UNIT 4 GENERAL PRINCIPLES OF CHROMATOGRAPHY

Structure

4.1 INTRODUCTION

In the beginning of this course in Unit 1 of Block 1, we have realized that chromatography as a collective science which encompasses a number of important separation techniques. The subject was introduced to the scientific world in a very modest way by M. Tswett in 1906. He employed the technique to separate various pigments such as chlorophylls and xanthophylls by passing a solution of these compounds through a glass column packed with finely divided calcium carbonate. Later on this technique was named as *liquid-solid adsorption chromatography*. About the same time, Thompson and Way had realized the ion exchange properties of soils. Almost after three decades, in 1935, Adams and Holmes observed ion exchange character in crushed phonograph. This particular observation opened up the field for synthetic organic resin exchangers. Thereafter, the subject of *ion exchange chromatography* started expanding in its utility. The concept of *gas-liquid chromatography* was first introduced by Martin and Synge in 1941. They were also responsible for the developments in liquid-liquid partition chromatography. Moreover, in 1944, from Martin's laboratory, the separation of amino acids by *paper chromatography was reported*. This made people to realize the importance of such a simple technique for tedious separations. Simultaneously the efforts were being made to understand the theoretical aspects of chromatography. The high point of all these activities reached in 1952 when the contributions of Martin and Synge were recognized and they were awarded the Nobel Prize for their work in chromatographic science.

The analytical scientists all around the world got actively engaged in improving the participating phases, detectors and the instrumentation in general. In 1959, a technique known as *gel filtration chromatography* to separate high and low molecular weight substances, originated at Biochemical Institute in Uppsala, Sweden. In the 1960s, further improvements in the conventional liquid chromatography led to the development of *high performance liquid chromatography*. The following decade of the seventies saw an important improvement in adsorption chromatography in the form of *affinity chromatography* which was mainly based on biological interactions. The researches for developments in chromatography were still being carried out and a new type of chromatography using superficial fluids appeared on the scene. *Supercritical fluid chromatography* is a hybrid of gas and liquid chromatography and combines the advantageous features of the two.

The above description on the growth of chromatography clearly points out that once the potential of some simpler versions of chromatography for separations was realized, the researches took place on a phenomenal pace. It will not be wrong to say that the entire twentieth century can be named as the century of chromatography. Even today, the efforts are being made to bring different types of improvements. At this point, one should not forget that the developments are not only because of variety of physicochemical principles that can be incorporated in chromatographic techniques to bring out separations, but are partly due to the compelling need of the scientific community to achieve a variety of tedious separations particularly related with life sciences.

The present unit aims at proposing a classification of chromatographic techniques. An effort will also be made to explain some of the general principles which lead to separation of compounds present in a mixture. Before discussing the theory or the concepts, it may be necessary to familiarize you with the terms which are used to assess the separation efficiency of a process.

Objectives

After studying this Unit, you should be able to

- discribe the different ways chromatographic techniques can be classified,
- explain processes taking place during elution on columns,
- realize the importance of terms like migration rate, distribution constant, retention time, retention factor, selectivity factor and resolution,
- explain the concept of theoretical plates and its importance, and
- discuss the rate theory.

4.2 DEFINITION AND CLASSIFICATION

In order to classify the different forms of chromatography, we will have to once again look into the definition of chromatography. An analysis of the definition will help us to visualize the different ways the techniques can be classified and finally evolve a system of classification so as to be able to include most of the forms of chromatography.

4.2.1 Definition

Chromatography is a method of separation in which the components to be separated are distributed between two phases, one of these is called a *stationary phase* and the other a *mobile phase* which moves on the stationary phase in a definite direction. The components of a mixture redistribute themselves between two phases by a process which may be *adsorption, partition, ion exchange or size exclusion*. The stationary

phase can be a solid or a liquid and the mobile phase a liquid, gas or a supercritical fluid. The stationary liquid is held on an inert solid support or coated on the walls of a capillary. The solid whether it is support or an active material is held in the form of a column, coated over a plate or presented as a paper. The latter two types come under the head of *two dimensional chromatography*.

4.2.2 Classification

If we look into the definition of chromatography and the situation spelt out above, chromatography can be classified on the basis of the following:

- The shape of the solid support.
- The nature of the mobile phase.
- The mechanism responsible for separation.

If we go by the *shape of support* in chromatography, there will be three dimensional (column) and two dimensional (thin layer and paper) chromatography. In two dimensional chromatography, there will only be *two* types of chromatography, thin layer and paper and all the other forms of chromatography will fall under the head of column chromatography. This is too simple a classification and does not take into consideration the nature of the mobile phase and the process responsible for separation. Moreover, *two dimensional chromatography* is operated using the liquid only as a mobile phase.

If the classification is done purely on the basis of the nature of mobile phase, there will be three types of chromatographic techniques – *liquid*, *gas* and *supercritical fluid chromatogra*phy. Here, the first two are well known and several advancements have already been made in these two forms of chromatography. *Supercritical fluid chromatography* is relatively new and does not figure so frequently. Inspite of some advantages it has still to become popular. Again, in this type of classification, a large number of chromatographic techniques with varied modes of separations fall under the head of *liquid chromatography*.

In case the *mechanism responsible for separation* is taken as the criteria for classification, there will be four distinct types of chromatography *viz*, *adsorption*, *partition*, *ion-exchange* and *size exclusion*. But we will have to separately mention the competing phases.

The above discussion clearly points out that no single criteria by itself will be able to effectively classify the various chromatographic techniques. We will have to take one as the main criteria and make a sub-classification based on the other yardstick. A simple approach seems to be to take the nature of the mobile phase as the main criteria and propose a sub-classification based on the various mechanisms responsible for separation. If any special feature exists within a sub-classification it may be pointed out right there.

For summing up the classification of chromatographic techniques, it emerges that there will be three types of chromatographic techniques.

i) Liquid chromatography.

ii) Gas chromatography, and

iii) Supercritical fluid chromatography.

We will discuss each one in brief and take into consideration the other competiting phase, the mechanism responsible for separation and the nature and shape of surface. The following sub-sections will be devoted to discussion on these lines. Finally, the conclusion will be presented in the form of a table.

SAQ 1

Based on the shape of support, what will be the main categories of chromatography?

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4.2.3 Liquid Chromatography

The simplest one with liquid as a mobile phase seems to be based on partition between the liquid supported on an inert support and the other liquid acting as a mobile phase. This is known as *liquid- liquid partition chro*matography. The support holding the liquid can be in the form of three dimensions (*column*) or two dimensions (*thin layer* and *paper*). In the two dimensional form, the mobile phase can move from top to bottom or bottom to top and thus, known as *descending* or *ascending chromatography*. The two types of partition chromatography are also distinguishable on the basis of relative polarities of the mobile and stationary phases. *Conventionally*, the highly polar solvent is held on the support and the mobile phase is relatively non-polar. This is known as *normal-phase chromatography*. If the stationary phase is non-polar and the mobile phase relatively polar, it is termed as *reversed-phase chromatography*. An important modification in terms of stationary phase is introduced by loading the extractant used for solvent extraction on a hydrophobized inert support and irrigating the support with aqueous solvents. This is known as *extraction chromatography*. At this point, it may be important to introduce liquid chromatography on bonded phases. Initially, the partition chromatography was only liquid-liquid type but now chromatography with bonded phase has gained prominence.

In liquid-liquid type, a liquid stationary phase is held on the support packing by physical adsorption while in bonded phase the stationary phase is bonded chemically to the support. One of the main disadvantages of the liquid-liquid system is the loss of stationary phase by dissolution in the mobile phase. This requires periodic recoating of the support particles. Furthermore, the solubility of the stationary phase prohibits the use of liquid-phase packing for gradient elution. However, with bonded-phase, these problems are mitigated to a large extent. In a majority of cases, the bonded- phase packings for partition chromatography are prepared from rigid silica or silica based compositions. Bonded phase packings are classified as reversed phase when the bonded coating is non polar and as the normal phase if the coating contains polar functional groups. Mostly in reversed phase the coating is a C_8 chain (*n*-octyl) or a C_{18} chain (*n*-octyldecyl). For normal phase polar bonded phases that have a diol ($-C_3H_6OCH_2CHOHCH_2OH$), cyano ($-C_2H_4CN$), amino ($-C_3H_6NH_2$) and dimethylamino ($-C_3H_6N(CH_3)_2$) functional groups are commercially available. We have seen that the nature of the stationary phase and the way it is held on the support are responsible for quite a few diversifications in liquid partition chromatography. If the solid support instead of holding the liquid acts as an active adsorbent, the related chromatography is known as *liquid-solid adsorption chromatography*. This statement is true for both column and two dimensional chromatography. Sometimes, it becomes difficult to distinguish whether it is the partition or the adsorption which is dominating in the separation.

An advancement in liquid adsorption chromatography was reported in the form of affinity chromatography. In this the column material, a gel like material, is attached with a biospecific material through a spacer arm. This biospecific material is known as a ligand. The ligand selectively picks up a biological molecule from the mixture to

form a complex. The desired biological molecule can be eluted by modest changes such as pH or ionic strength in the eluant.

An important branch of liquid chromatography is *ion exchange chromatography*. This involves exchange of ions, cations or anions, between an insoluble matrix and the solution which comes in contact with it. The insoluble material which carries exchangeable cations and anions is known as ion exchanger. Those carrying exchangeable cations are known as *cation exchangers* and those with exchangeable anions are known as *anion exchangers*. There are *amphoteric exchangers* which are capable of both cation and anion exchange. Ion exchange, with a very few exceptions, is a reversible process. In contrast to adsorption, ion exchange is a stoichiometric process. Every ion removed from the solution is replaced by an equivalent amount of another ionic species of the same sign. Ion exchangers are organic and inorganic, natural and synthetic. Most of the ion exchangers in daily use are organic resins.

There is another form of chromatography which is synonymous to ion- exchange chromatography and is known as *ion-pair* (or *paired-ion*) *chromatography*. It is a type of reversed phase chromatography and used for the separation of ionic species. In this, the mobile phase consists of an aqueous buffer containing an organic solvent (methanol/ acetonitrile) and an organic compound containing a counter ion of opposite in charge to the analyte. The counter ion combines with the analyte to form a neutral ion pair. The neutral ion pair is retained on the non- polar stationary phase. It is subsequently eluted with an aqueous solution of methanol or other water soluble organic solvents. Ionic or partial ionic compounds can be chromatographed on reversed phase columns by using ion pairing reagents. Typically these reagents are long-chain alkyl anions or cations. When these reagents are used in dilute solutions, the retention of the analytical ion can increase. C_5 to C_{10} alkyl sulphonates are commonly used cationic compounds. Tetra alkyl ammonium salts (tetramethyl-, tetrabutyl-, and ammonium salt or triethyl- (C_5 - C_8) alkyl ammonium salts are generally used in the case of anionic solutes.

The applications of ion pair chromatography frequently overlap with those of ion-exchange chromatography. Ion chromatography is generally used for the separations which are difficult to attain with ion exchange chromatography. A typical example is the separation of chlorate and nitrate which is difficult to achieve on an ion exchange but easily attained by ion pair chromatography.

Size exclusion chromatography is a very useful form of liquid chromatography employed for the separation of high molecular weight substances. This is also called *gel permeation* or *gel filtration chromatography*. Two important types of column packing material used for chromatography in aqueous phase are *cross-linked dextrans* and *polyacrylamide*. The process responsible for the separation is due to the distribution of solute between the aqueous phase with in the gel particles and external water. The selectivity is achieved on the basis of pore size of the gel. A solute which is small enough may enter the gel phase. In a column, this will have retarding effect. The molecules which have penetrated the gel, will spend their time sheltered from the moving phase. At the other extreme if the molecule is large enough not to be able to enter the pores will spend all the time in the mobile phase and move rapidly through the column. In between there will be molecules of intermediate size which can penetrate in some degree and their movement down the column will be somewhat retarded. This forms the basis for separating the molecules of different sizes.

While concluding the discussion on liquid chromatography, it will be important to point out that the most significant advancement in liquid chromatography came in the form of *high performance* or *high pressure liquid chromatography*. This improved version is more or less available for different forms of liquid chromatography. The separation is performed on a column under pressure. The column usually contains

particles of very small size, 3 -10 µm. The material may be an adsorbent or materials that permit partition, ion exchange or molecular permeation. The pressure generally used is around 6000 psi. The mobile phase may be composed of one solvent or a mixture of solvents with or without modifiers. Now high pressure thin layer chromatography (HPTLC) has also gained prominence.

SAQ 2

What is the main advantage of reversed phase liquid chromatography?

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SAQ 3

What is extraction chromatography?

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SAQ 4

What particular property of the gel is responsible for separations in gel chromatography?

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4.2.4 Gas Chromatography

In gas chromatography, the mobile phase is a gas. The gas does not react with the molecules of the analyte. It only carries the sample through the column. Two types of gas chromatography are encountered

- Gas-solid chromatography (GSC).
- Gas-liquid chromatography (GLC).

In GSC, the retention of the analyte is due to *physical adsorption* on the column material. In GLC, the separation takes place because of *partition* between the gaseous

mobile phase and the liquid immobilized on the surface of an inert solid. GSC is not as popular as GLC because of some limitations of the former mode. In GSC, the retention time may be unusually large and high temperatures may be required to desorb the species. There may be severe tailing due to non-linear character of adsorption. The active adsorbent may catalyze the adsorbed species and change it to some other compound. As a result of all these GSC has a limited utility and gas chromatography more or less refers to gas-liquid partition chromatography. Generally, two types of columns are used in gas chromatography, packed and open tubular or capillary columns. The chromatographic columns vary in length from less than 2m to 50m or more. They are constructed of stainless steel, aluminium glass or fused glass or teflon. Open tubular columns are two basic types, wall-coated open tubular and support-coated open tubular. Wall coated columns are capillary tubes coated with thin layer of stationary phase. In support coated open tubular columns the inner surface is lined with a thin film of support material. This support material holds the stationary phase. The most commonly used stationary phases are polydimethyl siloxanes. Organic species bonded to a solid surface are also used as stationary phases.

SAQ 5

What are the two types of columns used in gas chromatography?

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4.2.5 Supercritical Fluid Chromatography

During the last quarter of the twentieth century, another type of chromatography with an entirely new type of mobile phase, supercritical fluid, has come up on the forefront. It is basically partition chromatography. Here, instead of a liquid or a gas, we use a supercritical fluid. In order to understand a *supercritical fluid* we will have to know *critical temperature* and *pressure* of a substance. The *critical temperature* of a substance is the temperature above which a distinct liquid phase cannot exist regardless of pressure. The vapour pressure of a substance at its critical temperature is its **critical pressure**. At temperature and pressure above its critical temperature and pressure (**critical point**), a substance is known as **supercritical fluid**. The properties like density, viscosity and other characteristics of supercritical fluids are intermediate between the gaseous and liquid state. The most well known compound used as a supercritical fluid in chromatography is CO_2. The other compounds in use are nitrous oxide, ammonia and *n*- butane. There are about two dozen compounds which have been used as mobile phases in supercritical chromatography. The critical temperature and pressure at these temperatures is well within the operating conditions used in HPLC.

One remarkable property of supercritical fluids which is related to their high densities (0.2- 0.5 g/cm^3) is their capability to dissolve large non-volatile molecules. Another property which is associated with many supercritical fluids is that the dissolved analyte can be easily recovered by allowing the solutions to equilibrate with the atmosphere at a relatively low temperature. This is important if the analyte happens to be thermally unstable. A significant point which goes in the favour of many

supercritical fluids is that they are inexpensive and non- toxic. They can be easily allowed to evaporate in atmosphere with no harmful effects.

As a matter of fact supercritical fluid chromatography is a hybrid of gas and liquid chromatography. It combines some of the advantages of both liquid and gas chromatography. In supercritical fluid chromatography, the mobile has a low viscosity and high self diffusion coefficient. The mobile phase can be further modified by addition of polar modifier in small amounts. In this type of chromatography both packed and open tubular columns are used. In open tubular columns, the internal coating is of siloaxanes of various types and that of bonded phases.

Like gas chromatography, supercritical fluid chromatography is faster than liquid chromatography because of lower viscosity. Diffusion rates in supercritical fluid chromatography are intermediate between those in gases and in liquids. As a result of this, the band broadening is greater in supercritical fluids than in liquids but lesser than in gases. In nutshell, the intermediate diffusivities and viscosities of supercritical fluids may lead to faster separations than are obtained with liquid chromatography accompanied by lower zone broadening than observed in gas chromatography.

This new type of chromatography has started finding applications for a wide variety of materials like natural products, foods, pesticides, polymers, fossil fuels and explosives. In spite of gaining prominence, this particular technique has not been discussed in detail in this course. It is still to match in popularity with other separation techniques included in the course.

SAQ 6

Mention two important advantages of supercritical fluid chromatography.

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4.2.6 Conclusions

To sum up the discussion on classification of chromatographic techniques, it can be said that a large number of techniques fall under the head of liquid chromatography. This is mainly because of the fact that a large number of mechanisms operate with a liquid as a mobile phase. Moreover, there is the availability of a variety of types of surfaces as stationary phases. Also, there have been some improvements in the methodology. The most important in this regard is the introduction of high performance liquid chromatography. Gas chromatography, inspite of being a valuable tool for separations, remains mostly confined to gas-liquid chromatography because of certain limitations of its gas-solid version. Supercritical fluid chromatography has to still catch up liquid and gas chromatography in their popularity. The entire discussion on classification is being summed up in Table 4.1.

It is not possible to include all the different features discussed in the text in the table because it may become too congested. However, the salient features of the classifications are being presented in the table.

Table 4.1: Classification of Chromatographic Methods

General Category	Stationary Phase	Mechanism	Modes
Liquid Chromatography (LC)[Mobile Phase: liquid]	Liquid adsorbed on a solid or organic bonded to solid surface	Partition	Column, two dimensional; normal, reversed phase; loaded extractant; ion pair formation
	Solid adsorbent	Adsorption	Column, two dimensional, adsorbent, biospecific molecule attached to surface
	Ion Exchanger Liquid in interstices of a gel	Ion Exchange Partition/ Sieving	Column, two dimensional, batch, column, thin layer
Gas Chromatography (GC)[Mobile phase: gas]	Liquid adsorbed on a solid or organic bonded to solid surface Solid adsorbent	Partition Adsorption	Column Column
Supercritical Fluid Chromatography (SFC)[Mobile phase: supercritical fluid]	Organic species bonded to solid surface	Partition	Column

Note: The different forms of liquid chromatography can be carried in the high performance liquid chromatography mode; the exception being if the paper is used as the support.

4.3 ELUTION OF COLUMNS

After having learnt the classification of chromatographic methods, it is important to look into some concepts which may explain the separation process. From the discussion, it can be noted that the separations are conducted on columns or on planar surfaces. The equilibria upon which the two types of chromatography are based are identical. The theory developed for column chromatography is adapted to planar chromatography.

To start with we look into a simple picture as to how the two substances X and Y are separated on a column by elution with a liquid as a mobile phase. Actually, elution involves washing a species through a column by continuous addition of fresh solvent. The different stages of separation are schematically shown in Fig. 4.1. The sample is introduced at the head of column (time t_0) where the components of the sample distribute themselves between the two phases. Further addition of mobile phase pushes the solvent containing a part of the sample down the column. Here further partition between the mobile phase and fresh portion of stationary phase occurs (time t_1). Simultaneously, partitioning between the fresh solvent and the stationary phase is taking place at the site of the original sample.

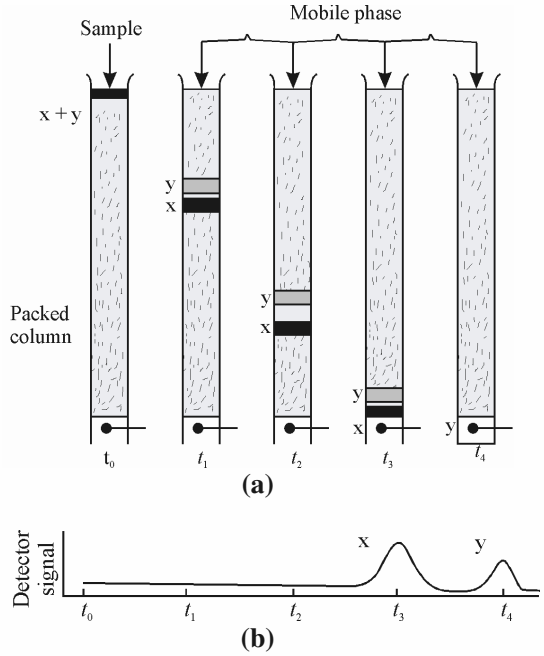

Fig. 4.1: (a) Column separation of a mixture of components X and Y; and (b) Output of signal detector at various stages

In this way, continued addition of solvent carries the solute molecules down the column in a continuous series of transfers between the mobile and stationary phases. The solute movement can only occur in the mobile phase, therefore, the average rate at which a solute zone migrates down the column depends upon fraction of time it spends in that phase. This fraction of time spent is small for solutes which are strongly retained by the stationary phase. If we refer to Fig. 4.1, this is the situation for Y. On the other hand, the retention of X is more in the mobile phase. As a result of this difference in retention rates, the components of mixture separate into bands or zones, (time t_2). If sufficient quantity of solvent is passed, the two species are isolated as individual zones and then pass out of the column where they can be detected or collected. (time t_3 and time t_4).

It should be noticed that during the separation procedure, the dilution of analyte takes place. The analytes get more dilute than they were present in the original mixture. Therefore, the detectors employed for the separated components should be invariably more sensitive than would be required if the separation was not needed. The detector responds to the concentration of the solute coming out of the column. When its signal is plotted against time or the volume of the mobile phase a series of peaks are plotted. Such a plot is known as a *chromatogram*. The chromatogram is useful for both qualitative and quantitative analysis. The position of the peak on time axis helps to identify the components and the areas under the peak provide the amount of each component.

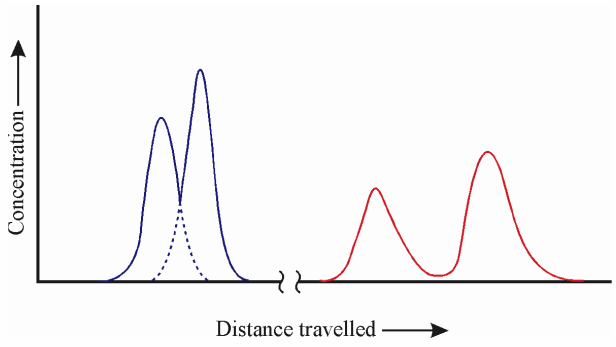

Fig. 4.2: Concentration profiles at different stages of elution

In the separation of X and Y, the species Y is more strongly held by the column and thus it lags. During the movement in the column the distance between the two zones increases. At the same time, broadening of two zones takes place. This lowers the efficiency of the separation method. There can be two approaches to increase the resolution of species.

1. One component moves at a faster pace and the other at a slower rate.
2. The rate of zone broadening for the two species is decreased.

The two situations are illustrated in Fig. 4.3.

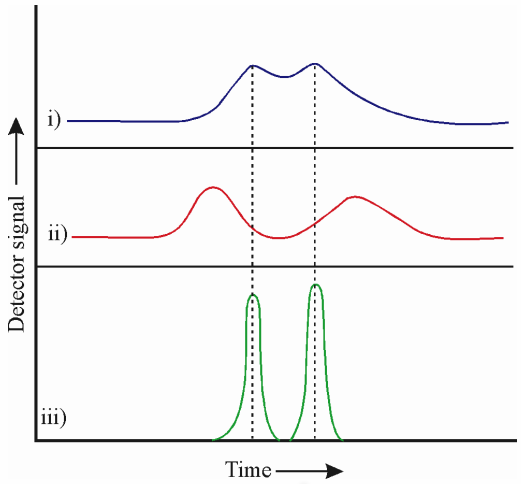

Fig. 4.3: Two approaches to improve the binary separation; (i) initial chromatogram with overlapping peaks improved by; (ii) increase in band separation; and (iii) decrease in band width

4.4 MIGRATION RATES

From the above example, it becomes clear that the effectiveness of a column in separating the two solutes depends in part on the relative rate at which the two are eluted from the column. The rates depend upon the magnitude of the equilibrium constants of reactions by which the solutes distribute themselves between the mobile and stationary phases.

4.4.1 Distribution Constant

In chromatography, the distribution equilibrium is often simple and is described by the equations that involve the transfer of an analyte between the mobile and stationary phase. Thus, for the solute X, we may write

$$X_{mobile} \Leftrightarrow X_{stationary}$$

The equilibrium constant, K, for the reaction is known as *distribution constant* and is given by the following expression

$$K = \frac{C_S}{C_M}. \qquad \ldots (4.1)$$

Where, C_S is the molar concentration of the solute in the stationary phase and C_M is its molar concentration in the mobile phase. Ideally, C_S is directly proportional to C_M over a wide range of concentration. If such a situation exists, the chromatography is known as *linear chromatography* and results in symmetric Gaussian type peaks. The retention times are independent of the amount of the compound injected. Ideal behaviour, on the other hand, is not attainable in any actual chromatographic process. It is actually

non-ideal behaviour which operates and the corresponding chromatography is known as *non-linear chromatography*.

SAQ 7

What conditions will lead to linear ideal chromatography?

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4.4.2 Retention Time

In the preceding sub-section, we have introduced a term known as *retention time*. It is time taken after sample injection for the analyte peak to reach the detector. It is denoted by the symbol t_R and in Fig. 4.4 is represented.

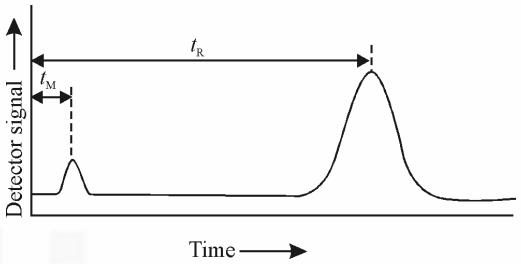

Fig. 4.4: Small peak at the left represents a species that is not retained and reaches the detector immediately. t_M is approximately equal to the time needed for the molecules of the mobile phase to pass through the column

The small peak in the beginning on the left is for the species that is not retained at the column. Sometimes, the sample may contain an unretained species. If it is there it may be added because it helps in peak identification. The time t_M for the unretained species is the same as the average rate of migration of mobile phase molecules. If L is the length of the column, the average rate of migration of solute molecules is given by the following expression:

$$v = \frac{L}{t_R} \quad \ldots (4.2)$$

Similarly, the average linear rate of migration of mobile phase molecule

$$u = \frac{L}{t_M} \quad \ldots (4.3)$$

where, t_M is called dead time. It is the time required for the average molecules of the mobile phase to pass through the column. After getting familiar with the terms distribution constant and retention time, it may be important to look into the relationship between the two terms. We express migration rate of a solute

$$v = u \times \text{fraction of time solute spends in the mobile phase} \quad \ldots (4.4)$$

This fraction is equal to average number of moles of a solute in the mobile phase at any instant divided by the total number of moles of the solute in the column.

$$v = u \times \frac{\textit{Moles of the solute in mobile phase}}{\textit{Total moles in the column}} \quad \ldots (4.5)$$

The number of moles of the solute in the mobile and stationary phases are the product of their concentration and respective volumes

$$v = u \times \frac{C_M V_M}{C_M V_M + C_S V_S} \quad \ldots (4.6)$$

where, C and V denote the concentration and volume, respectively

$$v = u \times \frac{1}{1 + C_S V_S / C_M V_M} \quad \ldots (4.7)$$

We know that $K = \frac{C_S}{C_M}$

Thus, we have a relation between solute migration and distribution constant

$$v = u \times \frac{1}{1 + K V_S / V_M} \quad \ldots (4.8)$$

4.4.3 Retention Factor

There is another term known as retention factor or capacity factor which is commonly used to describe the migration rates of the solutes. For a solute X, the retention factor k'_X is expressed as

$$k'_X = \frac{K_X V_S}{V_M} \quad \ldots (4.9)$$

K_X = distribution constant for X

Substituting Eq. 4.9 in Eq. 4.8, we get

$$v = u \times \frac{1}{1 + k'_X} \quad \ldots (4.10)$$

Now, substituting Eqs. 4.3 and 4.2 into Eq. 4.10, we get

$$\frac{L}{t_R} = \frac{L}{t_M} \times \frac{1}{1 + k'_X} \quad \ldots (4.11)$$

Rearranging the above equation, we get

$$k'_X = \frac{t_R - t_M}{t_M} \quad \ldots (4.12)$$

We can determine t_R and t_M from a chromatogram. If the retention factor is much less than unity, elution occurs so rapidly that the accurate determination of retention factor is difficult. It the retention factor is larger, somewhere 20 or 30, the elution times are unusually long. Ideally separations of solutes are carried out for which the retention factors are between 2 and 10.

SAQ 8

What are the problems if the retention factor is too small or too large?

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4.4.4 Selectivity Factor (Separation Factor)

The relative migration rates of the solutes are expressed in terms of *selectivity factor*. The *selectivity factor/ separation factor*, α, of a column for two solutes X and Y is expressed as follows:

$$\alpha = \frac{K_Y}{K_X} \quad \ldots (4.13)$$

where, K_Y is the distribution constant for the more strongly retained species Y, and K_X is the distribution constant for more rapidly eluted species X. The selectivity factor should always be greater than unity. Substituting Eq. 4.9 and the analogous equation for Y in Eq. 4.13, the following equation is obtained for selectivity factor in terms of retention factor

$$\alpha = \frac{k'_Y}{k'_X} \quad \ldots (4.14)$$

where, k'_y and k'_x are retention factors for Y and X, respectively. Eq. 4.14 can be converted into the form of retention times as follows:

$$\alpha = \frac{(t_R)_Y - t_M}{(t_R)_X - t_M} \quad \ldots (4.15)$$

Later, the selectivity factor and retention factor will be used to compute the resolving power of column.

4.4.5 Shapes of Peaks

At this stage, it may be important to examine the peaks in a chromatogram (see Fig. 4.4) and the bands on a column (Fig. 4.1). They show a similarity to normal error or Gaussian curves when the values of a measurement are plotted as a function of their occurrence. An individual solute molecule undergoes thousands of transfers between the stationary and mobile phase. In some cases, the residence time in a given phase may be transitory and in other case the period may be relatively long. The molecule is eluted during its residence in the mobile phase. Therefore, the migration is highly irregular down the column. Since there is a variability in the residence time the average rate at which the individual molecules travel relative to mobile phase varies considerably. Some molecules travel faster because of their accidental inclusion in the mobile phase for a majority of time. On the contrary, others lag behind because they spend more time in the stationary phase. As a consequence of these random processes, a systematic spread of velocities around the mean value is observed. The breadth of band increases because more time is allowed for spreading to take place. It can be concluded that the zone breadth is directly related to the residence time in the column and inversely related to the velocity of the mobile phase.

4.5 CONCEPT OF THEORETICAL PLATES

With all the concepts learnt up to this stage, we may be in a position to assess the performance of a column. When we say performance, it automatically implies efficiency in attaining a separation. The various factors contributing to efficiency are examined by a concept known as height equivalent to theoretical plate (HETP). This concept of theoretical plates is similar to one occurring in fractional distillation or countercurrent extraction. According to this, chromatographic column can be assumed to be made up of a large number of theoretical plates where equilibrium is achieved. Ideally, a theoretical plate is a fictitious concept which does not correspond to any actual entity in the column. However, it is a convenient parameter for evaluation. Thus there are two terms, plate height (H) and plate count or number of plates (N). The two are related as

$$N = \frac{L}{H} \quad \ldots (4.16)$$

where L is the length of column packing (usually in cm). H is defined as that length of the column which will yield an effluent in equilibrium with mean concentration over that length in the stationary phase. In other words, at each plate equilibrium of the solute between the mobile and stationary phase is assumed to take place. Movement of the solute down the column is then assumed to take place as stepwise transfer of equilibrated mobile phase from one plate to the next. For an efficient separation, we want more such transfers that is more number of plates. This amounts to the fact that the height equal to theoretical plates (HETP) should be minimum. Fig. 4.5 shows a Gaussian elution band and the parameters which are used to calculate N.

Retention time (t_R)

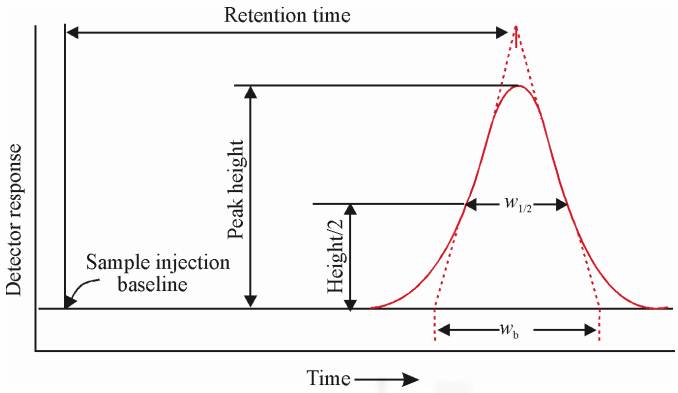

Fig. 4.5: A typical chromatogram showing retention time, peak height and band width for estimating the number of theoretical plates

The retention time t_R is the time taken from the injection of sample to the appearance of the peak of the elution band at the detector. The formula used to calculate N is

$$N = 16 \left(\frac{t_R}{W_b}\right)^2 \quad \ldots (4.17)$$

Tangents to the band are drawn at the two inflection points and the width, W_b, is the distance between the intersections of these tangents with the baseline. The other way is in terms of $W_{1/2}$, the width measured halfway between the baseline and the top of the band

$$N = 5.54 \left(\frac{t_R}{W_{1/2}}\right)^2 \quad \ldots (4.18)$$

The two methods give comparable results and the choice is a matter of personal preference. For the purpose of determining N, the units for abscissa make no difference. Although t_R is defined but actually may be measured on a the recorder chart in cm or mm. Actually, t_R and W, should be measured in the same units.

It should be kept in mind that the number of theoretical plates in a column is function of column preparation, the characteristics of the solute, temperature, flow rate, method of sample introduction and so forth. Moreover, the number of plates in a column is found to vary with the sample size in a regular way. Overloading of column deteriorates the performance of the column. As a result of all this N is only an approximate number that is useful for comparative purposes.

In a nutshell, the plate concept only successfully explains the Gaussian shape of the chromatographic peaks and their rate of movement down a column. It fails to explain peak broadening in a realistic way. The concept, somehow, gives an impression that

the column contains plates where equilibrium conditions exist. Actually, the equilibrium can never be visualized when the mobile phase is in constant motion. A more realistic picture is taken into consideration in the rate theory which is being discussed in the next section.

SAQ 9

Does the number of theoretical plates in a column change with the concentration of the sample?

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4.6 RATE THEORY

This concept actually visualizes the role of different kinetic variables on zone broadening. The migration of the solute down the column takes place as a result of several mass transfers and there is some time which these mass transfers take place. The kinetic effects depend upon the length of time, the mobile phase spends in contact with the stationary phase. This, in turn, depends upon the flow rate of the mobile phase. For this reason the studies on the efficiency of the column are carried out by determining HETP (H) as a function of mobile phase velocity (u). The shapes of two such plots, one for the liquid chromatography and the other for gas chromatography are shown in Fig. 4.6.

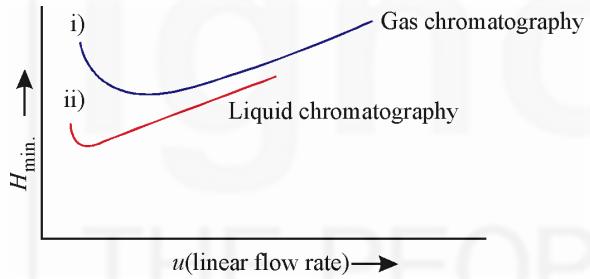

Fig. 4.6: Dependence of plate height on the flow rate of mobile phase i) liquid chromatography ii) gas chromatography

In both the cases, there is a $H_{min.}$ at a low flow rate. The minima for liquid chromatography occurs generally at flow rates well below those for gas chromatography. Also, in liquid chromatography, the flow rates for $H_{min.}$ is often so low that they are not observed under normal working conditions. In gas chromatography, the separations are achieved in shorter times than those attained in liquid chromatography.

Several attempts have been made to develop a quantitative relationship between the plate height and the column variables. One of the most accepted and in common use was given in 1950s by a Dutch chemical engineer van Deemeter. The equation given by him, known as *van Demeter equation*, is in the following form

$$H = A + B/u + Cu \qquad \ldots (4.19)$$

where, H is the plate height (cm), u is the linear velocity of the mobile phase (cm/sec) and A, B and C are the coefficients related to the phenomena of *eddy diffusion* or *multiple flow paths*, *longitudinal diffusion* and *mass transfer between phases*, respectively. The coefficient C can be divided in two coefficients, one pertaining to

the stationary phase (C_S) and the other related to mobile phase (C_M). It means the equation can be rewritten as

$$H = A+B/u+ (C_S+C_M)\, u \qquad \ldots (4.20)$$

Now, we have to examine the effect of variables on the four terms, A, B/u, $C_S u$ and $C_M u$.

4.6.1 Eddy Diffusion Term (A)

This factor arises from the multiplicity of pathways for a gas flowing through the column; therefore, it is also known as *'multiple flow paths'* term. The column is packed with particles of various sizes and shapes arranged in an irregular manner. As the carrier gas flows through the channels of the packing, it is divided into many streams, some of which may merge and again divide in a complex manner. Thus, the molecules travel through a series of tortuous pathways. The solute molecules carried by the gas will face a similar fate. They will follow many paths, some shorter, some longer than the average distance. The situation is illustrated by taking the example of two molecules passing through the column (see Fig. 4.7).

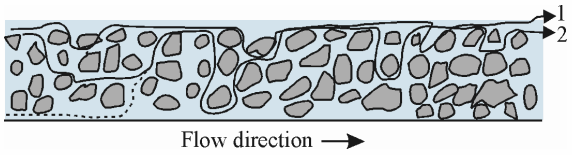

Fig. 4.7: Typical pathways of two molecules 1 and 2, 2 will travel longer distance than 1

This will mean that the original solute *plug* will spread out. Some molecules will reach the detector sooner, some later and many at the average time. The magnitude of contribution of eddy diffusion has to depend on the size, shape and uniformity of packing particles. Multiple path broadening may be partially offset by ordinary diffusion which results in molecules being transferred from a stream following one pathway to a stream following another. Furthermore, it seems that some of the band broadening which used to be attributed to eddy diffusion occurs outside the column say in the tubing that led from the column to the detector and in the detector itself. It could also be the glass wool that is sometimes used to plug the column. The contribution of eddy diffusion has now become very small with well designed gas chromatograph with good column. Moreover, it is almost negligible compared to other band broadening factors. This factor is independent of mobile phase velocity. If the velocity of mobile phase is low, a large number of transfers will occur spending a brief time in each. As a result of this, the molecule moves down the column tending to approach the average velocity. In the case of moderate or high velocities, sufficient time is not available for diffusion averaging to occur and the band broadening due to the different path length is observed.

From the above discussion, one can conclude that A will depend on irregularity of packing and diameter of the particles, i.e.

$$A = 2\, \lambda\, d_P \qquad \ldots (4.21)$$

λ = is a dimensionless parameter depicting the irregularity of packing, and
d_P = is the diameter of particles (cm).

SAQ 10

What particular factor mainly contributes towards the eddy diffusion term?

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4.6.2 Longitudinal Diffusion Term (B/u)

The second term in the equation arises because of longitudinal diffusion of the solute in the gas phase. Some of the solute molecules tend to diffuse along concentration gradients. Thus, a solute band will broaden as the molecules spread into regions of lower concentration ahead of the band and behind it. Actually, the solute molecules spend part of their time in the gas phase and part in the liquid phase. This type of diffusion is much faster in the gas phase than in liquids. Typically, the diffusion coefficient in the gas phase is about a million times more than in the liquids.

Generally, in GLC, the diffusion in liquids is neglected. The diffusion takes time and will increase with time required to elute the band from the column. Therefore, in the equation, the contribution due to longitudinal diffusion decreases as the velocity of the carrier gas increases. In other words, the *inverse* relationship between the longitudinal diffusion and the carrier gas velocity is not surprising because the solute spends lesser time in the column when the carrier velocity is high. The diffusion from the centre of the band to two edges has lesser time to occur. It may be important to point out here that in liquid chromatography, the effect of longitudinal diffusion is negligible because diffusion coefficients in liquids are several orders of magnitude lower than in gases. B will depend on diffusion coefficient of the solute in the mobile phase (D_M) and another factor (γ) accounting for the irregularity of diffusion pathways through the packing material.

$$B = 2\gamma D_M \qquad \ldots (4.22)$$

$$B/u = 2\gamma D_{M/\mu}$$

where, γ = is the *correction factor* accounting for irregularity of diffusion pathways through the packing material, and

D_M = is the *diffusion coefficient of solute in the gas phase*.

SAQ 11

Why does the solute band broaden and contribute towards the longitudinal term?

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SAQ 12

Is the longitudinal diffusion term more pronounced in liquid chromatography than in gas chromatography?

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4.6.3 Non-Equilibrium in Mass Transfer Term (Cu)

This term in nonideality arises due to the fact that equilibrium in the distribution of the solute between the stationary and the mobile phases cannot be attained because the mobile phase is continuously moving. If $Conc._L$ and $Conc._G$ are the concentrations of the solute in the liquid and gas phases, respectively and if we apply Henry's law to give the following expression:

$$\frac{Conc._L}{Conc._G} = K \qquad \ldots (4.23)$$

or, $Conc._L = K.Conc._G$

But Henry's law is valid in equilibrium condition and for cases where insufficient time is available for equilibration, we can write

$$Conc_L = K\, Conc_G \times f(t) \qquad \ldots (4.24)$$

where, $f(t)$ is some function of time which reflects the kinetics of mass transfer process between the two phases. If t is very large, i.e. when equilibrium is approached, $f(t)$ approaches unity and the validity of Henry's law is observed. In the actual situation the equilibrium is not instantaneous and band broadening is observed. Fig. 4.8 shows the ideal and non ideal conditions in the solute distribution between the two phases.

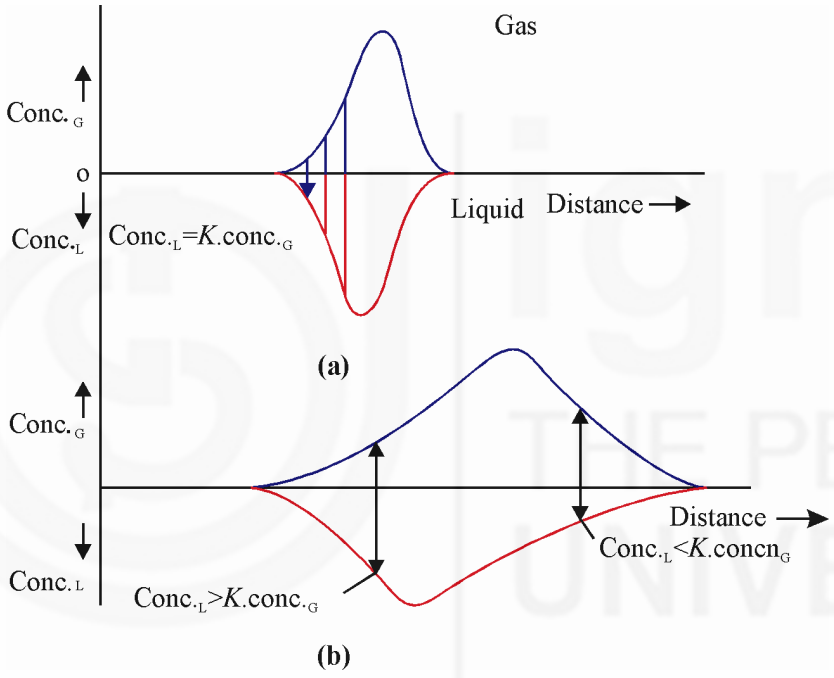

Fig. 4.8: Concentration profiles of a solute in gas and liquid phases
(a) Instantaneous equilibrium leading to ideal distribution
(b) Finite rate of mass transfer leading to actual distribution

Fig. 4.8(a) depicts where equilibrium is attained in all the plates before mass transfers are made. Fig. 4.8 (b) depicts where gas is continuously moving and equilibrium is never attained and in general, $Conc._L \neq K\ Conc._G$. Initially, the front of the solute zone in the gas encounters some of the solute dissolved in the liquid and we do not wait for the equilibrium to be established; therefore, $conc._L < conc._G$. At the other extreme end of this plot, the rear of the gas phase solute zone, the solute is unable to leave the liquid rapidly enough to equilibrate with the fresh carrier gas and $Conc._L > K.Conc._G$. This means that a part of the solute is retained more in the liquid phase than would have if the equilibrium was established instantaneously. This process continues

through the length of the column and the peak is broader than would have actually been observed.

In the above context, it may be important to visualize the exact situation leading to band broadening from mass transfer effects. There are many flowing streams of the mobile phase and the layer making up the stationary phase. Both of these have finite widths. As a result of this, there is some time required for the solute molecules to diffuse from the interior of these phases to their interface for the mass transfer to occur. This condition prevails throughout the column. If the rates of mass transfer are infinite, there will not be any band broadening.

In order to quantify the role of different factors in mass transfer, we will have to primarily consider three parameters: *carrier gas velocity* (u), *stationary phase mass transfer coefficient* (C_S) and *mobile phase mass transfer coefficient* (C_M). We can see that both longitudinal broadening and mass transfer broadening depend upon the velocity of the carrier gas. However, there is a marked difference in the direction of the diffusion in the two cases. In the case of longitudinal diffusion, the direction of movement of solute molecules tend to be *parallel* to the flow of the gas whereas in mass transfer it tends to be *perpendicular* to the flow. As a result of this, the effect of carrier gas velocity in mass transfer broadening is in contrast to that in longitudinal broadening. The *faster* is the rate of mobile phase movement, the *lesser* the time available for equilibrium to be attained. Therefore, as the velocity increases, the plate height increases. We will have to examine the situation in terms of characteristics of stationary phase. *Mass transfer term* (C_S) is a complex function $f_S(k')$ of retention factor k' and directly proportional to the square of the thickness of the film on the support particles d^2_f and inversely proportional to the diffusion coefficient D_S of the solute in the film. The thickness of the film for non equilibrium becomes important because the molecules have to travel thickness to reach to the interface for equilibrium. This means a solute in a thin film of liquid will be closer to equilibrium than that penetrated deep in the thick film. If the diffusion coefficient of the solute in the film is smaller the molecules will diffuse slower. Thus, C_S can be expressed as

$$C_S = \frac{f_s(k')d_f^2}{D_S} \quad \ldots (4.25)$$

C_S = coefficient of mass transfer in stationary phase,
$f_s(k')$ = complex function of retention factor k',
d_f = thickness of liquid film (cm), and
D_S = diffusion coefficient of the solute in the film

Likewise, we can consider the **mobile phase mass transfer coefficient** (C_M). It is a complex function of the retention factor k', i.e. $f_M(k')$, is directly proportional to the square of the particle diameter of the packing d^2_p and inversely proportional to the diffusion of coefficient (D_M) of the solute in the mobile phase.

Thus, C_M is expressed as

$$C_M = \frac{f_M(k')d_p^2}{D_M} \quad \ldots (4.26)$$

4.6.4 van Deemter plot and zone broadening

The effect of different factors discussed earlier is shown in Fig. 4.9. This is known as *van Deemter plot*. From the van Deemter plot depicting the effect of carrier gas velocity on the factors affecting the plate height, it is clear that A is not affected, B decreases and C increases with the carrier gas velocity. The combined effect on the plate height gives a minimum at a certain value of carrier gas velocity. The velocity at which HETP $(H)_{min}$ is obtained is known as *optimum carrier gas velocity* ($U_{opt.}$).

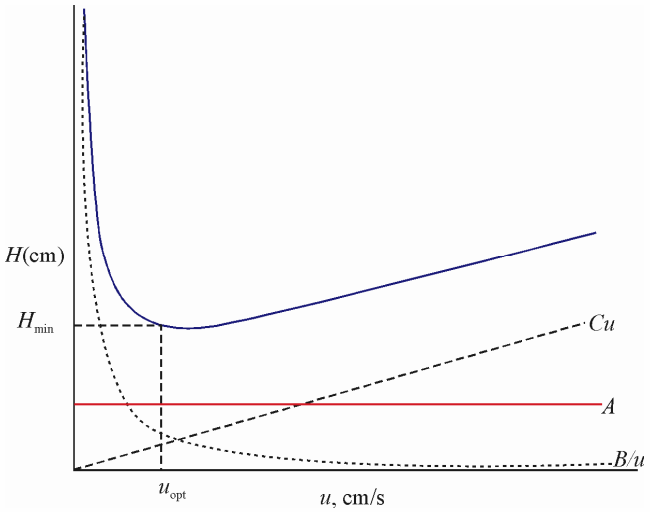

Fig. 4.9: A depiction of van Deemter equation showing u_{opt} for H_{min}.

Now, from the above discussion on reducing the zone broadening we can summarize the important points as under:

1. The column should be packed with smaller particles. The packing should be compact.
2. The column diameter should be narrow.
3. The thickness of the liquid layer in the column should be minimized.
4. With gaseous mobile phase, the longitudinal diffusion can be lowered by reducing the temperature. This effect is not noticeable in liquid chromatography because longitudinal diffusion has little effect on plate height.

SAQ 13

Why is Henry's law not valid in GLC?

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SAQ 14

Are the directions of longitudinal diffusion and mass transfer diffusion same?

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SAQ 15

Does the thickness of liquid film on the particles increase the contribution to the mass transfer term? Support your answer with reasoning.

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4.7 RESOLUTION

The foregoing discussion on reducing the broadening of the peaks is essentially aimed to achieve a minimum of overlap between the consecutive peaks of the analytes. In other words, the position of elution bands on the horizontal axis and their widths will determine the extent of separation. The quantitative measure of ability of a column to separate two analytes is expressed in terms of a factor *resolution* (R).

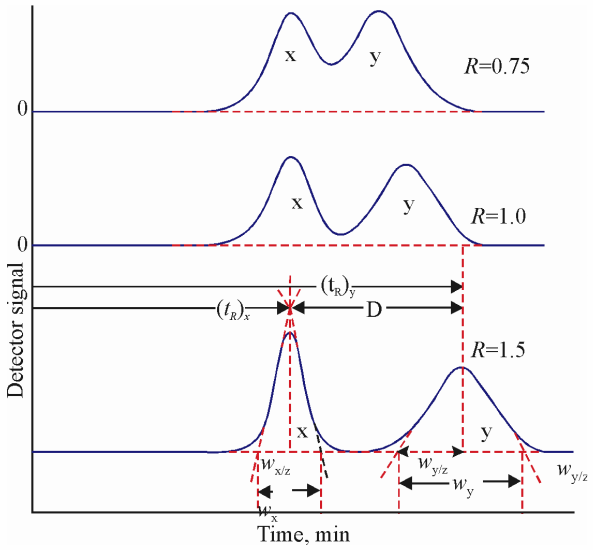

Fig. 4.10: Separation at three resolutions R= 0.75, 1.0 and 1.5

Fig. 4.10 shows the chromatogram of two species X and Y on three columns having different resolutions.

$$R = \frac{\Delta D}{W_{X/2} + W_{Y/2}} = \frac{2\Delta D}{W_X + W_Y} = \frac{2[(t_R)_Y - (t_R)_X]}{W_X + W_Y} \quad \ldots (4.27a)$$

$$R = \frac{2[(t_R)_Y - (t_R)_X]}{1.669(W_{X/2} + W_{Y/2})} \quad \ldots (4.27b)$$

It is clear from the figure that if $R = 1.5$, the solutes are virtually completely separated. There will be only 0.3% overlap of two elution bands. In the case of $R = 1$, the overlap is about 2% and the separation is considered adequate. As R decreases below 1, the overlap starts getting more severe say, at about $R = 0.75$, there is about 50% overlap and the separation is rated unsatisfactory for most of the purposes.

One thing which seems to be important for resolution under a certain set of conditions is the *length* of the column. One way to improve the resolution is to employ a longer column. This will mean that with a longer column, we will have more theoretical plates. The separation of two peaks $(t_R)_Y - (t_R)_X$ is directly proportional to the distance the two species migrate. On the other hand, the width of a peak increases with the square root of the distance. Thus, by increasing the length of a column the two bands will separate faster than they broaden and the resolution will improve. The two important drawbacks in making the column too long are requirement of high pressure of carrier gas and unusually long time for elution. In case we are not able to obtain a satisfactory separation by using a good column of reasonable length giving proper attention to operating parameters like temperature and flow rate of carrier gas, the reasonable approach is to change the stationary liquid phase. This can be viewed as, if the logical attempts to achieve resolution by narrowing the solute bands fail, we must move the peaks farther apart. For making the peaks apart, we have to change K values.

In the above context, you are advised to refer to sub-sec 4.4.4 where the term selectivity factor/ separation factor was introduced and the following expressions were given.

$$\alpha = \frac{K_Y}{K_X} = \frac{(t_R)_Y - t_M}{(t_R)_X - t_M} \approx \frac{(t_R)_Y}{(t_R)_X} \qquad \ldots \text{[from Eqs. (4.13) and (4.15)]}$$

You may remember that the *separation factor* is the ratio of distribution constants of the two solutes *X* and *Y* and is usually the same as the ratio of retention times of the two solutes. It can be seen that *α and R are not the same thing*. The ratio of retention times, measured at the peaks of elution bands does not itself represent the effectiveness of separation because it does not tell anything about the width of the peak. It seems logical that there will be a relation between R and α if the number of theoretical plates in the column is taken into account, i.e.

$$R = \frac{N^{1/2}(\alpha - 1)\alpha}{4} \qquad \ldots (4.28)$$

Earlier in this section, we have noted that a fairly good separation is obtained if $R = 1$, but if we plot the *number of plates required* vs. *separation factor*, a curve as shown in Fig. 4.11, is obtained.

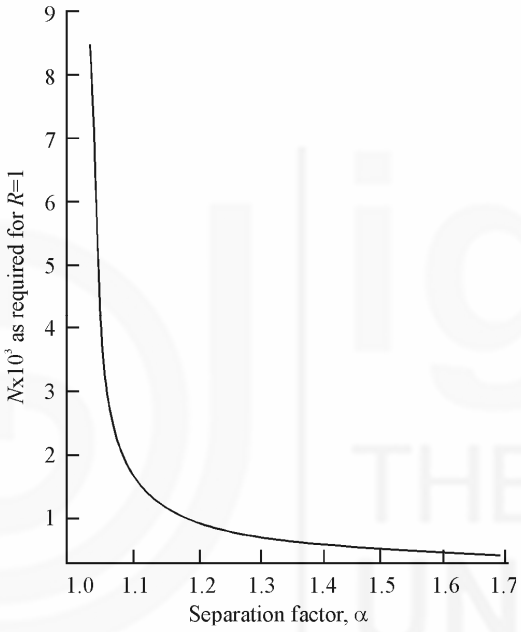

Fig. 4.11: Number of theoretical plates needed to achieve $R = 1$ plotted as a function of separation factor (α)

The curve has a very unique feature i.e., it approaches Y-axis asymptomatically, meaning thereby that if $\alpha = 1$, no separation is possible however large the number of plates is. This implies that *the separation will not be achieved whatever may be length of the column*. If α increases above 1, the number of plates required decreases rapidly. In other words, a better separation can be achieved by bringing a small change in α by changing the liquid rather than bringing a large increase in the length of the column.

4.8 SUMMARY

This unit in brief gives a chronological development of the important chromatographic techniques. It highlights the fact that the advancements in chromatographic science are prompted to a large extent by the compelling needs of the scientists. Taking a clue from the definition of chromatography, the different ways to classify chromatographic

methods are discussed. It emerges that no single criteria seems to be effective to classify all the different techniques logically. The difficulty arises mainly due to diversifications. A simpler approach seems to be by taking the nature of the mobile phase as the main criteria and propose a sub-classification based on the mechanisms responsible for separation. The underlined principles involved in different techniques are explained. A large number of variations are available in liquid chromatography primarily because of different operative mechanisms and availability of a variety of surfaces. Supercritical fluid chromatography combines the advantages of both liquid and gas chromatography but it has to still become popular. The migration of solutes in the column is viewed in terms of fraction of time they spend between the stationary and mobile phases. Depending on this they emerge out of the column at different intervals of time. The position of peak on time axis helps to identify the components and the areas under the peak provide the amount of the component. For separation, the relative migration rates are important and the rates depend upon the equilibrium constants of reaction by which the solutes distribute themselves between the two phases. The significance of retention and selectivity factors is explained. The shape of the peak is generally Gaussian. In order to assess the performance of a column, the concept of theoretical plates or height equivalent to theoretical plates is introduced. This is merely conceptual and there is no actual entity like this. However, it is able to successfully explain the Gaussian shape of the peaks but fails to explain the broadening of peak. A more realistic picture is presented in the form of rate theory. The rate theory, expressed in terms of van Deemter equation, takes into account the eddy diffusion, longitudinal diffusion and mass transfer between the phases. The effect of carrier gas velocity on the three terms is taken into consideration. Based on the conclusions, an optimum carrier gas velocity at which $(HETP)_{min.}$ is obtained is visualized. Moreover, the conditions which can reduce the peak broadening are concluded. The concept of resolution and its significance in terms of separation/ selectivity factor is discussed.

4.9 TERMINAL QUESTIONS

1. What are the different modes available in liquid-liquid partition chromatography?

2. What are the main drawbacks of GSC as compared to GLC?

3. What does HETP signify?

4. On what major parameters the number of plates depends?

5. What are the main factors which can reduce zone broadening in column chromatography?

6. Are the resolution factor and separation factor same? What will be the situation at $R = 1$ and the separation factor being unity?

7. Two substances X and Y were found to have retention time 18.50 and 19.80 min., respectively on a 32.0 cm column. An unretained species passed through the column in 1.10min. The peak widths (at base) for X and Y were 1.20 and 1.30 min., respectively. Calculate

   a) column resolution,

   b) the average number of plates in the column, and

   c) the plate height.

8. In a liquid column chromatographic separation of two components X and Y, the

following data were recorded:

i) Length of column packing = 25.7cm.

ii) Flow rate = 0.313ml/min.

iii) V_M = 1.37mL

iv) Vs = 0.164mL

v) The retention time and peak width data as under

	Residence time (min.)	Peak width(sec.)
Unretained	3.1	-
X	5.4	24.6
Y	14.1	69.6

Calculate

a) Number of plate from each peak.

b) The plate height for column.

c) Retention factor for X and Y.

d) Distribution constant for X and Y.

e) Resolution and selectivity factor.

9. In a 25.0 cm long column, the solvent took 2.35min. to run through whereas two compounds *X* and *Y* took 9.87min. and 10.63min. with peak half width 45.6sec. and 53.4 sec., respectively. Calculate

a) Capacity factor for X and Y.

b) Separation factor α .

c) Average number of plates and plate height.

d) Resolution.

4.10 ANSWERS

Self Assessment Questions

1. There are two types of chromatography on the basis of shape of the support

i) Column chromatography and

ii) Two dimensional (thin layer and paper) chromatography.

2. The main advantages of reversed phased liquid chromatography is that it does not require periodic recoating of the support particles.

3. In the extraction chromatography the extractant used for solvent extraction is coated or impregnated on the support. It can be carried in column or on two dimensional supports.

4. The particular property of the gel responsible for separation is its pore size.

5. The two types of columns used in gas chromatography are packed columns and open tubular or capillary columns.

6. The two main advantages of supercritical fluid chromatography are

i) The dissolved analyte can be easily recovered particularly when it is thermally unstable.

ii) It is faster than liquid chromatography accompanied by lower zone broadening than observed in gas chromatography.

7. For linear ideal chromatography the molar concentration of the solute in the stationary phase (C_S) is proportional to that in mobile phase (C_M) over a wide range of concentration. This will result in a symmetric Gaussian type peak.

8. If the retention factor is too small, say less than unity, elution occurs so rapidly that its accurate determination is difficult. If it is too large the elution times are unusually long.

9. Yes, the number of theoretical plates in a column is found to vary with sample size in a regular way.

10. The eddy diffusion term mainly arises due to irregular pathways by which the gas molecules travel through the column and so the solute molecules do. This mainly arises due to various sizes and shapes of the particles packed inside the column.

11. The solute band will broaden due to concentration gradient. The molecules spread in to regions of lower concentration ahead of the band and behind it.

12. No, it is not more pronounced in LC than GC. It is the other way round.

13. The Henry's law

$$\frac{\text{Conc.}_G}{\text{Conc.}_L} = K$$

is not valid in GLC because sufficient time has to be available for the equilibrium to be established. Here, in GLC the mobile phase, the gas, is continuously moving.

14. The directions of the longitudinal diffusion and mass transfer diffusion are not the same. In the case of longitudinal diffusion, the direction of the solute molecules tend to be parallel to the flow of gas whereas in mass transfer it tends to be perpendicular to the flow.

15. The thickness of the liquid film increases the contribution to the mass transfer term. The mass transfer term C_S is directly proportional to the square of thickness of film (d^2_f) on the support particles.

Terminal Questions

1. The different modes available in liquid-liquid partition chromatography are

   i) Column and two dimensional,

   ii) Normal and reversed phase,

   iii) Extractant loaded surface (extraction chromatography), and

   iv) Ion pair formation (ion pair chromatography).

2. The main drawbacks of GSC are

   i) The retention time may be unusually large and high temperatures may be required to desorb the species,

   ii) There may be severe tailing due to non-linear character of adsorption, and

   iii) The active adsorbent may catalyse the adsorbed species and change it to some other compound.

3. The column is assumed to be made up of a large number of plates where equilibrium is achieved. The plate height (HETP) is defined as that length of the column which will yield in equilibrium with mean concentration over that length in the stationary phase. In other words, equilibrium of the solute between the mobile and stationary phase is assumed to take place. Movement of solute down the column is then assumed to take place as stepwise transfer of equilibrated mobile phase from one plate to the next.

4. The major factors on which the number of plate depends are

   i) Column preparation (length included),

   ii) Characteristics of the solute,

   iii) Concentration of the solute,

   iv) Method of sample introduction,

   v) Flow rate, and

   vi) Temperature.

5. The main factors which can reduce zone broadening are

   i) The column should be packed with smaller particles and the packing should be compact,

   ii) Column diameter should be narrow,

   iii) The thickness of the liquid layer should be minimized; and

   iv) With gaseous mobile phase, the longitudinal diffusion can be lowered by reducing the temperature. This effect is not noticeable in liquid chromatography.

6. The resolution factor and the separation factor are not the same. They are related by the expression

$$R = \frac{N^{1/2}(\alpha - 1)\alpha}{4}$$

   R = resolution factor, N = number of plates and α = separation factor. R, the ratio of retention time measured at the peaks of elution bands, does not itself represent the effectiveness of separation because it does not tell the width of the peak. If $R = 1$, a good separation seems to be possible but with $\alpha = 1$ no separation seems to be feasible however large the number of plates may be.

7. a) $$R = \frac{2(t_{R_Y} - t_{R_X})}{W_X - W_Y}$$

$$R = \frac{2(19.80 - 18.50)}{(1.20 + 1.30)} = 1.04$$

   b) $$N_X = 16\left(\frac{18.5}{1.20}\right)^2$$

$$= 3.80 \times 10^3$$

$$N_Y = 16\left(\frac{19.8}{1.30}\right)^2$$

$$= 3.71 \times 10^3$$

$$N_{Av} = \frac{3.80 \times 10^3 + 3.71 \times 10^3}{2}$$

$= 3.76 \times 10^3$

c) $H = \frac{L}{N} = \frac{30}{3.76 \times 10^3}$

$= 7.98 \times 10^3$ cm

8. a) $N_X = \left(\frac{4xt_{R_x}}{W_x}\right)^2$

$= \left(\frac{4 \times 5.4 \times 60}{24.6}\right)^2$

$= 2776$

$N_Y = \left(\frac{4xt_{R_Y}}{W_Y}\right)$

$= \left(\frac{4 \times 14.1 \times 60}{69.6}\right)^2$

$= 2364$

b) $N_{Av} = \frac{2776 + 2364}{2}$

$= 2570$

$= 2.57 \times 10^3$

$H = \frac{25.7}{2.57 \times 10^3} = 10 \times 10^{-3}$ cm

c) $k'_x = \frac{t_{R_x} - t_M}{t_M}$

$= \frac{5.4 - 3.1}{3.1}$

$= 0.7419$

$k'_y = \frac{t_{R_y} - t_M}{t_M}$

$= \frac{14.1 - 3.1}{3.1}$

$= 3.548$

d) $k' = \mathrm{K} \frac{V_S}{V_M}$

$K_X = \frac{0.7419 \times 1.37}{0.164}$

$= 6.198$

$K_Y = \frac{3.54 \times 1.37}{0.164}$

$= 29.64$

e) $R = \frac{2(t_{R_Y} - t_{R_X})}{W_X + W_Y}$

$$= \frac{2(14.1\times60 - 5.4\times60)}{24.6 + 69.6}$$

$$= 2\left(\frac{522}{94.2}\right)$$

$$= 11.08$$

$$\alpha = \frac{t_{R_y} - t_M}{t_{R_X} - t_M}$$

$$= \frac{846 - 186}{324 - 186}$$

$$= 4.78$$

9. a) $k'_x = \frac{t_{R_x} - t_M}{t_M}$

$$= \frac{9.87 - 2.35}{2.35} = 3.2$$

$$k'_y = \frac{t_{R_y} - t_M}{t_M}$$

$$= \frac{10.63 - 2.35}{2.35}$$

$$= 3.52$$

b) $\alpha = \frac{t_{R_y} - t_M}{t_{R_X} - t_M}$

$$= \frac{10.63 - 2.35}{9.87 - 2.35}$$

$$= 1.10$$

c) $N_X = 5.54\left(\frac{t_{R_x}}{W_{x/2}}\right)^2$

$$= 5.54\left(\frac{9.87\times60\,\text{sec}}{45.6\,\text{sec}}\right)^2$$

$$= 934$$

$$N_Y = 5.54(\frac{t_{R_y}}{W_{y/2}})^2$$

$$= 5.54\left(\frac{10.6\times60\,\text{sec}}{53.4\,\text{sec}}\right)^2$$

$$= 786$$

$$N_{Av} = \frac{934 + 786}{2}$$

$$= 860$$

$$H = \frac{L}{N_{Av}}$$

$$= \frac{25}{860} = 0.029\ \text{cm}$$

d) $R = \frac{2(t_{R_y} - t_{R_x})}{1.699(W_{y/2} + W_{x/2})}$

$$= \left(\frac{2(10.63 \times 60 - 9.87 \times 60}{1.699(45.6 + 53.4)} \right)$$

$$= 0.54$$

Further Reading

1. *Chromatography and Separation*, By Satinder Ahuja, Academic Press.

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4. *Instrumental Methods of Chemical Analysis*, By G.W. Ewing, McGraw Book Company.

5. *Basic Concepts of Analytical Chemistry*, By S.M. Khopkar, Wiley Eastern Limited.

6. *Analytical Chemistry*, By G.D. Christian, John Wiley & Sons.

7. *Instrumental Methods of Analysis* By H. H. Willard, L. L.Merritt(Jr), J. A. Dean and F. A. Settle (Jr).