CHROMATOGRAPHY AND EXTRACTION

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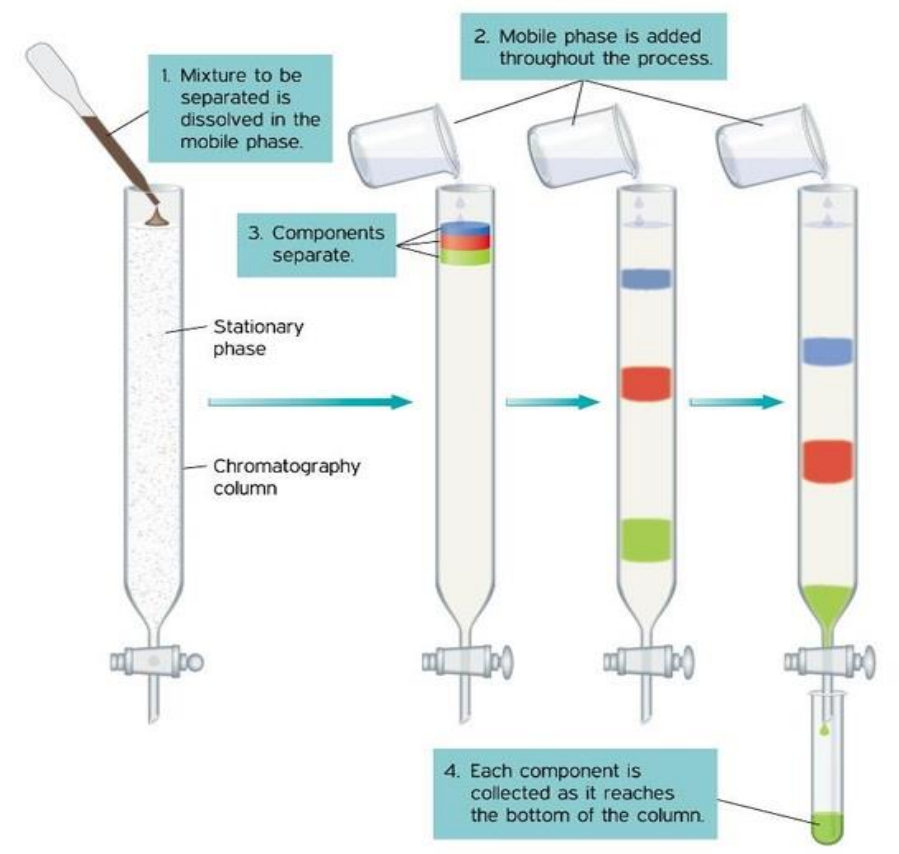
INTRODUCTION

- Chromatography is a physical process where the components (solutes) of a sample mixture are separated as a result of their differential distribution between stationary and mobile phases.



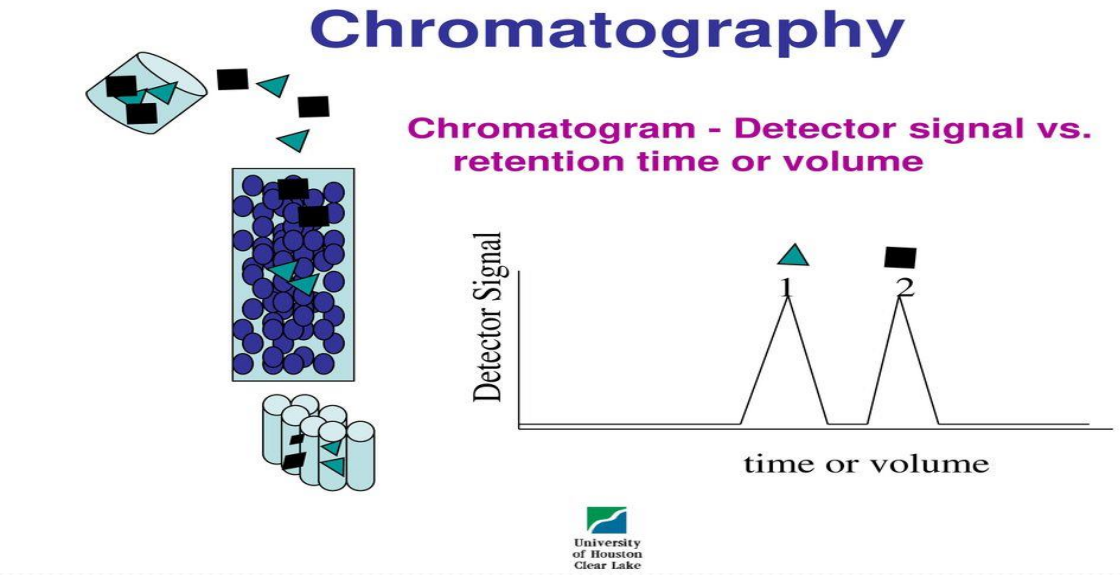
History

- Tswet, russian botanist (referred to as Father of chromatography) credited for the development of chromatography.
- He entailed separation of plant pigment into separate colored bands on a column Of calcium carbonate.
- Greek chroma meaning 'color' and graphein meaning 'writing

Principle:

- Chromatography is usually based on principle of partition of solute between two phases. It usually consists of a Mobile Phase and a Stationary Phase.
- ► The **Mobile Phase** usually refers to the mixture of the substances to be separated dissolved in a liquid or a gas
- ► The **Stationary Phase** is a porous solid matrix through which the sample contained in the mobile phase percolates.

- A graphical presentation of detector response, concentration of analyte in the effluent, or other quantity used as a measure of effluent concentration.
- The retention time or volume is a interval or volume required for a solute to pass from the injector , through the column and to the detector



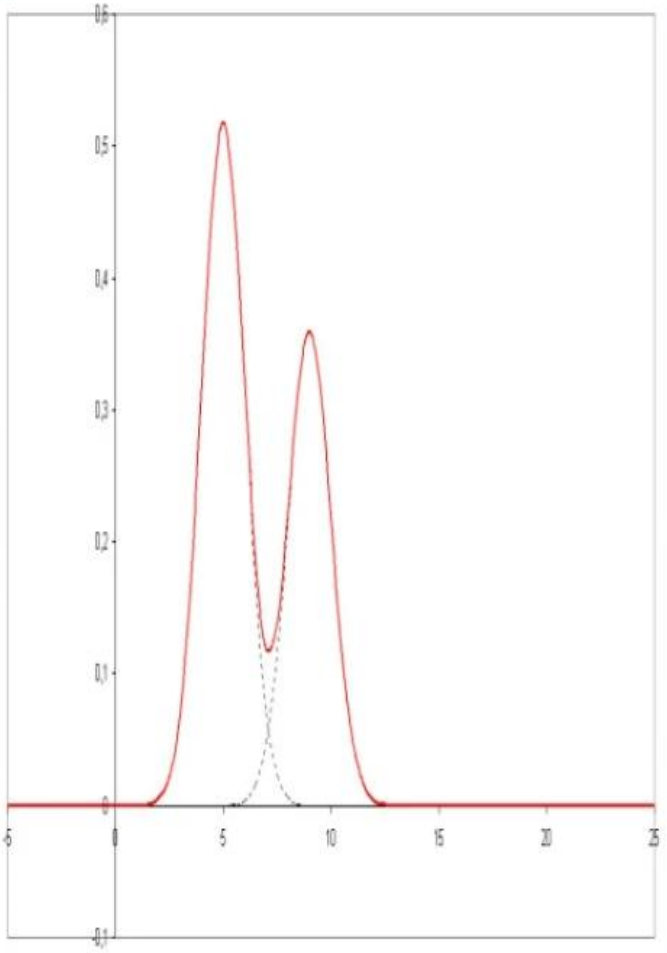
- Data represented by the chromatogram are used to help identify and quantify the solute(s).

- Because eluting solutes are displayed graphically as a series of peaks, they are frequently referred to as chromatographic peaks. These Peaks are described in terms of peak,

  (1) width,

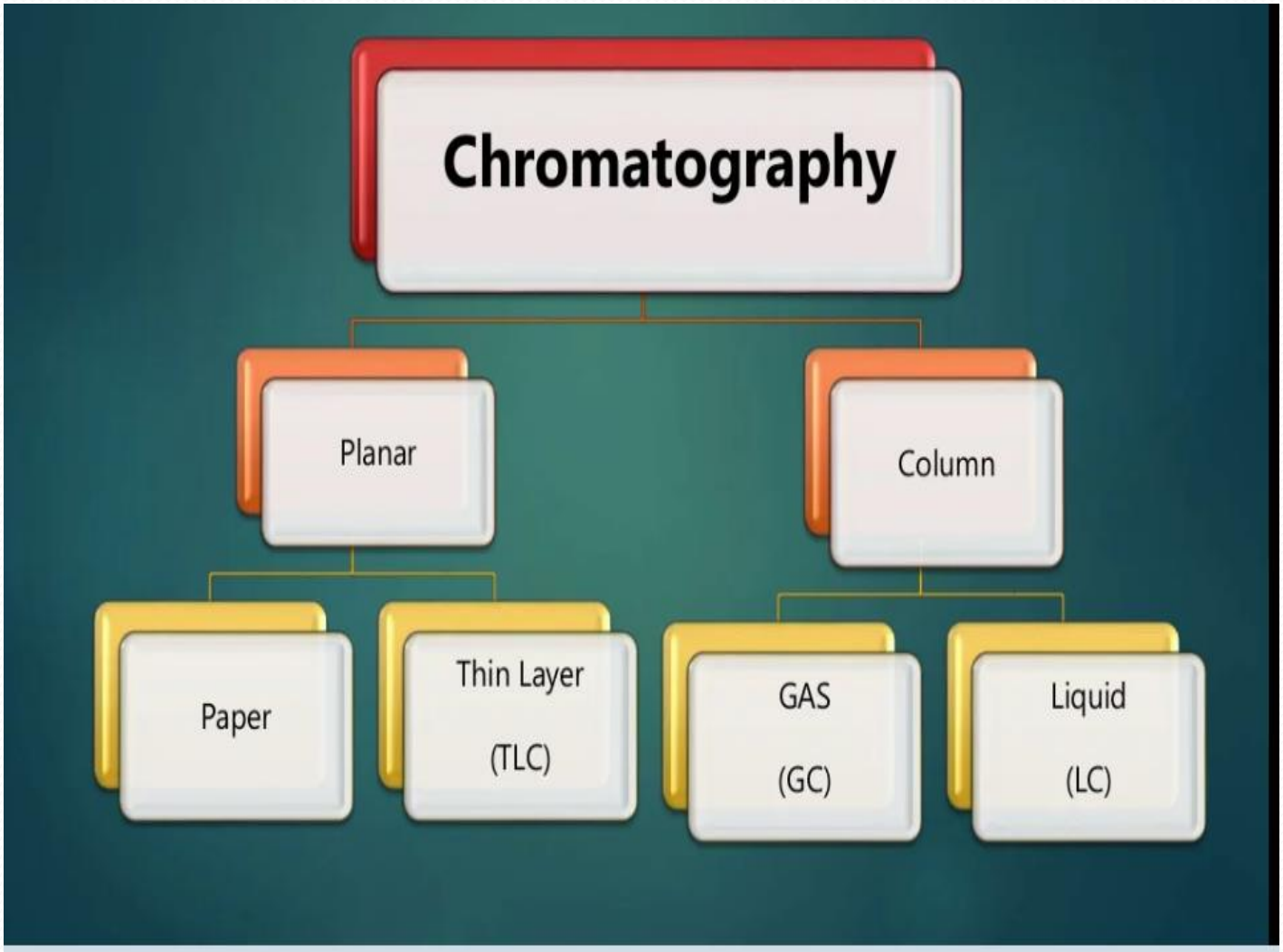
  (2)height,

  (3)area.



Classification

- Chromatographic methods can be classified in three different ways :
- a) Based on shape of chromatographic beds e.g.- Planar and column Chromatography
- b) Based on the physical state of mobile and stationary phase. Gas and liquid chromatography
- c) Based on mechanism of separation. e.g.- lon-exchange chromatography, partition, affinity and adsorption chromatography


Chromatography
Planar
Column
Paper
Thin Layer
(TLC)
GAS
(GC)
Liquid
(LC)

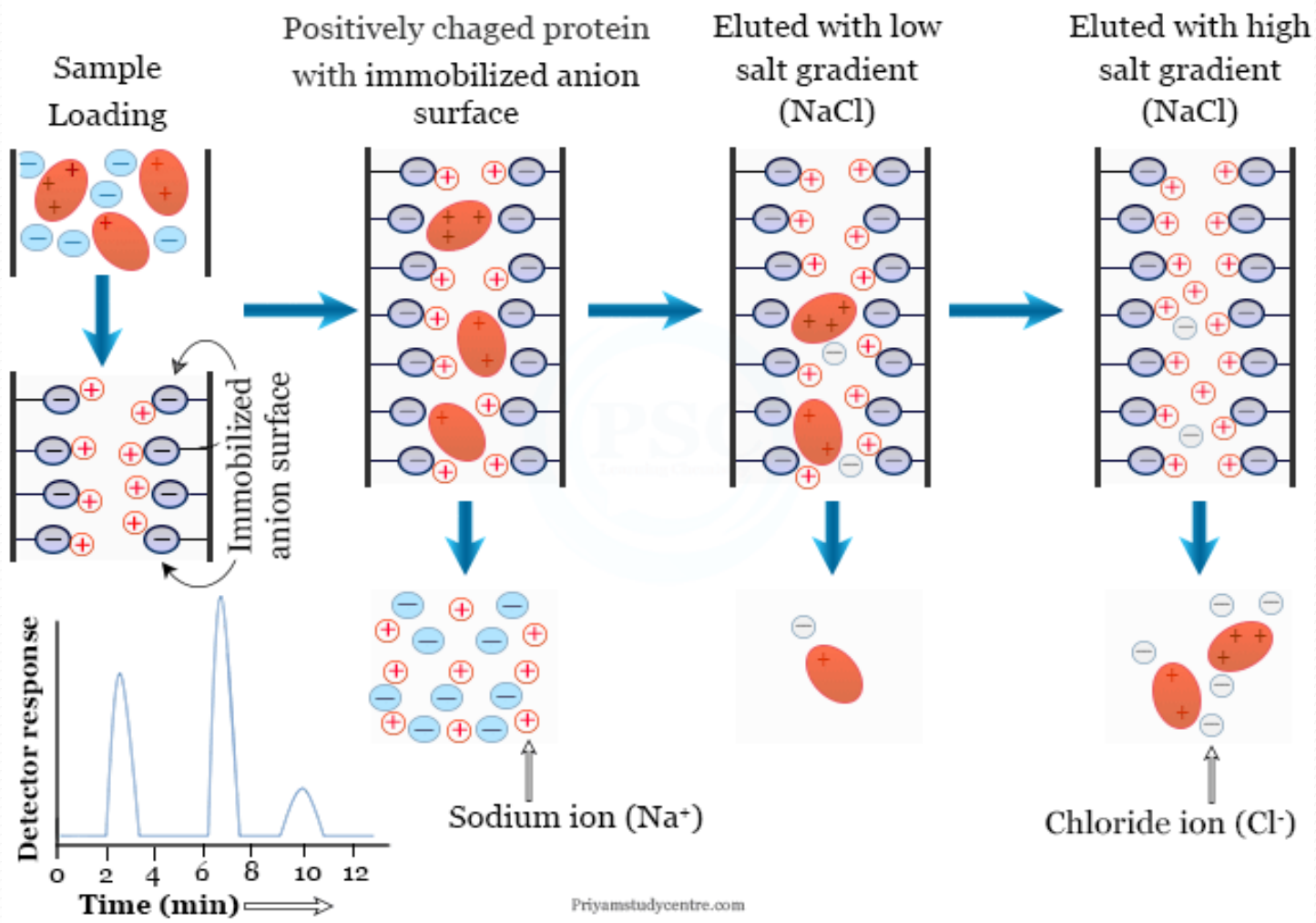
Separation Mechanism
Ion exchange chromatography

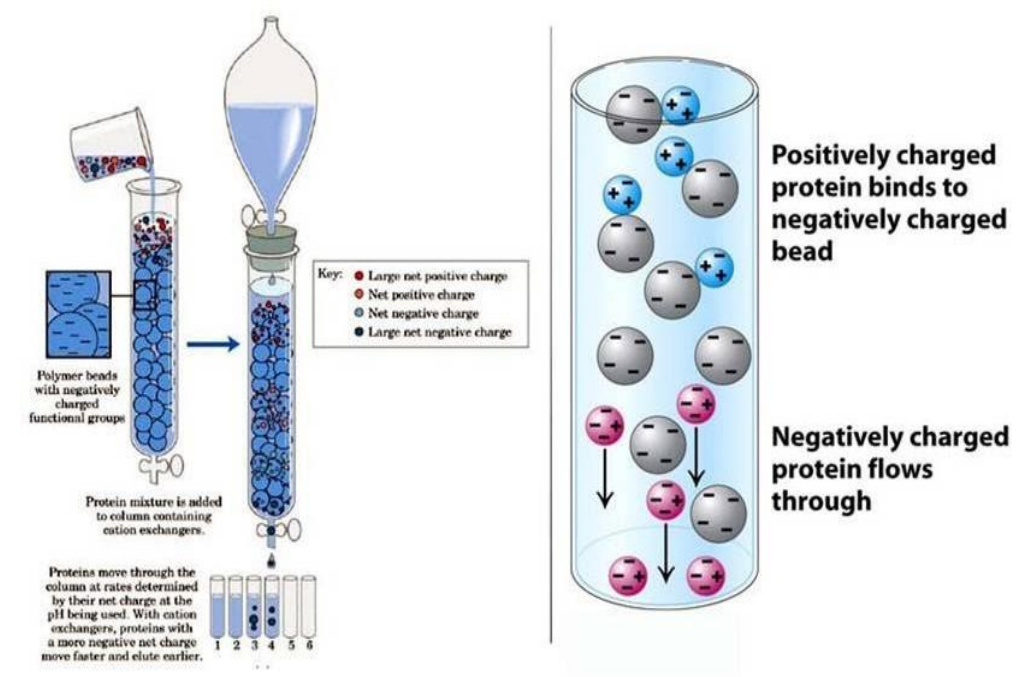
- **PRINCIPLE**

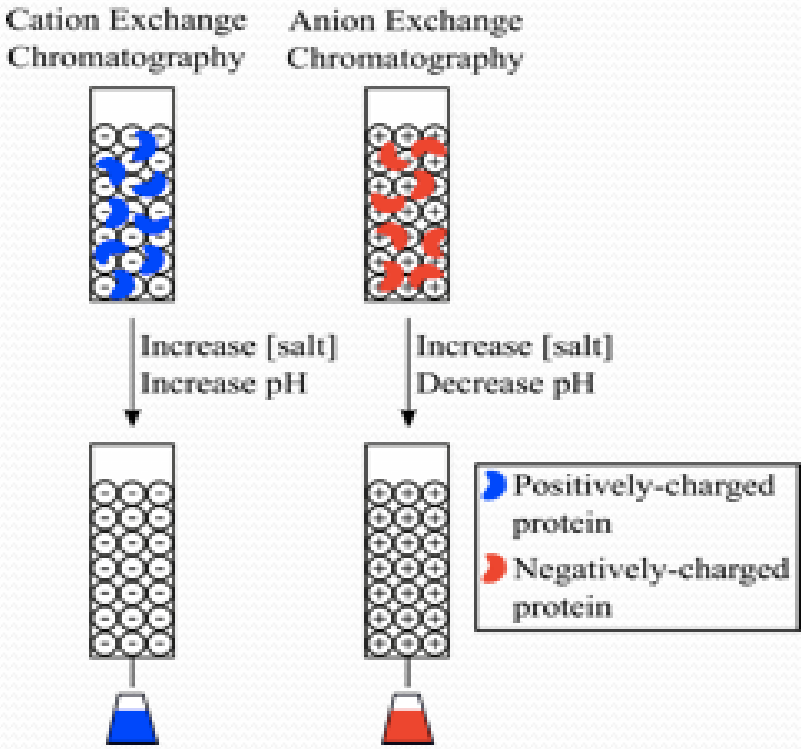
➢ Ion-exchange chromatography is based on an exchange of ions between a charged stationary surface and ions of the opposite charge in mobile phase.

➢ Depending on the conditions, solutes are either cations (positively charged) or anions (negatively charged). These are also known as ion exchangers.

Ion Exchange Chromatography

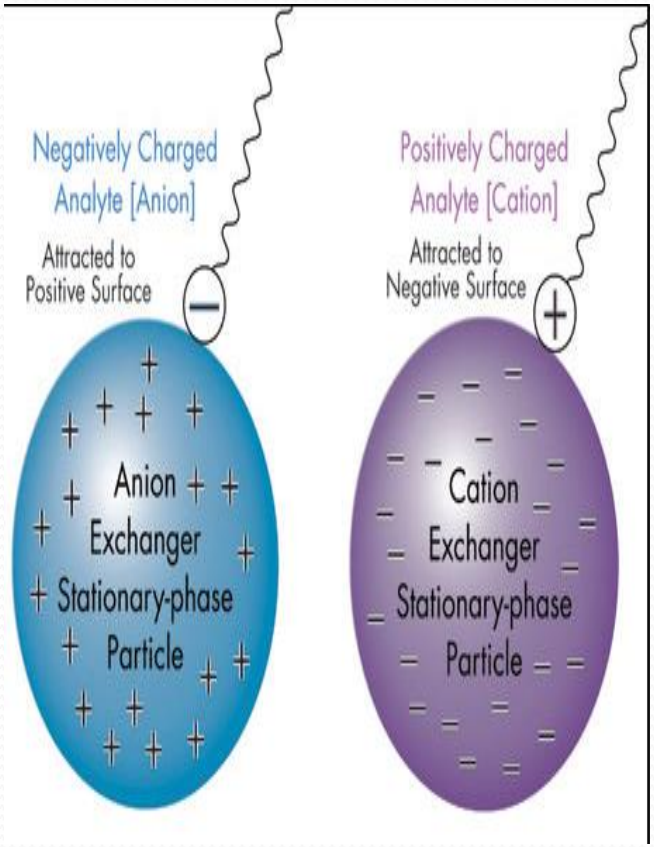



Key:
Large net positive charge
Net positive charge
Net negative charge
Large net negative charge
Polymer beads with negatively charged functional groups
Protein mixture is added to column containing cation exchangers.
Proteins move through the column at rates determined by their net charge at the pH being used. With cation exchangers, proteins with a more negative net charge move faster and elute earlier.
1 2 3 4 5 6
Positively charged protein binds to negatively charged bead
Negatively charged protein flows through


Cation Exchange Chromatography
Anion Exchange Chromatography
Increase [salt]
Increase pH
Increase [salt]
Decrease pH
Positively-charged protein
Negatively-charged protein

ion exchangers

- Ion-exchanger are made up of two parts an insoluble matrix and chemically bonded charged groups within and on the surface of the matrix.
- It is classified as cationic or anionic based on whether it exchanges cation or anions.
- Cation exchanger-also known as acidic ion-exchanger.
- Anion exchanger -also called basic ion exchanger.
- Each type of exchanger is also classified as strong or weak depending on the ionising strength of the functional group.



Type	Functional groups	Matrices
Weakly acidic (cation exchanger)	carboxy	Agarose
	carboxymethyl	Cellulose
Strongly acidic (cation exchanger)	sulpho	Cellulose
	sulphomethyl	Dextran
	sulphopropyl	Polystyrene
Weakly basic (anion exchanger)	aminoethyl	Agarose
Strongly basic (anion exchanger)	Trimethylaminomethyl	Cellulose
	Triethylaminoethyl	Dextran

- Bound molecules can be eluted by altering the pH of the eluting buffer or by increasing the salt concentration of the eluting buffer.
- A positively charged protein bound to cation exchanger can be eluted by increasing the salt concentration in the eluting buffer because cations present in the buffer compete with positively charged groups on the protein for binding to the ion exchanger.
- Proteins having a low density of net positive charge will tend to emerge first followed by those having a higher charge density.

Selection of ion exchanger

- The choice of ion exchanger for the purification of bio molecule largely depends on the isoelectric point of the bio molecule.
- A Solute having a positive charge will bind to a cationic exchanger and vice versa.
- Many solutes having more than one type of ionising group and may have both positively and negatively charged groups.
- The net charge on such molecules depend on pH. At isoelectric point, the solute has no net charge and would bind to any type of ion exchanger.

Choice of buffers

- Cationic buffers should be used with anionic exchangers and vice versa.
- Firstly, the pH chosen for the buffer depends on the range of stability of macromolecule to be separated.
- Second, the buffer pH should be chosen so that the desired macromolecule will bind to the ion exchanger.
- The ionic strength should be relatively low to avoid damping of interaction between solute and ion exchanger.
- Buffer concentrations in the range 0.05 to 0.1 M are recommended.

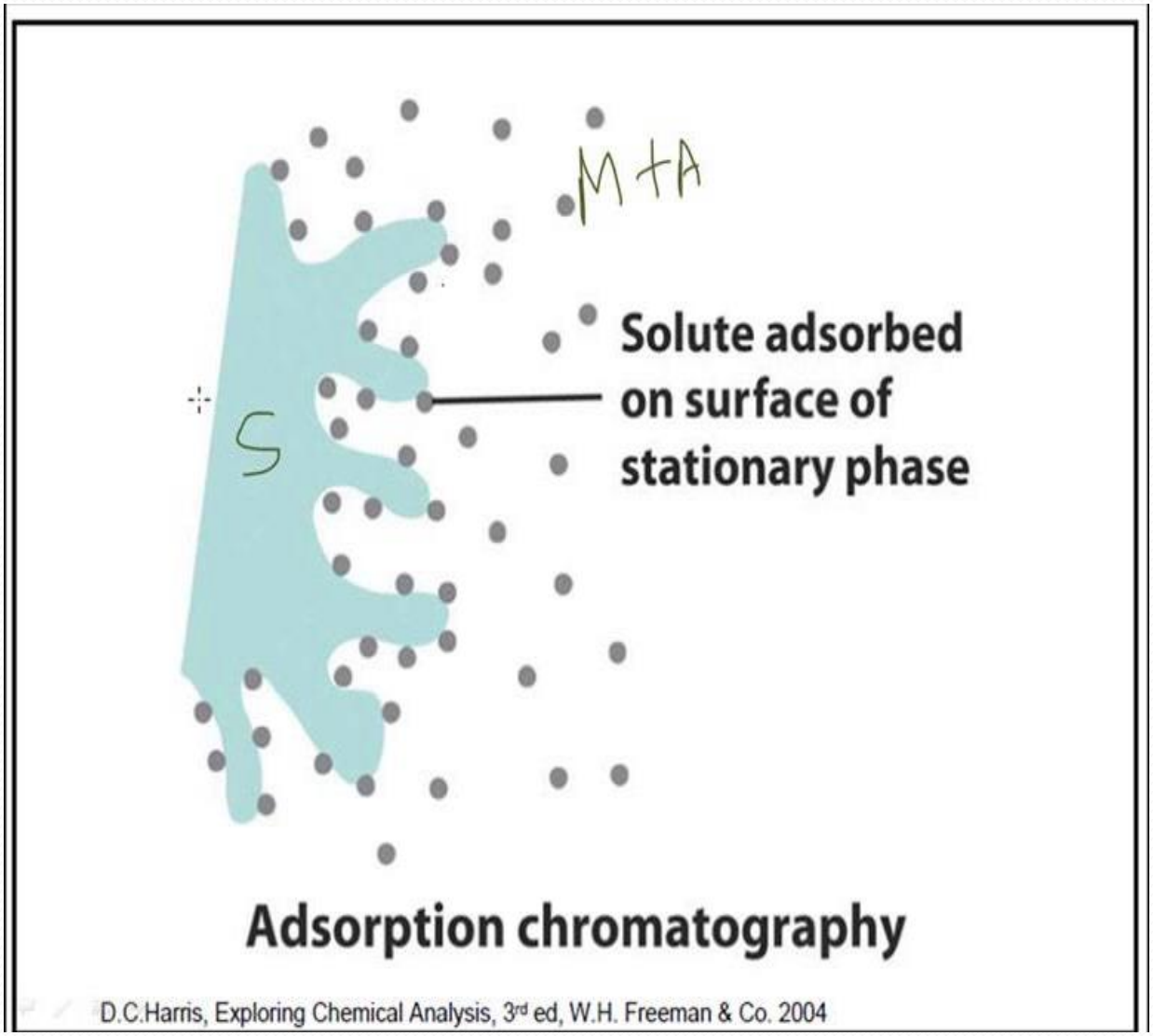
Applications

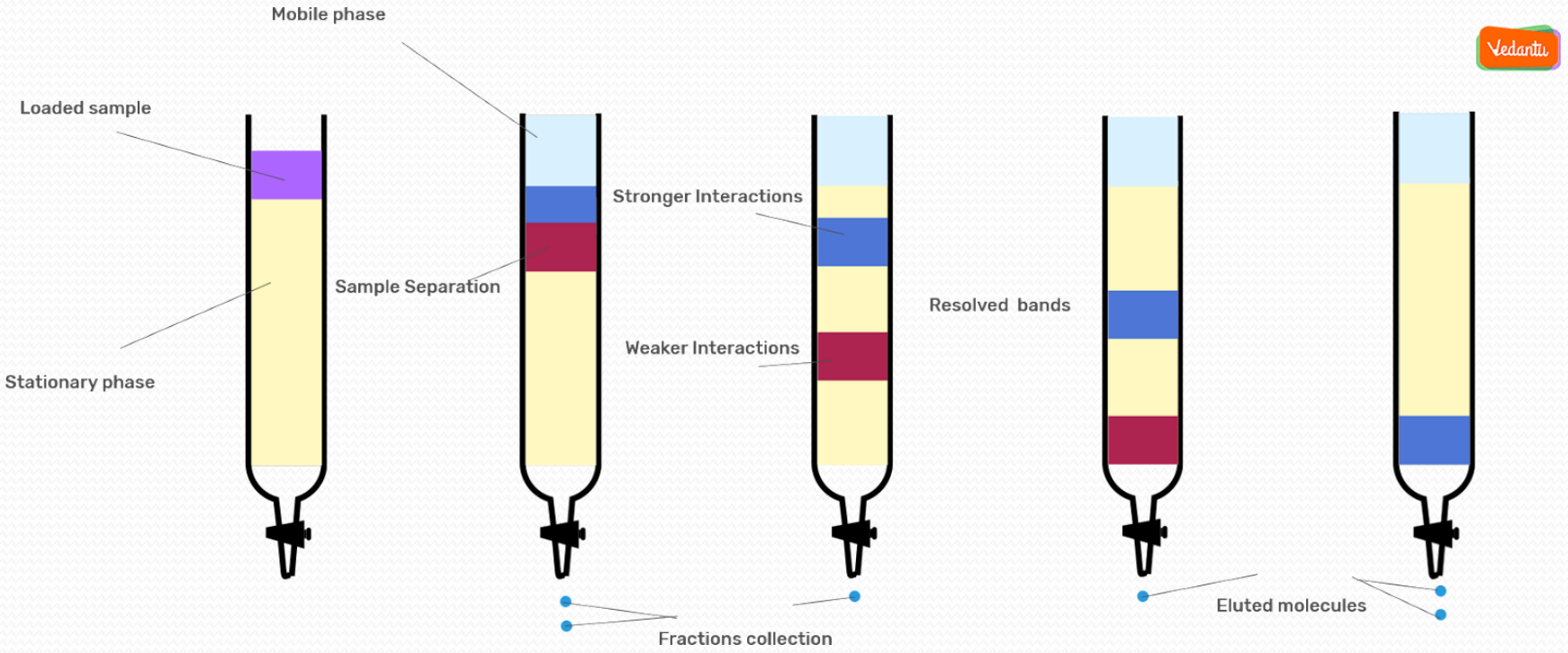
- The separation and purification of proteins, peptides, nucleic acids, polynucleotides and other charged molecules, mainly because of its high resolving power and high capacity.
- Used to separate DNA from cell extract.
- Separation and removal of inorganic ions from aqueous mixtures.
- As number of clinical application, including amino acid and hemoglobins.

Adsorption chromatography

Principle

- Certain solid materials, collectively known as adsorbents, have the ability to hold molecules at their surface.
- This adsorption process, which involves weak, non-ionic attractive forces of the van der Waals and hydrogen-bonding type occur at specific adsorption sites.
- Silica is a typical adsorbent. It has silanol (Si-OH) groups on its surface, which are slightly acidic, and can interact with polar functional groups of the analyte or eluent.
- Other commonly used adsorbents are alumina and carbon.


M+A
S
Solute adsorbed
on surface of
stationary phase
Adsorption chromatography
D.C.Harris, Exploring Chemical Analysis, 3rd ed, W.H. Freeman & Co. 2004


Mobile phase
Loaded sample
Stationary phase
Sample Separation
Stronger Interactions
Weaker Interactions
Fractions collection
Resolved bands
Eluted molecules

Applications of Adsorption Chromatography

- Separation of aromatic or aliphatic non-polar compounds such as lipids.
- Separation of high molecular weight compounds.
- Separation / purification of Nucleic acids.
- Analysis of plant pigments, Fat soluble vitamins.

Advantages of Adsorption Chromatography –

- It has a wide range of mobile phases for the separation of compounds.
- The complex sample mixtures can be easily separated by this method.
- Simple and low cost technique.

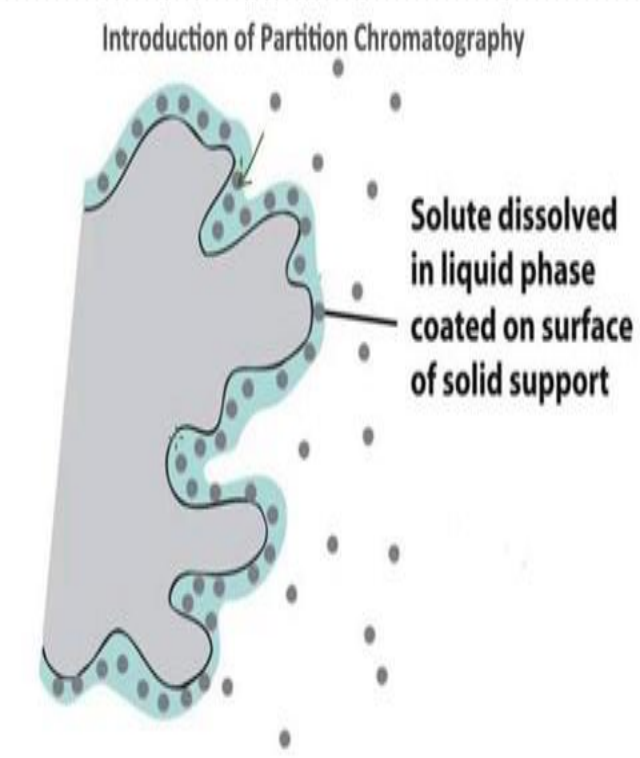
Disadvantages of Adsorption Chromatography –

- Results obtained are not reproducible.
- Some compounds may permanently retain on the stationary phase.

Partition chromatography

Partition Column Chromatography

- Here solute molecules partitions between two liquids, first liquid is stationary phase and second is mobile phase.
- Here separation of sample mixture components occurs differences in their partition coefficients. (relative solubilitis in stationary and mobile phase liquids).
- Here stationary phase is a liquid which is held in place by coating on solid support or by forming chemical bond with solid support (bonded phases) and then packed in column.



- The differential distribution of solutes between two immiscible liquids is the basis of separation of partition chromatography.
- It can be subdivided into :-

- liquid-liquid chromatography
- bonded-phase liquid chromatography.

- **liquid-liquid chromatography**, the liquid stationary phase is attached to a supporting matrix by purely physical means.
- **bonded-phase liquid chromatography**, the stationary phase is covalently attached to the matrix. Solute dissolved in liquid phase coated on surface of solid support.

- LLC is classified as :

1. Normal phase liquid chromatography
2. Reversed phase liquid chromatography

Normal Phase Liquid Chromatography

- The stationary phase is polar and the mobile phase relatively non-polar.
- Stationary phase is an alkylamine bonded to silica.
- The mobile phase is generally an organic solvent such as hexane,heptane, dichloromethane or ethyl acetate.
- Based on Polarity :n-hexane <cyclohexane < trichloromethane< dichloromethane < tetrahydrofuran < acetonitrile < ethanol <methanol <ethanoic acid < water.
- The order of elution of analytes is such that the least polar is eluted first and the most polar last.

Application

- Used to separate analytes that have low water solubility and those that are not amenable to reversed phase liquid chromatography.

Reversed Phase Liquid Chromatography

- The stationary phase is non-polar and the mobile phase relatively polar.
- Stationary phase Alkylsilane groups are chemically attached to silica.
- The mobile phase is commonly water or aqueous buffers, methanol, acetonitrile or tetrahydrofuran, or mixtures of them.
- The order of elution of analytes is such that most polar are eluted first and least polar elute last.
- Reversed-phase liquid chromatography differs from most other forms of chromatography, in that the stationary phase is essentially inert and only non-polar (hydrophobic) interactions are possible with analytes.

Application

- It is widely used to analyse drugs and their metabolites, insecticide and pesticide residues, and amino acids, peptides and proteins

Ion-pair reversed-phase liquid chromatography

- The separation of some highly polar analytes, such as amino acids, peptides, organic acids and the catecholamines, is not possible by reversed-phase chromatography. It is possible to achieve such separations by one of two approaches:
- Ion suppression: The ionic character of a weakly acidic or basic analyte is neutralised or "supressed" through modification of the mobile phase pH.
- Ion-pairing: A counter ion opposite-in charge to that of the analyte-is added to the mobile phase, where it forms ion pairs with ionic analytes, displaces the usual base pairs, and neutalize the analyte ions.

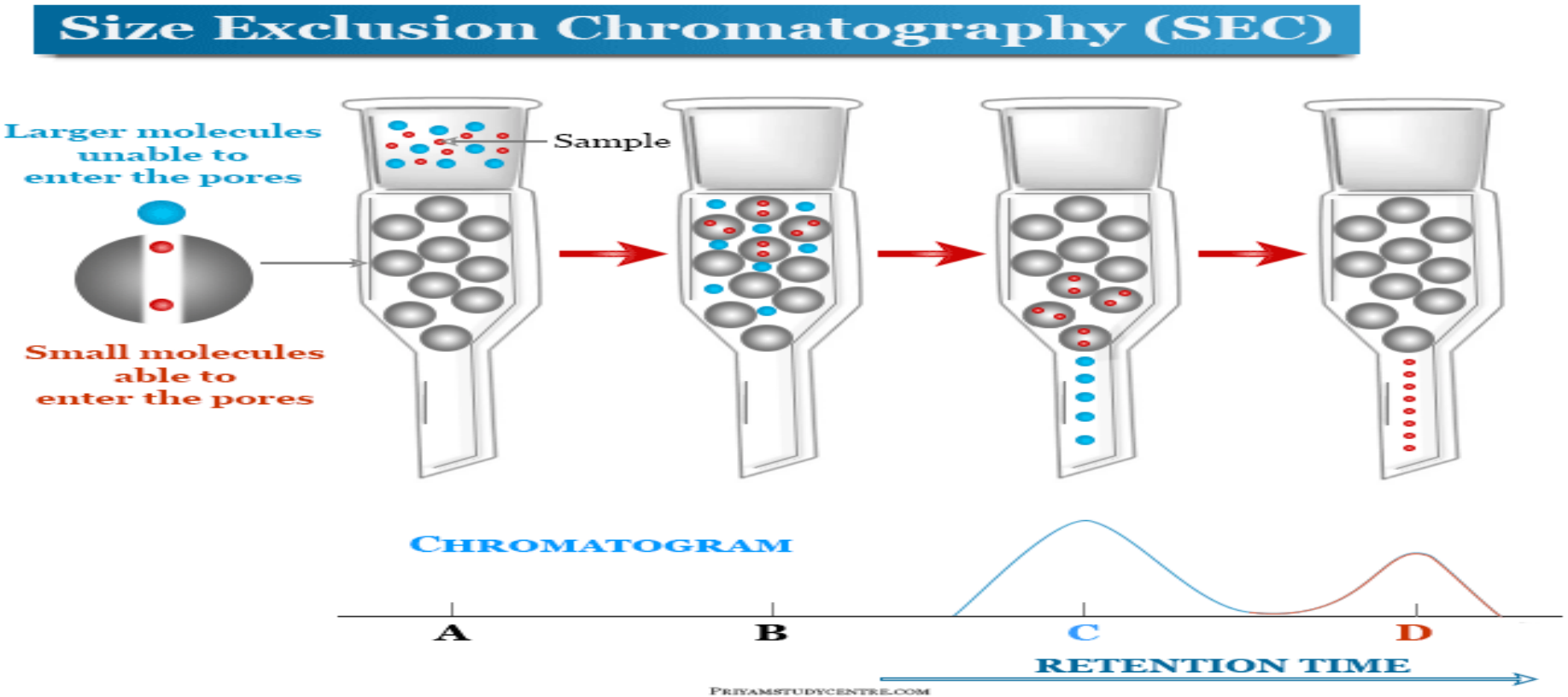
Size exclusion chromatography

Principle

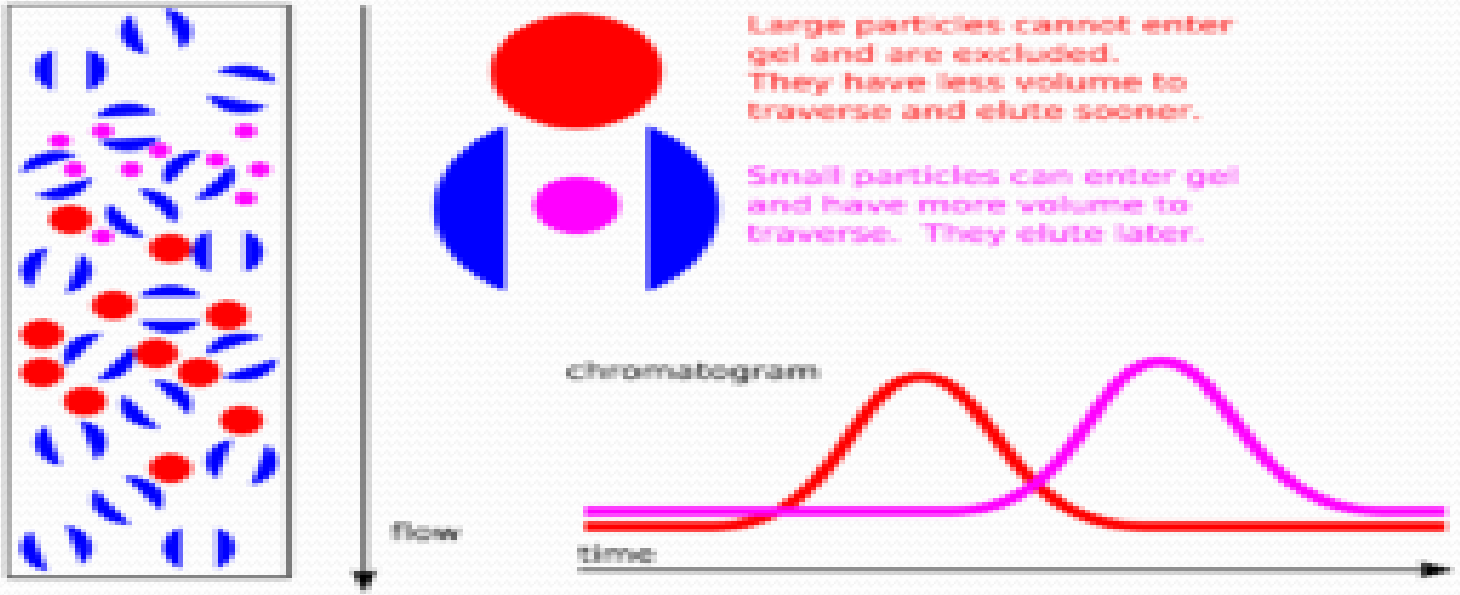
- The separation of molecules on the basis of their molecular size and shape exploits the molecular sieve properties of a variety of porous materials.
- Size exclusion chromatography includes:- Gel Permeation Chromatography and Gel Filtration Chromatography.
- Materials used for stationary phases include cross-linked dextran, polyacryl amide, agarose, polystyrene, and porous glass with an inert surface coating.

Large analytes that are completely excluded from the pores will pass through the interstitial spaces between the particles and will appear first in the elute.

- Smaller analytes will be distributed between the mobile phase inside and outside the particles and will therefore pass through the column at a slower rate, hence appearing last in the elute.



- Gel Filtration Chromatography can be used to separate compounds such as small molecules, proteins, polysaccharides and nucleic acids when in aqueous solution.
- When organic solvent is used as mobile phase, the processs known as Gel Permeation Chromatography



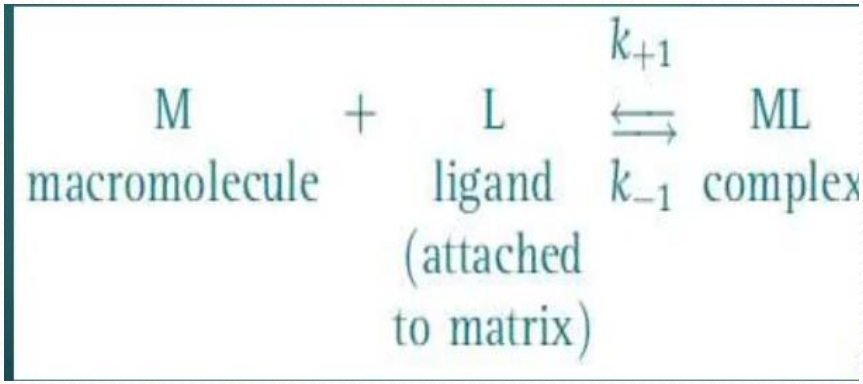
Applications

- It can be used for :
- Fractionation of molecules and complexes within a predetermined size range.
- Size analysis and determination Removal of large proteins and complexes
- Buffer exchange
- Desalting
- Removal of small molecules such as nucleotides, primers, dyes and contaminants
- Assesment of sample purity
- Separation of bound and unbound radioisotopes.

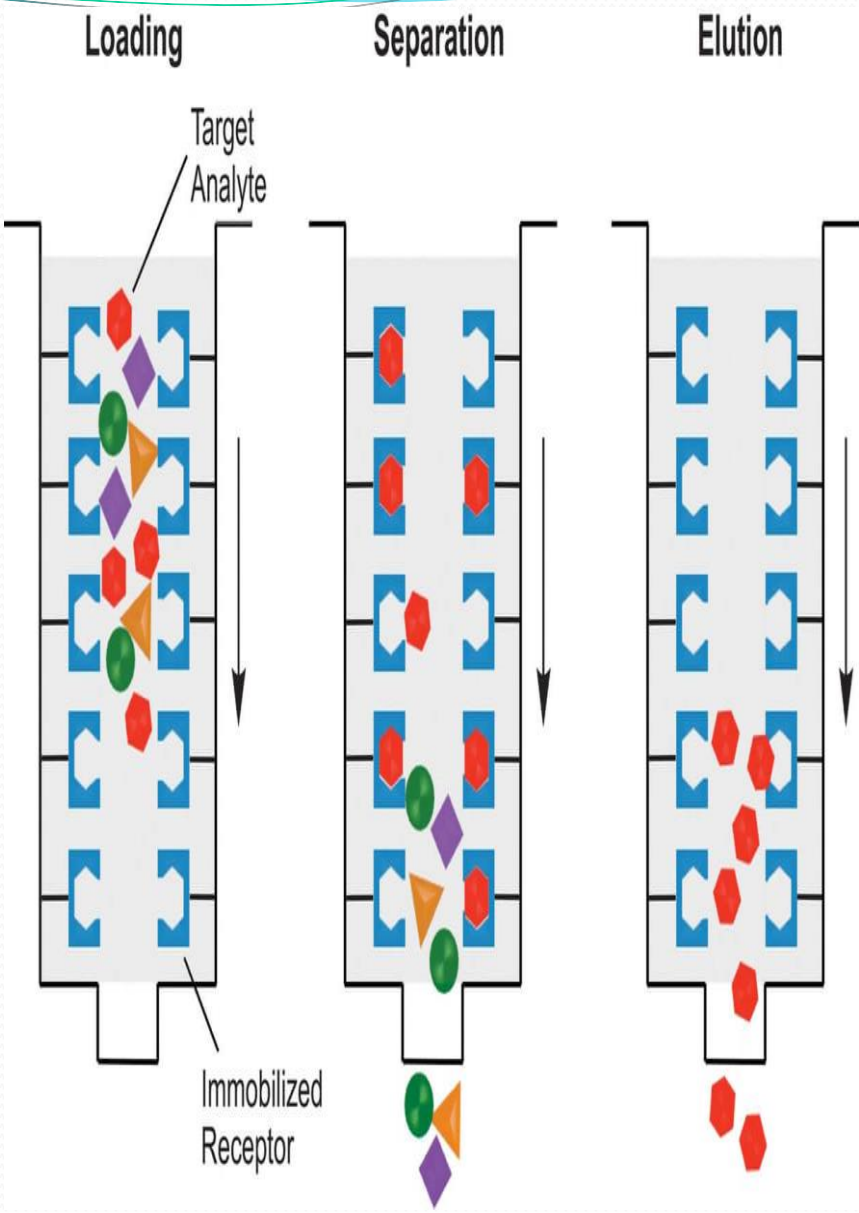
Affinity chromatography

Principle

- It does not rely on differences in the physical properties of the analytes instead, it exploits the unique property of extremely specific biological interactions to achieve separation and purification.
- The technique requires that the material to be isolated is capable of binding reversibly to a specific ligand that is attached to an insoluble matrix:



- When a complex mixture containing the specific compound to be purified is added to the immobilised ligand, generally contained in a conventional chromatography column, only that compound will bind to the ligand.
- All other compounds can therefore be washed away and the compound subsequently recovered by displacement from the ligand.



Steps

- Choice of appropriate Ligand
- Immobilization of the Ligand onto a support matrix.
- Binding of molecules of interest with the ligand.
- Removal of non-specifically bound molecules.
- Elution of molecules of interest in purified form

Choice of Ligand

Factors to be considered for the choice of Ligand are;

- **Specificity**-The Ligand should recognise only the molecule of interest to be purified.
- **Reversibility**-The ligand should form a reversible complex with the molecule of interest to be purified.
- **Stability**-The ligand should be stable to the condition to be used for immobilisation as well as the conditions of use
- **Size** - The Ligand should be large enough such that it contains several groups able to interact with the molecules of interest resulting in sufficient affinity
- **Affinity**-Binding affinity is the strength of the binding interaction between a molecule of interest to its ligand. Binding affinity is typically measured in terms of equilibrium dissociation constant.

Typical biological interactions used in affinity

Types of Ligand	Target Molecules
Enzymes	Substrate analogue, Inhibitor , Cofactor
Antibody	Antigen
Lectin	Polysaccharide, Glycoprotein, Cell surface receptor, Cell
Nucleic Acid	Complementary base sequence, Nucleic acid binding protein
Hormone	Receptor
Calmodulin	Calmodulin –binding Molecule
Glutathione	Glutathione-S-transferase
Proteins A and G	Immunoglobulins

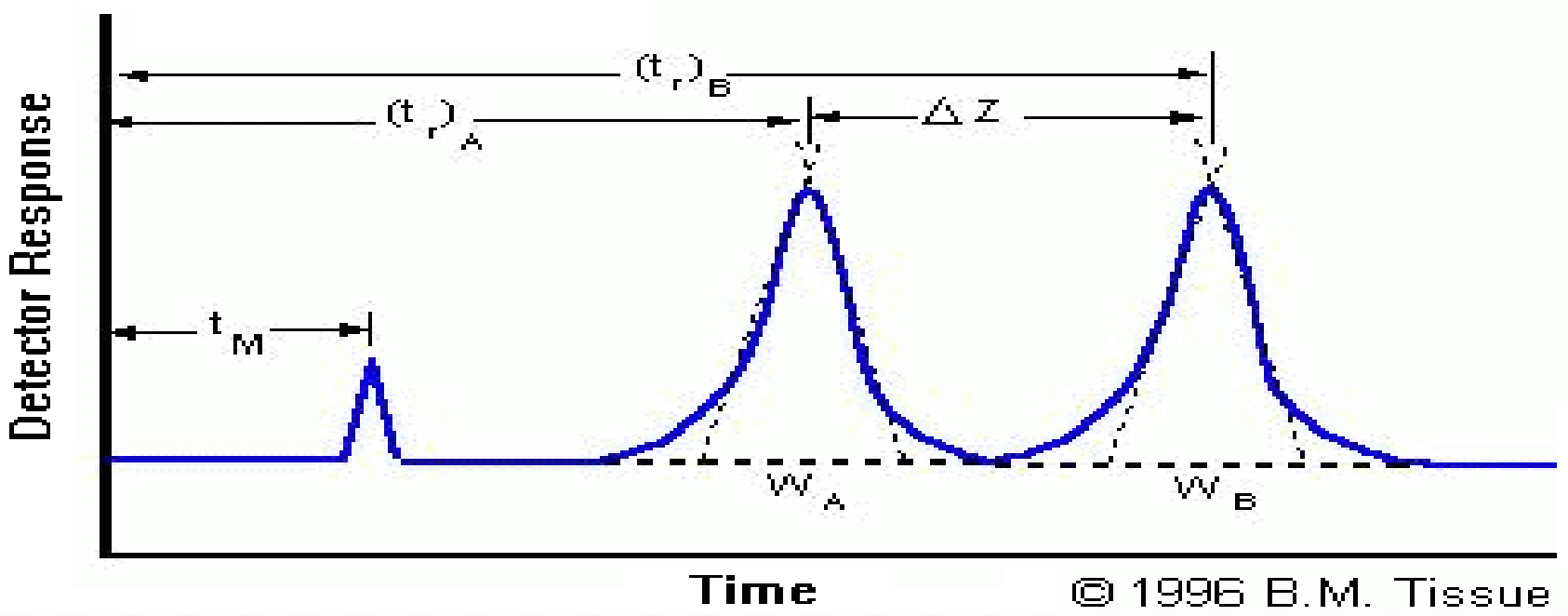
Applications

- Many enzymes and other proteins, including receptor proteins and immunoglobulins, are purified.
- Immobilised single-stranded DNA can be used to isolate complementary RNA and DNA.
- Immobilised nucleotides are useful for the isolation of proteins involved in nucleic acid metabolism.
- Lectin affinity chromatography offers a means of assessing changes in the oligosaccharide structure bound to glycoprotein.

Resolution

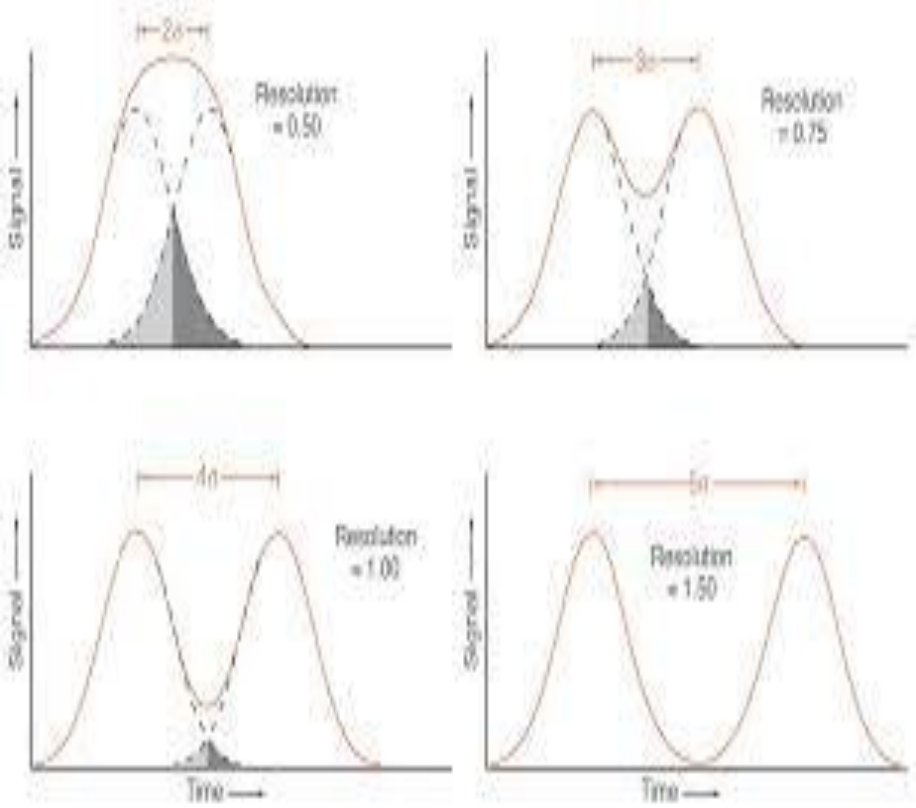
- Resolution (R) is a measure of the separation between two components.
- Improved resolution may result from sharper peaks (higher chromatographic efficiency) or from a greater separation between peaks (higher selectivity).
- It is expressed mathematically as follows:

$$R, = \frac{tr\,(B)-tr\,(A)}{\frac{w(A)-w(B)}{2}}$$



- t(A) =retention time for solute A
- t(B) =retention time for solute B
- W(A) =peak width (units of time) measured at base for solute A
- W(B)= peak width (units of time) measured at base for solute B

- resolution of 1.25 or greater reflects baseline separation of peaks; lower resolution represents increasing degrees of overlap between peaks,



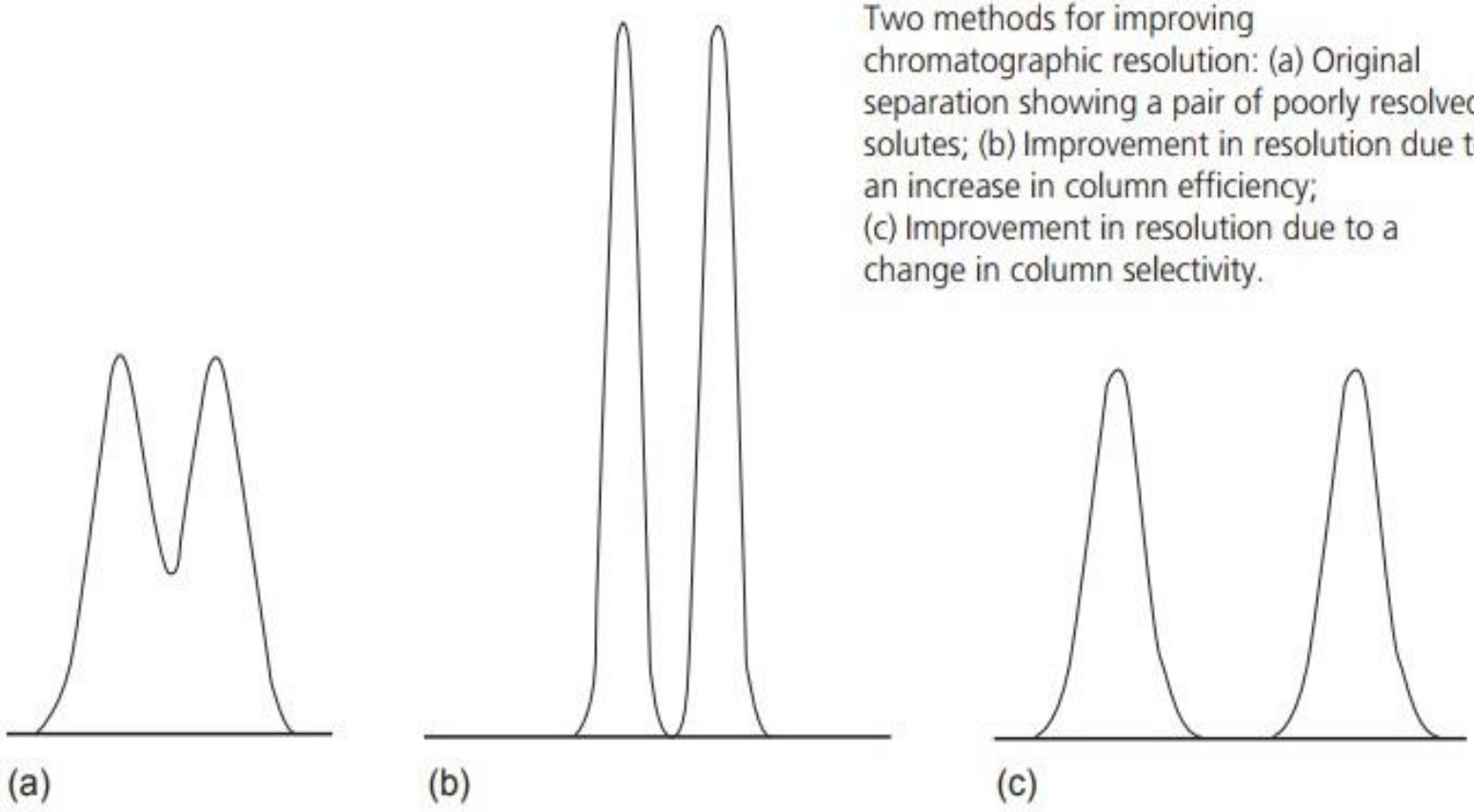


Figure 12.9

Two methods for improving chromatographic resolution: (a) Original separation showing a pair of poorly resolved solutes; (b) Improvement in resolution due to an increase in column efficiency; (c) Improvement in resolution due to a change in column selectivity.

Retention factor

- The retention factor k' is a measure of the time a solute resides in the stationary phase relative to the time it resides in the mobile phase.
- Mathematically, it is the ratio of the adjusted retention volume(v_r) or retention time (t_r) to the void volume (v_o) or hold-up time (t_o) (the time for unretained components to elute from the column).
- A k' of o indicates no binding between a solute and the stationary phase.

$$k' = \frac{V_r - V_o}{V_o} = \frac{t_r - t_o}{t_o}$$

- In planar chromatography, such as paper and thin-layer chromatography, all separation of solutes must occur within the distance traveled by the mobile phase. Thus, a solute's migration is expressed by its $R_{f(\text{relative front})}$ value, which is calculated as follows:

$$R_f = \frac{\text{distance from application point to solute center}}{\text{distance from application point to solvent front}}$$

- Therefore, the greater the solute affinity for the stationary phase, the smaller the R_f value.

Efficiency (N)

- Non uniformity of flow , diffusion and mass transfer effects between particles and the mobile phase produce band broadening.
- Chromatography efficiency is considered to be highest when band broadening is minimized
- Column efficiency is commonly expressed as N , the number of theoretical plates
- For peak forming a gaussian curve

$$N = \left\{ \frac{t_r}{\sigma} \right\}^2$$

t= retention time

σ = standard deviation

- Increase in N represent improved chromatographic efficiency and sharper peaks

- Efficiency is often expressed as the number of theoretical plates per column length(N/L)
- Column efficiency also is expressed as the height equivalent of a theoretical plate (HETP)

$$HETP = \frac{L}{N}$$

Selectivity: selectivity of a separation of two components reflects variable partitioning of different components between the two phases of an extraction or between the stationary and mobile phases of a chromatographic system.

- The selectivity factor (α) for two components A and B is the ratio of retention factors for two components.

$$\alpha = \frac{k'(B)}{k'(A)} = \frac{(t_r(B) - t_o)}{(t_r(A) - t_o)}$$

- If two component have same retention times , then

  $\alpha = 1$

- An α of 1.1 or greater for two components usually represents adequate chromatographic separation.

Peak capacity

peak capacity is the theoretical maximum of the number of components that are able to be separated in a single chromatographic analysis.

the peak capacity of HPLC separation is usually limited to several hundred peaks

- Some gains in peak capacity is achieved by:

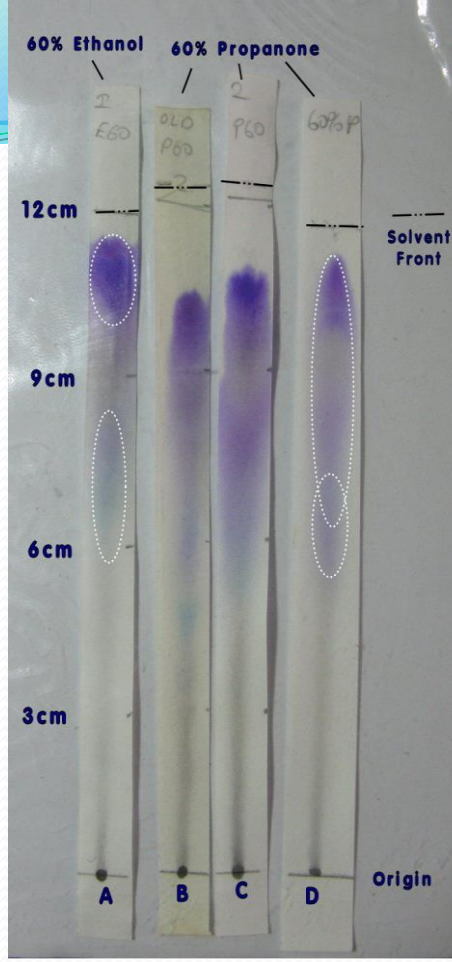
1. Increasing column length
2. Elution with solvent gradients
3. Extended run times
4. Increasing chromatographic efficiency

- Peak capacities of greater than 10,000 have been achieved for two dimensional HPLC separations

Planar chromatography

- In Planar Chromatography stationary phase is present on a plane.
- The Plane can be a paper impregnated by a substance acting as a stationary phase- **Paper Chromatography** OR a Thin layer of a substance acting as a stationary phase spread on a glass, metal or plastic plate- **Thin Layer Chromatography .**
- Planar chromatography is also termed as **Open Bed Chromatography**.

Paper chromatography



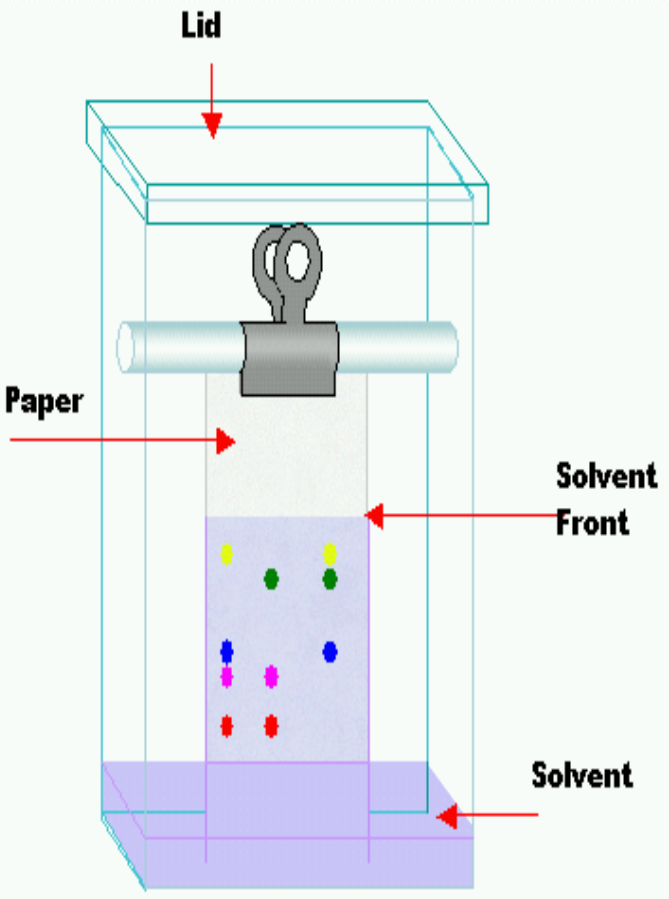
- Paper chromatography is a liquid partition chromatography
- In paper chromatography, the end of the paper is dipped in solvent mixture consisting of aqueous and organic components.
- The solvent soaks in paper by capillary action because of fibrous nature of paper.
- The aqueous component of the solvent binds to the cellulose paper and there by forms stationary phase with it.
- The organic component of the solvent binds continues migrating, thus forming the mobile phase.

Mechanism of Separation

- Mobile Phase rises up by capillary action.
- Testing sample is concentrated as a minute spot at the bottom of the filter paper.
- Sample mixture gradually rises up with the mobile phase which is liquid.
- Compounds in the mixture will be separated according to their ability of solubility.
- More Polar substances will move slower and less polar substances will travel faster.

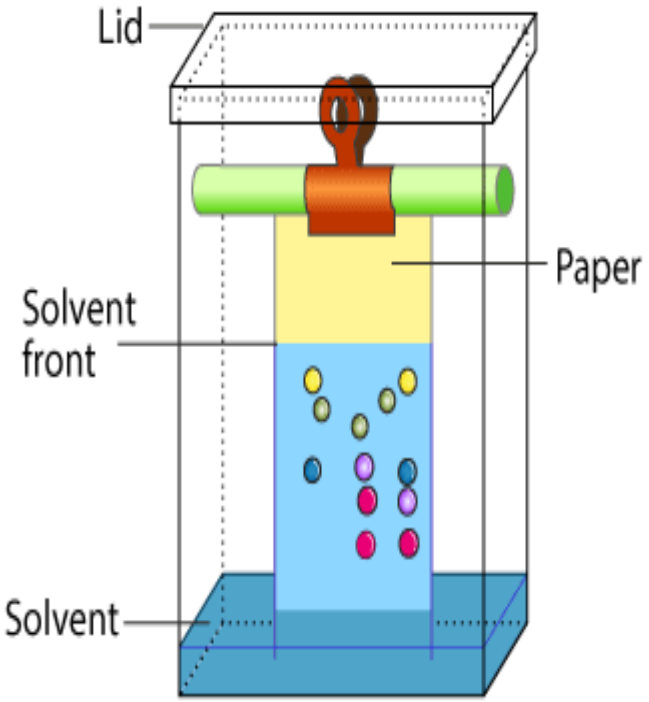
Procedure

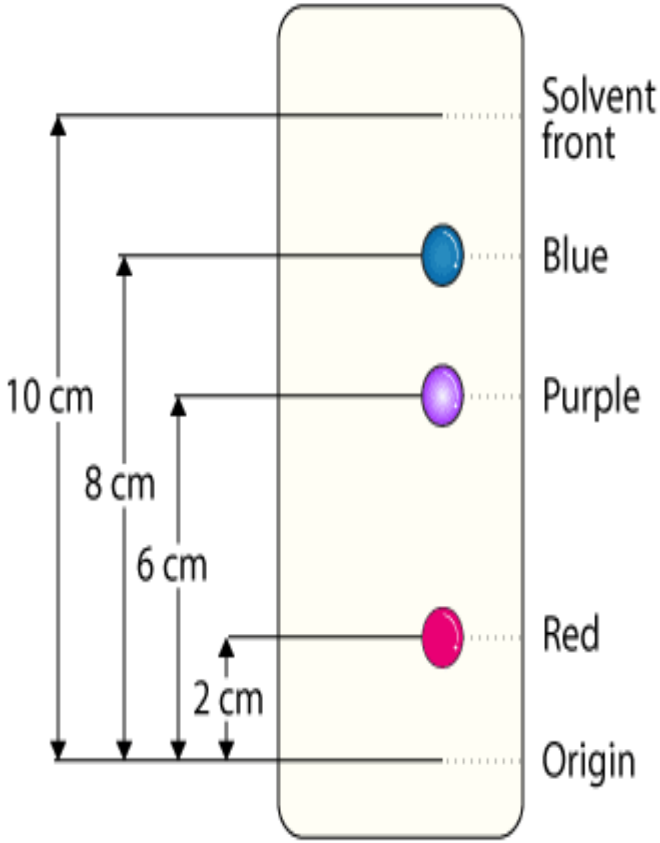
- A small spot of sample applied to a strip of chromatography paper about two centimeters away from the base of the plate.
- This sample is absorbed onto the paper and may form interactions with it.
- The paper is then dipped into a solvent, such as ethanol or water, taking care that the spot is above the surface of solvent, and placed in a sealed.



PAPER CHROMATOGRAPHY

BYJU'S The Learning App

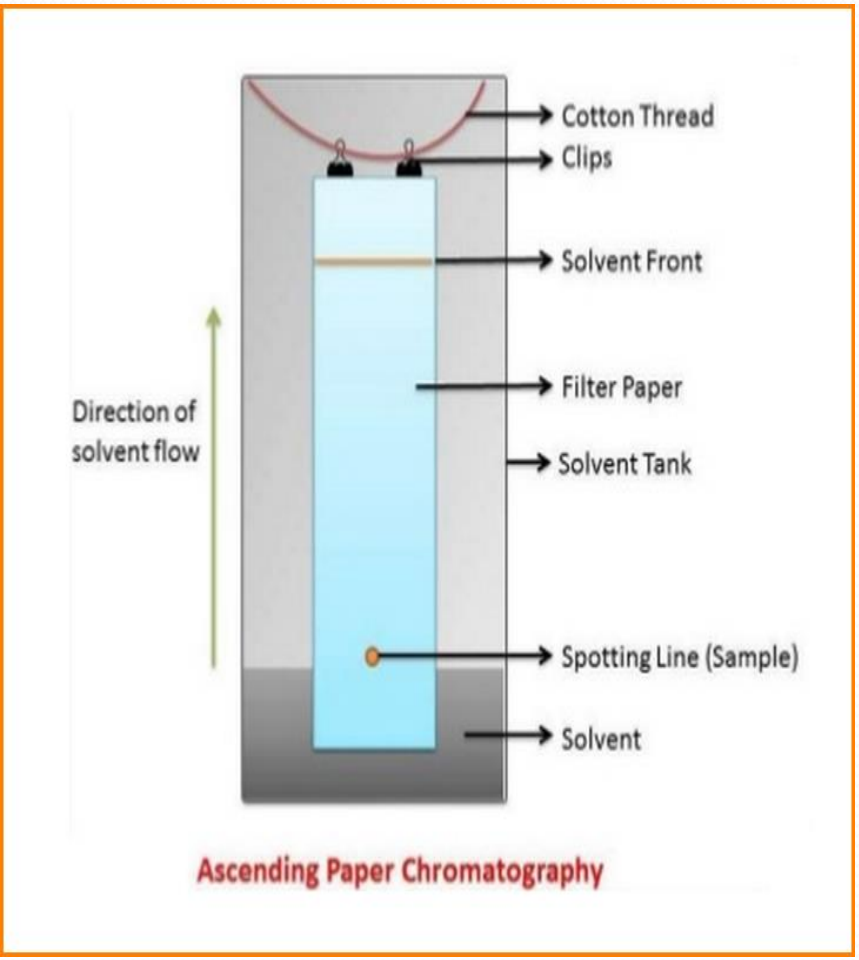


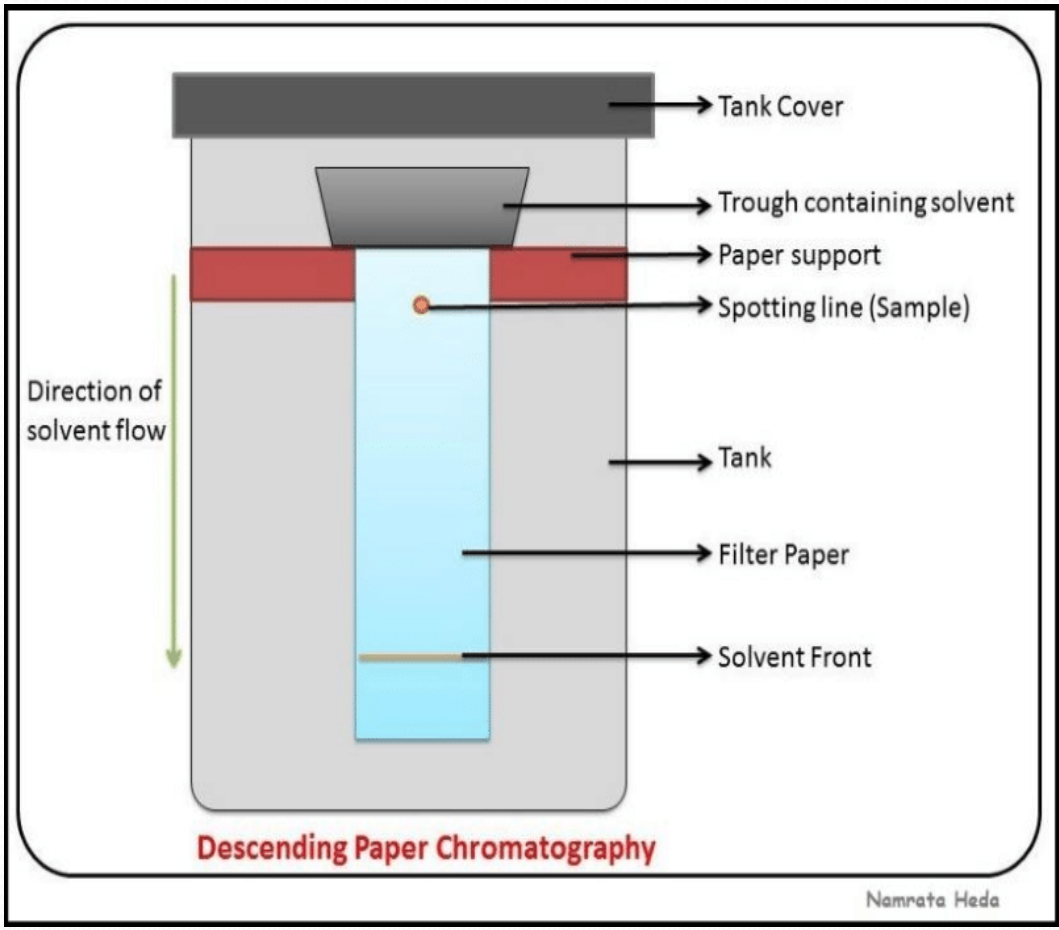


- The solvent moves up the paper by capillary action and dissolves the sample mixture, which will then travel up to the paper with the solvent solute sample.
- Different compounds in the sample mixture travel at different rates.
- it takes several minutes to several hours.
- **Analysis** - Spots corresponding to different compounds may be located by their color, UV light, Ninhydrin or by treatment with Iodine vapours.

Ascending and Descending Paper chromatography

- **Ascending Chromatography**- In this method, the solvent is in pool at the bottom of the vessel in which the paper is supported. it rises up the paper by capillary action against the force of gravity.
- **Descending Chromatography**- In this method, the solvent is kept in a trough at the top of the chamber and is allowed to flow down to the paper. The liquid moves down by capillary action as well as by the gravitational force.


Cotton Thread
Clips
Solvent Front
Filter Paper
Solvent Tank
Direction of solvent flow
Spotting Line (Sample)
Solvent
Ascending Paper Chromatography


Tank Cover
Trough containing solvent
Paper support
Spotting line (Sample)
Direction of solvent flow
Tank
Filter Paper
Solvent Front
Descending Paper Chromatography
Namrata Heda

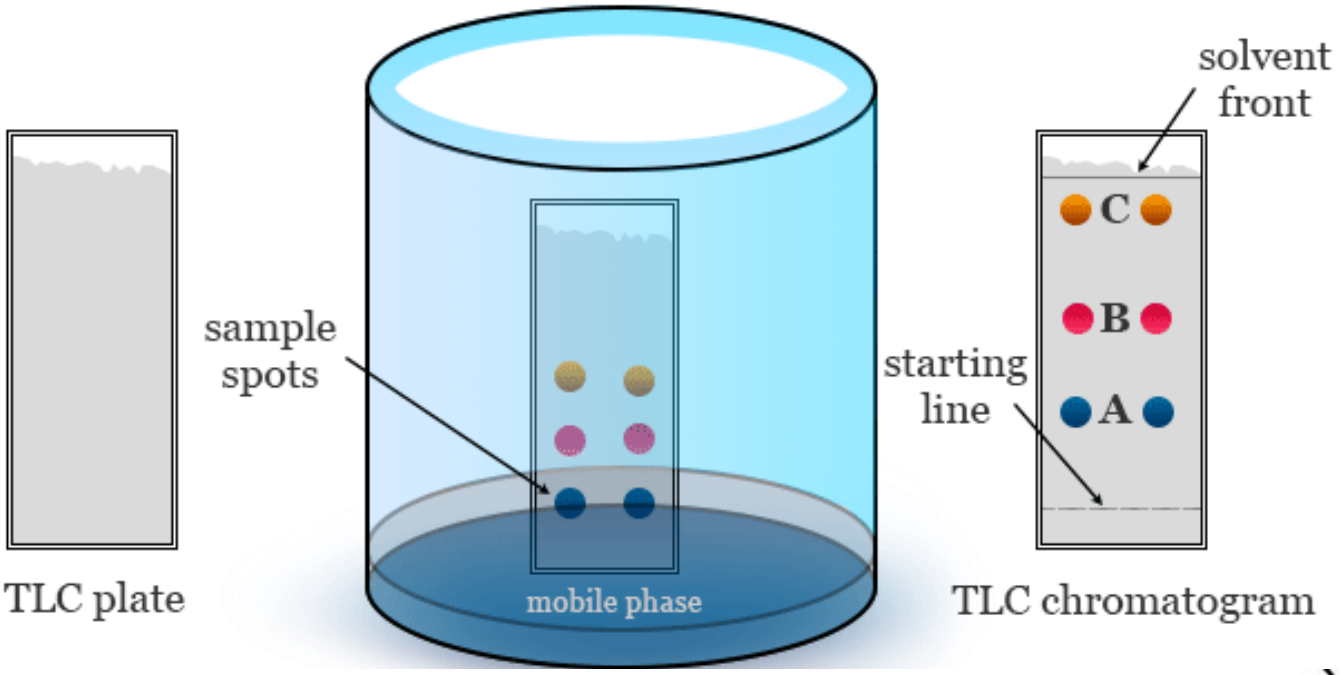
Significance of Paper Chromatography

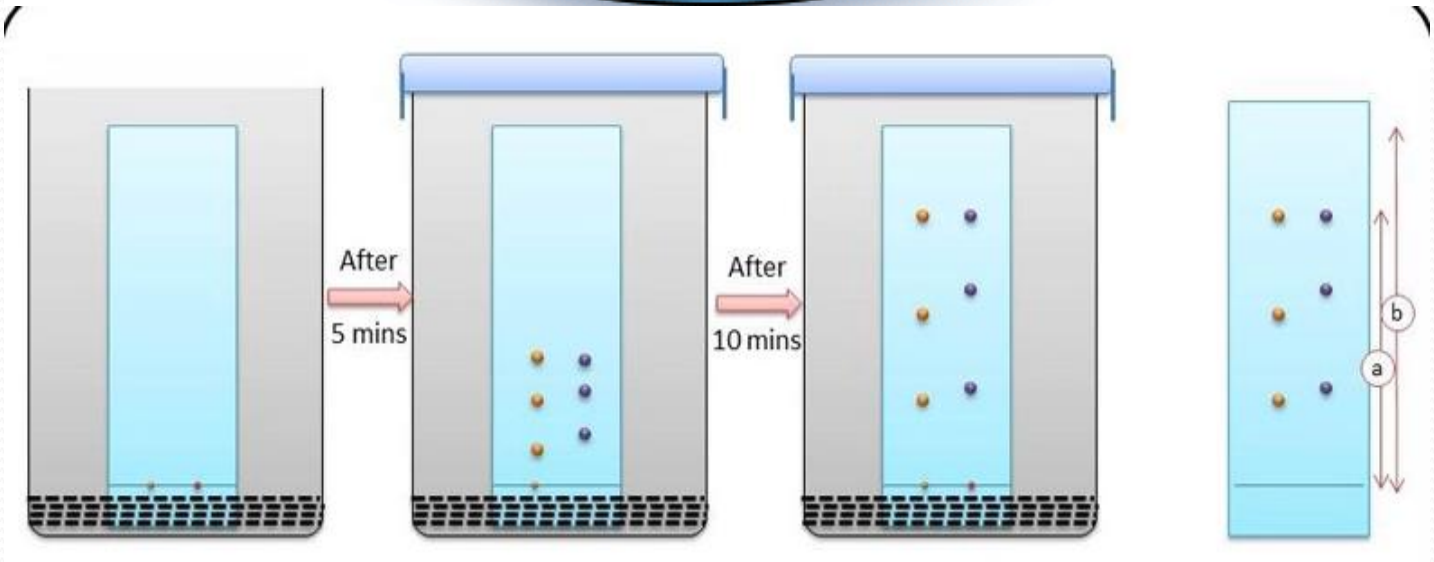
- It is very easy, simple, rapid and highly efficient method of separation.
- Can be applied in even in micrograms quantities of the sample.
- Can also be used for the separation of a wide variety of material like amino acids, oligosaccharides, glycosides, purines and pyrimidines, steroids, vitamins and alkaloids like penicillin, tetracyclin and streptomycin.

Thin Layer Chromatography (TLC)

- Stationary Phase consists of a thin layer of adsorbent material, usually silica gel aluminium oxide, or cellulose immobilized onto a flat carrier sheet.
- A Liquid Phase consisting of the solution an appropriate solvent and is drawn up the plate via capillary action, separating the solution based on the polarity of the compound.

Thin Layer Chromatography (TLC)





Significance

- Its wide range uses include –
- Determination of the pigments a plant contains.
- Detection of pesticides or insecticides in food.
- Identifying compounds present in a given substance.
- Monitoring organic reaction.

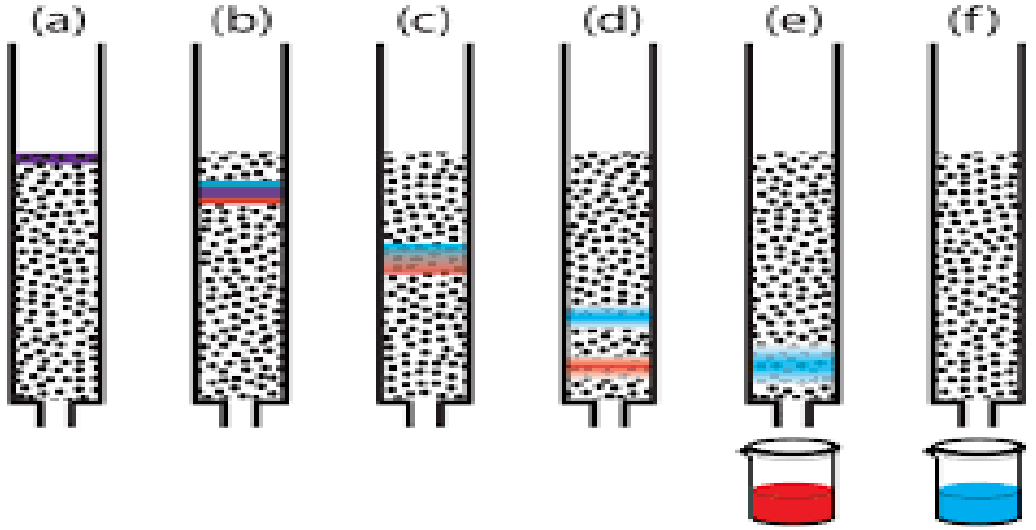
Advantages Of TLC over Paper Chromatography

- In case of Paper Chromatography, it takes 14-16 hrs for the separation of the components, but in TLC, it takes only 3-4 hrs.
- TLC has the advantage that the corrosive reagents like sulphuric acid can also be used which pose a limitation for the paper chromatography.
- It is easier to separate and visualise the components by this method.
- It has capacity to analyse multiple samples in a single run.
- It is relatively a low cost.

- The rate of migration of the various substances being separated are governed by their relative solubilities in the polar stationary phase and non polar mobile phase.
- The migration rate of a substances usually expressed as R_f(relative front).
- $R_f = \frac{\text{Distance travelled by the substance}}{\text{Distance travelled by the solvent front}}$

Column Chromatography

- The Stationary bed is within the tube.

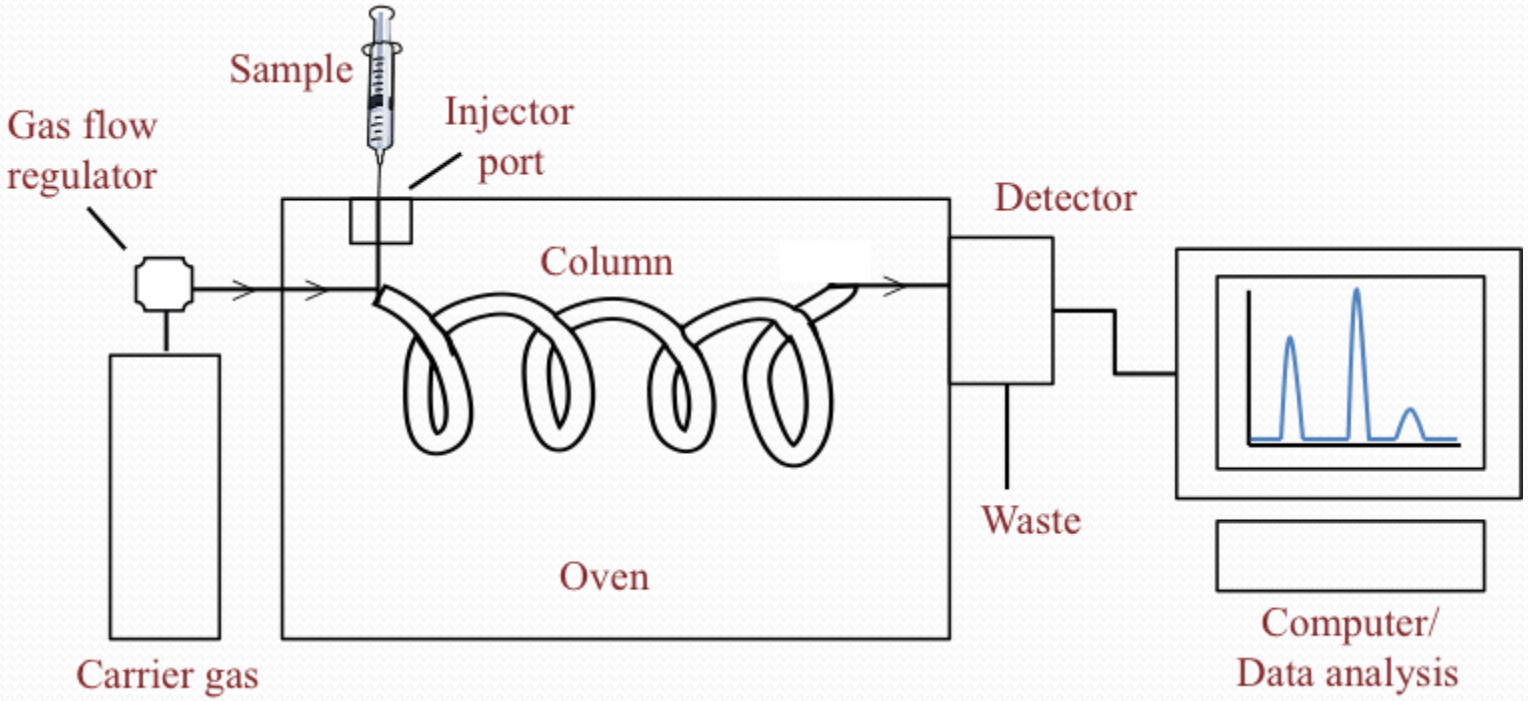


- In column Chromatography the stationary Phase may be pure silica or polymer, or may be coated onto chemically bonded support particles.
- Depending on whether mobile phase is a gas Chromatography or liquid Chromatography.
- When the Stationary phase in LC consists of small-diameter particles, the technique is High Performance Liquid Chromatography (HPLC).

Gas Chromatography

- In mobile phase, gas is used to pass a mixture of volatile solutes through a column containing the stationary phase.
- The mobile phase often referred to as the carrier gas, is typically an inert gas such as nitrogen, helium , or argon.
- Solute separation is based on the relative differences in the solutes vapour pressures and interactions with the stationary phase.
- Thus more volatile solute elutes from the column

- A solute that selectively interacts with the stationary phase elutes from the column after with lesser degree of interaction.
- The column effluent carries separated solutes to the detector in order of their elution.
- Solutes are identified qualitatively by their retention times.
- Peak size is proportional to the amount of solute detected and is used to quantify it.


Sample
Injector port
Gas flow regulator
Detector
Column
Waste
Oven
Carrier gas
Computer/ Data analysis

Instrumentation

- A basic gas Chromatograph consists of the following:
  - A chromatographic column to separate the solutes
  - A supply of carrier gas and flow-control apparatus to regulate the flow of carrier gas through the system.
  - An injector to introduce an aliquot of sample or derivatized analytes as they elute from the column.
  - A computer to control the system and process data.

Qualitative and quantitative analyses

- Chromatography is used to quantitatively identify and quantify the analyte's of interest

Analyte Identification

- The retention time or volume at which an unknown solute elutes from a column, or the distance traveled on a plate, is often compared and matched with that of a reference compound. The appearance of a solute peak, band, or spot at the same time, volume, or distance as that of a reference compound is consistent with the two compounds being the same.

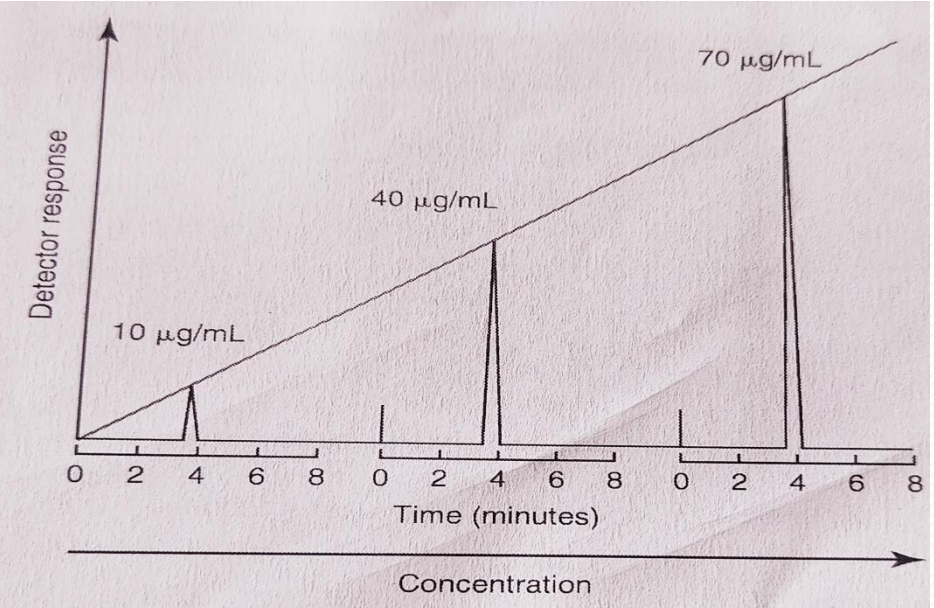
- In **planar chromatography**, reference compounds are chromatographed with the unknown sample. Tentative identification is made by comparison of migration distances and detection characteristics of the reference compounds with those of the unknown analytes.
- If the R_f of the unknown analyte and the R_f of the reference compound do not match, the compounds are judged to be different. If they match, the compounds are presumed to be identical.
- However, because more than one compound can have the same R_f in a particular chromatographic system, the presumptive identification should be confirmed by the use of specific spray reagents, antibody complexation, or isolation of the compound followed by chemical and/or instrumental analysis.

- *GC and LC columns*, it is possible to simultaneously introduce the components of a single injection into two columns made of dissimilar stationary phases. These columns are connected to separate detectors of the same or a different type.
- The most reliable analyte identification, however, is provided by a detector that features structural information, such as a mass spectrometer.

Analyte Quantification: Electronic signals from the detector(s) are also used to produce quantitative information. Both external and internal calibrating techniques have been used.

- With **external calibration**, reference solutions containing known quantities of analytes are processed in a manner identical to samples containing the analyte . A calibration curve of (1) peak height, (2) peak area, or (3) spot density versus calibrator concentration is constructed and used to calculate the conc. Of the analyte in the sample.

- With **internal calibration**, also called internal standardization, reference solutions of known analyte concentrations are prepared, and a constant amount of a different compound, the internal standard, is added to each reference solution and each sample. By plotting the ratio of the peak height (or area) or spot density of the analyte to the peak height (or area) or spot density of the internal standard versus the concentration of the analyte, a calibration curve that corrects for systematic losses is constructed.



igure 13-14 The use of external calibrators in the roduction of a calibration plot. *(From Krull I, Swartz M.*

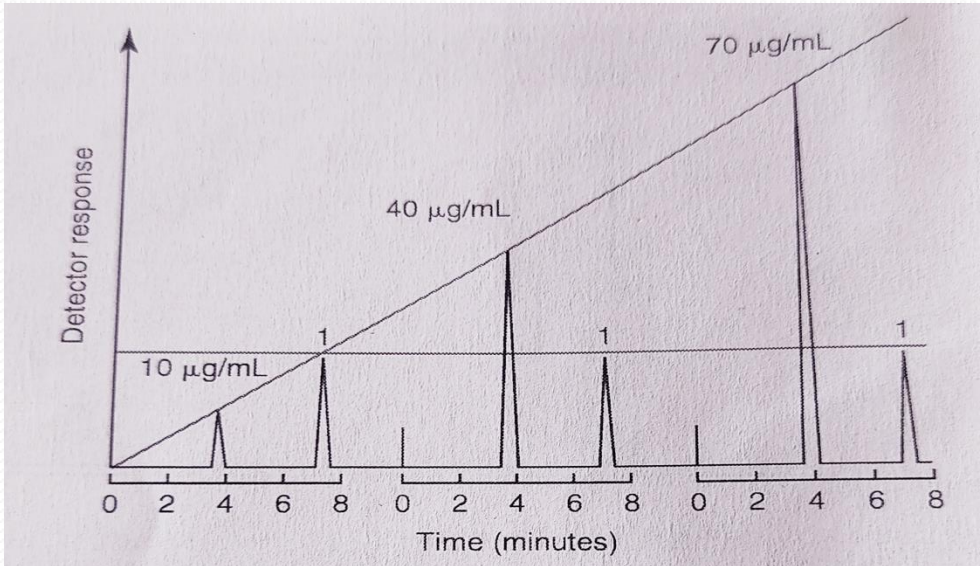


Figure 13-15 The use of internal calibrators in the production of a calibration plot (peak I being the internal standard). *(From Krull I, Swartz M. Quantitation in*

Extraction and differential precipitation

- Extraction and differential precipitation refer to the separation of components into discrete fractions, often two separate liquid fractions or a liquid and a solid fraction that may be separated by centrifugation or filtration.
- Extraction or differential precipitation is often used to simplify specimens and to remove components such as proteins that may interfere with subsequent chromatographic or analytical methods.
- Precipitation of proteins in biological fluids with acid or organic solvent is often used to separate them differentially from drugs or other small molecules that remain in solution.

- Use of solid-phase extraction has been increasing for many applications, and solid phases are packed in a variety of formats, including small open columns, cartridges, 96-well plates, and pipette tips. The use of particles with pore sizes smaller than 10 nm (100 Å) results in exclusion of most proteins from pores, leading to removal of proteins from the retentate.
- Solid-phase extraction can be performed on many specimens in parallel on a vacuum manifold or on extraction plates in 96-well formats.

Thank you