Instrumental Analysis II

Course Code: CH3109

Chromatographic &Thermal Methods of Analysis

Part 1: General Introduction

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☞**There are two major areas of analytical chemistry are:-**

☞**Qualitative analysis:** **where the constituents of an analytical sample are determined. The constituents in a sample are usually unknown or suspected so that analysis is done to reveal them.**

☞**Quantitative analysis:** **where the amount of the constituent in an analytical sample is determined. The constituents are known or suspected to be present so that the exact amount is determined.**

☞**It is therefore important to understand the analytical procedures** **that are the basis of the quantitative and qualitative determination.**

What is chemical analysis?

(1) A type of chemical analysis by which the analytes to be analyzed in a sample are **identified.**

(2) A type of chemical analysis by which the amount of each analyte or analytes in a sample is **determined.**

Analyte(s) is the species (component) to be determined in the sample.

Classification of Analytical Methods

1. Classical methods:

Qualitative: identification by; color, indicators, boiling or melting points, odors, ……

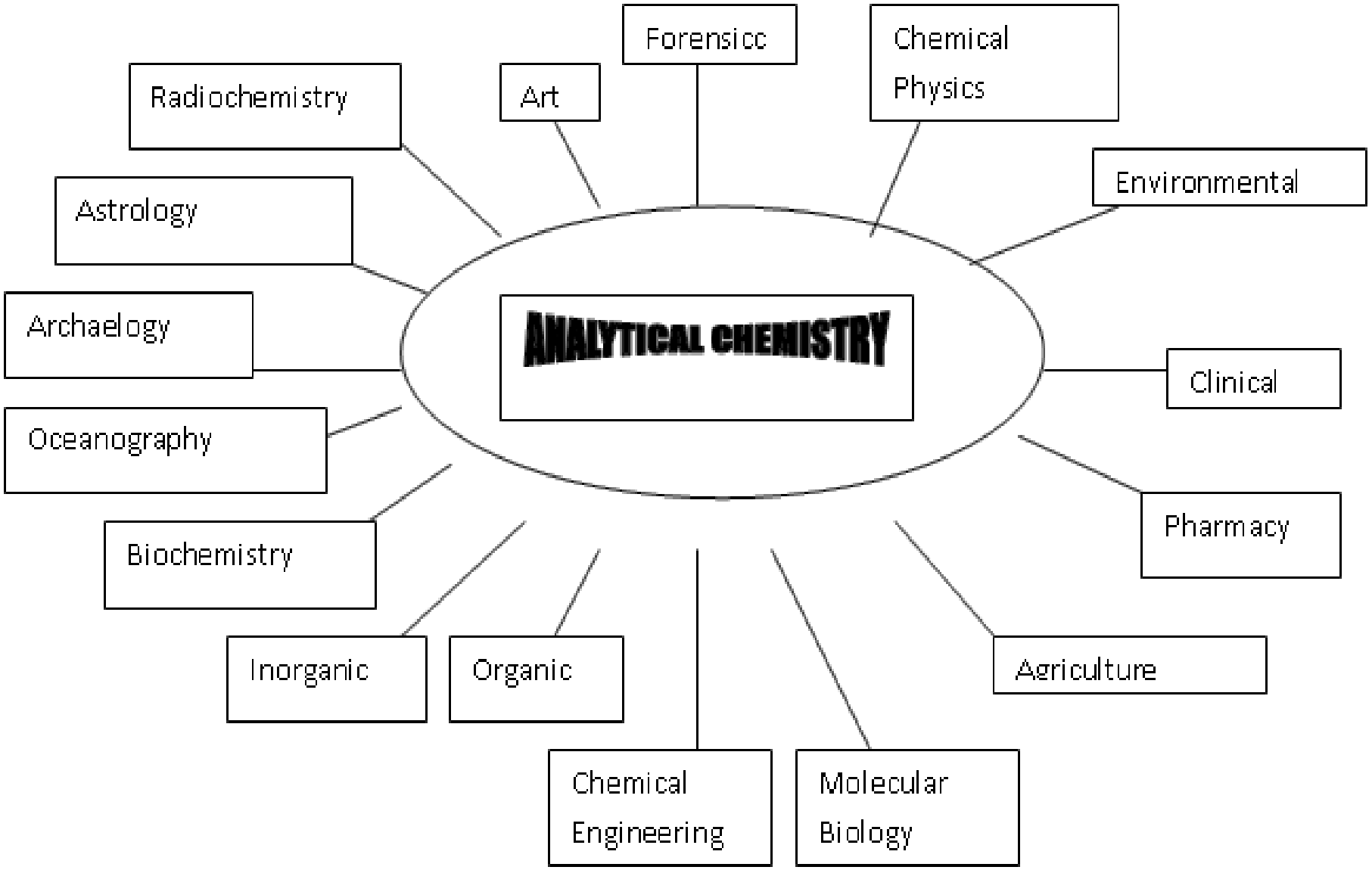
Quantitative: determination by mass or volume (e.g. gravimetric, volumetric methods)

2. Instrumental methods:

Qualitative: identification by measuring physical properties (e.g. spectroscopic (IR, NMR, Mass, UV-Vis.) chromatographic, electrochemical and thermal methods).

Quantitative: measuring the property and determining relationship to concentration (e.g. spectrophotometry, mass spectrometry, conductivity,…….)

The various areas where an analytical chemistry serves

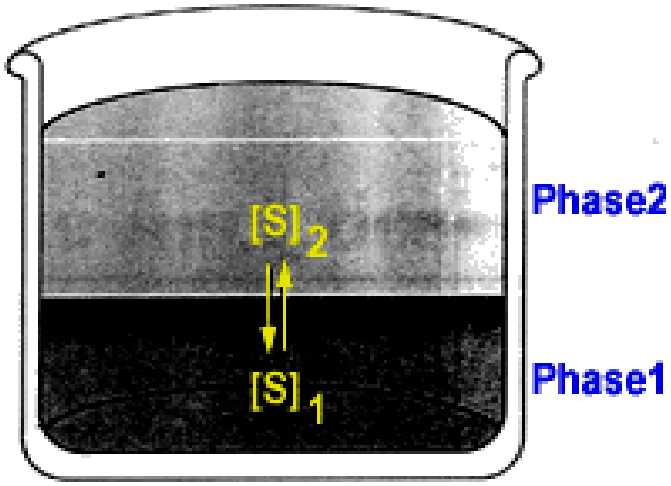


Basis of Chromatography

- In all chromatographic separations, the sample is transported in a **mobile phase**. The mobile phase can be **a gas, a liquid, or a supercritical fluid**. The mobile phase is then forced through a **stationary phase** held in a column or on a solid surface. The stationary phase needs to be something that **does not react** with the mobile phase or the sample. The stationary phase may be **a solid, a gel or a liquid supported on a solid.**

- The **sample components** then has the **opportunity to interact with the stationary phase** as it moves past it. Components that interact greatly, then appear to move more slowly. Components that interact weakly, then appear to move more quickly. Because of the difference in rates of flow, the samples can then be **separated into their components**.

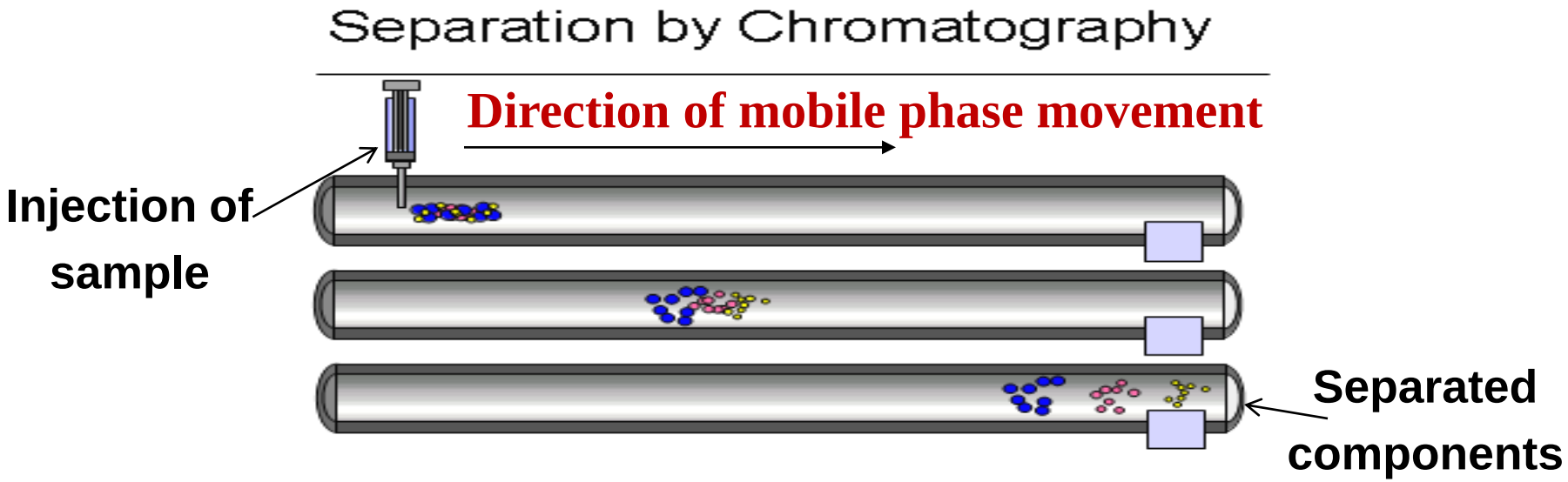
- That is to say, **chromatography is a physical method for separation** in which the components to be separated are distributed between two phases (one is the stationary phase and the other is the mobile phase).



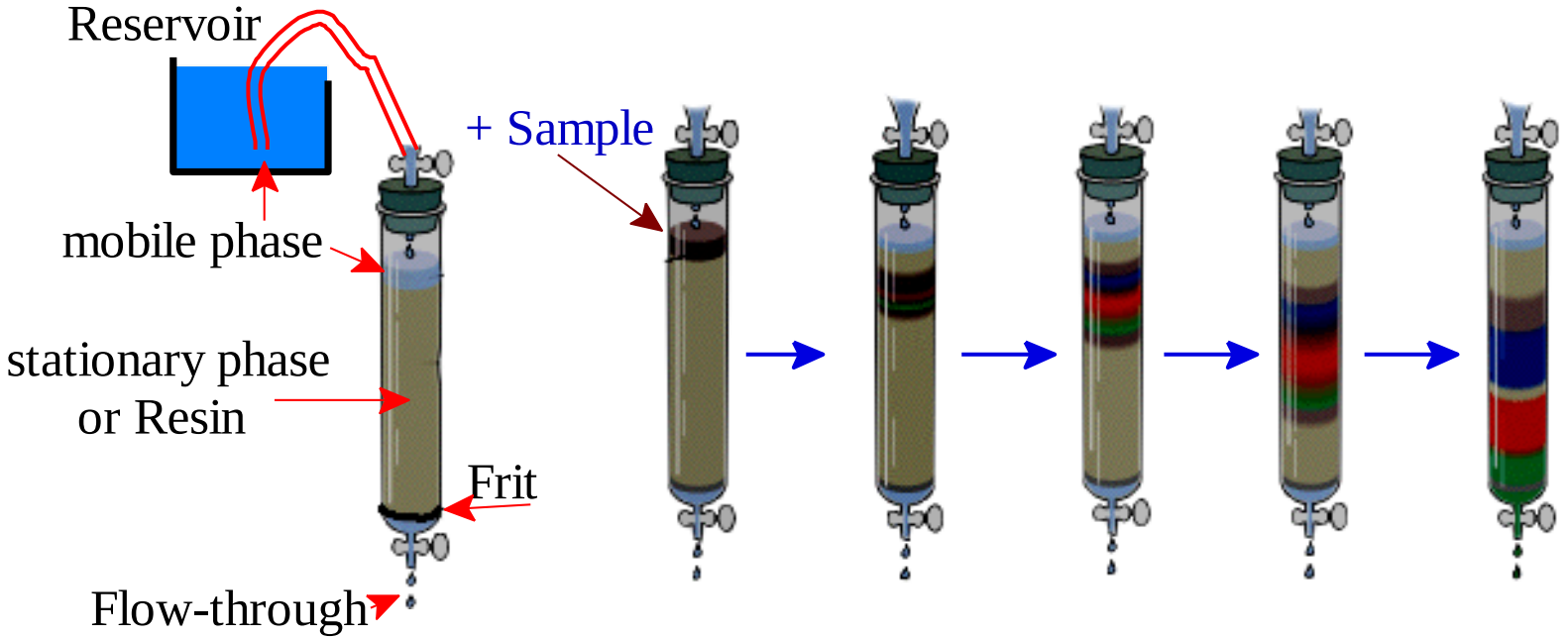
The distribution involves the transfer of an analyte between the mobile and stationary phase.

(A) in mobile phase ⇌ (A) in stationary phase

- **Chromatography is a dynamic process where the mobile phase moves in definite direction, by a controlled rate, on the stationary phase.**



- **Components are separated based on differences in their physical characteristics as; size, shape, charge, volatility, solubility, adsorption, partition.**
- **We can only control the stationary and mobile phase, while the mixtures to be separated are the problem we have to deal with.**

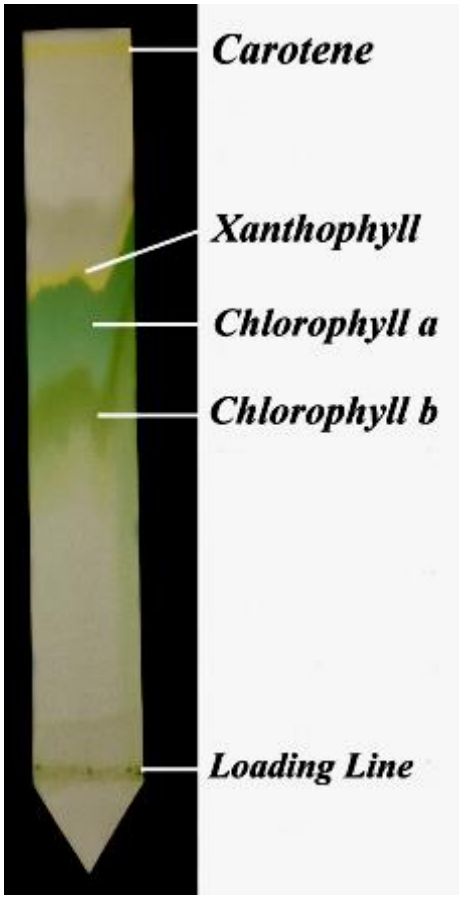


The analyte component which interacts most strongly with the stationary phase will take longer time to pass through the system (it has high retention and eluted later) than those with weaker interactions.

Eluent is the liquid / gas when it goes into the column.

Eluate (Effleunt) is the liquid / gas when it comes out at the end of the column.

- Russian scientist ‘ Tswett ’ in 1906 used a glass columns packed with finely divided $CaCO_3$ to separate the green plant pigments extracted by hexane. The pigments after separation appeared as colored bands (zones) that can come out of the column one by one.



- Tswett was the first to use the term "chromatography" derived from two Greek words; "Chroma" meaning color and "graphein" meaning to write or drew.

Theoretical Concept: Distribution Ratio

- **The distribution ratio, K_S (also called a partition ratio or partition coefficient), for a component A, is;**

$$K = \frac{C_S}{C_M} = \frac{\text{concentration of solute in the stationary phase}}{\text{concentration of solute in the mobile phase}}$$

K is constant over a wide range of solute concentration.

- **Each of separated components will have different values of K_s, reflecting their relative affinities for the stationary phase.**
- **The value of the distribution ratio tells us how high is the separation efficiency.**
- **Bigger K means that the material retained more time on the column i.e move slowly, while smaller K means faster movement.**

Classification of Chromatographic methods

- **Mobile phase:** a solvent that flows through the supporting medium to elute the mixture components (also called eluent).
- **Stationary phase:** a layer of solid, gel or liquid film coating on the supporting medium that interacts with the analytes to be separated.

Chromatographic methods are classified according to:

A – Mechanism of separation:

The mechanism of separation depends mainly on the nature of the stationary phase. Based on separation mechanisms, chromatographic techniques could be classified into:

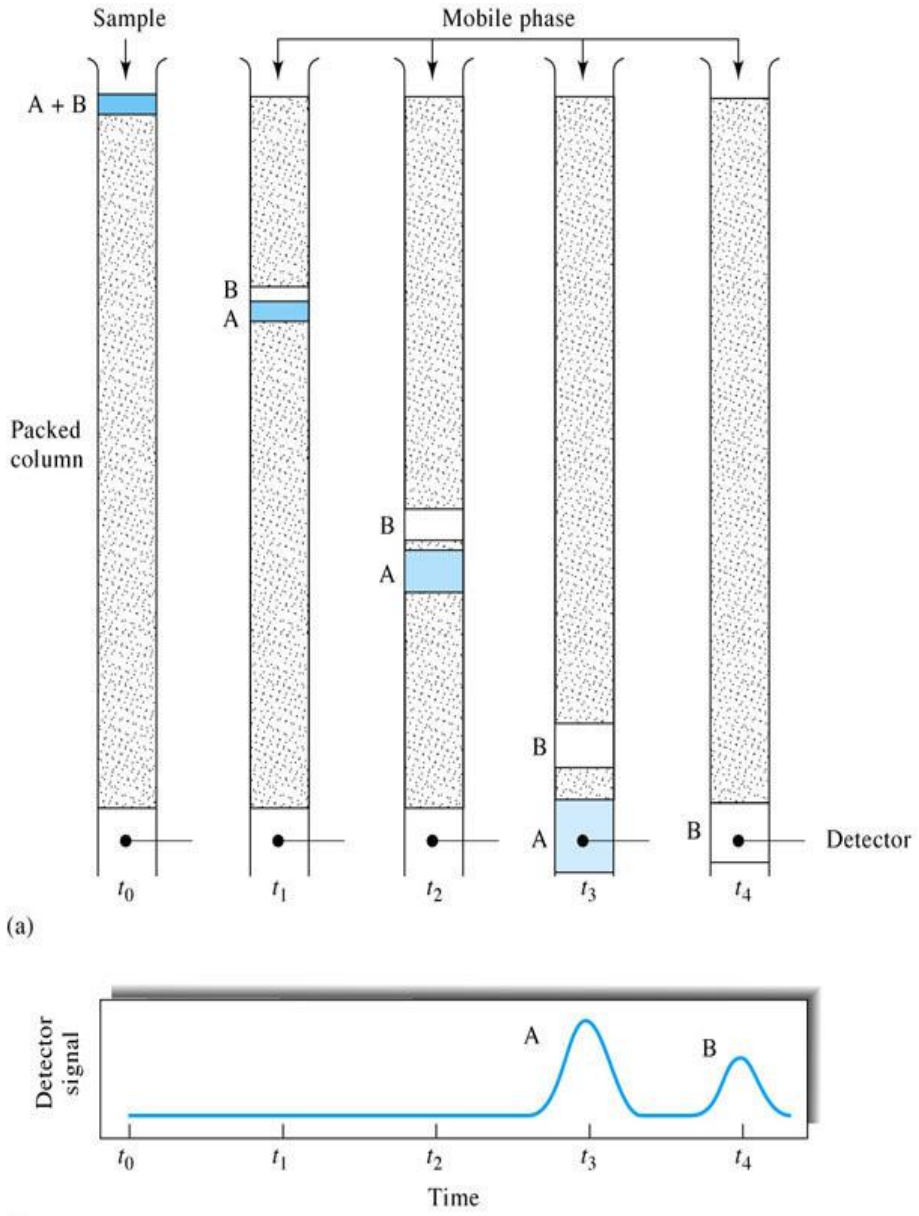
1- Adsorption Chromatography:

It is the oldest technique.

Separation is due to difference in the adsorption power of mixture components on solid stationary phase.

The stationary phase is a solid with adsorption characters.

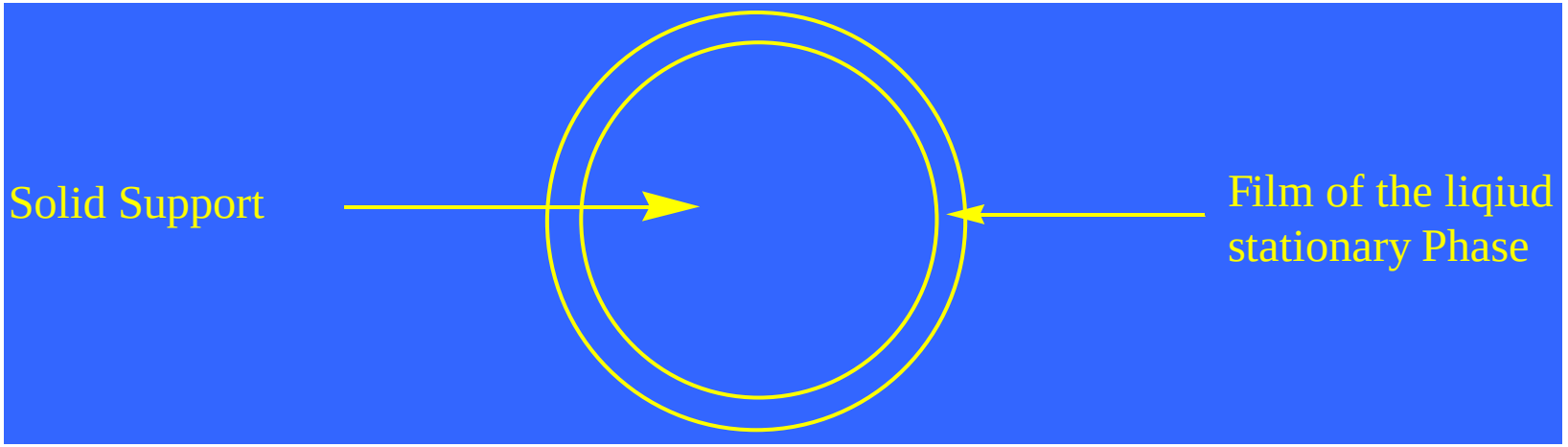
Silica gel and alumina are the most common stationary phases in adsorption chromatography.



2- Partition Chromatography:

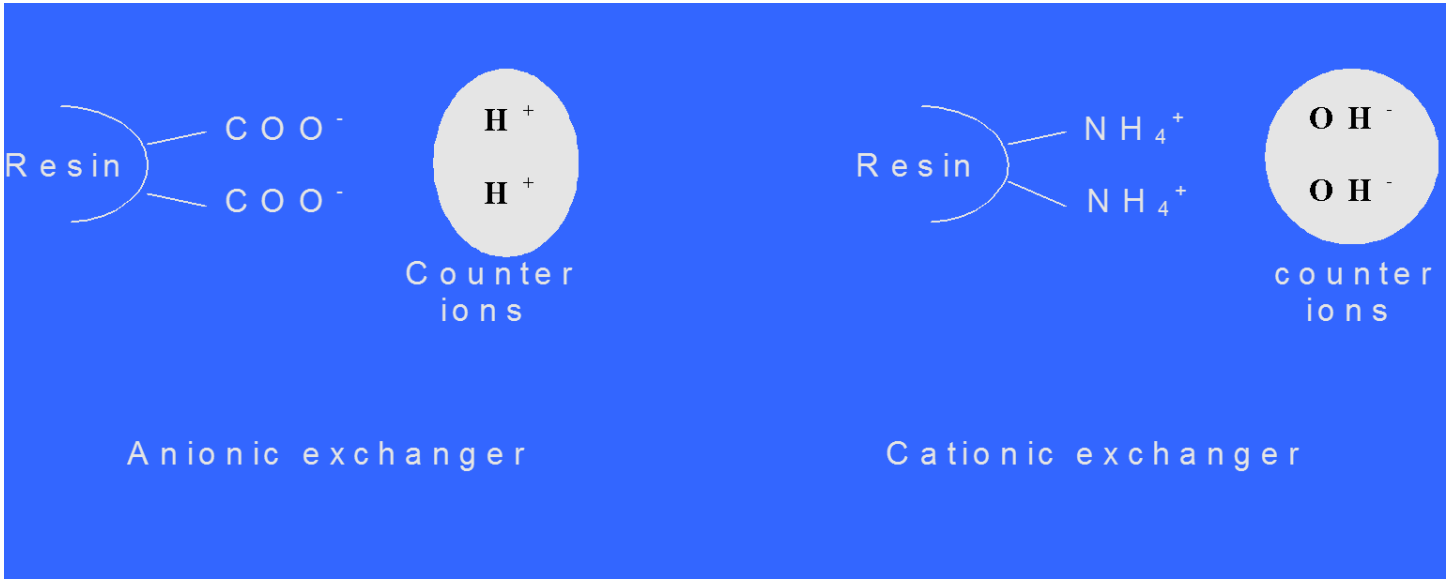
Separation is due to difference in solubility of components in two immiscible liquids.

The stationary phase is a liquid thin film on an inert solid support. The stationary liquid is usually more polar than the mobile phase. Cellulose powder and wetted silica gel are examples of supports in partition chromatography that carry film of water act as stationary phase.



3- Ion Exchange Chromatography (IEC):

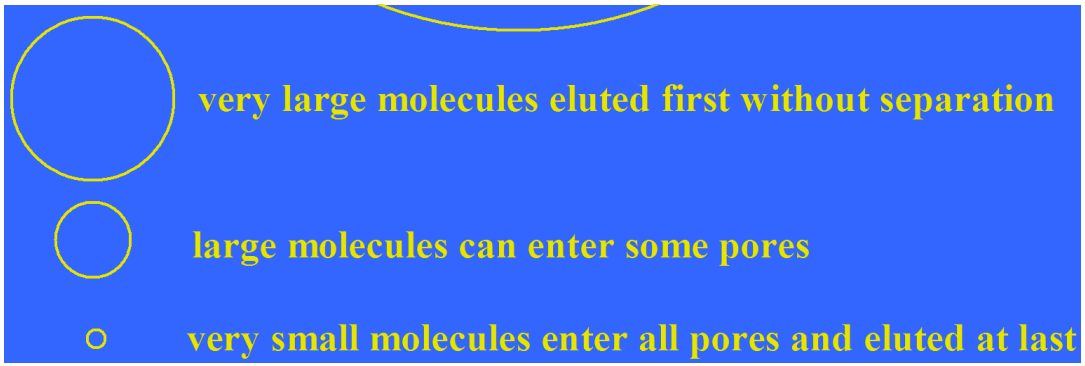
It is used for separation of charged molecules. The stationary phase is an ion exchange resin to which a cationic or anionic groups are covalently bonded. Ions of opposite charges (counter ions) in the mobile phase will be attracted to the resin and compete with the components of the mixture for the charged group on the resin. The mobile phase must be charged (electrolyte or buffer solution are most common).

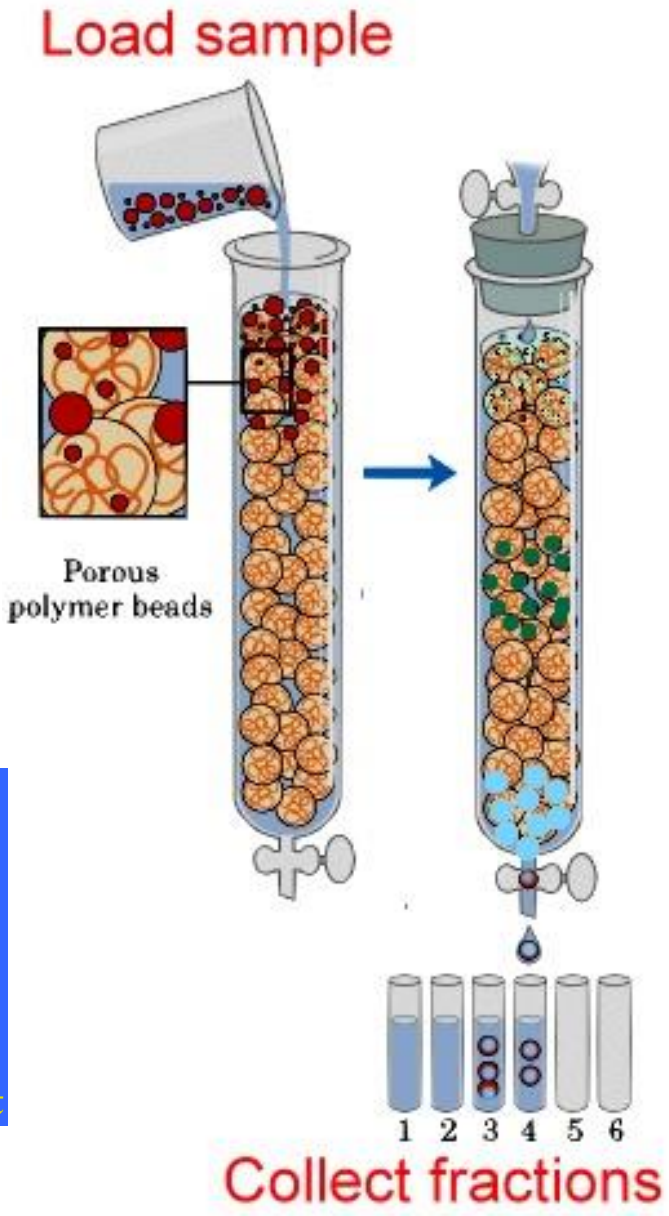


4- Gel Filtration Chromatography

Also called " Gel Permeation, Molecular Sieve or Size exclusion“: Separation is according to difference of size or Molecular weight (MW).

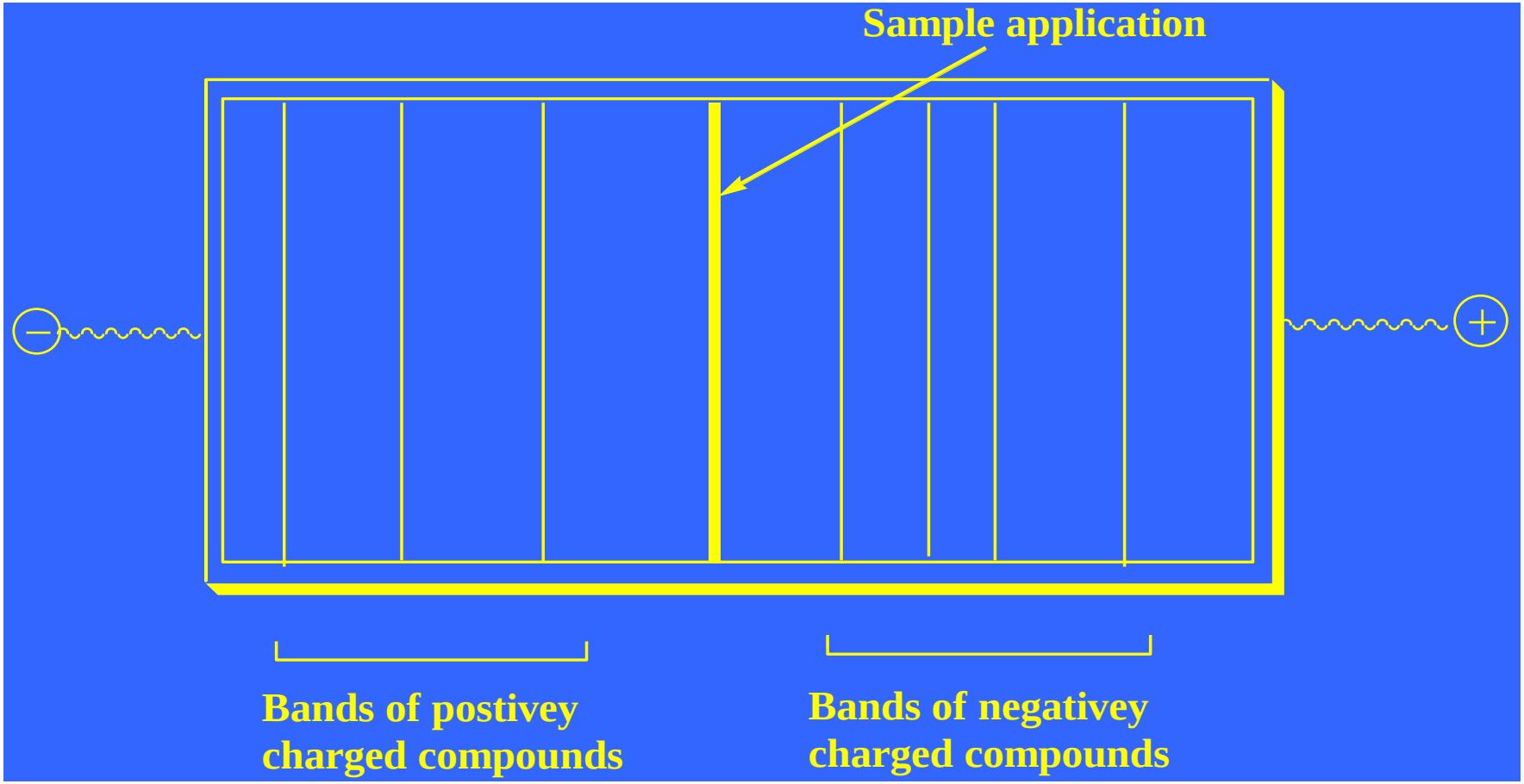
- **Stationary phase (column matrix): is beads of hydrated, porous polymer.**
- **Mobile phase: is buffer or solvent.**





5- Zone Electrophoresis:

Separation of electrically charged bio-molecules under the effect of an electric field of strength E, they will freely move towards the electrodes of opposite charge.



6- Affinity Chromatography:

The separation is based on the affinity of proteins to specific ligands such as enzymes. The ligand is attached to suitable polysaccharide polymer such as cellulose - agarose – dextran.

7- Chiral Chromatography:

In this type we can separate enantiomers – chiral stationary phase is used that react with one enantiomer more then the other so separation takes place.

B- According to the nature of the mobile and stationary phase:

In this regard chromatography is classified into:

1- Liquid Chromatography (LC):

The mobile phase is liquid. In case of separation by adsorption the stationary phase is solid so it is called: Liquid-Solid Chromatography (LSC). If separation occurs through partition, the stationary phase is liquid so it is called: Liquid -Liquid Chromatography (LLC).

2- Gas Chromatography (GC)

The mobile phase is an inert gas nitrogen or helium. Again if the stationary phase is solid it is called: Gas–Solid Chromatography (GSC). When stationary phase is liquid it is called: Gas-Liquid Chromatography (GLC).

C- According to the technique (process or methods of holding the Stationary Phase):

1- Planar or Plane Chromatography:

- In this type the stationary phase is used in the form of layer. Plane chromatography is further classified into:

a- Thin Layer Chromatography (TLC):

The stationary phase is spread on glass or plastic or aluminum sheets.

b- Paper Chromatography (PC):

A specific type of papers is used as stationary phase.

2- Columnar or Column Chromatography (CC):

The stationary phase is held in to a tube made of glass or metal (gel – ion exchange – adsorption).

Classification of Chromatography

General Classification	Specific Method	Stationary Phase	Type of Equilibrium
Liquid chromatography (LC) (mobile phase: liquid)	Liquid-liquid, or partition	Liquid adsorbed on a solid	Partition between immiscible liquids
	Liquid-bonded phase	Organic species bonded to a solid surface	Partition between liquid and bonded surface
	Liquid-solid, or adsorption	Solid	Adsorption
	Ion exchange Size exclusion	Ion-exchange resin Liquid in interstices of a polymeric solid	Ion exchange Partition/sieving
Gas chromatography (GC) (mobile phase: gas)	Gas-liquid	Liquid adsorbed on a solid	Partition between gas and liquid
	Gas-bonded phase	Organic species bonded to a solid surface	Partition between liquid and bonded surface
	Gas-solid	Solid	Adsorption
Supercritical-fluid chromatography (SFC) (mobile phase: supercritical fluid)		Organic species bonded to a solid surface	Partition between supercritical fluid and bonded surface

Arrangement of polar groups according to their binding to adsorbent		Elutropic series of solvents (increasing strength)
-COOH	carboxylic	Light petroleum & Hexanes
-OH	hydroxyl	Cyclohexane
$-NH_2$	amines	Carbon tetrachloride
-CHO	aldehydes	Trichloro ethylene
-C=O	ketones	Toluene
-COOR	esters	Benzene
$-OCH_3$	ethers	Dichloromethane
-C=C-	olifens	Chloroform
		Ethyl ether
		Ethyl acetate
		Acetone
		n-Propanol
		Ethanol
		Methanol
		Water

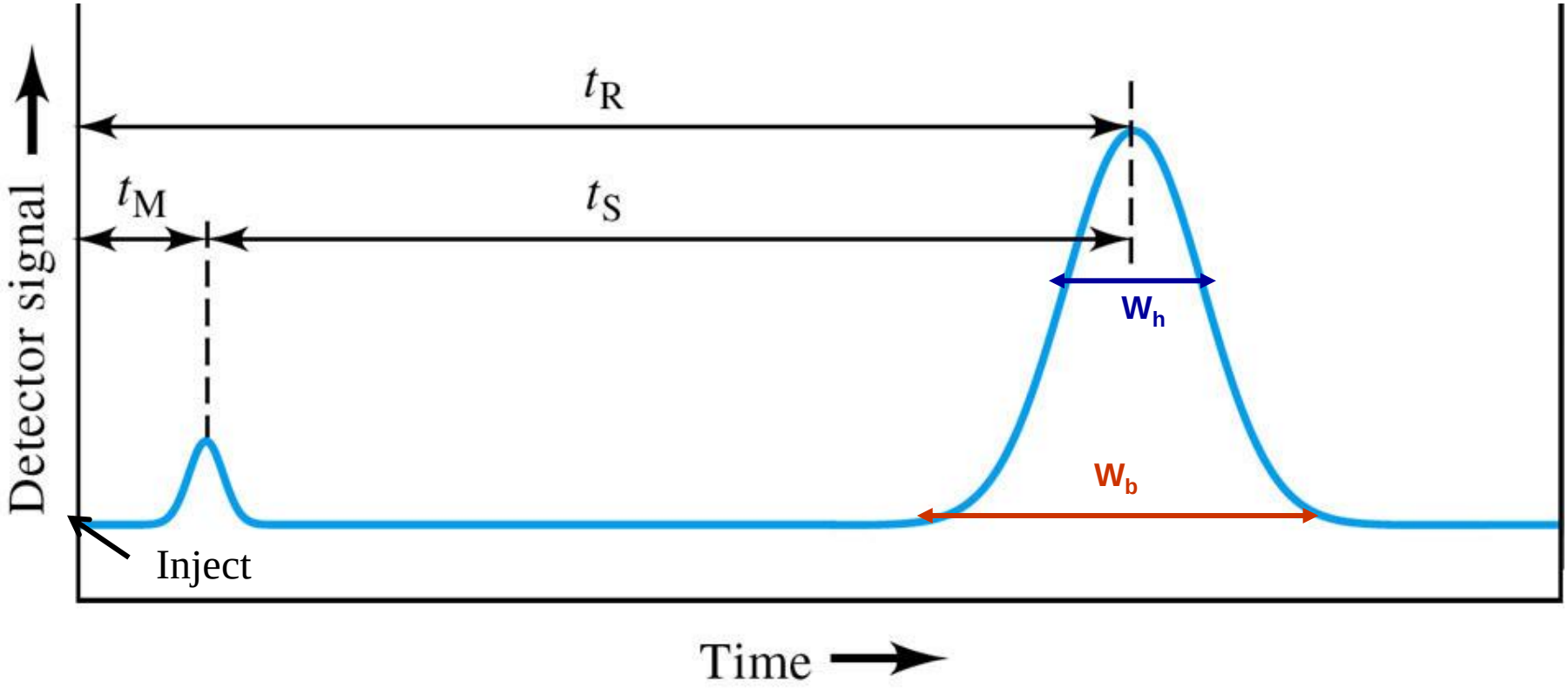
Forces Responsible for Separation

- **The affinity differences of the components for the stationary or the mobile phases can be due to several different chemical or physical properties including:**
  - Ionization state (no. of charges)
  - Polarity and polarizability
  - Hydrogen bonding / Van der Waals' forces
  - Hydrophobicity
  - Hydrophilicity
- **The rate at which a sample moves is determined by how much time it spends in the mobile phase.**

Chromatography Nomenclature

If a detector that responds to solute concentration is placed at the end of the column and its signal is plotted as function of time (or of volume of the added mobile phase), a series of peaks is obtained. Such a plot, called a chromatogram, is useful for both qualitative and quantitative analysis. The positions of peaks on the time axis may serve to identify qualitatively the components of the sample. The areas under the peaks provide a quantitative measure of the amount of each component.

Thus, the typical response obtained by chromatography is called a chromatogram: chromatogram represents the detector response versus elution time or elution volume (also called retention).



Where: t_R = retention time t_M = void time

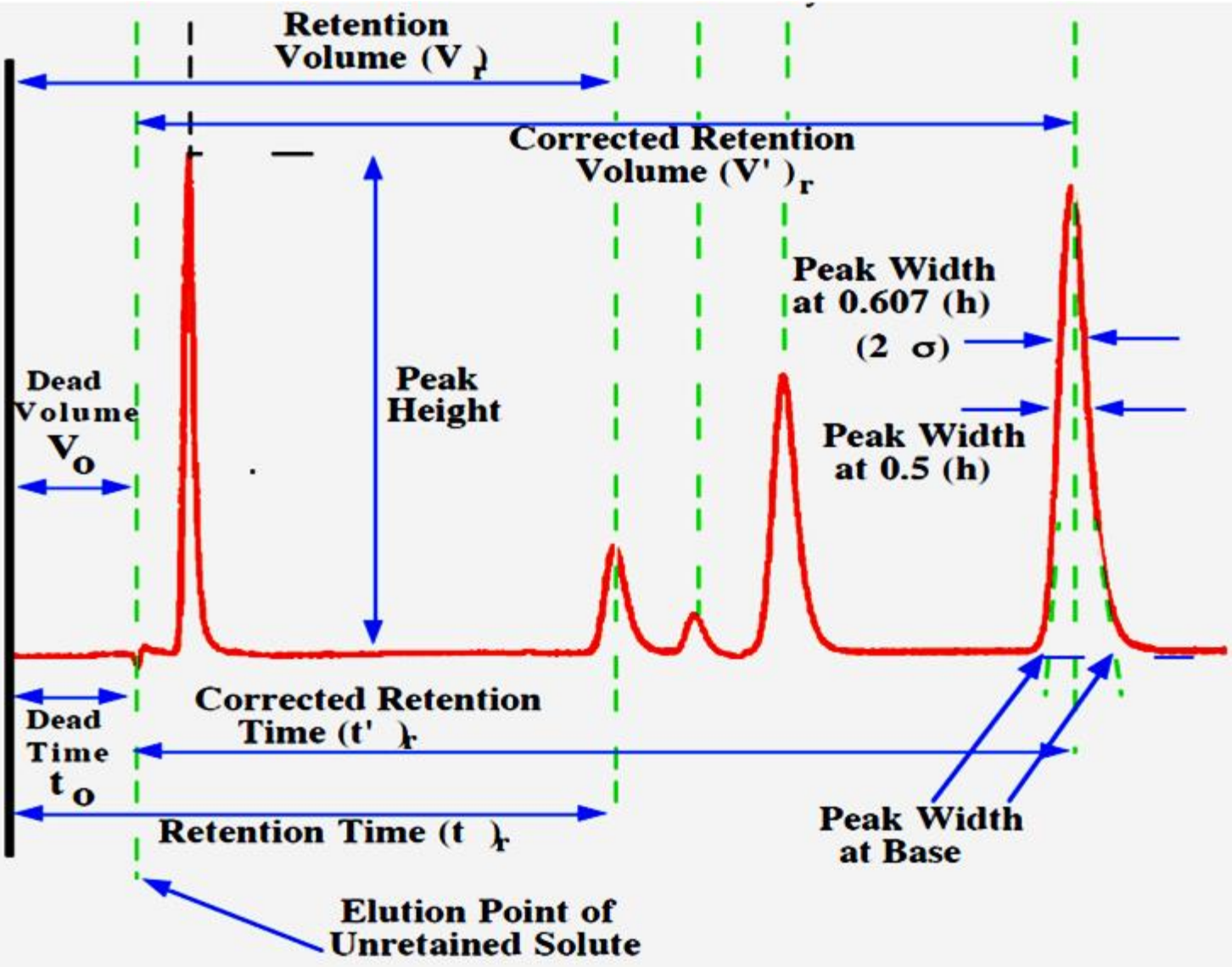
W_b = baseline width of the peak in time units

W_h = half-height width of the peak in time units

A summary of chromatographic terms are defined as follows:

- **The *baseline* is any part of the chromatogram where only mobile phase is emerging from the column.**
- **The peak maximum is the highest point of the peak.**
- **The injection point is that point in time/position time when/ where the sample is placed on the column.**
- **The dead point is the position of the peak-maximum of an unretained solute.**
- **The dead time (t_o) is the time elapsed between the injection point and the dead point.**

- **The dead volume (V_o) is the volume of mobile phase passed through the column between the injection point and the dead point.**
- **The retention time (t_r) is the time elapsed between the injection point and the peak maximum. Each solute has a characteristic retention time.**
- **The retention volume (V_r) is the volume of mobile phase passed through the column between the injection point and the peak maximum.**
- **The peak height (h) is the distance between the peak maximum and the base line geometrically produced beneath the peak.**



The Nomenclature of a Chromatogram.

The Development Procedures

- It is the process, by which the mixture components (substances) are moved through the chromatographic system (through a column or along a plate). The solute progresses through the chromatographic system, only while it is in the mobile phase. There are three types of chromatographic development:
- **Elution development,**
- **Displacement development**
- **Frontal analysis.**

Elution development is the only development technique employed in both GC and LC

Displacement Development

- Displacement development is only effective with a solid stationary phase where the solutes are adsorbed on its surface.
- The components array themselves along the distribution system in order of their decreasing adsorption strength.
- The sample components are usually held on the stationary phase so strongly that they are eluted very slowly or even not at all.
- The solute must be displaced by a substance more strongly held than any of the solutes (called displacer which is the mobile phase).
- In displacement development the solutes or sample components are never actually separated from one another.
- The solutes leave the system sequentially and somewhat mixed with its neighbor.

Frontal Analysis

- It can be effectively employed in a column chromatography. The sample is fed continuously onto the column as a dilute solution in the mobile phase.
- Frontal analysis can only separate part of the first component in a relatively pure state, each subsequent component being mixed with those previously eluted.

For a three components mixture, containing solutes (A), (B) and (C) as a dilute solution is fed continuously onto a column:

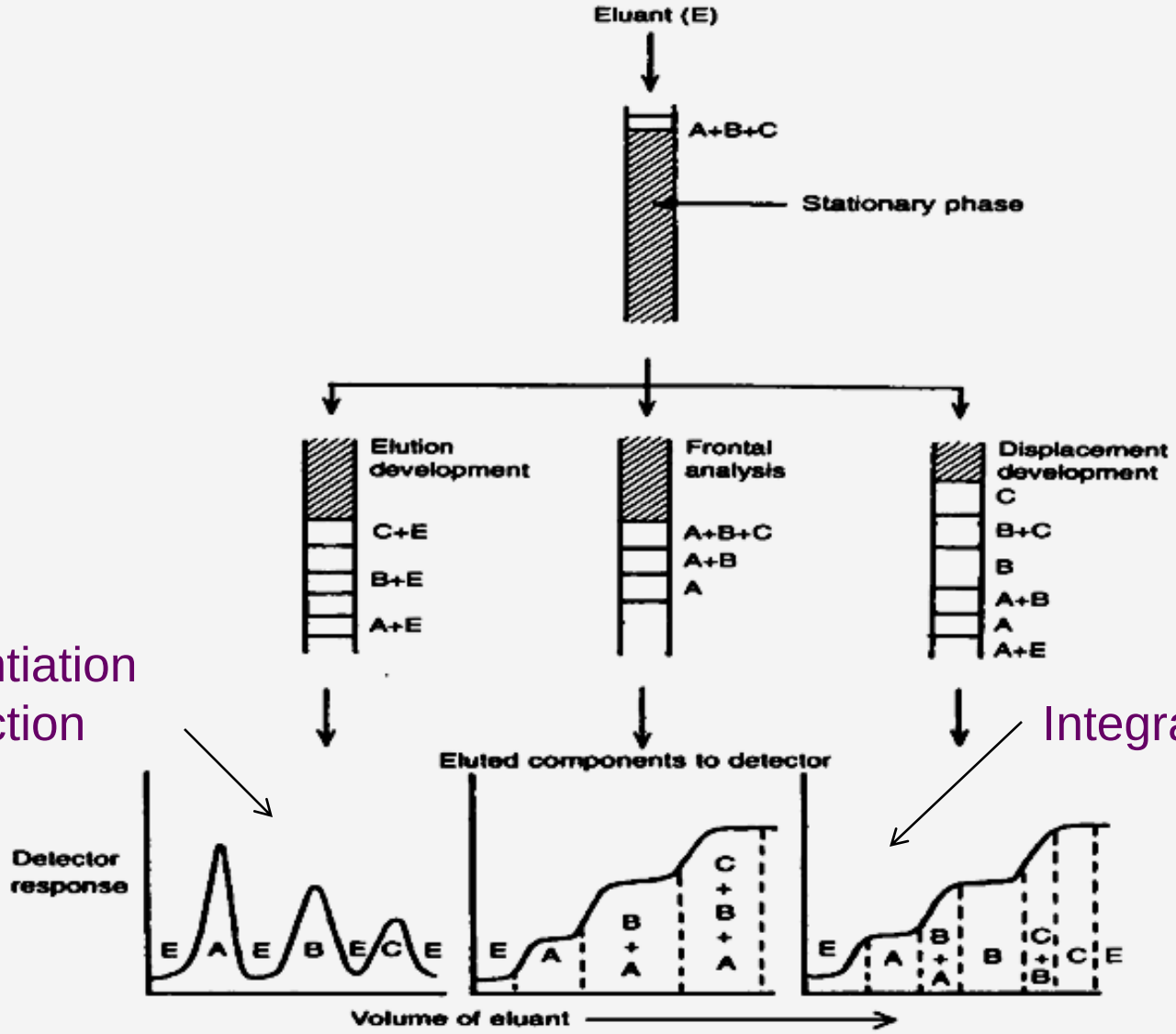
The first component to elute, (A), will have less affinity to the stationary phase. Then the second solute, (B), will elute but it will be mixed with the first solute. Finally, the third solute (C), will elute in conjunction with (A) and (B).

It is clear that only solute (A) is eluted in a pure form.

Thus, frontal analysis is not suitable for most practical analytical applications.

Elution Development

- Elution development is best described as a series of adsorption-extraction processes which are continuous from the time the sample is injected.
- As the elution proceeds, the moving phase will continuously displace the concentration profile of the solute in the mobile phase forward, relative to that in the stationary phase.



Classification of chromatographic methods according to development procedure, for components A, B, C and eluant E.

What is Elution

Elution involves washing of the species through a column by continuous addition of fresh solvent. The sample is introduced at the head of a column, whereupon the components of the sample distribute themselves between the mobile and stationary phases. Introduction of additional mobile phase (the eluent) forces the solvent containing parts of the sample to move down the column, where further partition between the mobile phase and fresh portions of the stationary phase occurs.

Types of elution techniques

- **Simple elution**: the column is eluted with the same solvent all the time. This is suitable when the components have similar affinities for the stationary phase and are therefore eluted rapidly, one after another.
- **Stepwise elution**: the eluent (solvent) is changed after a predetermined period of time. The eluents are chosen to have increasing elution power (increasing polarity), that is, increasing affinity to the remaining components, and therefore releasing them from the stationary phase.

- **Gradient elution:** the composition of the eluting solvent is changed gradually to achieve separation of widely varying affinities for the stationary phase. The ratio of two solvents is gradually changed to increase slowly the eluting power of the mobile phase. Thus enhancing the resolution (narrows the zones and reduces tailing). The solvent composition gradient may be a concentration, pH, polarity or ionic strength gradient.
- **Isocratic elution:** in isocratic elution mixed solvent with a fixed composition is used along the elution process (as an example; using a mixture of 30% EtOH – 70% Ether as an eluent)

Peak Resolution or separation

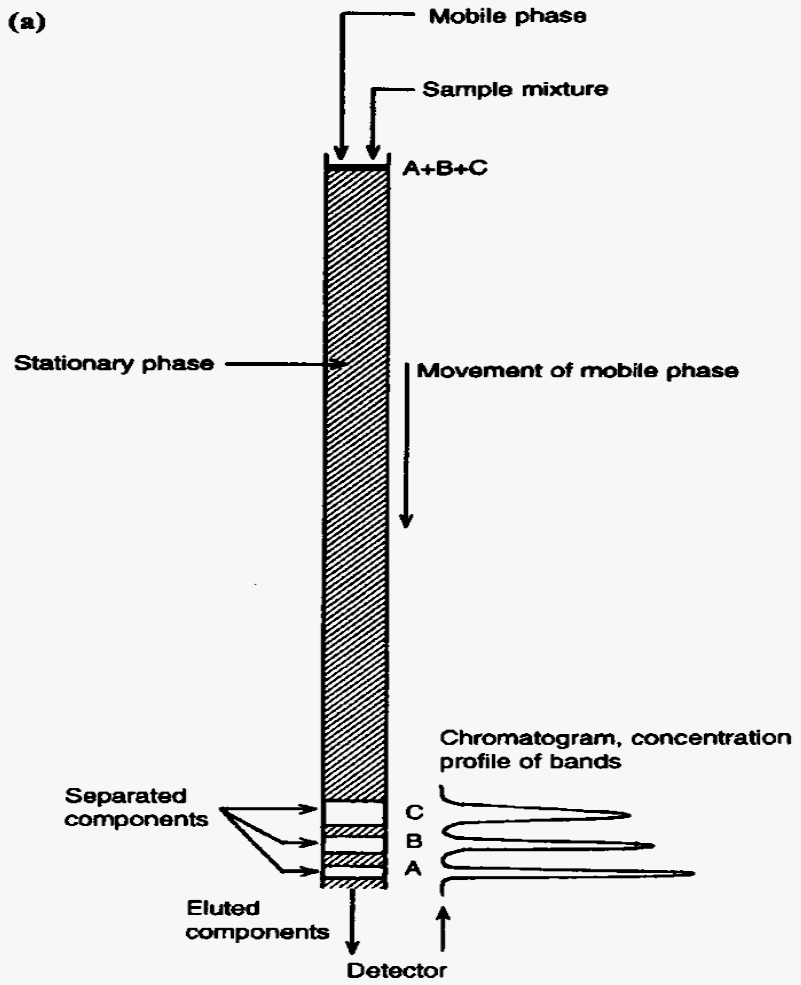


Figure 1.2 Separation using (a) column chromatography, and (b) planar chromatography (p. 12).

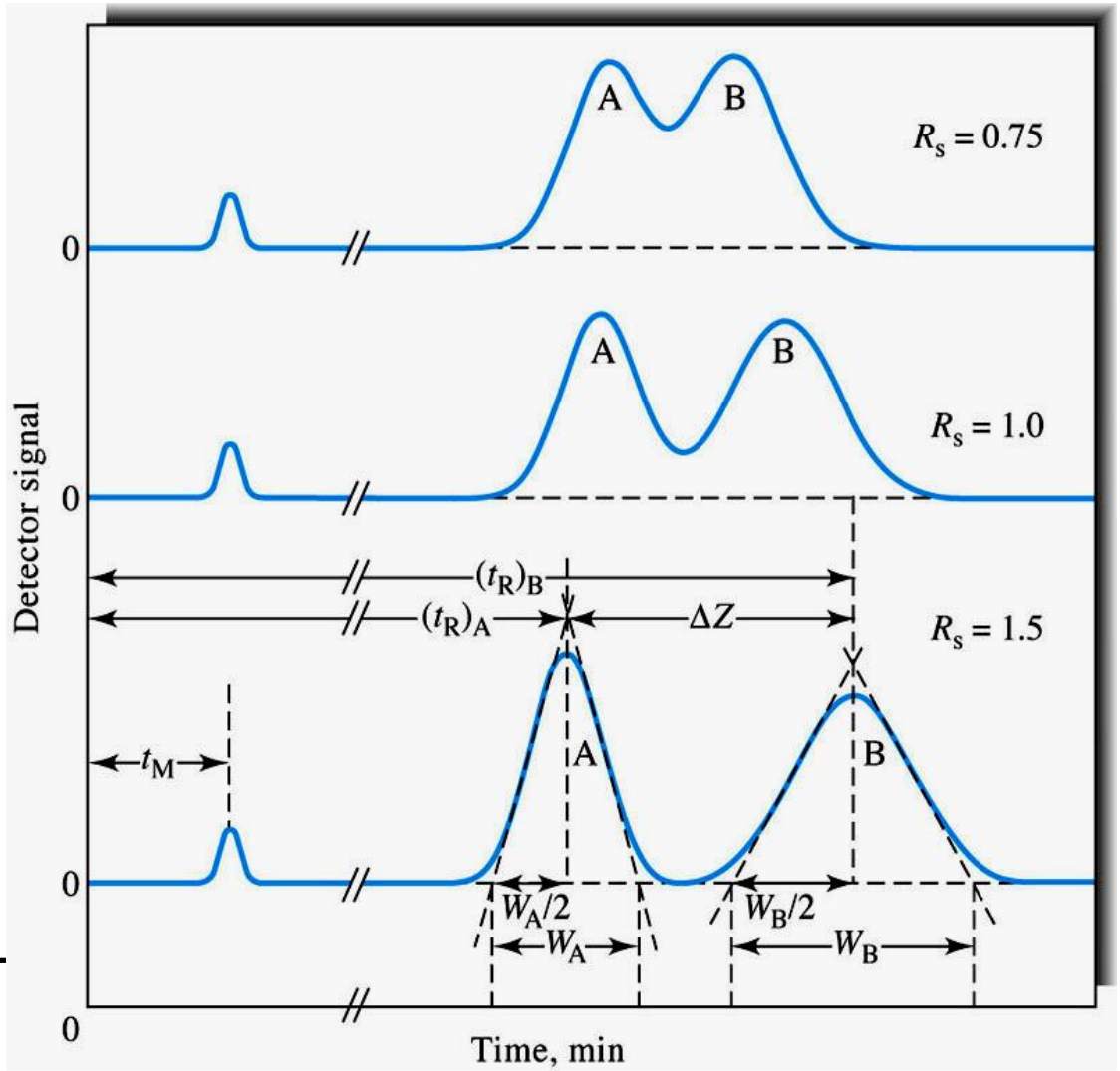
Peak Resolution or separation (R)

Poor resolution

More separation

High resolution;
less band overlap

$$R = \frac{V_2 - V_1}{1/2(W_1 + W_2)}$$



V is the retention volume, and W is the peak width at base

Selectivity Factor (α): parameter used to describe how well two components are separated by a chromatographic system.

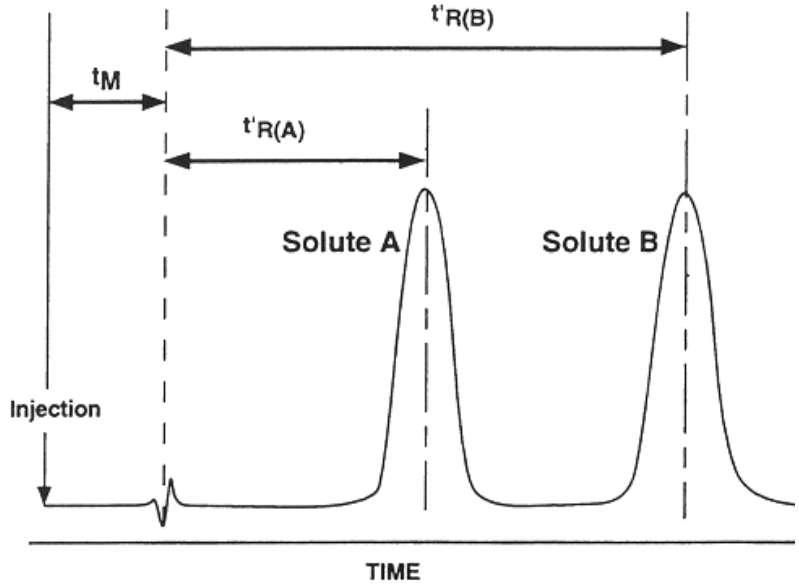
$$\alpha = \frac{K_B}{K_A}$$

K = Distribution Constant

$$\alpha = \frac{k_B}{k_A}$$

Where; k = $(t_R - t_M)/t_M$

Separation or Retention factor



$$k_A = \frac{(t_R)_A - t_M}{t_M} \quad and \quad k_B = \frac{(t_R)_B - t_M}{t_M}$$

$$\alpha = \frac{(t_R)_B - t_M}{(t_R)_A - t_M}$$

Where; t = Retention time

For two components mixture where B is more retained than A, then; $\alpha > 1$

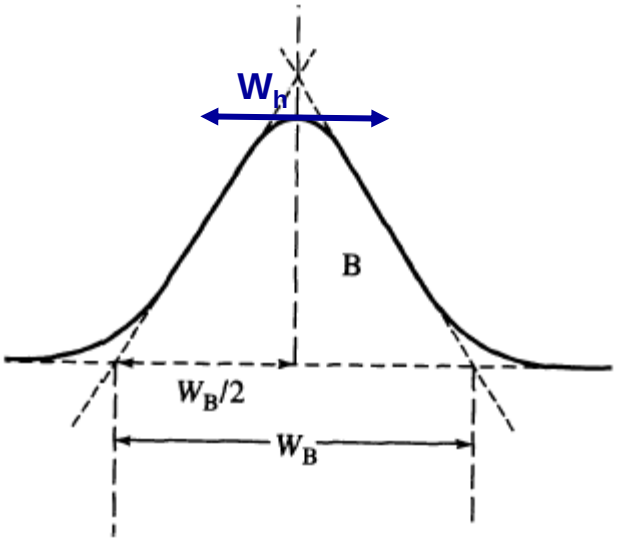
Separation (column) Efficiency (σ):

The efficiency of separation is related experimentally to a component's peak width.

- an efficient system will produce narrow peaks,

- narrow peaks → smaller difference in interactions

It is determined by the processes that are involved in solute retention and transport in the column



Estimate σ from peak widths, assuming Gaussian shaped peak:

$$W_b = 4\,\sigma$$

$$W_h = 2.354\,\sigma$$

Dependent on the amount of time that a solute spends in the column (k' or t_R)

Factors affecting separation effeciency (Peak Broadening)

A chromatographic column is made up of numerous discrete but contiguous narrow layers called theoretical plates. At each plate, equilibration of the solute between the mobile and stationary phase was assumed to take place. Movement of the solute down the column was then treated as a stepwise transfer of equilibrated mobile phase from one plate to the next.

Number of theoretical plates (N):

compare efficiencies of a system for solutes that have different retention times, and can be determined experimentally from;

$N = (t_R/\sigma)^2$ or for a Gaussian shaped peak

$N = 16\,(t_R/W_b)^2$ or $N = 5.54\,(t_R/W_{1/2})^2$

Where w is the peak width at base (W_b) or half-height ($W_{1/2}$)
The larger the value of N is for a column, the better the column will be able to separate two compounds.

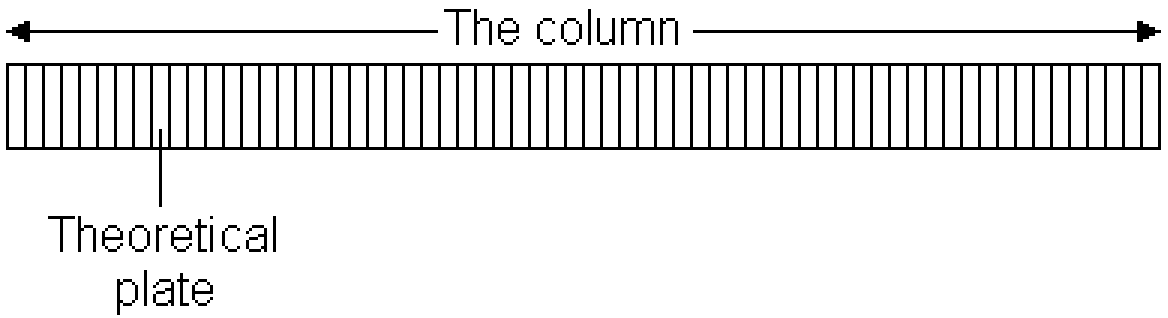


Plate height (H): compare efficiencies of columns with different lengths;

$$H = L / N$$

where: L = column length N = number of theoretical plates for the column

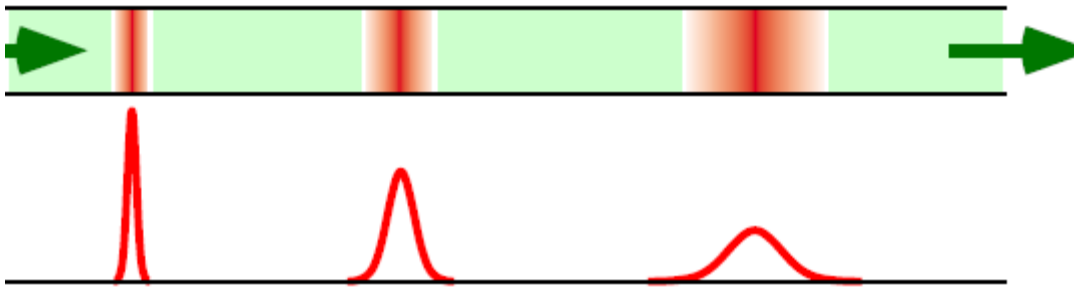
H can be also used to relate various chromatographic parameters (e.g., flow rate, particle size, etc.) to the kinetic processes that give rise to peak broadening.

Why Do Bands Spread (become broad)?

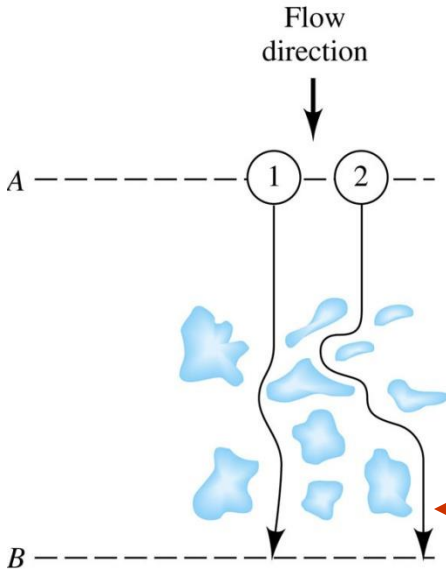
a. Eddy diffusion

b. Mobile phase mass transfer

c. Longitudinal diffusion



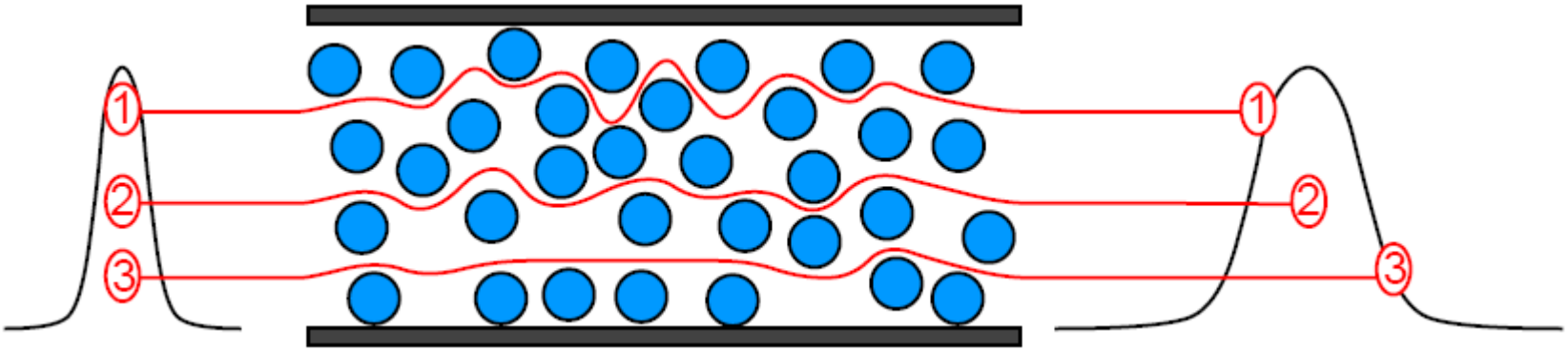
a.) Eddy diffusion : a process that leads to peak (band) broadening due to the presence of multiple flow paths through a packed column.



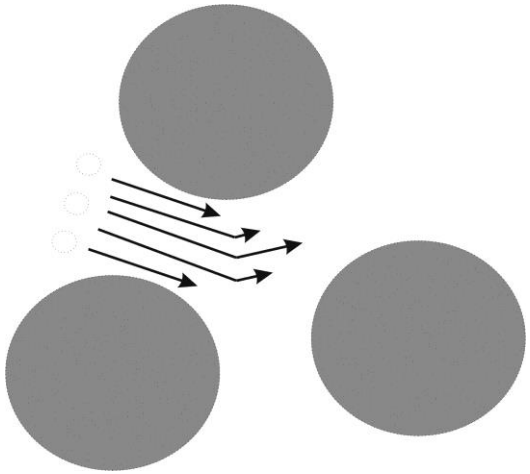
As solute molecules travel through the column, some arrive at the end sooner then others simply due to the different path traveled around the support particles in the column that result in different travel distances.

Longer path arrives at end of column after (1).

Eddy diffusion is directly proportional to the diameter of the particles making up the column packing.



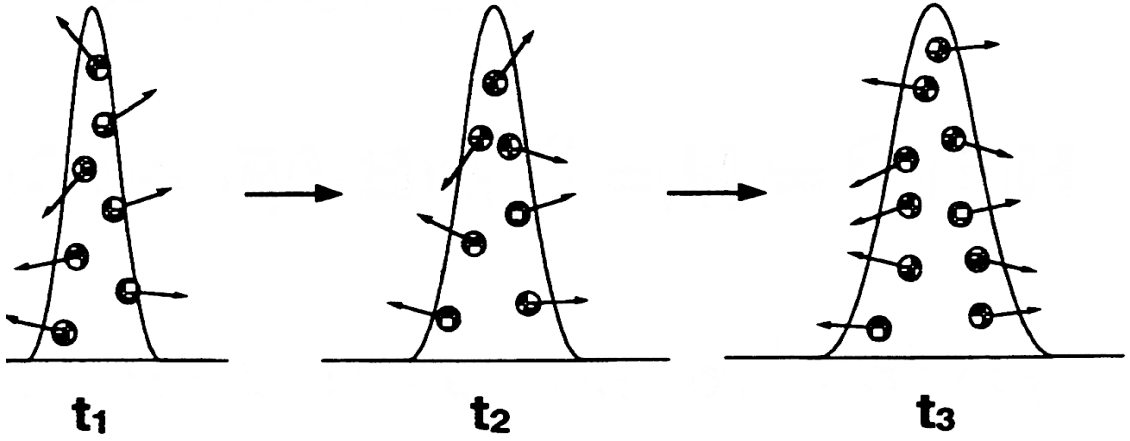
b.) non equilibrium mass transfer: a process of peak broadening caused by non equilibrium attainment due to the transfer of solute from the higher concentration region to the low concentration one.



The degree of band-broadening due to eddy diffusion and mobile phase mass transfer depends mainly on:

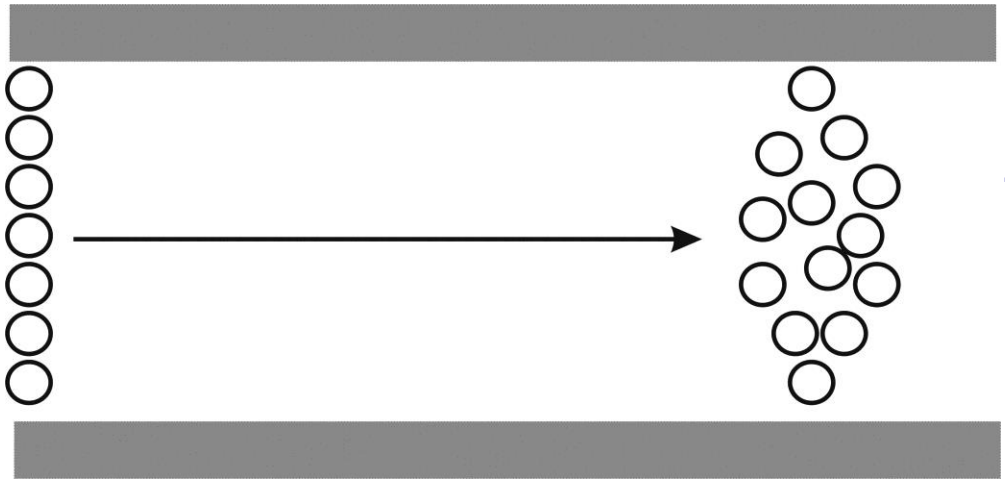
1) the size of the packing material
2) the diffusion rate of the solute

c.) Longitudinal diffusion: band-broadening due to the diffusion of the solute along the length of the column in the flowing mobile phase, ahead of and behind the zone center.



The degree of band-broadening due to longitudinal diffusion depends on:

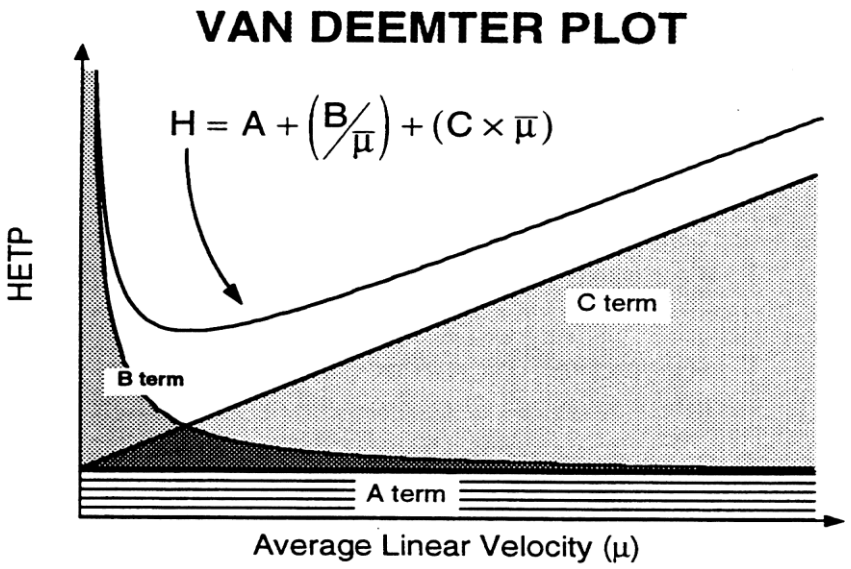
1) the diffusion of the solute
2) the flow-rate of the solute through the column



A solute in the center of the channel moves more quickly than solute at the edges, it will tend to reach the end of the channel first leading to band-broadening

Van Deemter equation: relates flow-rate or linear velocity to H:

$$H = A + B/\mu + C\mu$$



where:

μ = linear velocity (flow-rate x V_m/L)
H = total plate height of the column
A = constant representing eddy diffusion & mobile phase mass transfer

B = constant representing longitudinal diffusion
C = constant representing stagnant mobile phase & stationary phase mass transfer

This relationship is used to predict what the resulting effect would be of varying this parameter on the overall efficiency of the chromatographic system.

Factors increasing peak resolution

1. Increasing of column length
2. Decreasing of column diameter
3. Decreasing of flow-rate
4. Packing of column uniformly
5. Using of uniform stationary phase (packing material)
6. Decreasing of sample size
7. Selecting of proper stationary phase
8. Selecting of proper mobile phase
9. Using of proper pressure
10. Using of gradient elution

Factors due to Stationary Phase (Continue):

1- Particle size of the stationary phase: Reducing the particle size increases the surface area and improve separation. However, reduction of the particle size will decrease the flow rate of the mobile phase.

- In HPLC we use very fine particles to get very good separation. The flow rate problem is solved by the use high pressure pumps to push the mobile phase through the stationary phase. Columns are made of stainless steel to withstand the high pressure.

2- Adsorbent activity: The choice of the suitable adsorbent is very important.

3- Uniformity of packing of the column: If the stationary phase is not packed uniformly then the bands will be irregular and less uniform resulting in poor separation.

4- Concentration of the mixture: the proper ratio between sample to be separated and the amount of stationary phase is very important too much samples resulted in bad separation.

Factors due to Mobile Phase:

1- Selection of the proper mobile phase: Very polar mobile phase will wash out all components without any separation. On the other hand very non polar mobile phase will result in broad band and poor separation.

2- Flow rate: Slower flow rate usually resulted in a better separation and narrower bands.

3- Consistency of flow: The continuous flow of the mobile phase during the whole experiment gives better separation than interrupting the flow then continue it later.

Factors due to Columns:

- Column dimensions: Increasing the length of the column improve separation. However, this usually leads to slower flow rate. Also increasing the column length some times is impractical.
- Column temperature: Increasing the temperature usually reduces the adsorption power of the stationary phase and increase elution speed. This may leads to decrease in the efficiency separation.

Factors affecting solutes separation (Factors affecting column efficiency)

Factor	Effect
Particle size of solid stationary phase (or of support)	Decrease of size improves separation (but very small particles need high pressure).
Column dimensions	Efficiency increases as ratio length / width increases.
Uniformity of packing	Non uniform packing results in irregular movement of solutes through column & less uniform zone formation, (i.e. band broadning or tailing).
Column temperature	Increase in column temperature results in speed of elution but does not improve separation (tailing).
Eluting solvent	Solvents should be of low viscosity (to give efficient resolution) & h igh volatility (to get rapid recovery of the substances).
Solvent flow rate	Uniform & low flow rate gives better resolution.
Continuity of flow	Discontinuous flow disturbs resolution
Condition of adsorbent	Deactivation of adsorbent decreases separation.
Concentration of solutes	Substances of high concentration move slowly.