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M.Sc. chemistry

Semester 3 Material

CHAPTER -1

GENERAL INTRODUCTION OF CHROMATOGRAPHY

PAPER CODE C-301

ORGANIC CHEMISTRY

Chromatography

Basic concepts and its application

General Introduction

The most general method for removal of interference involves its physical separation from the analyte. Well-known methods for separations include distillation, crystallization, sublimation, solvent extraction, and chemical or electrolytic precipitation. However, the most widely used to eliminate interferences is **chromatography.** Chromatography was invented and named by the **Russian botanist M. Tswett**. He used the techniques to separate various plant pigments such as chlorophylls and xanthophyll by passing a solution of these compounds through a glass column packed with finely divided calcium carbonate. The separated species appeared as colored bands on the column, which accounts for the name he chose for the method. Chromatographic methods have a wide importance that allow the separation, identification and determination of closely related components of mixtures. Chromatographic separations are relatively simple, fast and be completed in a few minutes. It give an accurate and reproducible result so that they are also useful for method development and impurity profiling of various synthesized compounds.

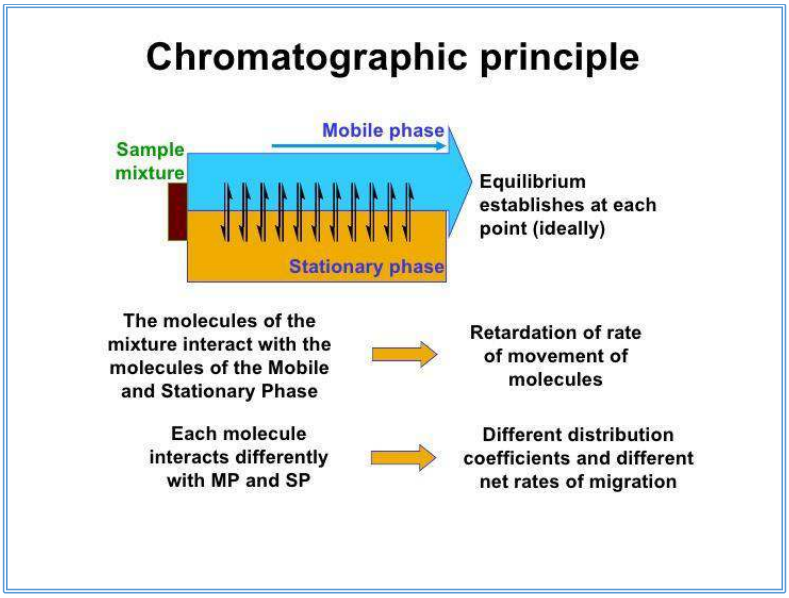
Principal

"Chromatography is a physical method of separation in which components in a mixture is separated based on different migration rates or different partition coefficient that can be possible by using two phases one is stationary phase and second is mobile phase".

- In all chromatographic separation, the sample is dissolved in a solvent called **mobile phase** which may be gas, liquid and super critical fluid. This

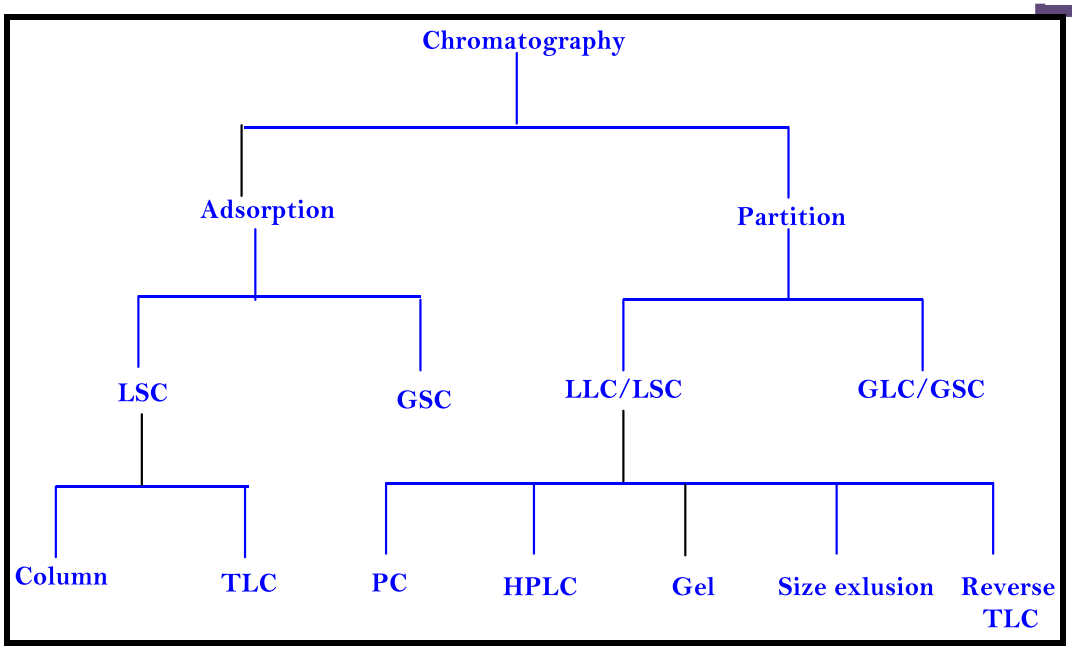
mobile phase is then forced through an immiscible phase which is fixed in a column called **stationary phase**. Between this two phases the components of the sample separated themselves.

- Those components strongly retained by stationary phase (their affinities towards the stationary phase is strong) move slowly (retention time is high), it is less eluated while other components that are weakly held by stationary phase travel rapidly (retention time is less), it is eluated first.



Classification

- Common classification of chromatographic methods according to the nature of the stationary phase and mobile phase. It is also according to the mechanism of separation (principal) of the chromatography.



Distribution Coefficient (K)

- All chromatographic separations are based upon differences in the extent to which solutes are partitioned between the mobile and stationary phase. The equilibria involved can be described quantitatively by means of a temperature-dependent constant, the **partition or distribution coefficient K:**

$$K = C_s / C_M \quad \text{.....(1)}$$

Where Cs is the analytical concentration of a solute in the stationary phase and C_M is its concentration in the mobile phase. In the ideal case, the partition ratio is constant over a wide range of solute concentrations that is, Cs is directly proportional to C_M.

- If K is less than 1 then the separation peak of the solute would be below the centre of the column (migration rate of the solute molecule is higher).
- If K is greater than 1 the separation peak of the solute would be the centre of the column (migration rate of the solute molecule is lower as compared to eluated peak).

- It is noted that K is affected by changing the distribution of components between the phases so that separation of components is also affected. By appropriate change in stationary phase, mobile phase or both, K can be also altered.

Chromatographic Terms

Chromatogram – It is an electronic visualization of chromatograph or a plot of the detector's signal as function of elution time or volume.

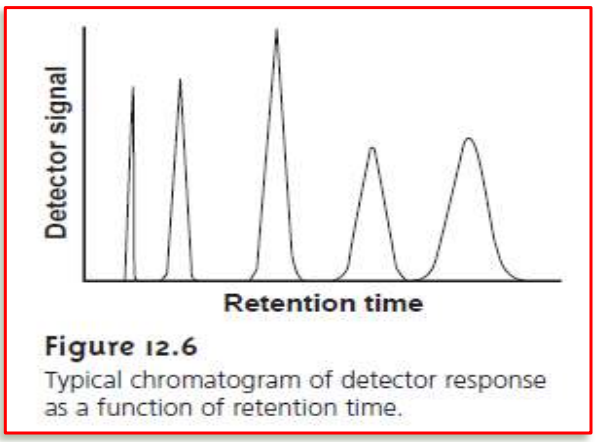


Figure 12.6
Typical chromatogram of detector response as a function of retention time.

Retention Time (t_R): It is a time taken by a solute molecules to move from the point of injection to the detector. The retention time is assigned to the corresponding solute peak. The retention time is a measure of the amount of time a solute spends in a column.

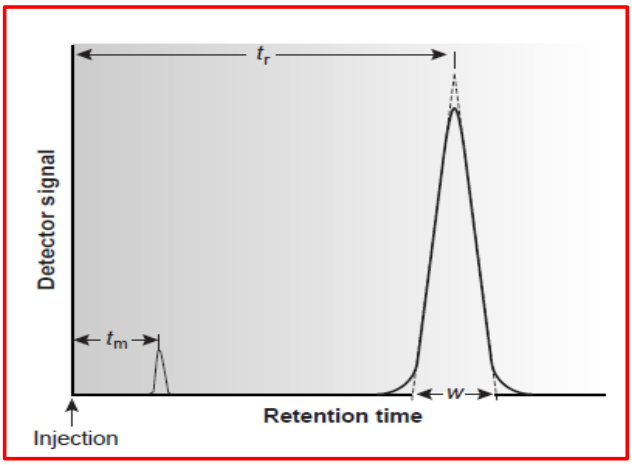
Retention volume (V_R): The volume of mobile phase required to move the solute molecules from the point of injection to the detector.

Dividing the retention volume by mobile phase's flow rate (u) gives retention time.

Retention time = retention volume / flow rate of mobile phase.

Baseline width – The width of a chromatographic peak of solute molecules measured at the baseline (w). It is measured by the intersection with the

baseline of tangent line drawn through the inflection points of either side of chromatographic peak. Baseline width is measured in units of retention time or volume.



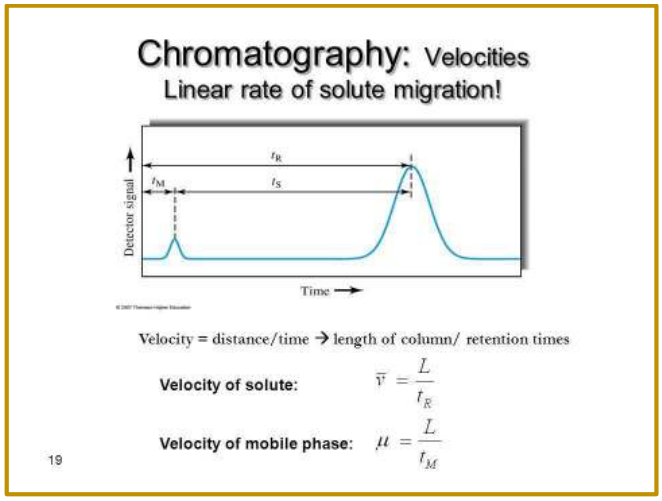
Beside the solute peak, small peak eluted soon after the sample injection into mobile phase. This small peaks moves through the column at the same rate as mobile phase because they do not interact with the stationary phase, they are considered as **unretained molecules**. The retained molecules spends a time (t_s) in the stationary phase.

Void time or void volume (t_M) – It is a time required for unretained molecules to move from the point of injection to detector.

The volume of mobile phase needed to move an unretained solute molecules from the point of injection to detector is called void volume.

Retention time (t_R) = Time of a retained solute spend on the stationary phase (t_s) + Time of a unretained molecule moves with the mobile phase (t_M)

$$t_R = t_s + t_M \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$



The average linear migration rate (v) of the solute molecule through the column can be calculated by

$$v = L/ t_R \quad \text{.....(3)}$$

L = length of the column and t_R = retention time of the solute molecule

Similarly average linear rate or velocity (u) of the mobile phase can be calculated by

$$u = L/ t_M \quad \text{.....(4)}$$

L = length of the column and t_M = retention time of the unretained solute molecule

Retention factor (k) or capacity factor

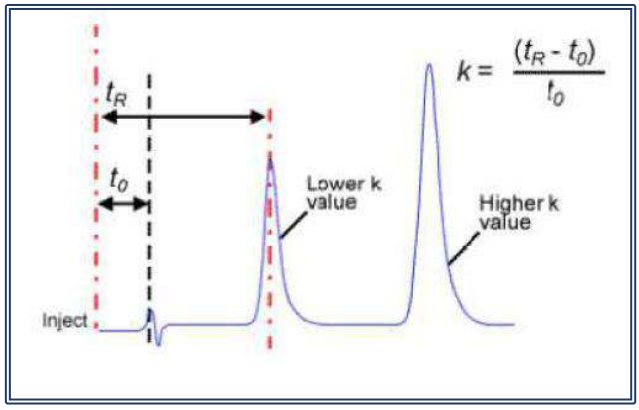
- The retention factor (k) is another measuring of retention of an analyte on the column. **It is the ratio of retention time of a solute spends on the column to the retention time of an unretained solute.**
- An unretained solute molecules has no affinity towards a stationary phase so that elutes with the solvent front at a time (t_M) which is known as "dead time" or "hold up time".
- Retention factor is independent of some of variable factors including small flow rate variables and column dimensions. Because of this useful

parameter when comparing retention of chromatographic peak obtained using different columns.

- Retention factor (k) can be given equation

$$k = \frac{t_R - t_M}{t_M} = \frac{tR'}{t_M} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(5)$$

t_R = retention time, t'_R = adjusted retention time, t_M = retention time of unretained compound

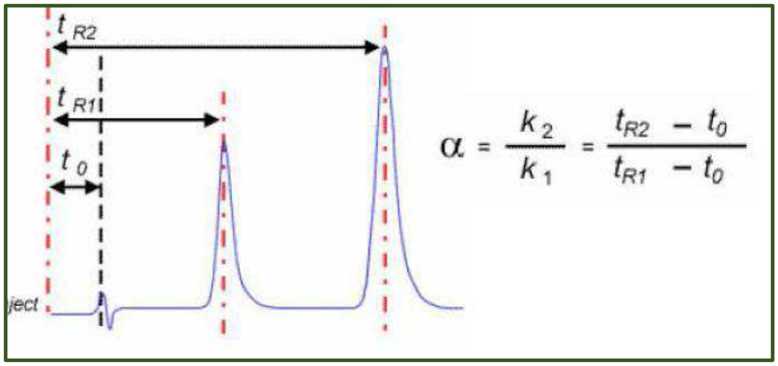


- Retention factor (k) = Zero for the unretained solute. Retention factor (k) is lower for the solute molecule which has less affinity towards stationary phase while k is higher for the solute which has high affinity towards stationary phase.

Selectivity or separation factor (α):

- The separation factor is a measure of the time or distance between the maxima of two peaks. It is usually measured as a ratio of the retention factors of the two peaks.

- Selectivity is always greater than one. When it is equal to one than the peaks are co-eluting. The greater selectivity value, separation between two peaks are higher.



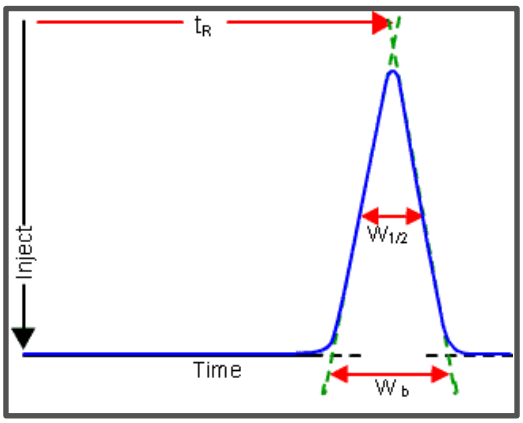
- Selectivity can be affected by changing the different solvents, stationary phase and temperature.

Plate theory (efficiency of column):

- The efficiency of the column can be explained by plate theory. According to the plate theory developed by Martin and Synge, a chromatographic column consists a no of theoretical plates and equilibration of the solute between stationary and mobile phase take place at each of these plates. The movement of the solute down the column is assumed by a series of stepwise transfers between one plates to other.
- The efficiency of separation of the column is increased as no of theoretical plate increases. This is because the no of equilibrations will also increases means better quality of separation. Efficiency of the column is depends on no of theoretical plates (N) and plate height (H) or HETP (Height Equivalent to a theoretical Plate) given by

$$N = L/H \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(6)$$

- The efficiency of the column increases as no of theoretical plates (N) becomes greater and as the plate height (H) becomes smaller.



- The plate theory successfully accounts for the Gaussian shape (due to dispersion of the peaks) of chromatographic peak and their relative movement down a column. The breadth (width) of Gaussian curve is described by the standard deviation (σ) or its variance (σ^2). So it is convenient to define efficiency of the column in terms of variance per unit length of the column. That is, the plate is given by

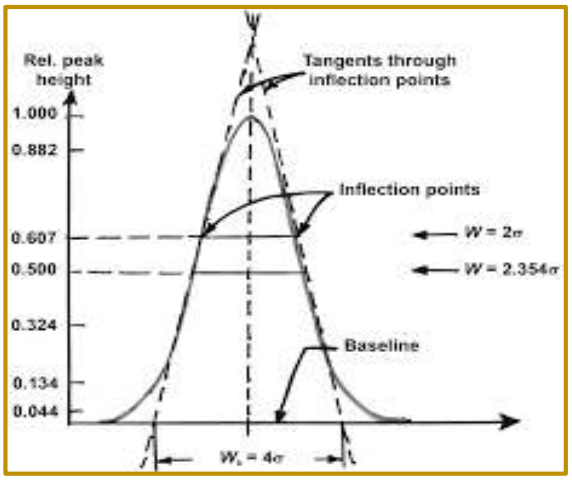
$$H = \sigma^2 / L \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

Note: Column efficiency is a function of the column dimensions (diameter, length and film thickness), the type of carrier gas and its flow rate or average linear velocity, and the compound and its retention. For column comparison purposes, the number of theoretical plates per meter (N/m) is often used.

- The theory was ultimately abandoned in favour the rate theory because its fails to accounts the peak broadening. Put the equation (7) in equation (6)

$$N = L^2 / \sigma^2 \quad \text{.....(8)}$$

Calculation of Chromatographic peak width



- The breadth (width) of Gaussian curve is described with units of length and time. If breadth of curve is described with units of length then σ sign is used while for time, τ sign is used. The relation between this two standard deviations are related by

$$\tau = \frac{\sigma}{L/tr} \quad \text{.....(9)}$$

The width of chromatographic peak is W=4 τ.

$$\frac{W}{4} = \frac{\sigma}{L/tr} \quad \text{..... (10)}$$

Rearrange the equation (10)

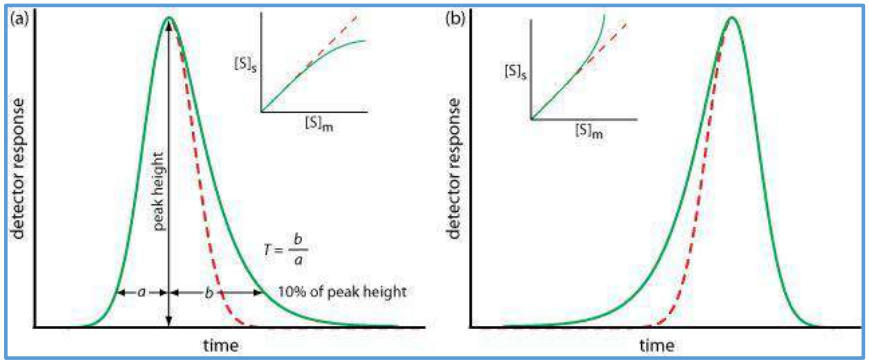
$$\sigma = \frac{LW}{4tr} \quad \text{..... (11)}$$

Making a square of equation (11) and substitute the value of (11) into equation (8)

$$N = 16\left(\frac{tr}{W}\right)^2 \qquad \ldots\ldots\ldots\ldots\ldots\ldots(12)$$

Rate Theory (Band Broadening)

- Band Broadening of chromatographic peak can be explained by rate theory. It describes the more realistic description of what actual processes work inside a column. We know that the chromatographic peaks look like Gaussian or normal error curves but some peaks are non-ideal and exhibit tailing and fronting.



- Band broadening reflects a loss of column efficiency. Band broadening takes due to the slower the mass transfer processes occurs while a solute migrates through a column. (Time taken for the solute to equilibrate between the stationary and mobile phase). The resulting band shape of a chromatographic peak is affected by the rate of elution.

- Note: The chromatographic peak in (a) is an example of tailing, which occurs when some sites on the stationary phase retain the solute more strongly than other sites. The peak in (b) is an example of fronting, which most often is the result of overloading the column with sample. For both (a) and (b) the green chromatogram is the asymmetric peak and the red dashed chromatogram shows the ideal.

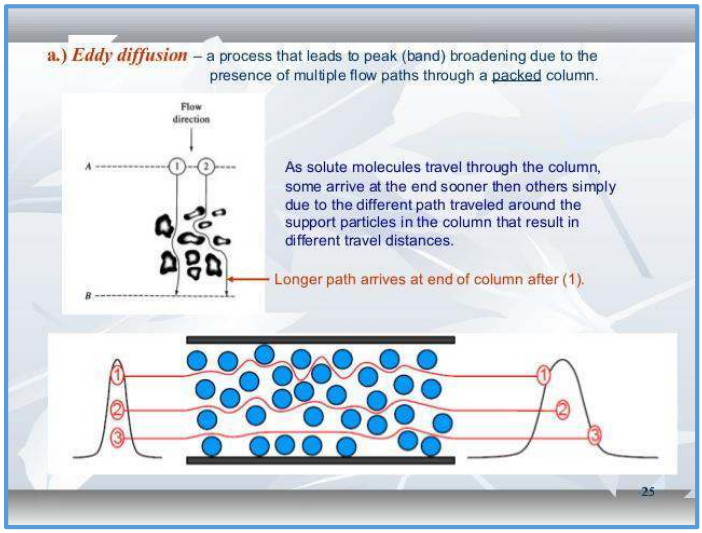
❖ If we consider the various mechanisms which contribute to band broadening, we arrive at the Van Deemter equation for plate height;

$$\text{HETP} = A + B / u + C\,u \qquad \text{.....(13)}$$

Where u is the average velocity of the mobile phase. A, B, and C are factors which contribute to band broadening.

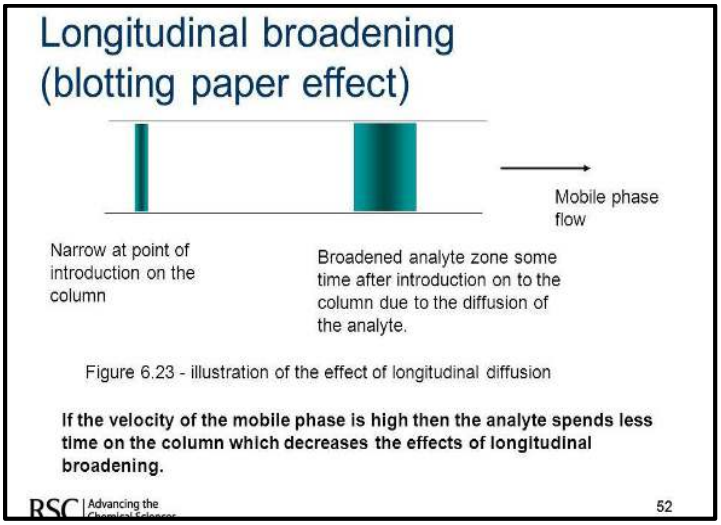
A. Eddy Diffusion (multipath diffusion)

❖ Solute molecules will take different paths and travel through the stationary phase to reach at end of the column. This multiple path effect is called eddy diffusion which would be independent of solvent velocity.



B – Longitudinal diffusion

❖ The concentration of solute molecules is lower at the edges of the band than at the center. Solute molecules diffuses out from the center to the edges. This causes band broadening. If the velocity of the mobile phase is high then the analyte spends less time on the column, which decreases the effects of longitudinal diffusion.

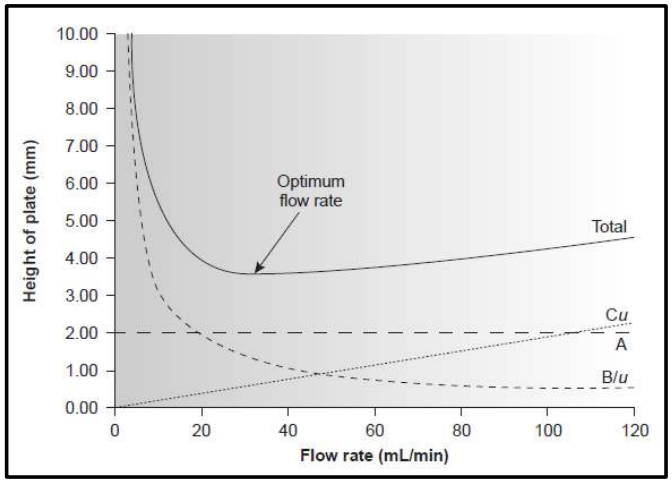


C. Mass transfer

- A chromatographic separation occurs solutes move between stationary phase and mobile phase. For the movement of the solute from one phase to other phase, it must diffuse first it is called **mass transfer.**
- The solute molecule takes a certain amount of time to equilibrate between the stationary and mobile phase.
- The band broadening occurs whenever movement of solute molecules is not fast enough to maintain the equilibration. If 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. The band of analyte is broadened. The higher the velocity of mobile phase, the worse the broadening becomes.

Van Deemter Plots

- A plot of plate height vs. average linear velocity of mobile phase.

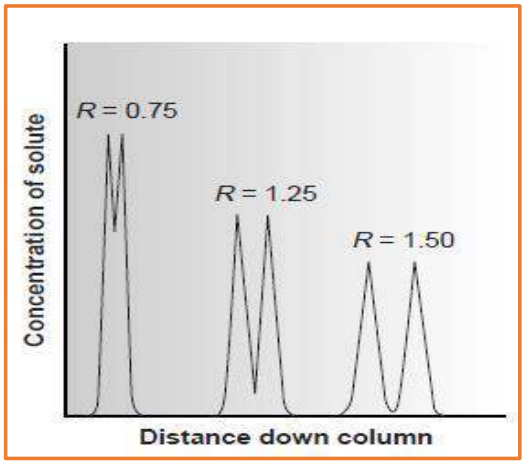


Chromatographic resolution (R_s)

- Resolution is quantitative measurement of degree of separation between two chromatographic peaks. It can be defined as

$$R = \frac{t_{r,B} - t_{r,A}}{0.5(w_B + w_A)} = \frac{2\Delta t_r}{w_B + w_A}$$

- As Shown in Figure, the degree of separation of peaks with increase in R_s



Calculation

(1) The following data were obtained for three compounds separated on a 20 meter column:

Compounds	Retention Time (min)	Peak widths (min)
A	8.04	0.15
B	8.26	0.15
C	8.43	0.16

(i) Calculate no of theoretical plates for each compounds and average no of theoretical plates for the column

(ii) Calculate average no of height of theoretical plates

(2) In chromatographic analysis for organic compounds give a peak with retention time of 8.66 min and base line of 0.29 min. How many theoretical plates are involved in this separation? If the length of column is 2.0 m long what is the height of the theoretical plates?

(3) In liquid chromatographic column following data are found: Length of column = 24.7 cm. Flow rate = 0.313 ml/min, V_m = 1.37 ml and V_s = 0.164 ml. A chromatogram of natural product mixture of species A, B, C and D provided following data:

Compounds	Retention Time (min)	Peak widths (min)
Not retained	3.1	-
A	5.4	0.41
B	13.3	1.07
C	14.1	1.16
D	21.8	1.72

(i) The no of plates from each of peak

(ii) The plate height for the column

(iii) The capacity factor for A, B, C and D.

(4) In HPLC separation of two drugs X and Y the following data are found :

Length of column = 24.7 cm.

Flow rate = 0.313 ml/min,

V_m = 1.37 ml and

V_s = 0.164 ml

A chromatogram of natural product mixture of species X and Y provided following data:

Compounds	Retention Time (min)	Peak widths (Second)
Not retained	3.1	-
X	5.4	24.6
Y	14.1	69.6

(i) The no of plates from each of peak

(ii) The plate height for the column

(iii) The Retention factor for X and Y.

(5) In a chromatographic analysis, two substance A and B were found to have retention time 9.25 and 9.90 min on 16 cm column. An unrestrained species passed through the column in 0.55 min. The peak with for A and B were 0.60 and 0.65 min. Calculate: Column resolution, plate height and Average no of plates in column.

Gas chromatography- Infrared (GC-IR)

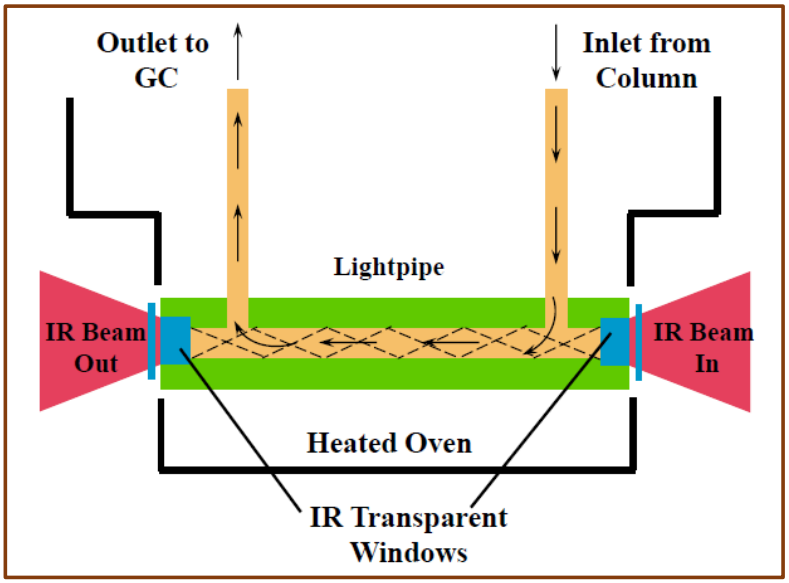
Introduction

- This is a separation technique followed by an identification similar to GC-MS.
- Coupling of GC with IR provides an effective method of separating compounds moves from GC column and identifying these components.
- GC-IR is preferred method for identifying isomers, which may be incorrectly identified using GC-MS.
- In addition, GC-IR may be used as complementary analytical technique to confirm GC-MS results. Typical application of GC-IR include analysis of drugs, fragrances and other organic compounds found in mixtures.
- GC-MS is a mass selective technique which is used to determine the molecular weight of the components.
- It also provide the information regarding to elemental composition and isotope. Whereas GC-IR is a functional group selective. It is specially used to identify the geometrical isomer (ring isomers, cis and trans isomers) where GC-MS does not give the information about the position of the functional group.

Instrumentation

Figure is a block diagram of GC-IR. The spectrometer head contains an interferometer which modulated the IR source beam. The modulated beam is focused into a heated "light pipe" cell through which separated GC components are directed. An interferogram is generated when the IR signal

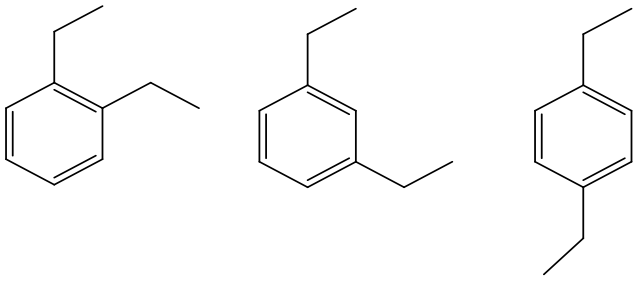
is fed to the computer. Currently, two GC interfaces are commercially available. The **light pipe** interface and GC/matrix-isolation/IR uses **matrix isolation** to trap the GC effluent to measuring the IR spectra of each components.



The light pipe cell is 120 mm long and having a 1.0 mm internal diameter. The cell is thermally heated, maximum temperature of cell is 300 ^{0}C. The light pipe cell having two windows which is made by KBr or ZnSe.

Application

1) **Conformation of geometrical isomers**



(A) (B) (C)

If the compound A, B and C is separated and identify by GC-MS. But Molecular formula ($C_{10}H_{14}$), molecular weight (134.11g/mol) and mass fragmentation pattern of each of the compounds are similar at that time we cannot identify the position of the functional groups by the GC-MS.

For the conformation of the position of the functional groups, GC-IR is used which give the different absorption frequencies regarding to the position.

The absorption frequencies of the aromatic compounds is shown below

Ar. C-H Stretching (sp^2)	3050 and 3010 cm^{-1}
Ar. C=C Stretching (sp^3)	1600 and 1475 cm^{-1}
Ar. C-H out of plane bending (Useful for the determination of the ring substituent)	900-690 cm^{-1}
Overtone and combination bands	2000 to 1667 cm^{-1}
Monosubsituted ring	750 to 690 cm^{-1}
Ortho substituted ring	750 cm^{-1} (strong band)
Meta substituted ring	880-690 cm^{-1}
Para substituted ring	850-800 cm^{-1} (strong band)

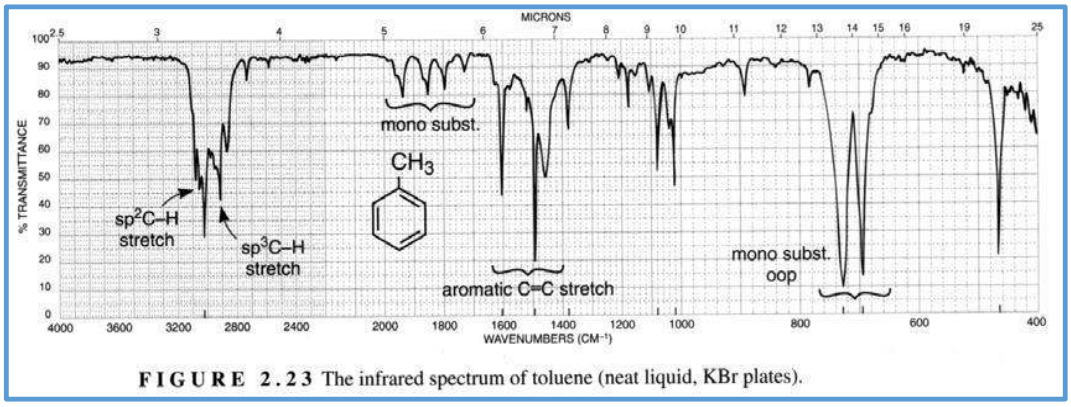


FIGURE 2.23 The infrared spectrum of toluene (neat liquid, KBr plates).

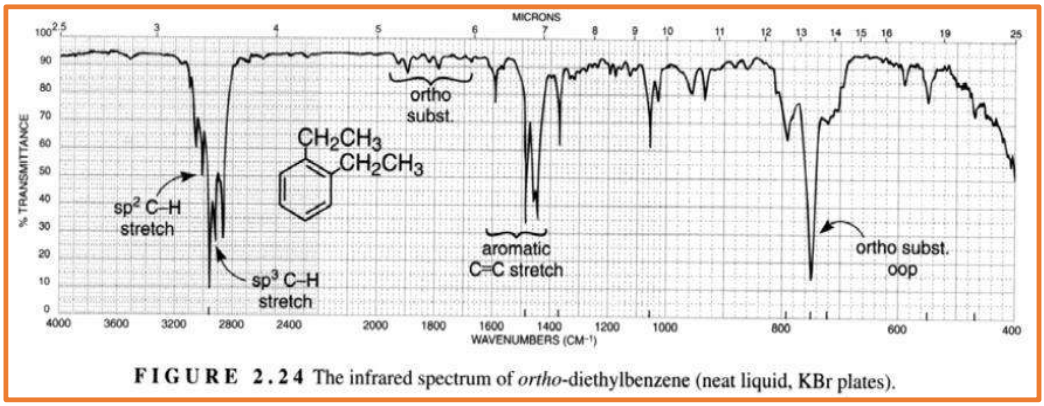


FIGURE 2.24 The infrared spectrum of *ortho*-diethylbenzene (neat liquid, KBr plates).

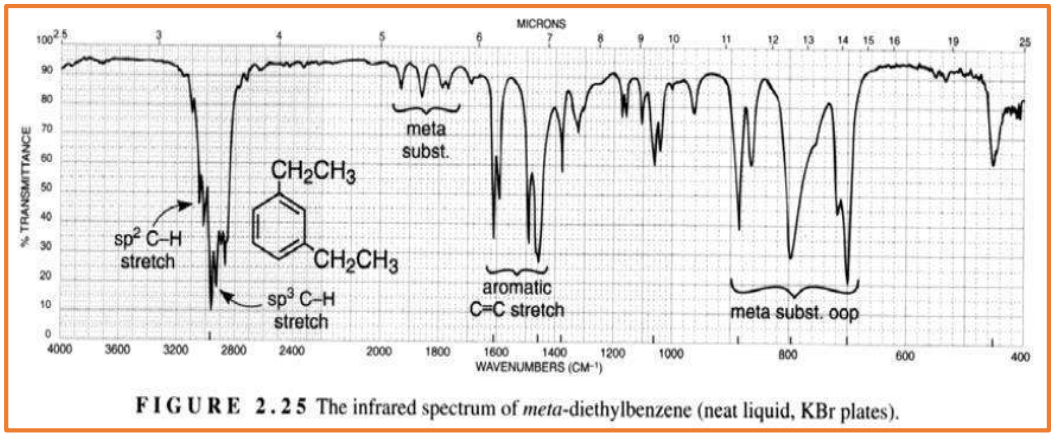


FIGURE 2.25 The infrared spectrum of *meta*-diethylbenzene (neat liquid, KBr plates).

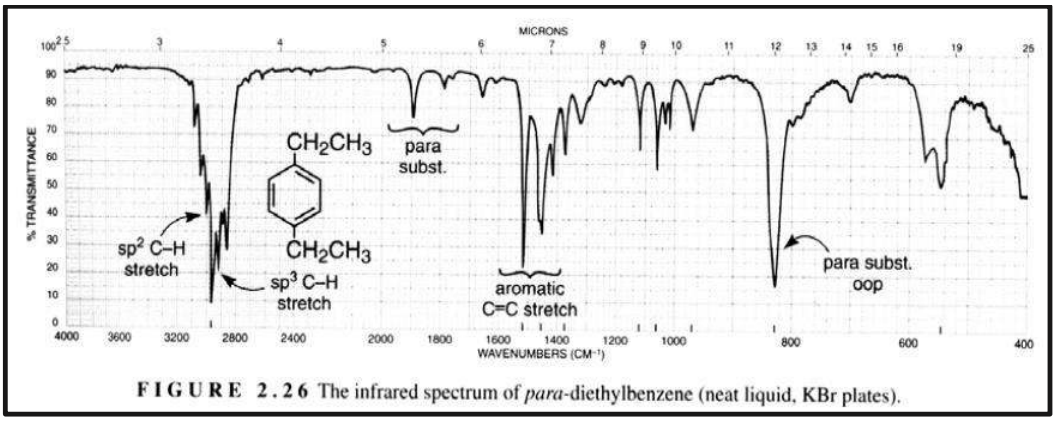
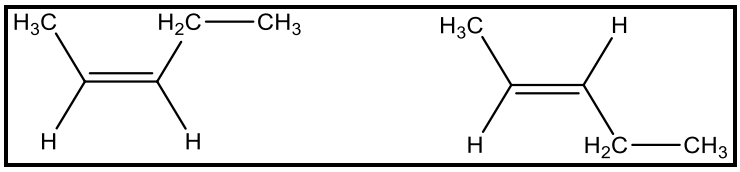


FIGURE 2.26 The infrared spectrum of *para*-diethylbenzene (neat liquid, KBr plates).

2) Distinguishing of double bond configuration of saturated compounds (cis and trans isomers)

For IR absorption, the absorption must result in change in dipole moment. In case of cis and trans isomers, trans isomers are more symmetrical than cis isomers. Trans isomer give rise to little or no change in dipole moment. Hence, Trans isomers give a simple IR spectrum with lower absorption frequency than cis isomer.



Cis isomer (C-H bending)	Trans isomer (C-H bending)
700-650 cm^{-1}	990-960 cm^{-1}

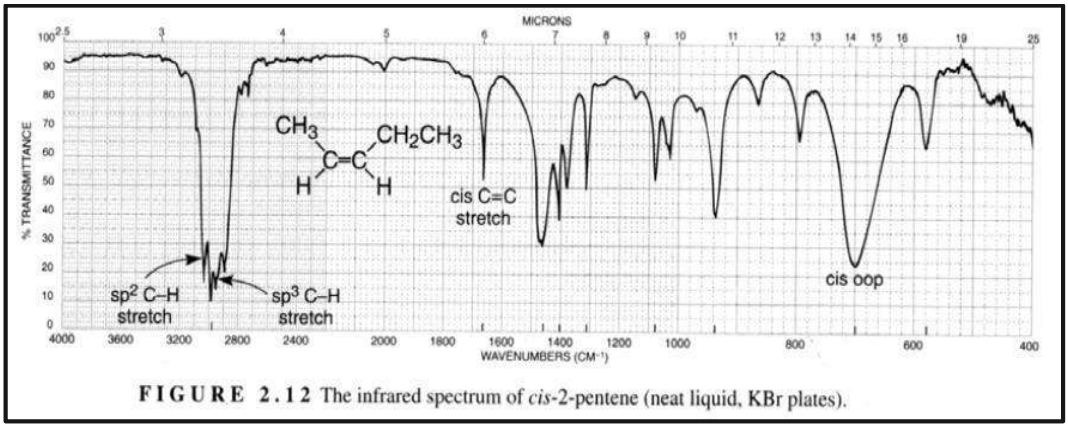


FIGURE 2.12 The infrared spectrum of *cis*-2-pentene (neat liquid, KBr plates).

In these IR spectrum, intense C=C stretching is observed at 1650 cm^{-1}. The broad band at 700 cm^{-1} confirm cis isomer.

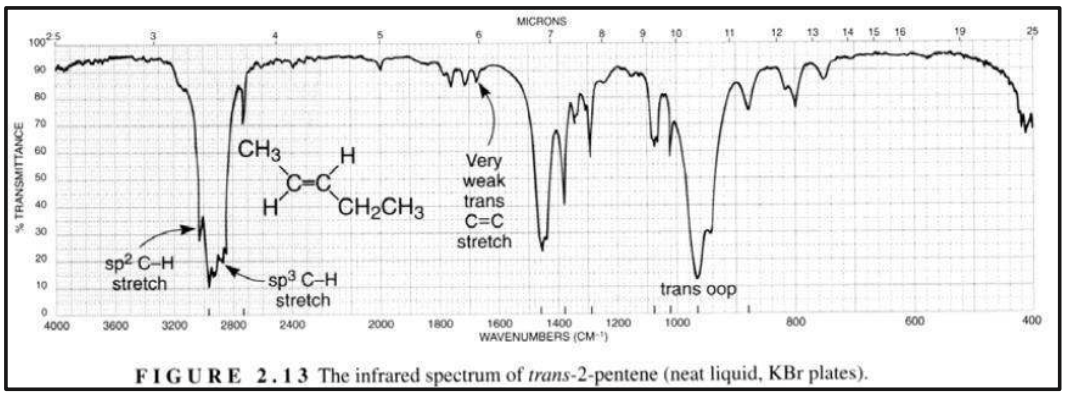


FIGURE 2.13 The infrared spectrum of *trans*-2-pentene (neat liquid, KBr plates).

In these IR spectrum, weak C=C stretching is observed at 1650-1750 cm^{-1}. The weal single peak at 970 cm^{-1} confirm trans isomer.