

B.PHARMA SEM-7

UNIT-4

PART- 2

INSTRUMENTAL METHODS OF ANALYSIS

CHROMATOGRAPHY

GAS CHROMATOGRAPH (GC)



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GAS CHROMATOGRAPHY

INTRODUCTION

- Specifically, gas-liquid chromatography- involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid.
- **Principal Adsorption (GSC) or partition (GLC)**
- **Main requirement Thermal stability and volatile nature of compound**



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• Derivatization in GC

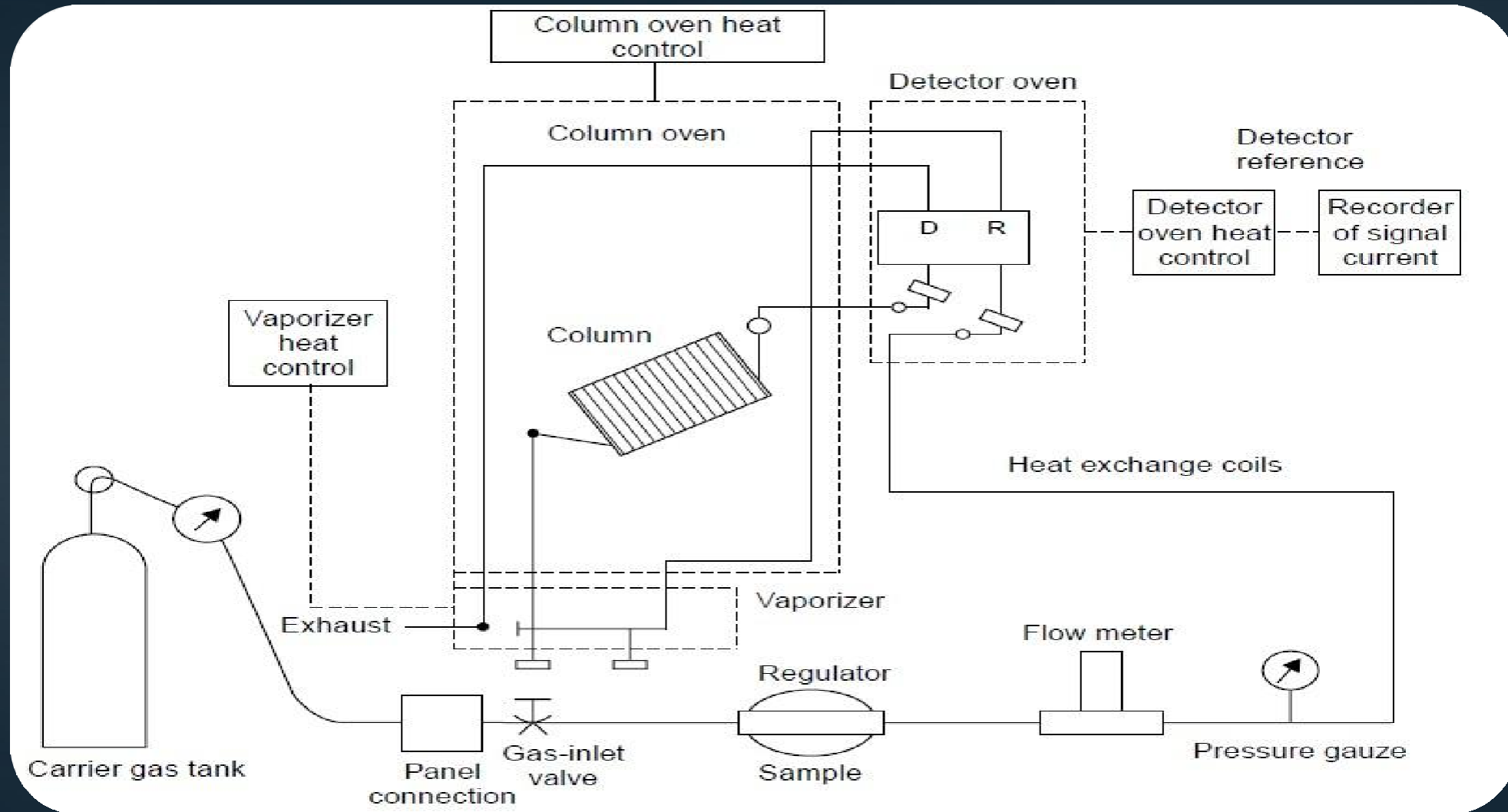
1. To improve thermal stability of compound (polar compound to non-polar compound).
2. To introduce a detector-oriented tag in molecule.
3. for purposeful adjustment of volatility.

Most common stationary phases

4. Separation of mixture of polar compounds Carbowax 20M (polyethylene glycol)
5. Separation of mixtures of non-polar compounds OV101 or SE-30 (polymer of methylsilicone)
6. Methylene ester of fatty acids □ DEGS (diethylene glycol succinate)



INSTRUMENTATION



Carrier gas

The carrier gas must be chemically inert. Commonly used gases include nitrogen, helium, argon, and carbon dioxide.

The choice of carrier gas often depends upon the type of detector used.

Sample injection port

The most common injection method is where a micro syringe is used to inject sample through a rubber septum into a flash vaporizer port at the head of the column.



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The temperature of the sample port is usually about 50°C higher than the boiling point of the least volatile component of the sample.

For packed columns, sample size ranges from tenths of a microliter up to 20 microliter.

Capillary columns, on the other hand, need much less sample, typically around 10–3 microliter.

For capillary GC, split/split less injection is used.



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Columns

- There are two general types of column packed and capillary (also known as open tubular).

O Packed columns

- a. Contain a finely divided, inert, solid support material (commonly based on diatomaceous earth) coated with liquid stationary phase.



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b. Most packed columns are 1.5–10m in length and have an internal diameter of 2–4mm.

0 Capillary columns

a. Have an internal diameter of a few tenths of a millimeter.

b. They can be of one of the two types:

Wall-coated open tubular (WCOT)

Support-coated open tubular (SCOT).



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- b. Wall-coated columns consist of a capillary tube whose walls are coated with liquid stationary phase.**
- Support-coated columns the inner wall of the capillary is lined with a thin layer of support material such as diatomaceous earth, onto which the stationary phase has been adsorbed. It is also known as PLOT (Porous layer open tubular column).**



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- **SCOT columns are generally less efficient than WCOT columns. Both types of capillary column are more efficient than packed columns.**
- **Tubular Column**
 - These have much thinner walls than the glass capillary columns, and are given strength by the polyimide coating.
 - **These columns are flexible and can be wound into coils.**
 - **They have the advantages of physical strength, flexibility and low reactivity.**



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Column temperature

- For precise work, column temperature must be controlled to within 10 oC.
- The optimum column temperature depends upon the boiling point of the sample.
- As a rule of thumb, a temperature slightly above the average boiling point of the sample results in an elution time of 2–30 minutes.
- Minimal temperatures give good resolution, but increase elution times.



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- If a sample has a wide boiling range, then temperature programming can be useful.
- The column temperature is increased (either continuously or in steps) as separation proceeds.
- Detectors
- A non-selective detector responds to all compounds except the carrier gas; a selective detector responds to a range of compounds with a common physical or chemical property and a specific detector responds to a single chemical compound



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DETECTORS	TYPE	SUPPORT GASES	SELECTIVITY	DETECTABILITY
Flame ionization (FID)	Mass flow	Hydrogen and air	Most organic compounds	100pg
Thermal conductivity (TCD)	Concentration	Reference	Universal	1ng
Electron capture	Concentration	Make up	Halides, nitrates, nitriles, peroxides, anhydrides, organometallics	50fg
Nitrogen-phosphorus	Mass flow	Hydrogen and air	Nitrogen, phosphorus	10pg
Flame photometric	Mass flow	Hydrogen and air possibly oxygen	Sulphur, phosphorus, tin, boron, arsenic, germanium, selenium, chromium	100pg
Photoionization	Concentration	Make up	Aliphatics, aromatics, ketones,	2pg



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❖ Applications of GC

1. Qualitative Analysis

- When 2 substance gives coincident peak (one known and one unknown), it is evidence that the compounds may same.
- Retention characteristics of unknown compound determined by:

a) Specific Retention volume (V_g) –

- Flow rate of carrier gas X Adjusted Retention time)
- But in this, reproducibility is very low due to varying packing density, liquid loading, activity of support, age etc.

b) Relative retention (r_A / B) –

- Adjusted retention volume of substance A related to that of reference standard B.
- Here reproducibility is good



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1. In gas chromatography which detector is most sensitive to halogenated compounds

- (A) TCD
- (B) FID
- (C) ECD
- (D) NPD



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2. The most commonly used carrier gas in gas chromatography

- (A) Hydrogen
- (B) Argon
- (C) Helium
- (D) Neon



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3. In gas chromatography column length is

- (A) 5-10 meter
- (B) 1-4 meter
- (C) 10-15 meter
- (D) 15-20 meter



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4. Which is the most common mobile phase for gas chromatography

- (A) Oxygen
- (B) Carbon dioxide
- (C) Ethane
- (D) Argon



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5. In gas chromatography, the basis of separation of the components is based on which of the following parameters

- (A) Conductivity
- (B) Partition coefficient
- (C) Mobility
- (D) Molecular weight



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6. The stationary phase in gas chromatography, as per I.P. is in the form of

- (A) Gas, liquid or combination of both**
- (B) Liquid, solid or combination of both**
- (C) Gas, solid or combination of both**
- (D) Gas, solid and liquid**



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7. The volume of carrier gas required to elute one half of the compound from the column is expressed as

- (A) Retention time
- (B) Revalue
- (C) Retention volume
- (D) Retention value



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8. Homologous series in hydrocarbons are determined by

- (A) R_f Value
- (B) R_r Value
- (C) R_s Value
- (D) R_c Value



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9. Squalane, the stationary phase used in GLC, is chemically

- (A) A high molecular weight saturated hydrocarbon
- (B) A high molecular weight unsaturated hydrocarbon
- (C) A low molecular weight complex
- (D) A high molecular weight complex



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10. The separation of compounds in GC is based on:

- A) Polarity
- B) Partition coefficient
- C) Boiling point
- D) All of the above



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ION EXCHANGE CHROMATOGRAPHY

INTRODUCTION

- Ion-exchange chromatography is a process that allows the separation of ions and polar molecules based on their affinity to the ion exchanger.
- It can be used for almost any kind of charged molecule including large proteins, small nucleotides and amino acids.
- Cations or Anions can be separated using this method.

Principle

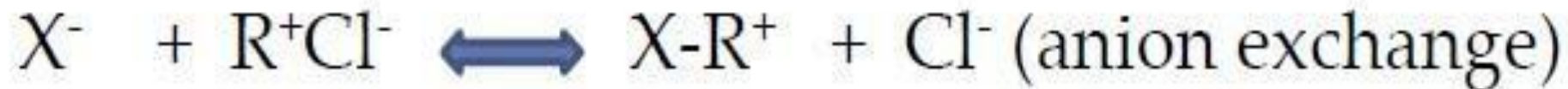
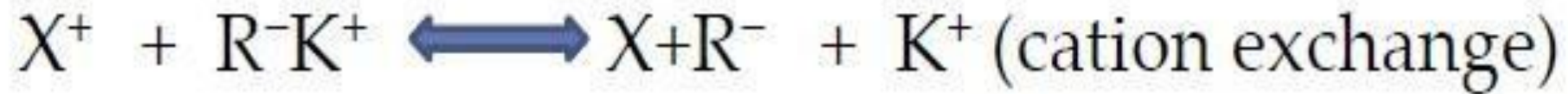
- It is based on the reversible electrostatic interaction of ions with the separation matrix (i.e.)
- The separation occurs by reversible exchange of ions between the ions present in the solution and those present in the ion exchange resin.

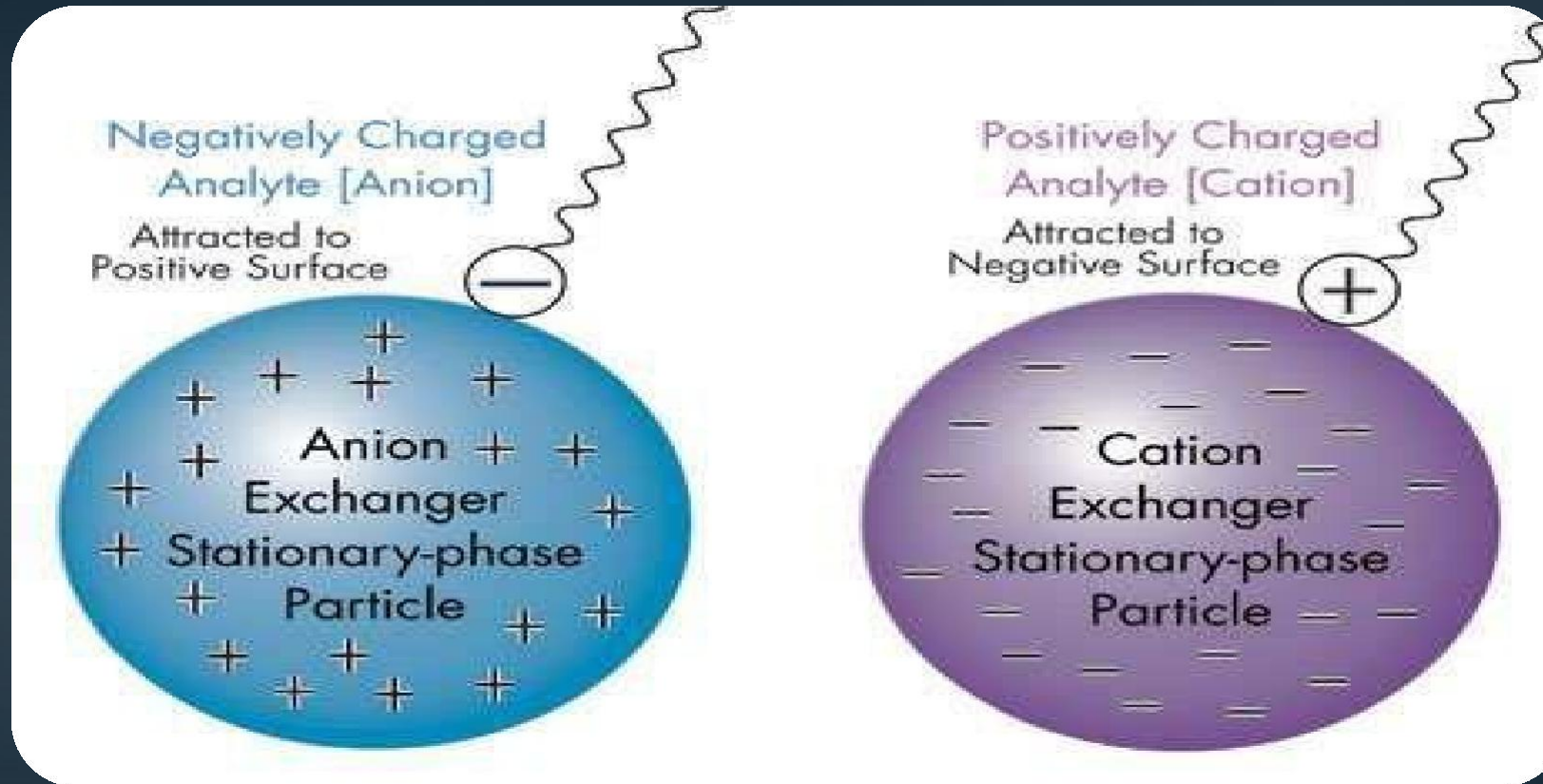


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- **Anion exchangers contain bound positive groups, whereas cation exchangers contain bound negative groups.**
- If the stationary phase is represented by R⁻ or R⁺ and the sample by X⁺ and X⁻, retention in IEC can be represented as





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❖ CLASSIFICATION OF RESINS

❑ According to the chemical nature they classified as-

1. Strong cation exchange resin sulphonic acid groups attached to styrene and di vinyl benzene copolymer.
2. Weak cation exchange resin carboxylic acid groups attached to acrylic and divinyl benzene co-polymer.
3. Strong anion exchange resin quaternary ammonium groups attached to styrene and divinyl benzene co-polymer.
4. Weak anion exchange resin poly alkyl amine groups attached to styrene and divinyl benzene copolymer.



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□ According to the source

1. Natural resins : Cation - Zeolytes, Clay; Anion - Dolomite
2. Synthetic resins: Inorganic & Organic resins
3. Organic resins: polymeric resin matrix
 - The resin composed of –
 - Polystyrene (sites for exchangeable functional groups)
 - Divinyl benzene (Cross linking agent)-offers stability.



❖ Points to remember

- More highly charged molecules more tightly bound to the resin travel slowly and eluted later.
- Moderately charged molecules equilibrating between the resins and moving buffer more readily
- Less charged molecules bounds less strongly to the resin equilibrate with the moving buffer more readily travels rapidly and eluted faster.



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❖ APPLICATIONS

- **Softening and demineralisation of water.**
- For extraction of enzymes from tissues.
- **Purification of solutions free from ionic impurities.**
- Separation of inorganic ions.
- **Separation of sugars, amino acids and proteins.**
- Ion exchange column in HPLC.



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SIZE EXCLUSION CHROMATOGRAPHY

❖ Introduction

- Size exclusion chromatography (SEC), also called as gel-filtration chromatography.
- Gel-permeation chromatography (GPC) uses porous particles to separate molecules of different sizes.
- It is generally used to separate biological molecules and to determine molecular weights and molecular weight distributions of polymers
- It is usually applied to large molecules or macromolecular complexes such as proteins and industrial polymers.



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- When an aqueous solution is used to transport the sample through the column, the technique is known as gel filtration chromatography.
- When an organic solvent is used as a mobile phase, the technique is known as Gel-permeation chromatography.
- The separation of molecules is called fractionation.
- The size of pores in beads determines the exclusion limit (what goes through the beads and what goes around the beads)



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❖ Principle

- A mixture of molecules dissolved in liquid (the mobile phase) is applied to a chromatography column which contains a solid support in the form of microscopic spheres, or “beads” (the stationary phase).
- The mass of beads within the column is often referred to as the column bed.
- The beads act as “traps” or “sieves” and function to filter small molecules which become temporarily trapped within the pores.



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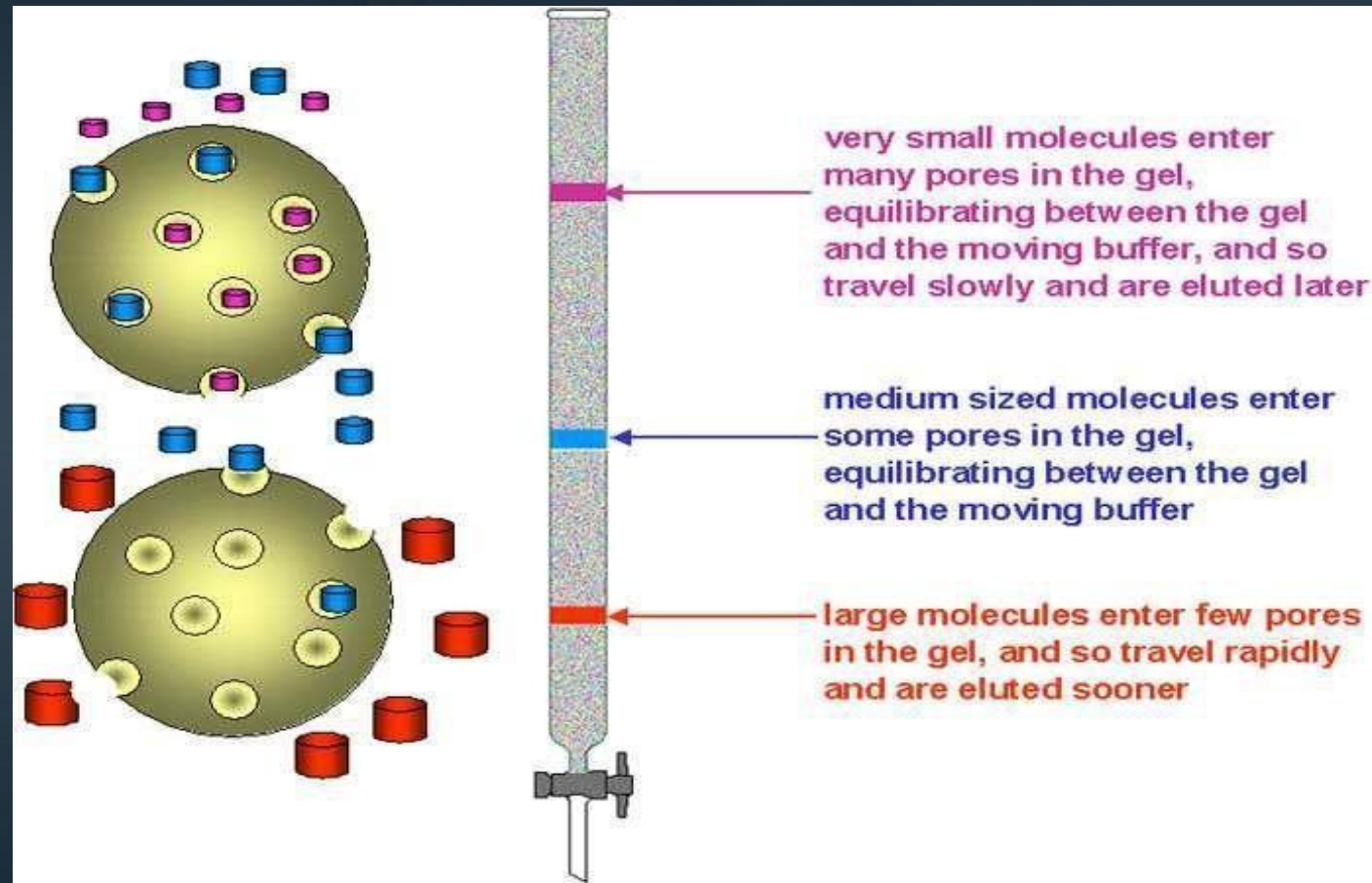
- Larger molecules pass around or are “excluded” from the beads.
- Large sample molecules cannot or can only partially penetrate the pores, whereas smaller molecules can access most or all pores.
- Thus, large molecules elute first, smaller molecules elute later, while molecules that can access all the pores elute last from the column.



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- Particles of different sizes will elute (filter) through a stationary phase at different rates.



❖ Total column volume (V_t)

$$V_t = V_g + V_i + V_0$$

Where, V_g --is the volume occupied by the packing V_i --is the volume of solvent in the pores

V_0 --is the free solvent volume (similar to injection volume)

❖ Stationary Phase



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1. Soft gel e.g. – dextran (Sephadex), Polyacrylamide gels; Separation of proteins.
2. Semi-rigid gel e.g. - bio beads; Separation of non-polar polymers in non-polar solvents.
3. Highly rigid gels and glasses; Separation of polar systems.



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❖ Mobile Phase

Material

Synthetic elastomers (polybutadiene , polyisoprene)

Poly styrene, PVC, Styrene-Butadiene Rubber , Epoxy resins

Polyolefins

Polyurethane

Proteins, polysaccharides

Solvent

Toluene

Tetrahydrofuran

Tri- chloro -benzene

Di- methylformamide

Water / Buffers



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❖ Application

- Proteins fractionation
- Purification
- Molecular weight determination.
- Separation of sugar, proteins, peptides, rubbers and others on the basis of their size.



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- This technique can be for determining the quaternary structure of purified proteins.
- SEC is a widely used technique for the purification and analysis of synthetic and biological polymers, such as protein, polysaccharides and nucleic acid.
- Various species of RNA and viruses have been purified using agarose gels.
- For Desalting
- For copolymerisation studies



1. The upper surface of a column should be protected by usingin gel filtration

- (A) Adsorbents**
- (B) Charcoal**
- (C) Filter paper**
- (D) Absorbents**



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2. An example of a strongly acidic cation exchange resin is

- (A) Quaternary polymethacrylate
- (B) Phenol formaldehyde
- (C) Quaternary Polystyrene
- (D) Sulphonated polystyrene



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3. Which of the following is the bulk property detector

- (A) Diode array detector
- (B) UV detector
- (C) Fluorescence detector
- (D) Refractive index detector



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4. Which of the following is used for the determination of molecular weight

- (A) Gas chromatography**
- (B) Paper chromatography**
- (C) Gel permeation chromatography**
- (D) Partition chromatography**



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5. Which one of the following chromatographic techniques can be used to determine sucrose after silylation

- (A) High Performance Liquid Chromatography**
- (B) Gel Chromatography**
- (C) Gas Liquid Chromatography**
- (D) Paper Chromatography**



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6. In size exclusion chromatography of a mixture of molecules, which one will elute first

- (A) The largest molecule**
- (B) The smallest molecule**
- (C) The most polar molecule**
- (D) The most non polar molecule**



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7. Ion-exchange chromatography is used for the separation of

- (A) Aldehydes
- (B) Amino acids
- (C) Fatty acids
- (D) Hydrocarbons



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8. In adsorption chromatography, the stationary Phase is

- (A) Liquid
- (B) Gas
- (C) Solid
- (D) All of the above



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9. In gel permeation chromatography, molecules are separated on the basis of them

- (A) Size and shape**
- (B) Chemical nature**
- (C) Adsorptive, properties**
- (D) Partition coefficient**



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10. Which of the following material is not used in the stationary phase of size exclusion chromatography

- (A) Dextran
- (B) Agarose
- (C) Polyacrylamide
- (D) Polyvinyl Alcohol



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Number of theoretical plates (N) :

It indicates the efficiency of the column

$$N = 16 (t_r/W)^2$$



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PART-1

INSTRUMENTAL METHODS OF ANALYSIS

CHROMATOGRAPHY

**HIGH PERFORMANCE
LIQUID CHROMATOGRAPHY
(HPLC)**



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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

INTRODUCTION

- High performance liquid chromatography is basically a highly improved form of column chromatography.
- Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures. That makes it much faster.



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- It also allows you to use a very much smaller particle size for the column packing material which gives a much greater surface area for interactions between the stationary phase and the molecules flowing fastly and allows a much better separation of the components of the mixture.
- **These new HPLC instruments could develop up to 6,000 psi [400 bar] of pressure, and incorporated improved injectors, detectors.**



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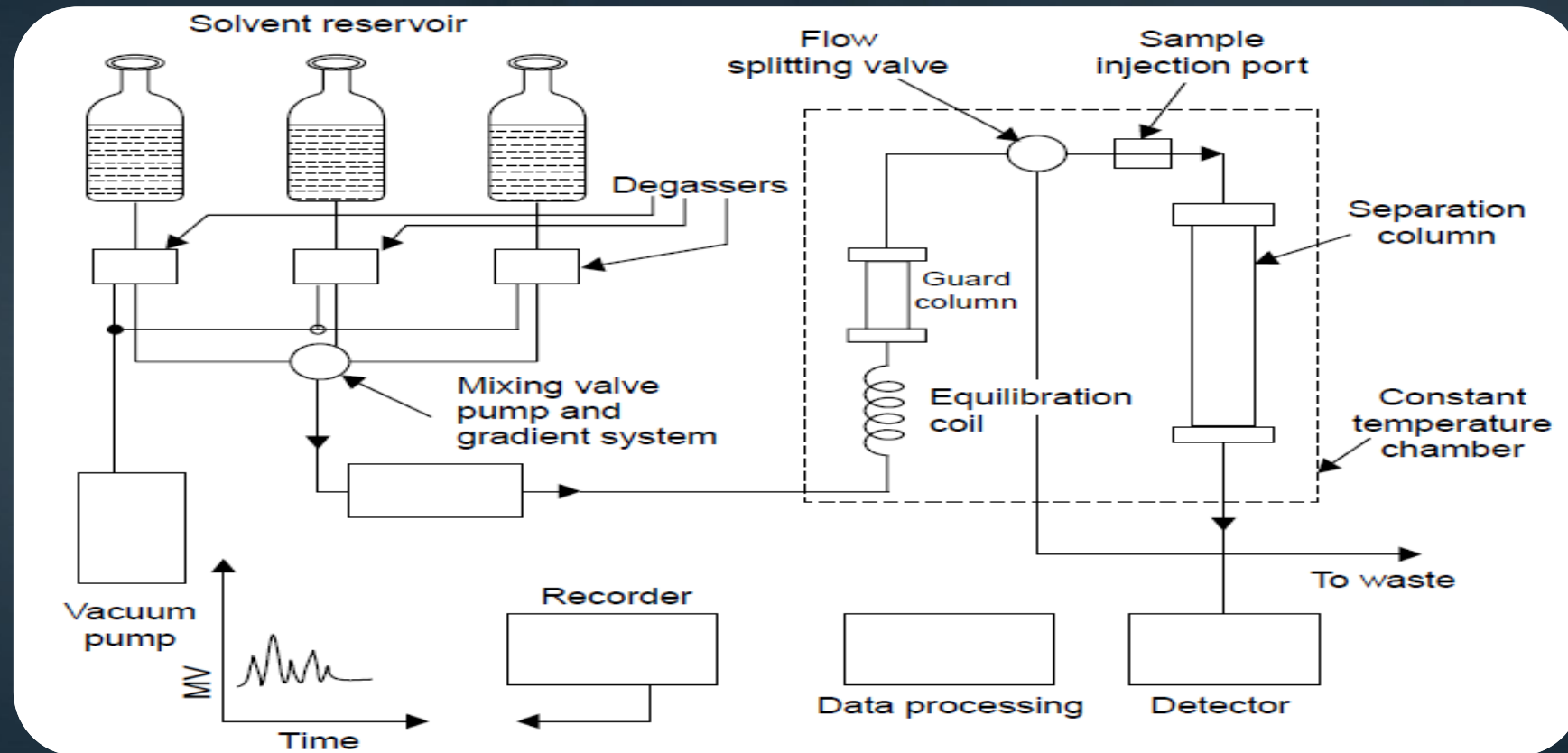
- **High-performance liquid chromatography [HPLC] is now one of the most powerful tool in analytical chemistry.**
- **It has the ability to separate, identify, and quantitate the compounds that are present in any sample that can be dissolved in a liquid.**
- **Today, compounds in trace concentrations as low as parts per trillion [ppt] may easily be identified.**
- **HPLC can be, and has been, applied to just about any sample, such as pharmaceuticals, food, nutraceuticals, cosmetics, environmental matrices, forensic samples, and industrial chemicals.**



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INSTRUMENTATION



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1. Solvent reservoir:

- Glass or stainless steel (1 lt.)
- Stainless steel should be avoided for use with solvents containing halide ions.
- Degassing of mobile phase is done to prevent the formation of gas bubbles in the pump or detector by sonication, sparging with helium.



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2. Pumps:

- 5000 psi force is needed.
- Flow rate 0.1 to 10mL/min.
- Types -
 - a. Mechanical pump: constant flow rate (by reciprocating piston)



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0 Syringe (Displacement pump)

0 Reciprocating Pump (RP)

i. Single piston RP

ii. Double piston RP

iii. Reciprocating diaphragm pump

b. Pneumatic pump: Constant pressure



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i. Direct pressure pump

ii. Amplifier pump

- Pulse damping device is used to correct the variation in base line.

3. Gradient analysis: for mixing the two different solvents.

4. Solvent conditioning column (5-10 cm): mobile phase is saturated with silica gel.



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5. Injection:

- Septum injector (microliter syringe) is injected to self sealing rubber or Teflon disk.
- Stop flow septum less injection
- Microvolume sampling valve automatic injection
- Rheodyne injector
- Sample injection is mainly done by using rotary valve or loop injector.



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6. Precolumn (2-10 cm):

- Optional, stationary phase coated with liquid. For the particulate rapping.
- **Mainly used to remove the impurities from the solvent.**
- **Prevents contamination of analytical column.**
- **Also called guard column or protective column.**
- **Having large particle size.**



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7. Analytical column (25 – 100cm length, 2-4.6 mm diameter):

- Particles are $> 10\mu\text{m}$, pressure 6000 psi.
- Actual separation is carried out here.
- Stainless steel tube
- The solid support can be silica gel, alumina.



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8. Retention time (R_t): The time taken for a particular compound to travel through the column to the detector is known as its retention time. This time is measured from the time at which the sample is injected to the point at which the display shows a maximum peak height for that compound.



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9. The detector:

- **Ultra-Violet absorption:** Many organic compounds absorb UV light of various wavelengths. The simplest of these are fixed at one wavelength usually 254nm.
- **Photodiode array detector:** used if more than one substance is co-eluting.
- **Fluorescence spectrometer:** more sensitive (pictogram).
- **Refractive index detector**
- **Differential refractometer:** measures refractive index (10^{-4} to 10^{-5} RI units).



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Application:

- **Therapeutic drug monitoring**
- Qualitative and quantitative analysis
- **Structural determination**
- **Biochemical genetics**
- **Clinical application**
- **Diagnostic studies**
- **Drug discovery**
- **Proteomics**
- **Drug metabolism study**
- **Cosmetics**
- **Environmental analysis**



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1. High Performance Liquid Chromatography is characterized by

- (A) Liquid mobile phase and stationary phase with a very finely divided solid particle
- (B) Liquid mobile phase and stationary phase with coarse particles
- (C) Both the mobile phase and stationary phase are in liquid state
- (D) Both the mobile phase and stationary phase are in solid state



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2. Scanning densitometer is a part of

- (A) High pressure liquid chromatograph
- (B) High pressure thin layer chromatograph
- (C) Gas liquid chromatograph
- (D) Gas solid chromatography



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3. What is the first eluted compound in normal, reversed phase chromatography respectively

- (A) Polar, Non-polar
- (B) Polar, Semi-polar
- (C) Non-polar, Polar
- (D) Semi-polar, Non-polar



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4. Which of the following is used as a detector in HPLC?

- (A) UV-Visible Detector
- (B) Flame Ionization Detector
- (C) Thermal Conductivity Detector
- (D) Electron Capture Detector



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5. Which one of the following is most commonly used as the stationary phase in reverse-phase HPLC?

- (A) Silica gel
- (B) C18 bonded silica
- (C) Alumina
- (D) Polyamide



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6. In reverse-phase HPLC, the mobile phase is:

- (A) Polar
- (B) Non-polar
- (C) Amphoteric
- (D) Hydrophobic



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7. Which of the following parameters is used to measure column efficiency in HPLC?

- (A) Resolution
- (B) Retention time
- (C) Theoretical plates
- (D) Selectivity factor



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8. Retention time (t_R) in HPLC depends on:

- (A) Flow rate of mobile phase
- (B) Nature of analyte
- (C) Interaction with stationary phase
- (D) All of the above



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9. Gradient elution in HPLC refers to:

- (A) Using only water as mobile phase
- (B) Decreasing column temperature
- (C) Changing mobile phase composition during separation
- (D) Constant flow rate



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10. Which of the following improves resolution in HPLC?

- (A) Increasing particle size of stationary phase
- (B) Increasing flow rate
- (C) Increasing column length
- (D) Decreasing temperature



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- **Main requirement Thermal stability and volatile nature of compound**



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- **Derivatization in GC**

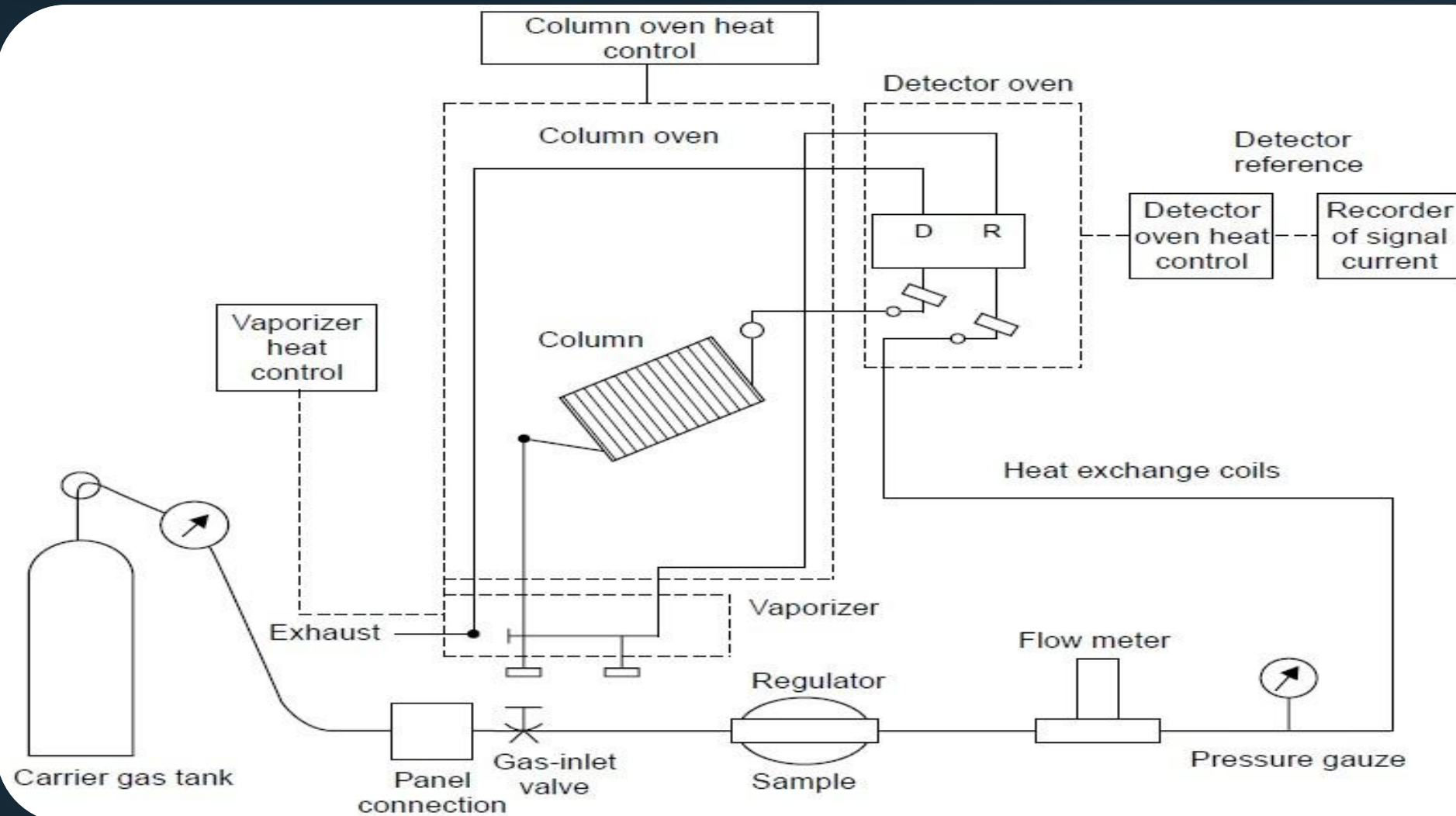
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2. To introduce a detector-oriented tag in molecule.
3. **for purposeful adjustment of volatility.**

Most common stationary phases

1. Separation of mixture of polar compounds Carbowax 20M (polyethylene glycol)
2. Separation of mixtures of non-polar compounds OV101 or SE-30 (polymer of methylsilicone)
3. Methylene ester of fatty acids □ DEGS (diethylene glycol succinate)



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The choice of carrier gas often depends upon the type of detector used.

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The most common injection method is where a micro syringe is used to inject sample through a rubber septum into a flash vaporizer port at the head of the column.



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The temperature of the sample port is usually about 50°C higher than the boiling point of the least volatile component of the sample.

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b. Most packed columns are 1.5–10m in length and have an internal diameter of 2–4mm.

0 Capillary columns

a. Have an internal diameter of a few tenths of a millimeter.

b. They can be of one of the two types:

Wall-coated open tubular (WCOT)

Support-coated open tubular (SCOT).



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- b. Wall-coated columns consist of a capillary tube whose walls are coated with liquid stationary phase.**
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- **Tubular Column**
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- A non-selective detector responds to all compounds except the carrier gas; a selective detector responds to a range of compounds with a common physical or chemical property and a specific detector responds to a single chemical compound



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DETECTORS	TYPE	SUPPORT GASES	SELECTIVITY	DETECTABILITY
Flame ionization (FID)	Mass flow	Hydrogen and air	Most organic compounds	100pg
Thermal conductivity (TCD)	Concentration	Reference	Universal	1ng
Electron capture	Concentration	Make up	Halides, nitrates, nitriles, peroxides, anhydrides, organometallics	50fg
Nitrogen-phosphorus	Mass flow	Hydrogen and air	Nitrogen, phosphorus	10pg
Flame photometric	Mass flow	Hydrogen and air possibly oxygen	Sulphur, phosphorus, tin, boron, arsenic, germanium, selenium, chromium	100pg
Photoionization	Concentration	Make up	Aliphatics, aromatics, ketones,	2pg



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❖ Applications of GC

1. Qualitative Analysis

- When 2 substance gives coincident peak (one known and one unknown), it is evidence that the compounds may same.
- Retention characteristics of unknown compound determined by:

a) Specific Retention volume (V_g) –

- Flow rate of carrier gas X Adjusted Retention time)
- But in this, reproducibility is very low due to varying packing density, liquid loading, activity of support, age etc.

b) Relative retention (r_A / B) –

- Adjusted retention volume of substance A related to that of reference standard B.
- Here reproducibility is good



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1. In gas chromatography which detector is most sensitive to halogenated compounds

- (A) TCD
- (B) FID
- (C) ECD
- (D) NPD



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2. The most commonly used carrier gas in gas chromatography

- (A) Hydrogen
- (B) Argon
- (C) Helium
- (D) Neon



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3. In gas chromatography column length is

- (A) 5-10 meter
- (B) 1-4 meter
- (C) 10-15 meter
- (D) 15-20 meter



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4. Which is the most common mobile phase for gas chromatography

- (A) Oxygen
- (B) Carbon dioxide
- (C) Ethane
- (D) Argon



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5. In gas chromatography, the basis of separation of the components is based on which of the following parameters

- (A) Conductivity
- (B) Partition coefficient
- (C) Mobility
- (D) Molecular weight



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6. The stationary phase in gas chromatography, as per I.P. is in the form of

- (A) Gas, liquid or combination of both**
- (B) Liquid, solid or combination of both**
- (C) Gas, solid or combination of both**
- (D) Gas, solid and liquid**



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7. The volume of carrier gas required to elute one half of the compound from the column is expressed as

- (A) Retention time
- (B) Revalue
- (C) Retention volume
- (D) Retention value



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8. Homologous series in hydrocarbons are determined by

- (A) R_f Value
- (B) R_r Value
- (C) R_s Value
- (D) R_c Value



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9. Squalane, the stationary phase used in GLC, is chemically

- (A) A high molecular weight saturated hydrocarbon
- (B) A high molecular weight unsaturated hydrocarbon
- (C) A low molecular weight complex
- (D) A high molecular weight complex



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10. The separation of compounds in GC is based on:

- A) Polarity
- B) Partition coefficient
- C) Boiling point
- D) All of the above



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ION EXCHANGE CHROMATOGRAPHY

INTRODUCTION

- Ion-exchange chromatography is a process that allows the separation of ions and polar molecules based on their affinity to the ion exchanger.
- It can be used for almost any kind of charged molecule including large proteins, small nucleotides and amino acids.
- Cations or Anions can be separated using this method.

Principle

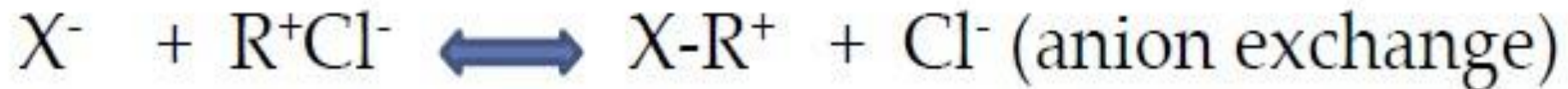
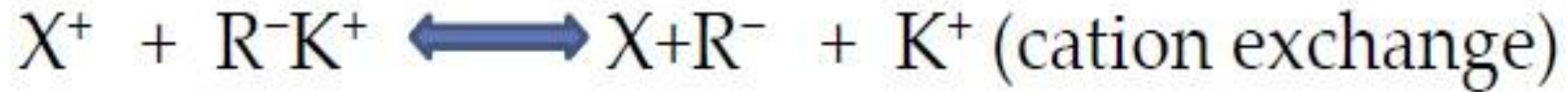
- It is based on the reversible electrostatic interaction of ions with the separation matrix (i.e.)
- The separation occurs by reversible exchange of ions between the ions present in the solution and those present in the ion exchange resin.

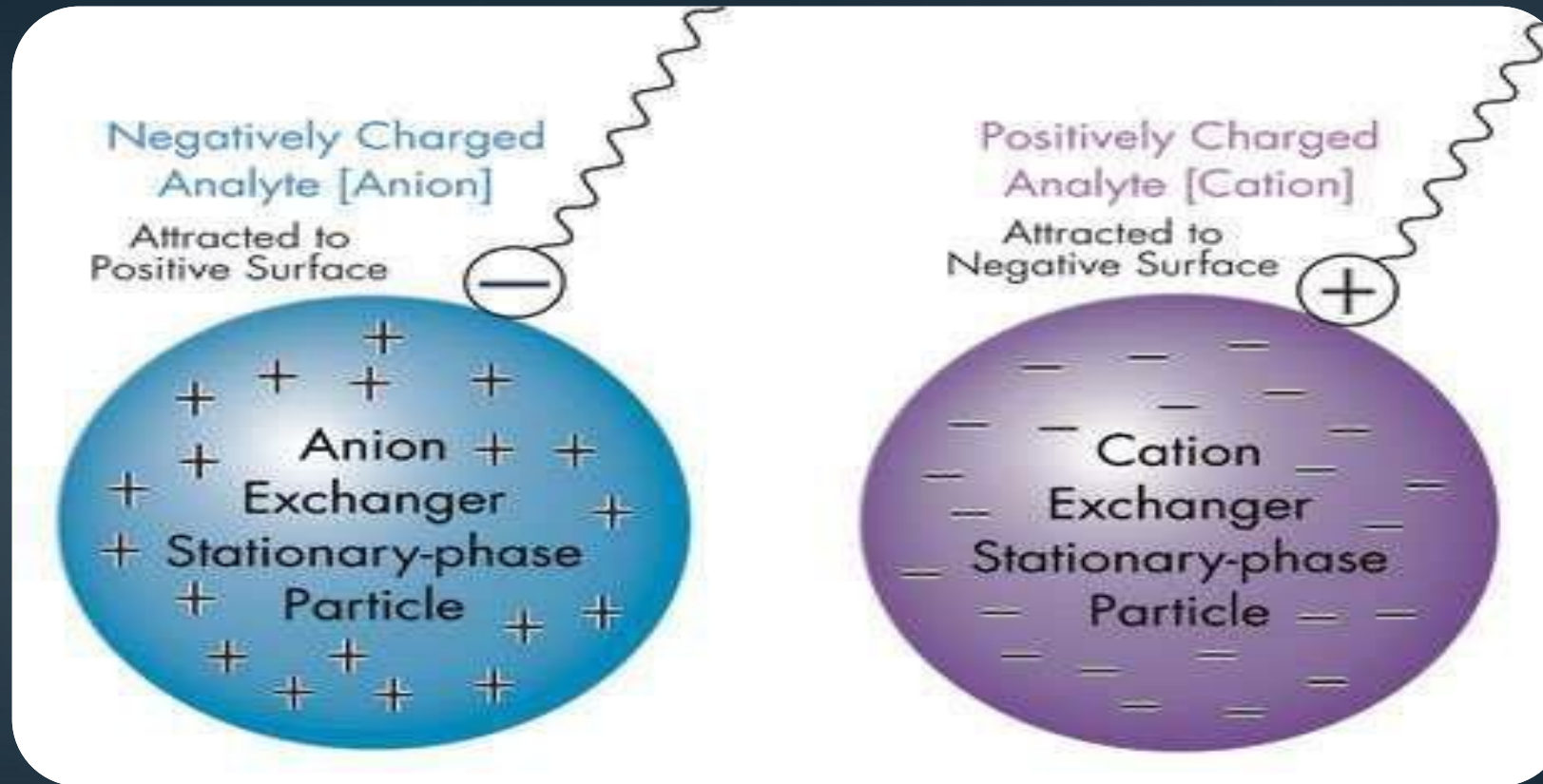


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- **Anion exchangers contain bound positive groups, whereas cation exchangers contain bound negative groups.**
- If the stationary phase is represented by R⁻ or R⁺ and the sample by X⁺ and X⁻, retention in IEC can be represented as





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❖ CLASSIFICATION OF RESINS

❑ According to the chemical nature they classified as-

1. Strong cation exchange resin sulphonic acid groups attached to styrene and di vinyl benzene copolymer.
2. Weak cation exchange resin carboxylic acid groups attached to acrylic and divinyl benzene co-polymer.
3. Strong anion exchange resin quaternary ammonium groups attached to styrene and divinyl benzene co-polymer.
4. Weak anion exchange resin poly alkyl amine groups attached to styrene and divinyl benzene copolymer.



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❑ According to the source

1. Natural resins : Cation - Zeolytes, Clay; Anion - Dolomite
2. Synthetic resins: Inorganic & Organic resins
3. Organic resins: polymeric resin matrix
 - The resin composed of –
 - Polystyrene (sites for exchangeable functional groups)
 - Divinyl benzene (Cross linking agent)-offers stability.



❖ Points to remember

- More highly charged molecules more tightly bound to the resin travel slowly and eluted later.
- Moderately charged molecules equilibrating between the resins and moving buffer more readily
- Less charged molecules bounds less strongly to the resin equilibrate with the moving buffer more readily travels rapidly and eluted faster.



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❖ APPLICATIONS

- **Softening and demineralisation of water.**
- For extraction of enzymes from tissues.
- **Purification of solutions free from ionic impurities.**
- Separation of inorganic ions.
- **Separation of sugars, amino acids and proteins.**
- Ion exchange column in HPLC.



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SIZE EXCLUSION CHROMATOGRAPHY

❖ Introduction

- Size exclusion chromatography (SEC), also called as gel-filtration chromatography.
- Gel-permeation chromatography (GPC) uses porous particles to separate molecules of different sizes.
- It is generally used to separate biological molecules and to determine molecular weights and molecular weight distributions of polymers
- It is usually applied to large molecules or macromolecular complexes such as proteins and industrial polymers.



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- When an aqueous solution is used to transport the sample through the column, the technique is known as gel filtration chromatography.
- When an organic solvent is used as a mobile phase, the technique is known as Gel-permeation chromatography.
- The separation of molecules is called fractionation.
- The size of pores in beads determines the exclusion limit (what goes through the beads and what goes around the beads)



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❖ Principle

- A mixture of molecules dissolved in liquid (the mobile phase) is applied to a chromatography column which contains a solid support in the form of microscopic spheres, or “beads” (the stationary phase).
- The mass of beads within the column is often referred to as the column bed.
- The beads act as “traps” or “sieves” and function to filter small molecules which become temporarily trapped within the pores.



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- Larger molecules pass around or are “excluded” from the beads.
- Large sample molecules cannot or can only partially penetrate the pores, whereas smaller molecules can access most or all pores.
- Thus, large molecules elute first, smaller molecules elute later, while molecules that can access all the pores elute last from the column.

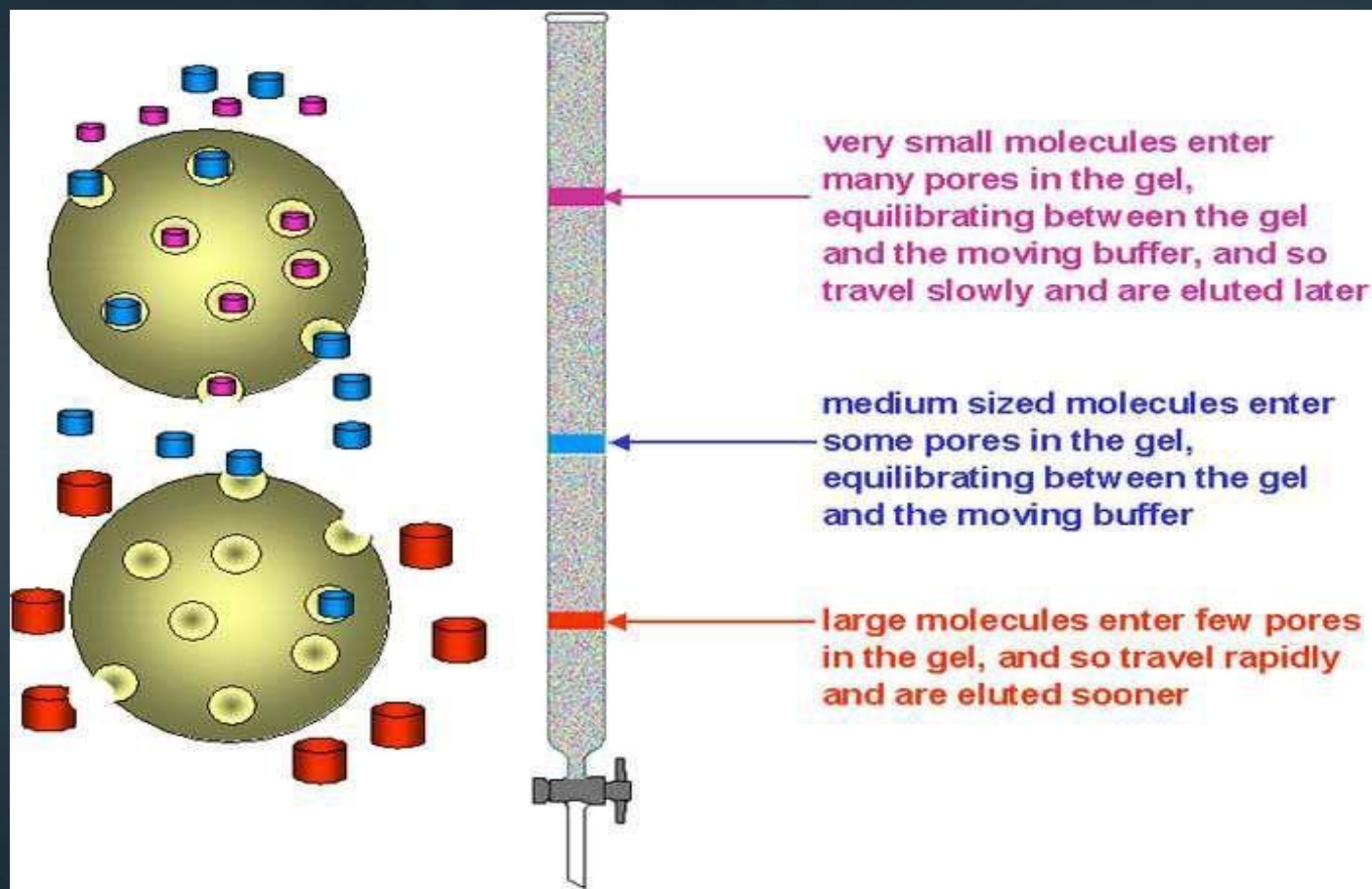


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- Particles of different sizes will elute (filter) through a stationary phase at different rates.



❖ Total column volume (V_t)

$$V_t = V_g + V_i + V_0$$

Where, V_g --is the volume occupied by the packing V_i --is the volume of solvent in the pores

V_0 --is the free solvent volume (similar to injection volume)

❖ Stationary Phase



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1. Soft gel e.g. – dextran (Sephadex), Polyacrylamide gels; Separation of proteins.
2. Semi-rigid gel e.g. - bio beads; Separation of non-polar polymers in non-polar solvents.
3. Highly rigid gels and glasses; Separation of polar systems.



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❖ Mobile Phase

Material

Synthetic elastomers (polybutadiene , polyisoprene)

Poly styrene, PVC, Styrene-Butadiene

Rubber , Epoxy resins

Polyolefins

Polyurethane

Proteins, polysaccharides

Solvent

Toluene

Tetrahydrofuran

Tri- chloro -benzene

Di- methylformamide

Water / Buffers



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❖ Application

- Proteins fractionation
- Purification
- Molecular weight determination.
- Separation of sugar, proteins, peptides, rubbers and others on the basis of their size.



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- This technique can be for determining the quaternary structure of purified proteins.
- SEC is a widely used technique for the purification and analysis of synthetic and biological polymers, such as protein, polysaccharides and nucleic acid.
- Various species of RNA and viruses have been purified using agarose gels.
- For Desalting
- For copolymerisation studies



1. The upper surface of a column should be protected by usingin gel filtration

- (A) Adsorbents**
- (B) Charcoal**
- (C) Filter paper**
- (D) Absorbents**



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2. An example of a strongly acidic cation exchange resin is

- (A) Quaternary polymethacrylate
- (B) Phenol formaldehyde
- (C) Quaternary Polystyrene
- (D) Sulphonated polystyrene



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3. Which of the following is the bulk property detector

- (A) Diode array detector
- (B) UV detector
- (C) Fluorescence detector
- (D) Refractive index detector



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4. Which of the following is used for the determination of molecular weight

- (A) Gas chromatography**
- (B) Paper chromatography**
- (C) Gel permeation chromatography**
- (D) Partition chromatography**



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5. Which one of the following chromatographic techniques can be used to determine sucrose after silylation

- (A) High Performance Liquid Chromatography**
- (B) Gel Chromatography**
- (C) Gas Liquid Chromatography**
- (D) Paper Chromatography**



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6. In size exclusion chromatography of a mixture of molecules, which one will elute first

- (A) The largest molecule**
- (B) The smallest molecule**
- (C) The most polar molecule**
- (D) The most non polar molecule**



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7. Ion-exchange chromatography is used for the separation of

- (A) Aldehydes
- (B) Amino acids
- (C) Fatty acids
- (D) Hydrocarbons



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8. In adsorption chromatography, the stationary Phase is

- (A) Liquid
- (B) Gas
- (C) Solid
- (D) All of the above



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9. In gel permeation chromatography, molecules are separated on the basis of them

- (A) Size and shape**
- (B) Chemical nature**
- (C) Adsorptive, properties**
- (D) Partition coefficient**



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10. Which of the following material is not used in the stationary phase of size exclusion chromatography

- (A) Dextran**
- (B) Agarose**
- (C) Polyacrylamide**
- (D) Polyvinyl Alcohol**



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Number of theoretical plates (N) :

It indicates the efficiency of the column

$$N = 16 (t_r/W)^2$$



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