- **Reproducible sample sizes for both liquid & gases are obtained by means of a rotary sample value.**



(a) - is for filling the sample loop ACB (b) – for introduction of sample into column

- **Solid samples are introduced as solutions or sealed into thin walled vials that can be inserted at the head of the column & punctured or crushed from outside.**

GAS CHROMATOGRAPHIC COLUMNS :

Two general types of columns are encountered in GC :

a. **Packed columns**
b. **Capillary columns or open tubular columns.**

- **Packed column length 1.5 -10 m and Capillary column 10-100 m**
- **Constructed of stainless steel, glass, fused silica or Teflon.**
- **To fit into an oven for thermostating, they are usually formed into coils having diameters of 10 to 30 cms.**

a. **Packed columns :**

- **Have inside diameter of about 2 to 4mms.**
- **These tubes are densely packed with a uniform, finely divided packing material or solid support (diatomaceous earth, glass beads), that is then coated with a thin layer of stationary liquid phase.**

Solid support materials :

Purpose – It serves to hold the liquid stationary phase in place so that as large a surface area as possible is exposed to the mobile phase. Egs – naturally occurring diatomaceous earth, glass beads.

Ideal characters :

- **Should be small, uniform, spherical.**
- **Good mechanical strength.**
- **Specific surface area of at least $1m^2/g$.**
- **Inert at elevated temperatures.**
- **Uniform wetability.**

- Support materials are often treated chemically with dimethylchlorosilane.
- The HETP is proportional to average particle diameter so that smallest possible particles should be preferred in terms of column efficiency.
- Particle size is 60 to 100 mesh (250 to 170 um).

b. Open tubular or capillary columns :

Inside diameter --- 1mm.

Capillary columns are of 2 basic types :

1. **Wall-coated open tubular columns (WCOT)**
2. **Support -coated open tubular columns (SCOT).**

1. **WCOT : They are simply capillary tubes coated with a thin layer of the stationary phase.**

WCOT columns are constructed with stainless steel, aluminum, copper, plastic & glass.

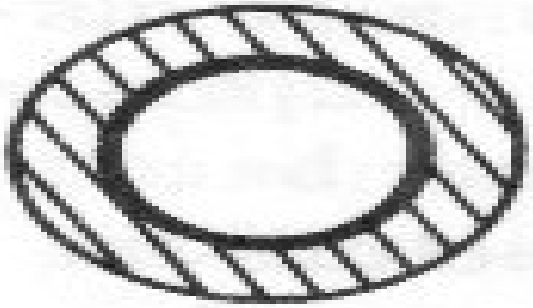
- **The column is treated with gaseous HCl, strong aqueous HCl, or potassium hydrogen fluoride to give a rough surface, which bonds the stationary phase more tightly.**

2. SCOT : The inner surface of capillary is lined with a thin film (30um) of a support material such as diatomaceous earth on which stationary phase is coated.

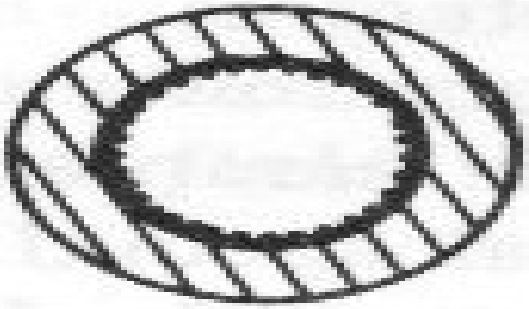
- **SCOT columns are less efficient than WCOT columns but have a higher sample capacity which enable them to be used without a sample splitter.**

- **A recent advancement in capillary columns are fused-silica open tubular columns (FSOT) columns. They are drawn from specially purified silica that contains minimal amounts of metal oxides.**

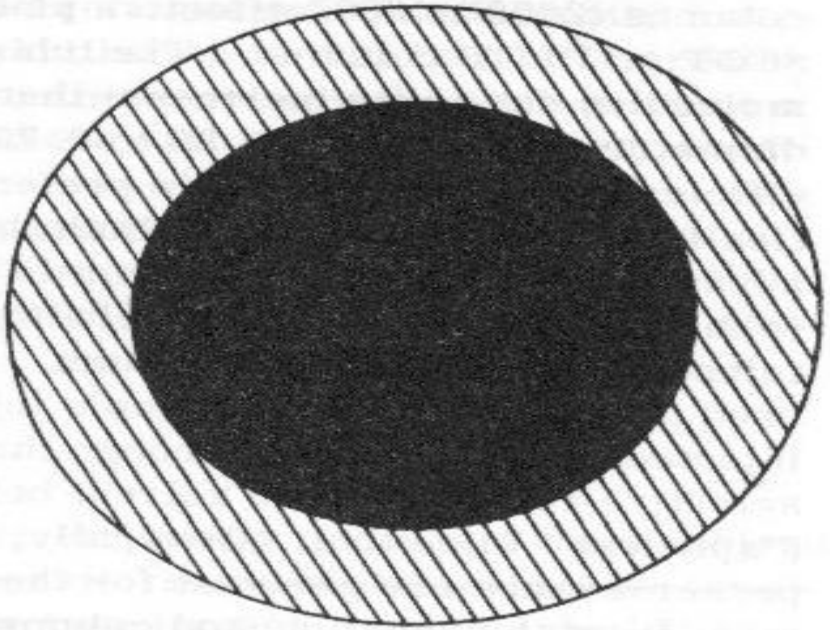
WCOT



SCOT
or
PLOT



Packed Column



LIQUID STATIONARY PHASES :

Desirable properties :

a. **Low volatility (ideally the B.P. of the liquid stationary phase should be at least 100^{o}C higher than the maximum operating temperature of the column).**

b. **It should be thermally stable.**

c. **Chemically inert.**

- **The proper choice of stationary phase is often crucial to the success of a separation.**

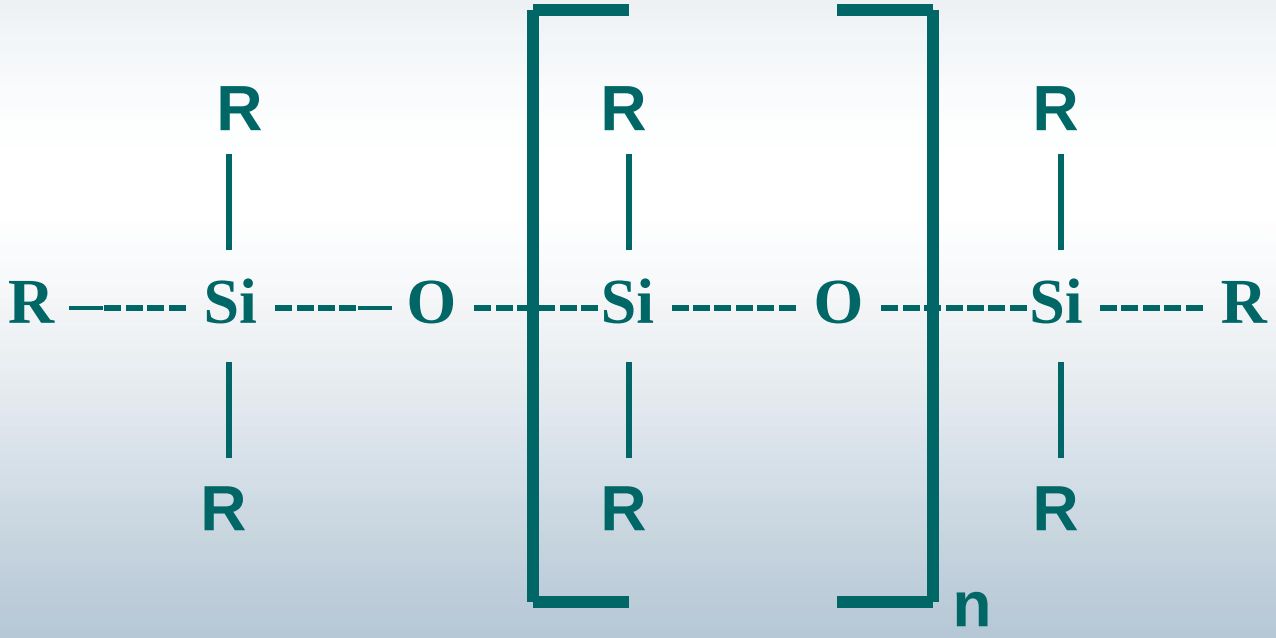
Some important facts about stationary phase :

- **The retention time for an analyte on a column depends on its distribution constant, which in turn is related to the chemical nature of the liquid stationary phase.**
- **To separate various sample components, their distribution constants must be sufficiently different to accomplish a clean separation.**
- **At the same time, these constants must not be extremely large or extremely small.**
- **To have a reasonable residence time in the column, an analyte must show some degree of compatibility (stability) with the stationary phase.**
- **Principle involved ⟶ "Like dissolves Like".**
- **Generally the polarity of the stationary phase should match with that of the sample components. When the match is good, the order of elution is determined is determined by the boiling point of eluents.**

Some common liquid stationary phases used in GLC :

STATIONARY PHASE	TRADE NAME	MAX. TEMP, °C	APPLICATIONS
Polydimethyl siloxane	OV-1, SE-30	350	General purpose nonpolar phase, hydrocarbons, polynuclear aromatics, steroids.
5% Phenyl - Polydimethyl siloxane	OV-3, Se-52	350	Fatty acid methyl esters, alkaloids, drugs, halogenated compounds.
50% phenyl - Polydimethyl siloxane	OV-17	250	Drugs, steroids, pesticides, glycols.
50% Trifluoroproply - Polydimethyl siloxane	OV-210	200	Chlorinated aromatics, nitro aromatics, alkyl substituted benzenes
Polyethylene glycol	Carbowax 20M	250	Free acids, alcohols, ethers, essential oils, glycols.
50% Cyanopropyl - Polydimethyl siloxane	OV-275	240	Polyunsaturated fatty acids, rosin acids, free acids, alcohols.

General structure of Poly siloxanes :



If R = CH_3 gives Polydimethyl siloxane.

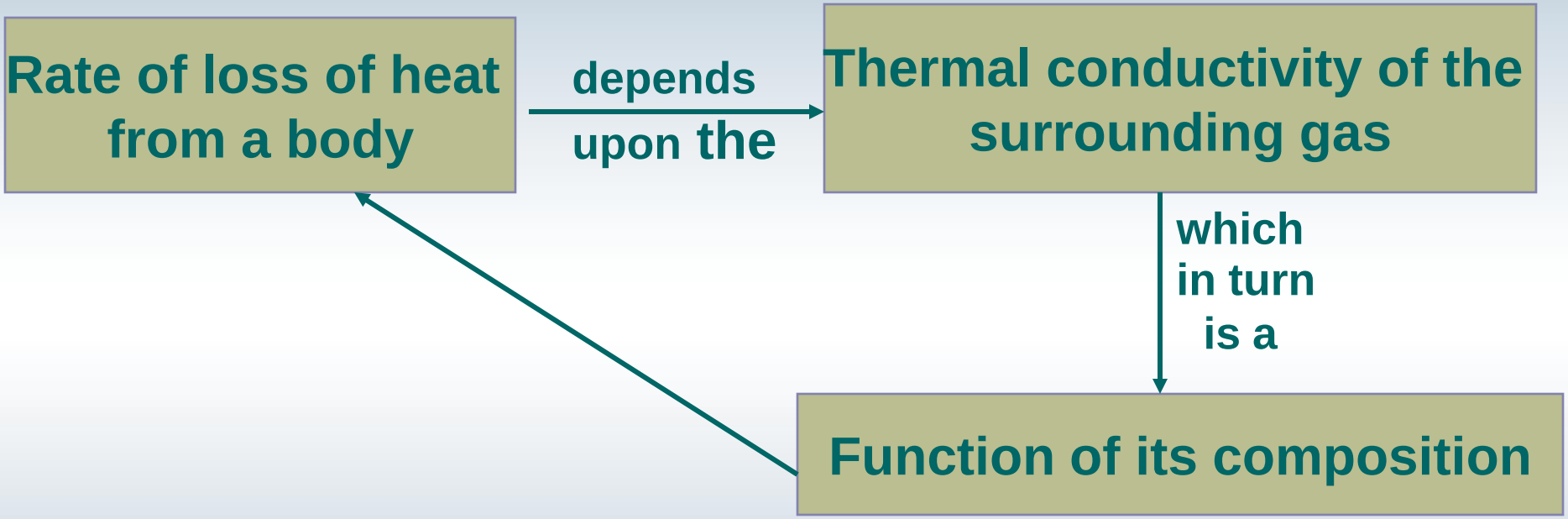
Detectors :

Characteristics of an Ideal detector :

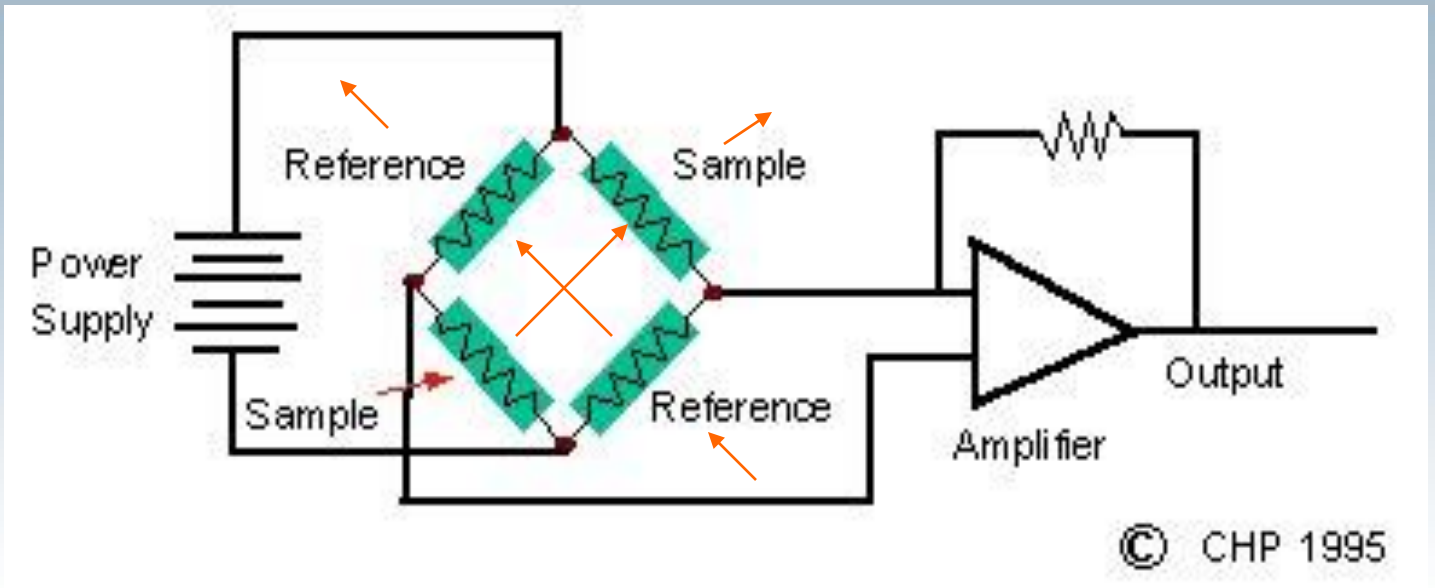
1. **Adequate sensitivity : Generally a detector should detect the solute in picogram quantities.**
2. **Good stability and reproducibility.**
3. **A linear response to solutes that extends over several orders of magnitude.**
4. **A temperature range from room temperature to atleast 400^{o} C.**
5. **A short response time that is independent of flow rate.**
6. **High reliability & ease of use.**
7. **Similarity in response toward all solutes or, alternatively, a highly predictable & selective response toward one or more classes of solutes.**
8. **Non destructive of sample.**

Thermal conductivity detector :

- **Also called as Katharometer or Hot wire detector.**
- **Principle :**



- **Thus the rate of loss of heat is related to the composition of the surrounding gas.**



- **TCD filaments are made up of fine platinum, gold or tungsten wire.**
- **TCD thermistors are made of Oxides of manganese, Cobalt or nickel to which some trace elements are added.**

<u>Advantages</u> :

- **Simple.**
- **Large linear dynamic range.**
- **It gives general response to both organic & inorganic species.**
- **Non destructive character, which permits collection of solutes after detection.**

<u>Limitation</u> : Low sensitivity.

Relative Thermal Conductivity

Compound	Relative Thermal Conductivity Ohm^{-1}
Carbon Tetrachloride	0.05
Benzene	0.11
Hexane	0.12
Argon	0.12
Methanol	0.13
Nitrogen	0.17
Helium	1.00
Hydrogen	1.28

Flame Ionization Detector :

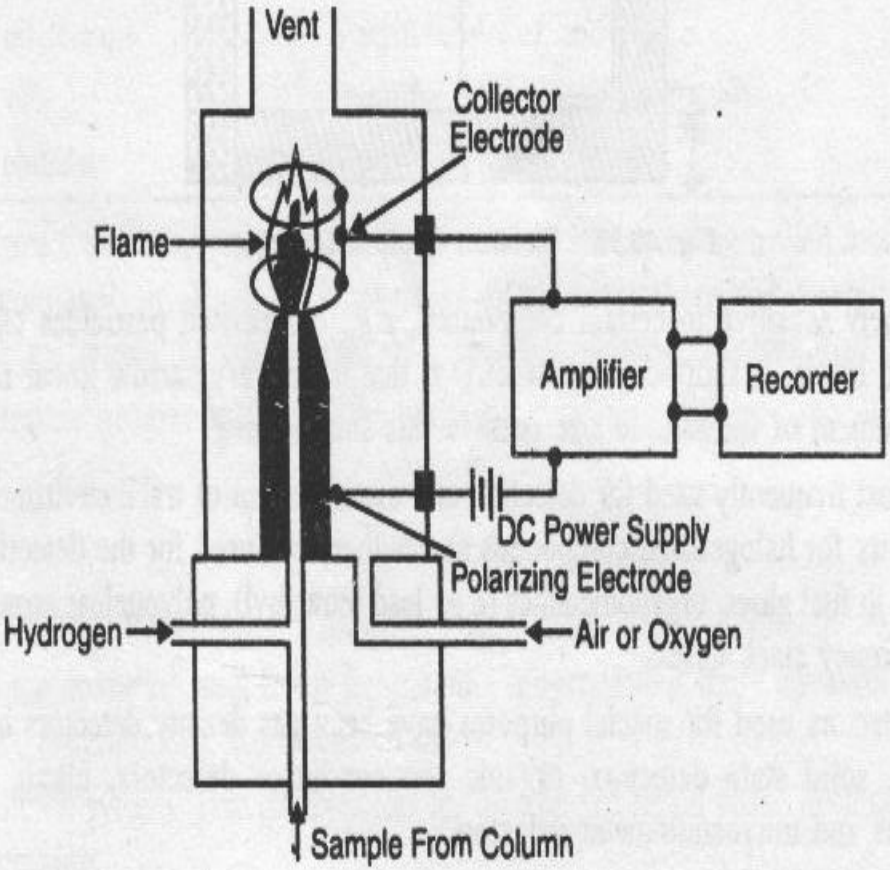


Fig. 40.9 : Flame Ionization Detector (schematic)

Advantage : FID is not concentration sensitive but is rather mass sensitive. That is it gives response proportional to total mass of component entering the detector & therefore independent of carrier gas flow rate.

Electron capture detector :

- **It responds to only those compounds whose molecules have an affinity for electrons.**
- **E.g. : halogen containing organic compounds such as pesticides & polychlorinated biphenyls; peroxides; quinones & nitro group containing compounds.**
- **On the other side, it responds very little to compounds such as hydrocarbons, alcohols, amines.**

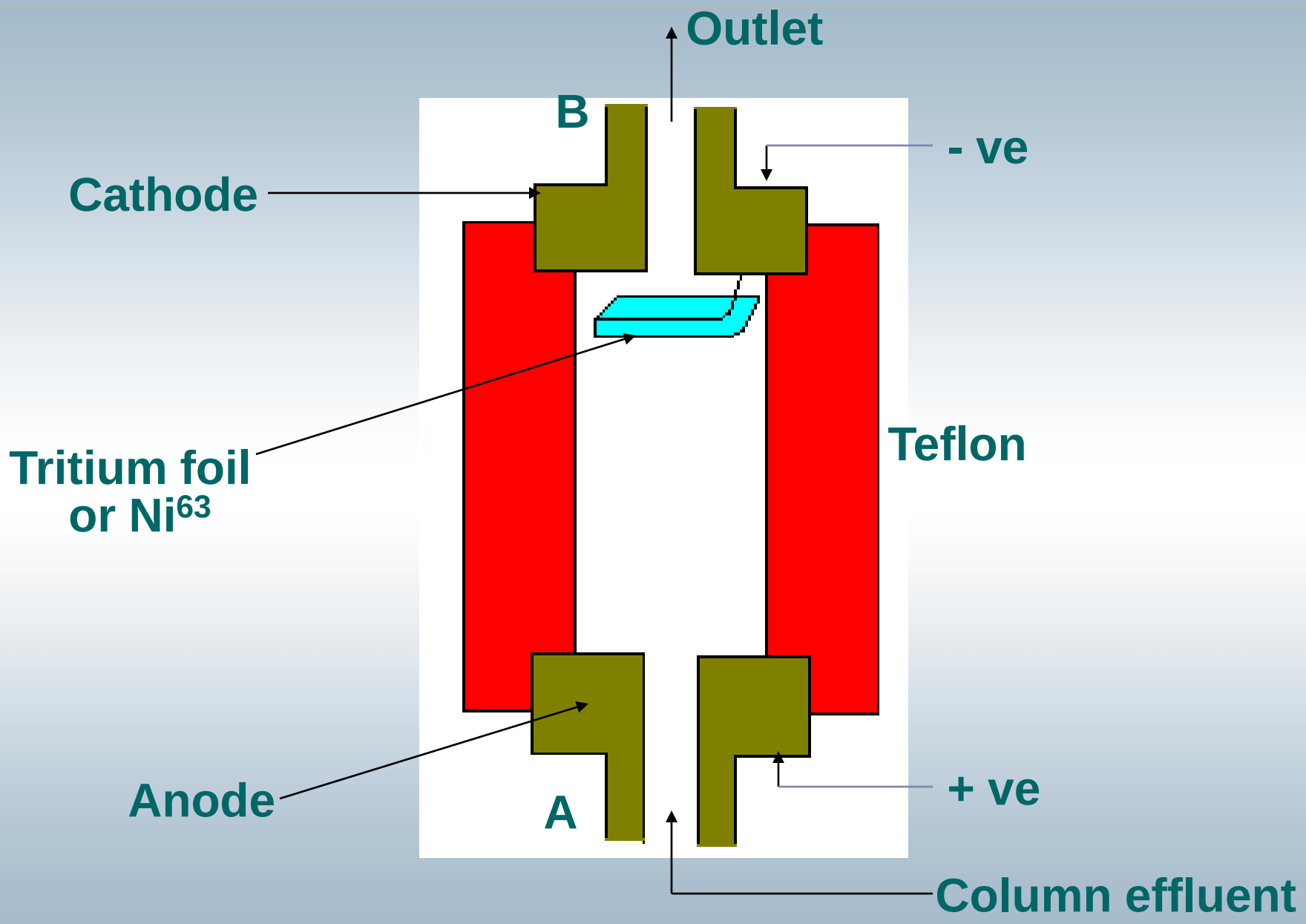

Outlet
B
- ve
Cathode
Tritium foil
or Ni63
Teflon
Anode
A
+ ve
Column effluent

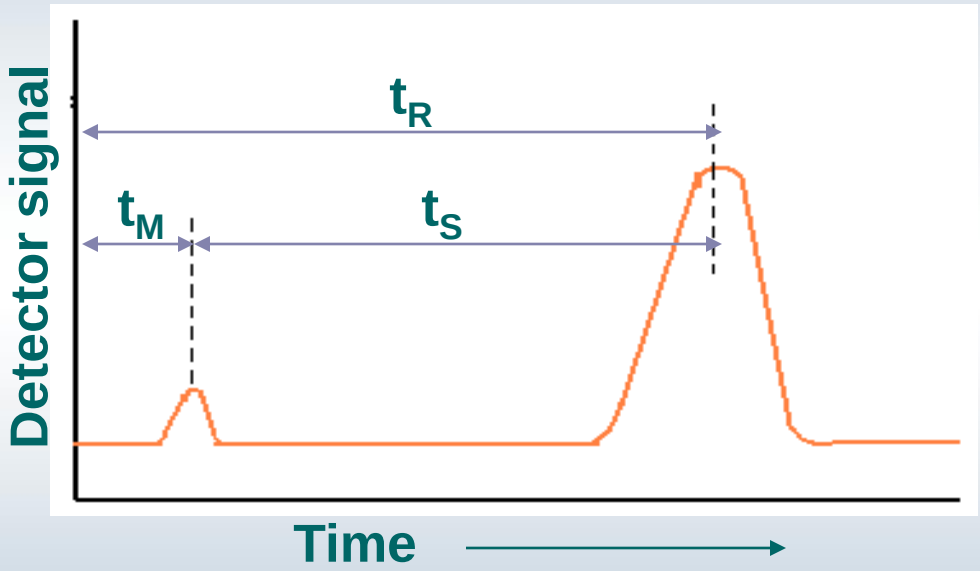
TABLE 3 Important Characteristics of Gas Chromatographic Detectors

Detector	Compounds detected	Linear dynamic range	Minimum detectable quantity (g)
Thermal conductivity (TCD)	Most compounds	10^3–10^4	10^{-6}–10^{-8}
Flame Ionization (FID)	Most organic	10^6–10^7	10^{-10}–10^{-11}
Thermionic (NP-FID)	Phosphorus, nitrogen sulfur, halogen compounds	10^3	10^{-12}
Electron capture (ECD)	Halogen, oxygenated, highly conjugated compounds	$>10^4$ (variable-frequency-pulsed)	10^{-12}–10^{-13}
Flame photometric (FPD)	Phosphorus, sulfur compounds	S—5×10^2; P—10^4 on log-log scale	S—2×10^{-10} P—4×10^{-11}
Photoionization (PID)	All with ionization potential < 10.2 eV	$>10^7$	2×10^{-12}

Theory of gas chromatography :

Retention time (t_R) :

The time gap between the sample injection & the appearance of chromatographic peak for a particular analyte is called as retention time.



Retention time is then :

$$t_R = t_M + t_S$$

Uses of dead time or void time :

- Provides a measure of the average rate of migration of the mobile phase.
- Is an important parameter in identifying analyte peaks.
- All components spend time t_M in the mobile phase.

Resolution (R) :

Resolution is the degree of separation between the two adjacent peaks i.e. the distance between the two adjacent peaks divided by the mean of the peak widths.

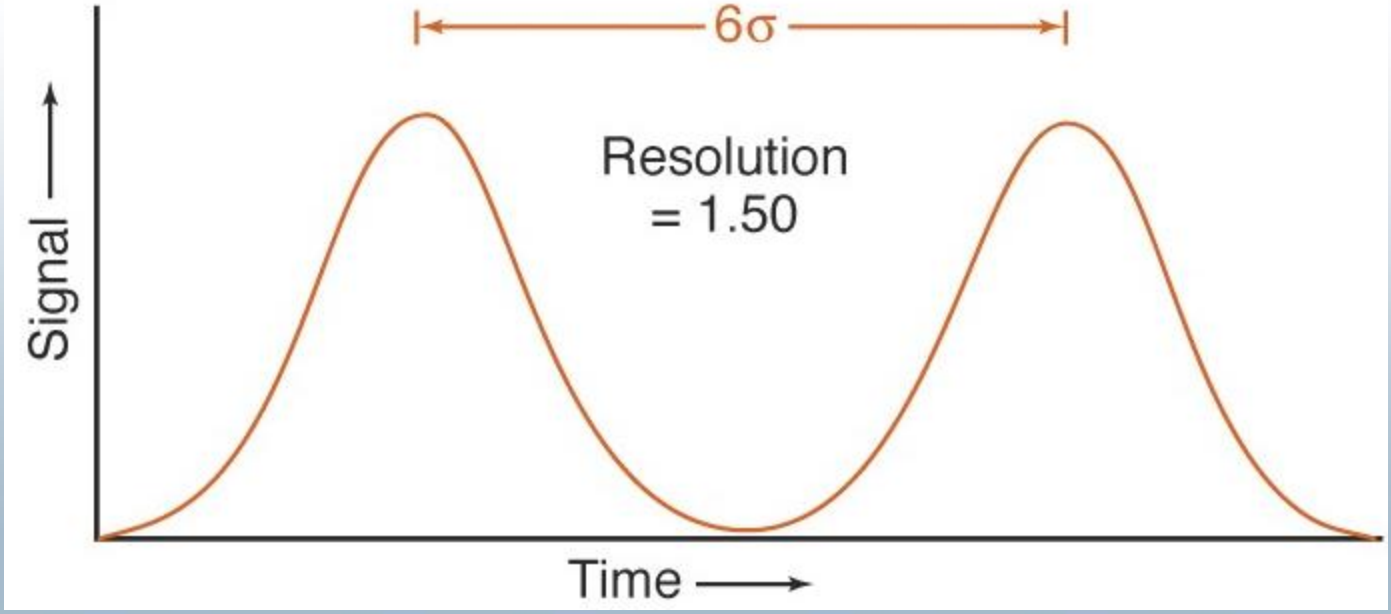
$$R = \frac{2d}{tw_1 + tw_2}$$

where, d = distance between two peaks.

tw1 = width of first peak.

tw2 = width of second peak.

If R = 1.5, baseline separation (99.7% resolution) is achieved.



The resolution of chromatographic peaks is dependent on two parameters viz column efficiency & solvent efficiency.

Column Efficiency :

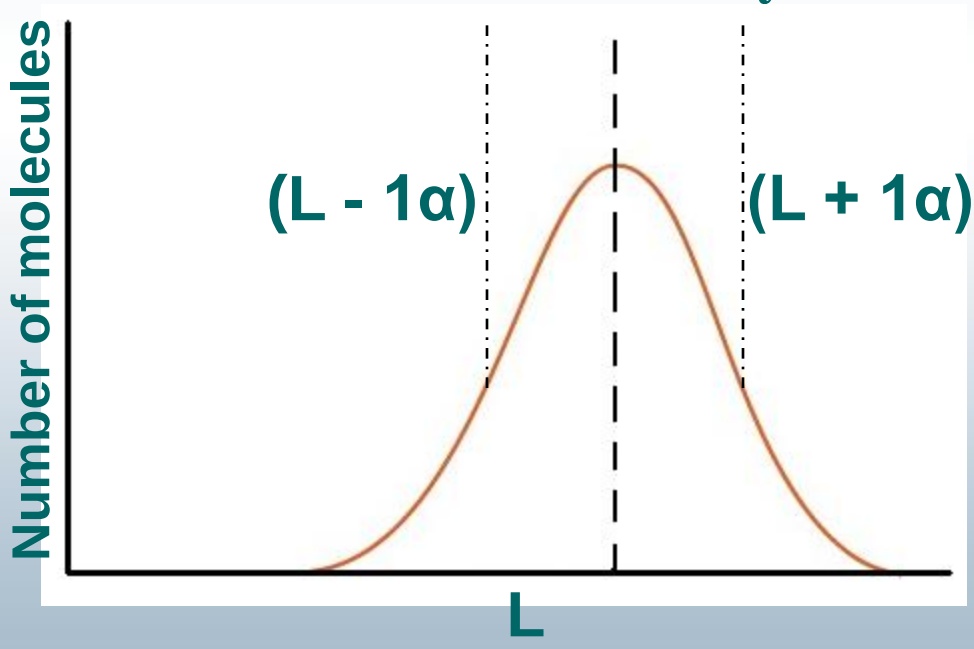
Chromatographic bands are usually Gaussian curves or normal error curves & the efficiency of the column is reflected in the breadth of chromatographic peak. Hence column efficiency "H" can be defined as :

$$H = \frac{\sigma^2}{L}$$

Where, σ = standard deviation

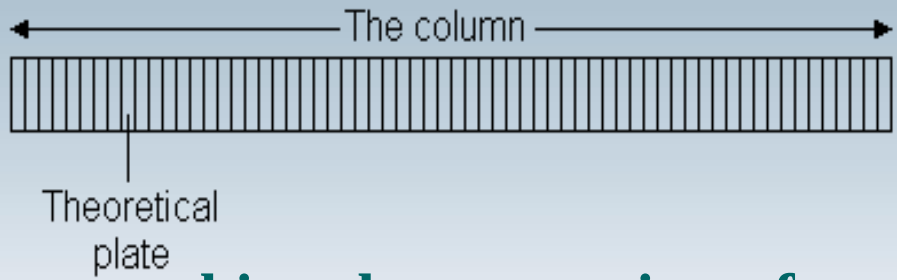
σ^2 = Variance

L = length of column.



The factors affecting column efficiency can be explained with the help of 2 theories : Plate theory

Rate theory.



1. Plate theory :

According to this theory, the chromatographic column consists of discrete, but continuous, narrow, horizontal plates of equal units called as "theoretical plates".

Following assumptions are made :

- **All the solute particles are placed on the first theoretical plate.**
- **During the chromatographic process the equilibrium of the solute between mobile & the stationary phase takes place at each theoretical plate with the step wise transfer of solute & solvent from one plate to next.**
- **The partition coefficient of all components remain constant.**

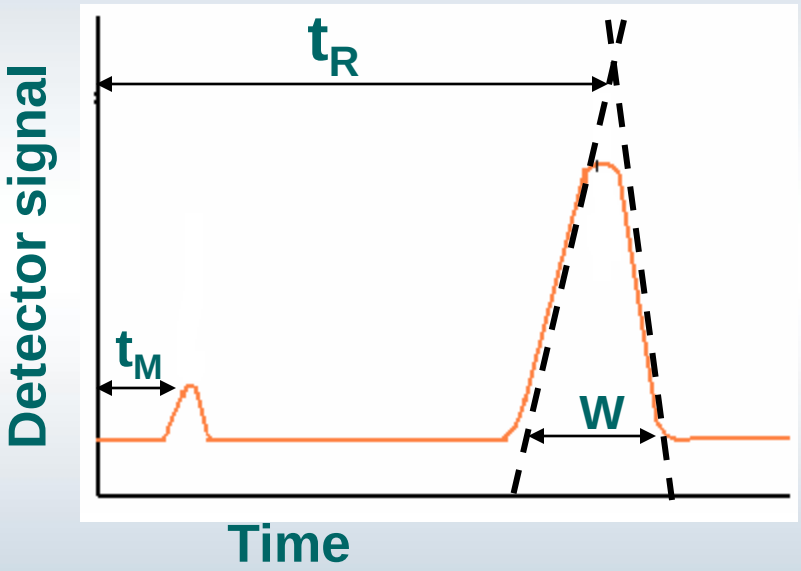
Thus the separation efficiency of the column increases with increasing number of theoretical plates.

The number of theoretical plates ‘N’ present in a column can be measured by :

$$N = 16\left(\frac{t_R}{W}\right)^2$$

wherw, t_R = retention time

W = peak width at its base (in units of time).



<u>Height equivalent to theoretical plates (HETP) or plate height (H)</u>:

It is defined as the height of the column required by the analyte in order to achieve equilibrium between the stationary & mobile phase.

$$H = \frac{L}{N} \qquad \text{or} \qquad H = \frac{L}{16}\left(\frac{W}{t_R}\right)^2$$

Where, L = length of the column usually in cms.

HETP calculations are useful in comparing columns of different lengths.

<u>Conclusions</u> :

- **The efficiency of chromatographic column increases as the plate count N becomes greater & as the plate height H becomes smaller.**
- **An increase in *N*, the number of theoretical plates, by lengthening the column leads to an increase in retention time and increased band broadening - which may not be desirable.**
- **Instead, to increase the number of plates, the HETP can be reduced by reducing the size of the stationary phase particles.**

Advantages of plate theory :

- **Plate model successfully accounts for the Gaussian shape of chromatographic peaks.**
- **Accounts for factors that influence differences in solute migration rates.**

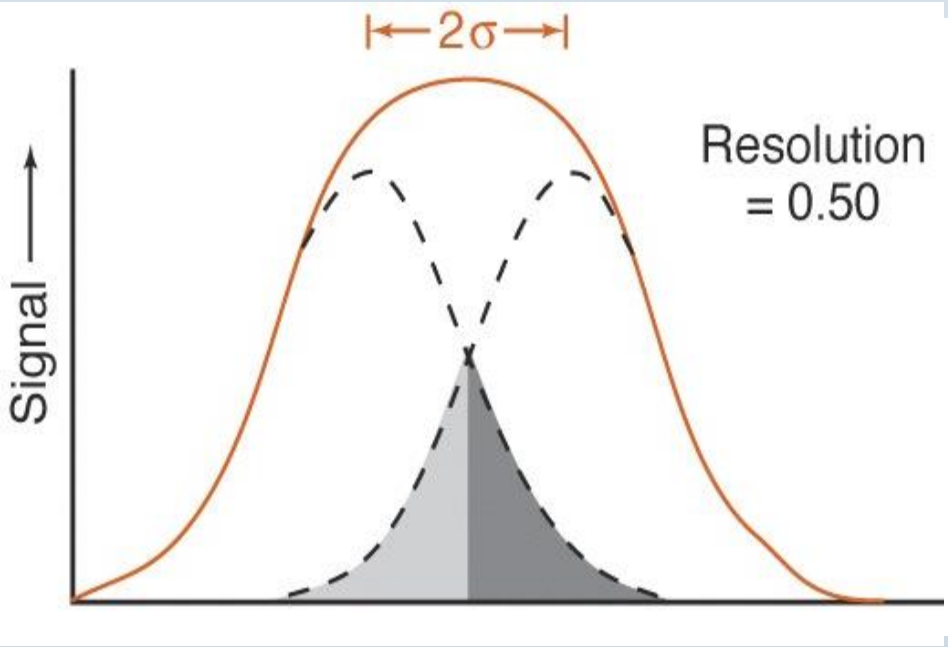
Disadvantages :

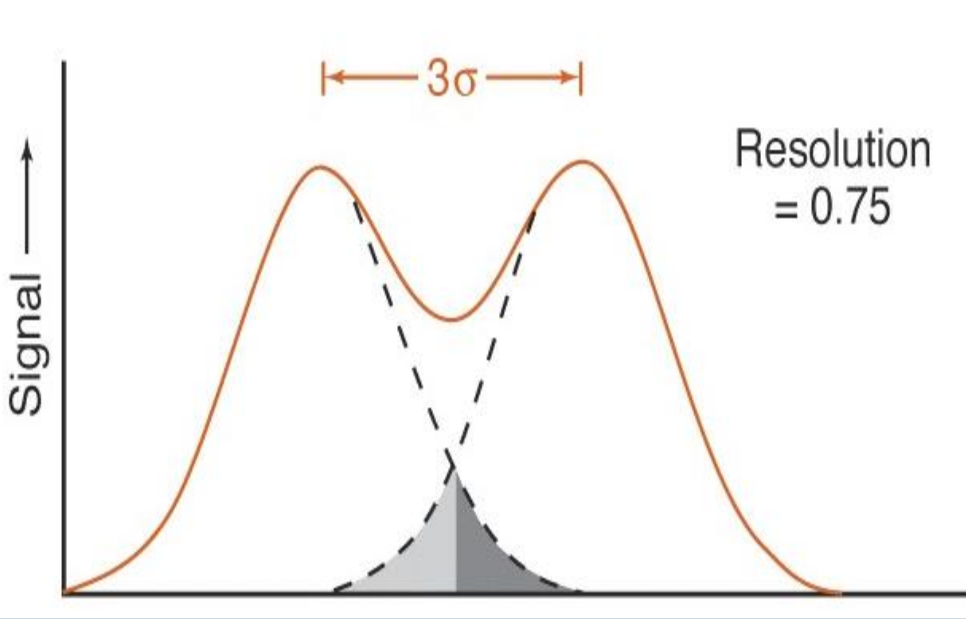
The plate model fails to explain band broadening because of its basic assumption that equilibrium conditions prevail throughout a column during elution.

The mobile & stationary phases are moving past one another at such a pace that sufficient time is not available for equilibrium.

2. Rate theory :

- **Mainly deals with Band broadening.**
- **Band broadening or zone broadening reflects a loss of column efficiency.**





Variables that influence column efficiency :

Variable	Symbol	Usual units
Linear velocity of mobile phase	u	Cm s^{-1}
Diffusion coefficient in mobile phase*	D_m	Cm^2 s^{-1}
Diffusion coefficient in stationary phase*	D_s	Cm^2 s^{-1}
Retention factor	k	Unit less
Diameter of packing particals	d_p	Cm
Thickness of liquid coating on stationary phase	d_f	Cm

* Increases as temperature increases

Theory of Band Broadening :

The efficiency of capillary & packed chromatographic columns at low mobile phase velocities can be approximated by the equation :

$$H = \frac{B}{u} + C_s u + C_m u \longrightarrow (1)$$

Where, H – plate height in Cms.

B – longitudinal diffusion coefficient.

C_S & C_m – Mass transfer coefficients for stationary & mobile phase respectively.

At high flow velocities in packed column where flow rates dominate diffusion, the efficiency can be approximated by :

$$H = A + B/u + Cu$$

This equation is popularly called as Van Deemter equation.

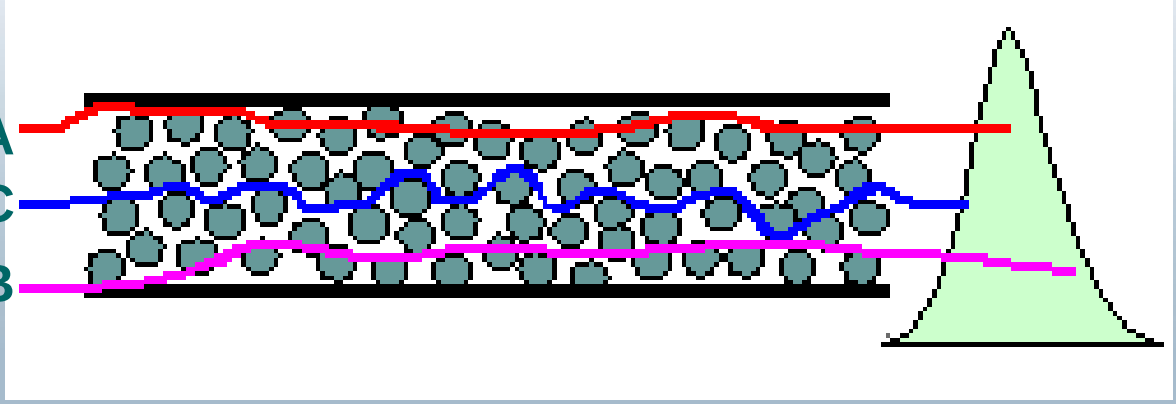
Where, A – Coefficient of multiple path effects (Eddy diffusion).

B - Coefficient of Longitudinal diffusion.

C - Coefficient of Mass transfer.

Eddy diffusion (Coefficient of multiple path effects) 'A' :

Eddy diffusion is concerned with the flow patterns of the mobile phase & it states that "each solute particle will have its own residence time in the column which results in band broadening & the band broadening depends on the size of the packing particles, the packing manner of stationary phase & the column diameter".



- **A is independent of flow rate.**

$$A = 2 \lambda d_p$$ **Where, λ – Multitude of pathway**

d_p – diameter of packing particles.

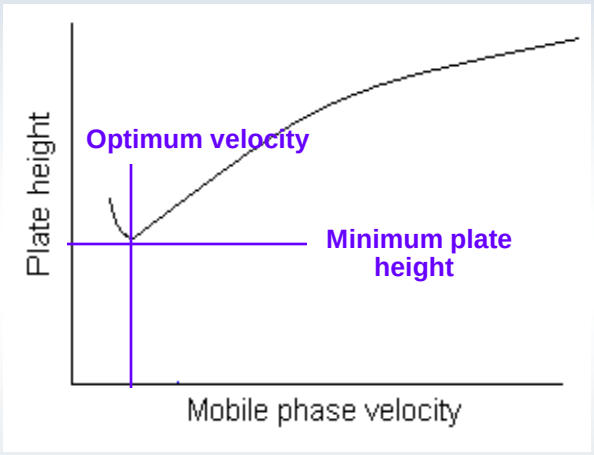
- **Eddy's diffusion can be minimized by using packing particles of small & uniform diameter, the manner of packing must be regular.**
- **Particle size must be atleast 100 – 200 mesh size.**

The longitudinal diffusion term (B/u) :

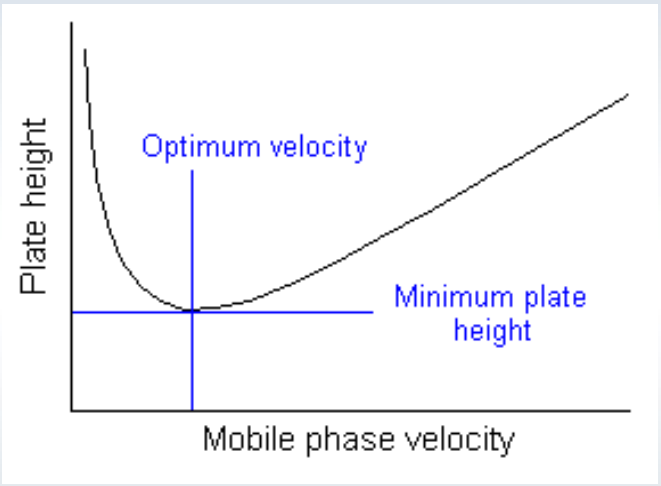
- **Diffusion is a process in which species migrate from a more concentrated part of a medium to a more dilute region.**
- **The concentration of the analyte is less at the edges of the band than at the center. Hence analyte diffuses out from the center to the edges causing band broadening.**
- **The longitudinal diffusion α cont^n difference between the regions & to the diffusion coefficient D_m of the species.**

- **Longitudinal diffusion is inversely proportional to the linear velocity of the eluents.**
- **This can be explained with the help of two graphs :**

A typical Van Deemter plot



Liquid chromatography



GLC

- **Longitudinal diffusion is a common source of band broadening in gas chromatography, where the rate at which molecules diffuse is high.**

The phenomenon is of little significance in liquid chromatography, where diffusion rates are much smaller.

Mass transfer coefficients (C) :

- There are two mass transfer coefficients C_s & C_m.

  C_s -- mass transfer coefficient in stationary phase.

  C_m -- mass transfer coefficient in mobile phase.

- The requirement of two mass transfer terms is evident from the fact that equilibrium between mobile & stationary phase is established so slowly that a chromatographic column operates under nonequilibrium conditions.
- When the velocity of the mobile phase is high, and the analyte has a strong affinity for the stationary phase, then the analyte in the mobile phase will move ahead of the analyte in the stationary phase. Hence the band of analyte is broadened.

  The higher the velocity of mobile phase, the worse the broadening becomes.

Stationary phase mass transfer term (C_s) :

- When the stationary phase is immobilized liquid on solid support particles :

$$C_s \ \alpha \ \frac{d_f^2}{D_s}$$

d_f^2 = square of the thickness of the film on the support particles.

D_s = diffusion coefficient of the solute in the film.

- When the stationary phase is solid surface :

Cs α time required for a species to be adsorbed or desorbed.

Mobile phase mass transfer term (C_m) :

- For packed columns :

$C_m \ \alpha \ d_p^2$ (square of the particle diameter of the packing material).

- For capillary columns :

$C_m \ \alpha \ d_C^2 \ u$ d_C^2 = square root of column diameter.

u = flow rate.

Summary of methods of reducing band broadening :

- **For packed columns, band broadening is minimized by small particle diameter.**
- **For capillary columns, small column diameters reduce band broadening.**
- **With gaseous mobile phases, the rate of longitudinal diffusion can be reduced appreciably by lowering the temperature & thus the diffusion coefficient. The consequence is significantly smaller plate heights at lower temperature.**
- **With liquid stationary phases, the thickness of the layer of adsorbed liquid should be minimum since C_s is proportional to the square of the variable.**

DERIVATIZATION TECHNIQUES :

It is a process that converts molecules with polar functional groups into less polar derivatives which are then volatile & elute as symmetrical peaks.

Derivatization reactions are meant to transform an analyte for detectability in Gas Chromatography (GC) or other instrumental analytical methods. Derivatization in GC analysis can be defined as a procedural technique that primarily modifies an analyte's functionality in order to enable chromatographic separations. A modified analyte in this case will be the product, which is known as the derivative. The derivative may have similar or closely related structure, but not the same as the original non-modified chemical compound.

Advantages : Derivatization can –

- **Increase the thermal stability of a compound.**
- **Increase volatility**
- **Increase its ability to be detected.**
- **Aid in identification of an unknown.**

Derivatization can be made before injection into the gas chromatograph or directly in the injection port ("on – column" Derivatization or "flash alkylation".)

Derivatization reagent

Derivatization reagent is the substance that is used to chemically modify a compound to produce a new compound which has properties that are suitable for analysis in GC or LC. The following criteria must be used as guidelines in choosing a suitable Derivatization

Reagent for GC analysis.

i. The reagent should produce more than 95 % complete derivatives.

ii. It should not cause any rearrangements or structural alterations of compounds during

formation of the derivative.

iii. It should not contribute to loss of the sample during the reaction.

iv. It should produce a derivative that will not interact with the GC column.

v. It should produce a derivative that is stable with respect to time.

Objectives for Derivatization

The following outlined objectives among others can be achieved by application of proper Derivatization procedures;

i. Improvement of resolution and reduce tailing of polar compounds which may contain –OH, –COOH, =NH, –NH2, –SH, and other functional groups.

ii. Analysis of relatively nonvolatile compounds.

iii. Reduction of volatility of compounds prior to GC analysis.

iv. Improvement of analytical efficiency and hence increase detectability.

v. Stabilization of compounds for GC analysis.

Types of DERIVATIZATION TECHNIQUES :

1. **Esterification :**

- It is used to prepare derivatives of carboxyl group.
- Egs of drugs ⟶ prostaglandins, analgesics, amino acids & anti-inflammatory agents.
- Conversion of carboxyl group to the ester increases volatility by decreasing hydrogen bonding.
- Esterification can be carried out by 2 methods :

➢ using strong acid with alcohol ⟶ Fischer esterification process

➢ Using BF_3 or BCl_3 (8 – 14%) with an aliphatic alcohol.

$$R - OH + R^1 - COOH \xrightarrow[BF_3]{H^+ \text{ or}} R^1 - COOR$$

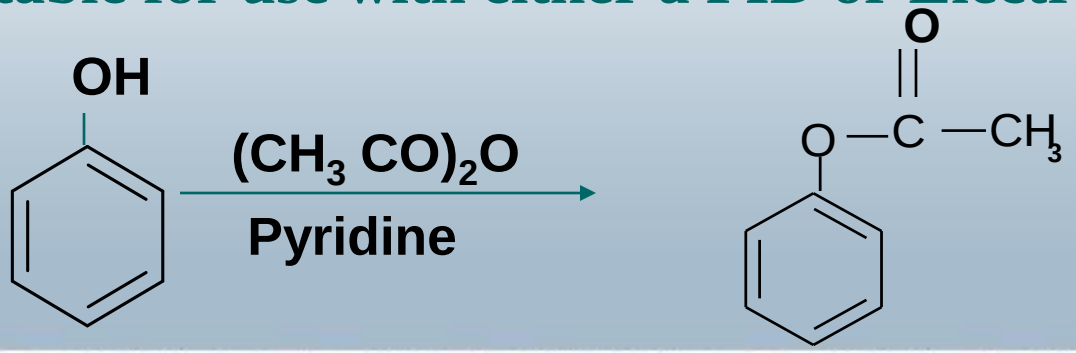
AMINO ACID

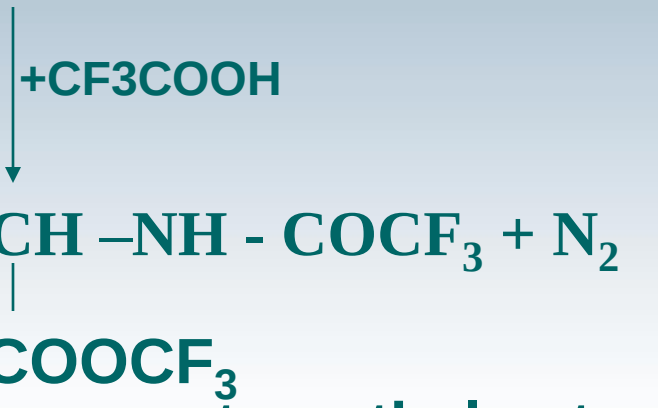
$$R - \underset{\displaystyle NH_2}{\underset{|}{CH}} - COOH \xrightarrow[CH_3OH]{HCl \,/\, HNO_3} R - \underset{\displaystyle Cl}{\underset{|}{CH}} - \underset{\displaystyle O}{\underset{||}{C}} - OCH_3$$

Chloro methyl ester

2. Acylation & perfluoroacylation :

- When the sample under study contains a phenol, primary or secondary alcohol or amine, derivatization by acylation is frequently used.
- Acetylation can be performed by use of acetic anhydride & a catalyst (acetic acid, p-toluene sulfonic acid, pyridine).
- Although acetylation is usually adequate to give good GC characteristics, trifluroacetates (TFA), pentafluoropropionates (PFP), or heptafluorobutyrates (HFB) are currently used to increase the sensitivity of the analysis.
- By these methods hologen incorporated in the derivative so that it is suitable for use with either a FID or Electron Capture D.

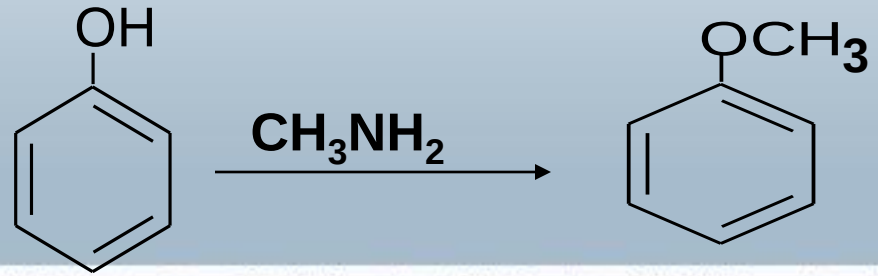




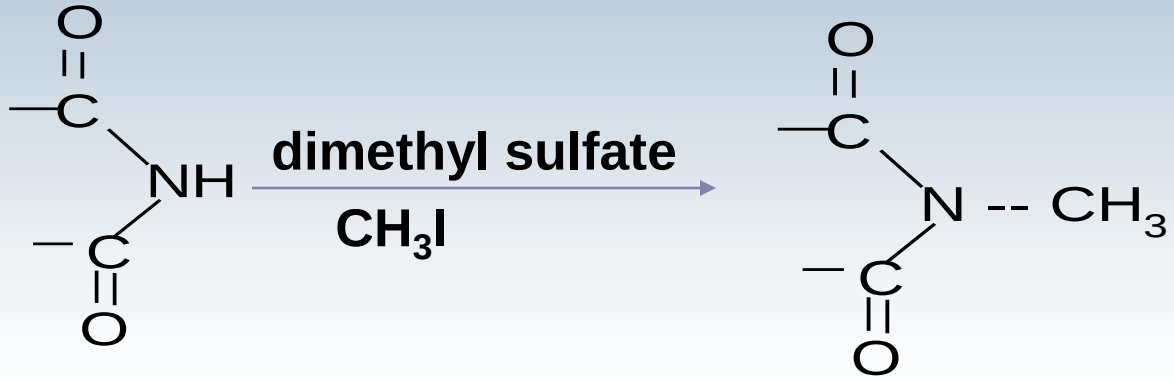
N - trifluoroacetomethyl ester

3. Alkylation :

- Alkylation is used to derivatize functional groups such as alcohols, phenols,, amines, imides, & sulfhydryl groups in which there is a labile hydrogen.
- Reagents used are Diazomethane, methylamines & pentafluorobenzylamide.



The imide nucleus is present in a number of drugs such as the barbiturates & anticonvulsants which can be derivatized to form methyl imide by using dimethyl sulfate.



4. Silylation :

- **The ACTIVE HYDROGEN of –OH, -COOH, -NH_2, -SH etc can be replaced by silyl groups to give more volatile derivatives.**
- **Several silylating agents are used & all have a general formula $(CH_3)_3$ – Si – X**

R-OH + $(CH_3)_3$ – Si - Cl ⟶ R – O – Si - $(CH_3)_3$ + HCl

Trimethylchlorosilane **Trimethylsilylether**

TABLE 7 Silylating Reagents Listed by Reactivity[a]

Trimethylsilylimidazole (TMSIM)
N, O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA)
N, O-bis-(trimethylsilyl)acetamide (BSA)
N-methyl-N-trimethylsilyltrifluoroacetamide (MSFTA)
N-trimethylsilyldiethylamine (TMSDEA)
N-methyl-N-trimethylsilylacetamide (MSTA)
Trimethylchlorosilane (TMCS)
Hexamethyldisilazane (HMDS)

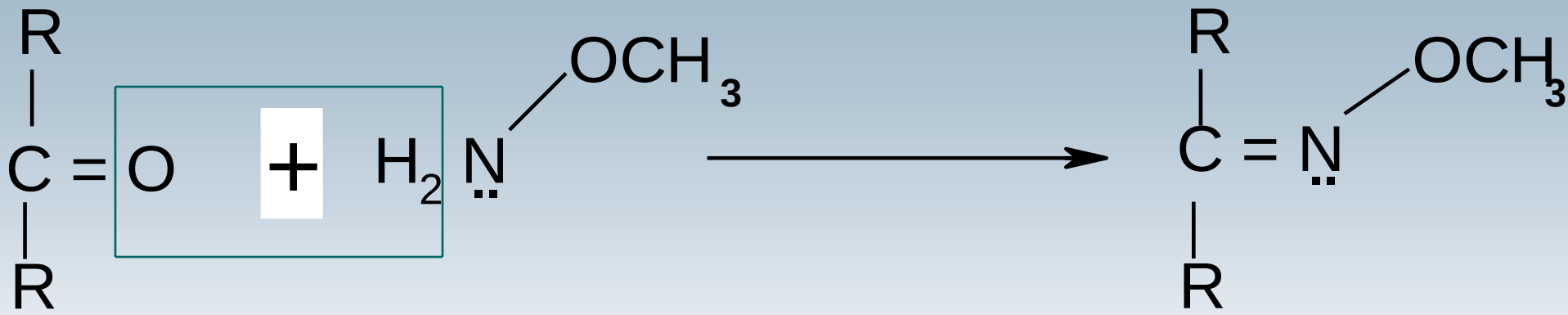
[a]Most reactive at top, least reactive at bottom.

5. Condensation :

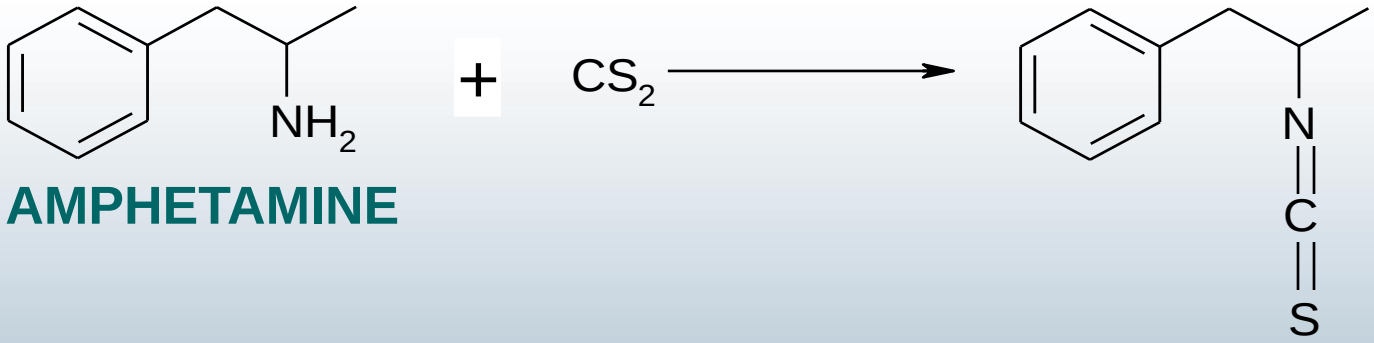
If a ketone or aldehyde is present in a sample, it is frequently derivatized :

- **To prevent hydrogen bonding due to enolization.**
- **Aid in resolution from am interfering substance.**
- **Increase its sensitivity of detection.**

Commonly used reagent is methoxylamine to protect enolizable keto groups in steroids.



- **Condensation reactions are also used to derivatizs amines, primary amines react with ketones to form enamines or with carbon disulfide to form isothiocyanates.**



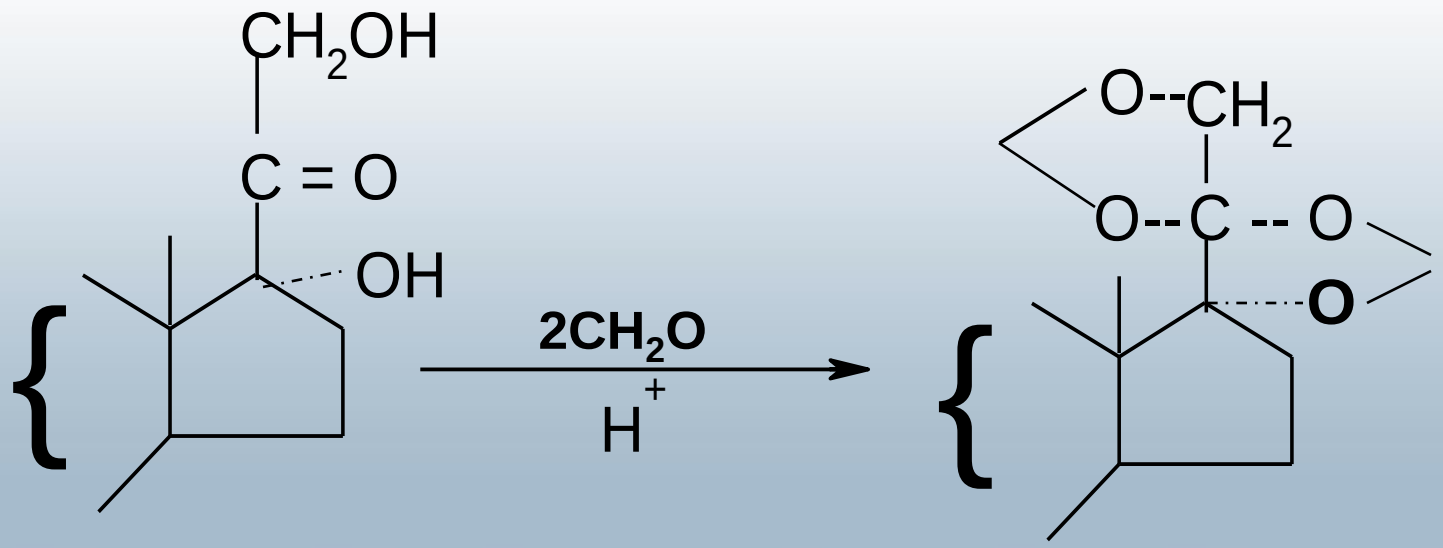
AMPHETAMINE

- **The isothiocyanates are thermally stable & tailing is minimal.**

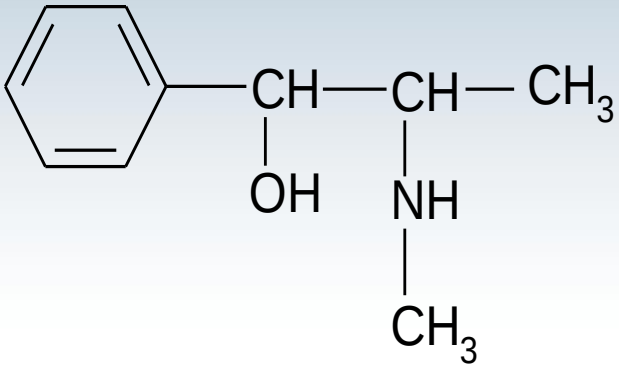
6. Cyclization :

- **Cyclization is performed on compounds containing two functional groups in close proximity so that 5- or 6- membered heterocycles can be formed. Heterocycles formed are ketals, boronates, triazines, & phosphites.**

E.g. 1: Cyclization of α – OH ketones (present in corticosteroids) with formaldehyde forms bismethylene dioxy derivatives which are thermally stable & permit resolution of corticosterone from a mixture of steroids.

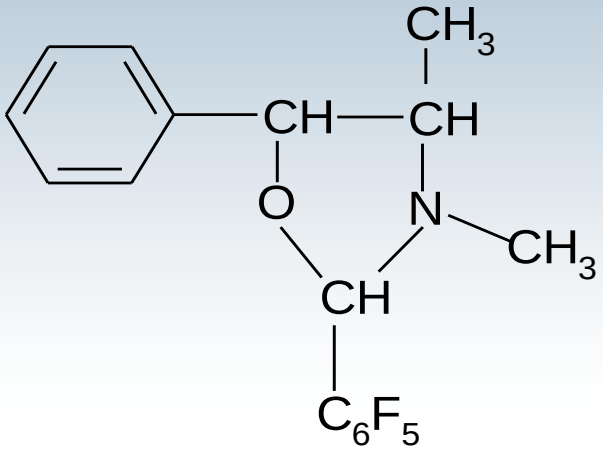


E.g. 2: Oxazolidones are formed from amino – alcohols such as those present in the catecholamines & propanolamines.



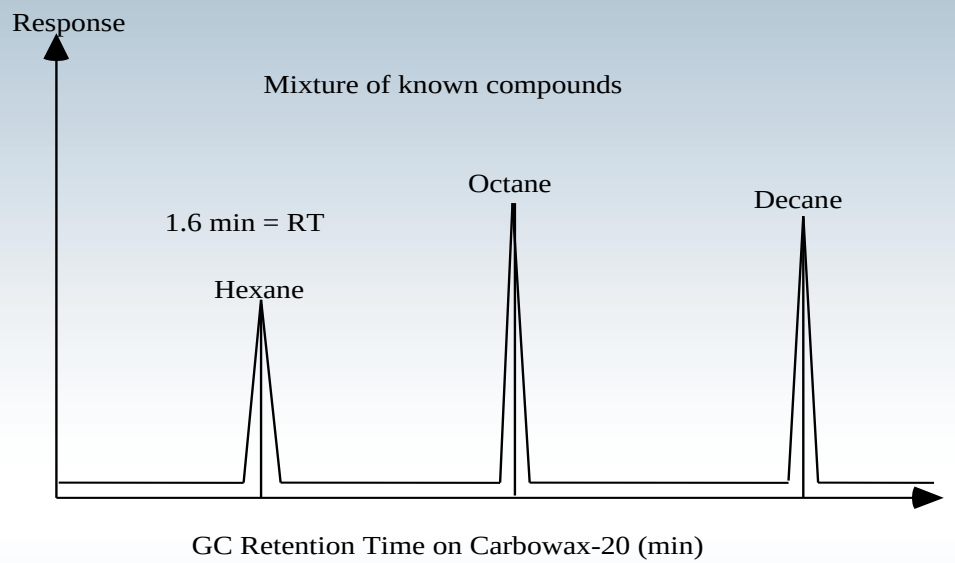
PHENYLPROPANOLAMINE

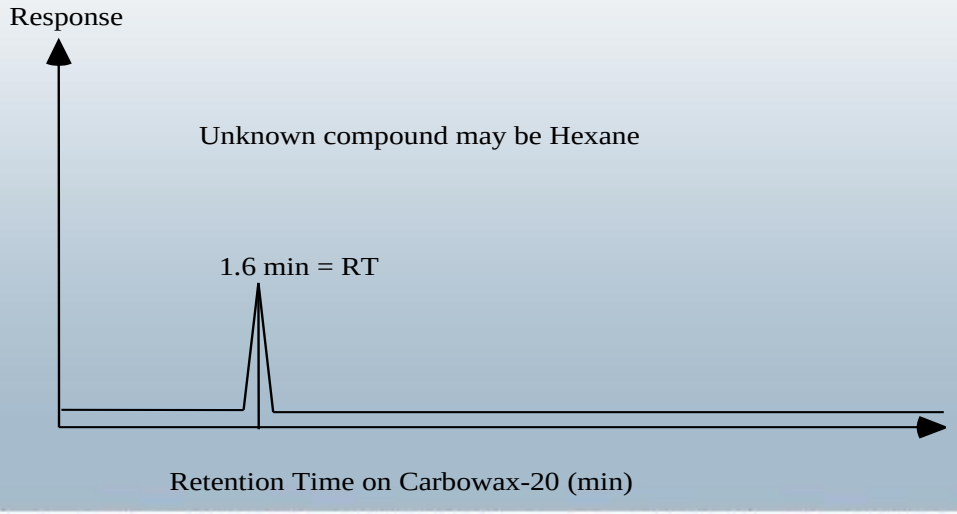
C_6F_5CHO



OXAZOLIDONE

TENTATIVE IDENTIFICATION OF UNKNOWN COMPOUNDS





Applications of GC in pharmaceutical analysis

APPENDIX Applications of Gas Chromatography in Pharmaceutical Analysis

Class and name of compound	Column	Column temperature (°C)	Derivatization	Detector	Purpose	Reference
I. Antibiotics						
A. Sulfonamides						
Sulfadiazine	1.82 m × 4 mm I.D. 5% OV-17	285	Diazomethane	ECD	Biological	221
B. Penicillins						
Penicillin G, penicillin V, phenethicillin, methicillin, oxacillin, cloxacillin, and dicloxacillin	61 cm × 3 mm I.D. 3% OV-17	215	HMDS + pyridine	FID	Formulation	222
C. Aminoglycosides						
Kanamycin	183 cm × 3 mm I.D. 3% OV-1	300	TMSIM + pyridine	FID	Formulation	223
Neomycin	61 cm × 3 mm I.D. 3% OV-1	290	TMSDEA + TMSIM + pyridine	FID	Formulation	224
Gentamicin	61 cm × 3 mm I.D. 3% OV-1	240	TMSDEA + TMSIM + pyridine	FID	Formulation	225
D. Others						
Chloramphenicol	1.22 m × 2 mm I.D. 3% OV-1	190 → 270	HMDS + TMCS + pyridine	FID	Biological	226
Tetracycline	185 cm × 3 mm I.D. 3% JX-R	260	BSA + TMCS + pyridine	FID	Formulation	227

APPENDIX (continued)

Class and name of compound	Column	Column temperature (° C)	Derivatization	Detector	Purpose	Reference
VIII. Antineoplastic agents (continued)						
Fluorouracil	2 m x ? 0.75% Carbowax 20 M + 5% KOH	210	TMAH on-column at 300° C	NP-FID	Biological	192
6-Mercaptopurine	5 ft x 0.25 in. O.D. 10% SE-30	135	TMAH	FID	Biological	240
Doxorubicin	92.3 cm x 2 mm I.D. 3% OV-101	260	Hydrolysis (acid), then methoxime + BSTFA + TMCS + TMSIM	FID	Formulation	241
IX. General anesthetics						
Ethanol	6 ft x 0.125 in. I.D. 50% Porapak Q 50% Porapak R	150	--	FID	Biological	252
Cyclopropane, diethyl-ether, and halothane	6 ft x 0.125 in. I.D. Porapak T	160-170	--	FID	Biological	253
Methoxyflurane and chloroform	275 cm x 4 mm I.D. 15% FFAP	100	--	FID	Biological	254
Nitrous oxide	95 cm x 0.25 in. O.D. Polypak 2	50	--	TC	Biological	255
Ketamine	-- 3% OV-1	220	--	FID	Biological	256

a) **Qualitative analysis :**

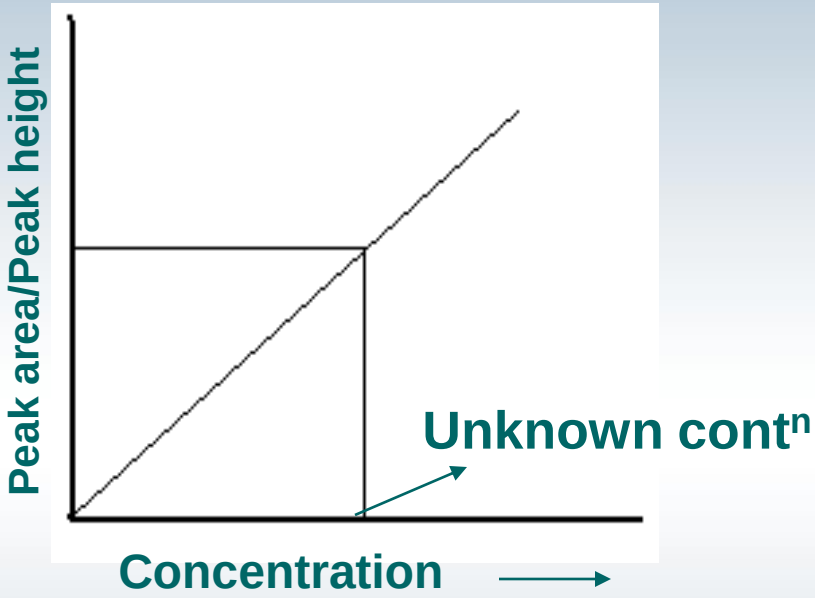
- **Gas chromatograms are widely used to establish the purity of organic compounds.**
- **Contaminants, if present, are revealed by the appearance of additional peaks.**
- **The areas under these peaks provide rough estimate of the extent of contamination.**

E.g. : Gas chromatography is used to determine the identity & composition of propellants that are widely used in aerosols.

The purity & acceptability of the propellants is tested with respect to moisture, halogen & non volatile residue determination by using GC.

b) Quantitative analysis :

1. External standardization :



1. Internal standardization :

- this method is used & preferred method in GC for quantitative analysis.
- It involves addition of a known weight of compound called as internal standard, which is not already present in the sample.

- **The internal standard should be :**

- structurally similar to the compound to be analyzed.

- separable from the test compound.

- internal standard peak should be similar to the sample peak.

Now we know that amount of compound in the sample is proportional to the area of the chromatographic peak.

Therefore, $A_1 = f_1 C_1$ **for sample**

$A_2 = f_2 C_2$ **for internal standard.**

$$\frac{A_1}{A_2} = K \frac{C_1}{C_2}$$

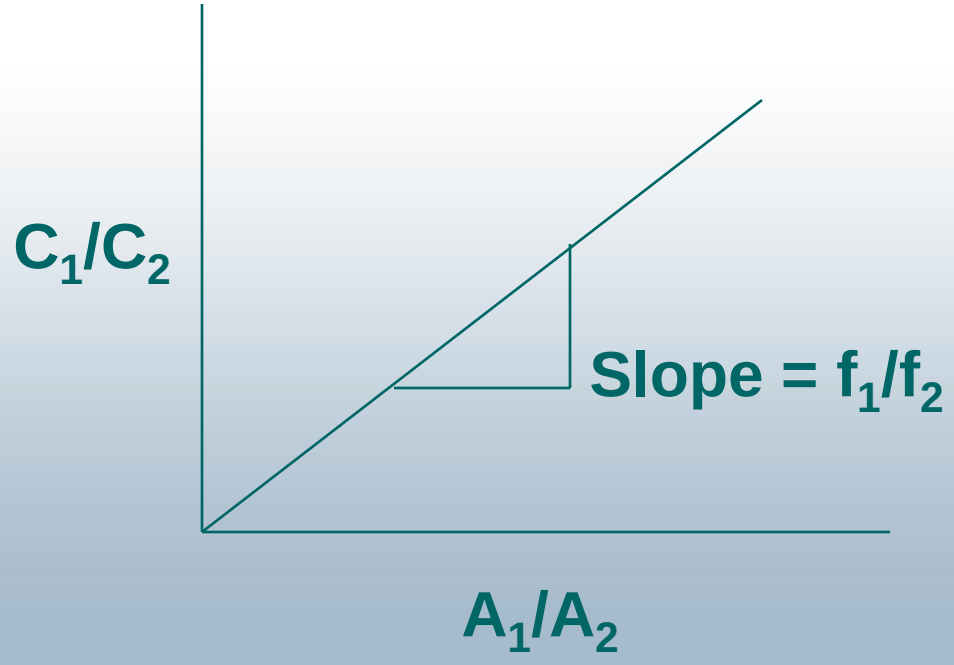
Where $K = f_1/f_2$ f_1 **– proportionality factor for A1**

f_2 **- proportionality factor for A2.**

C_1 **– weight of the sample.**

C_2 **– weight of internal std. added.**

- **Thus a graph of peak area ratio (A_1/A_2) versus weight ratio (C_1/C_2) will be a straight line with a zero intercept & a slope of f_1/f_2, which is used as a calibration curve for the analysis.**



References:

1. *Fundamentals of Analytical chemistry by Skoog, West, Holler, Crouch.*
2. *Pharmaceutical analysis by James. W. Munson.*
3. *Pharmaceutical Analysis - Dr. A. V. Kasture.*
4. *Internet source.*


THANK YOU