

GEL CHROMATOGRAPHY

Definition:

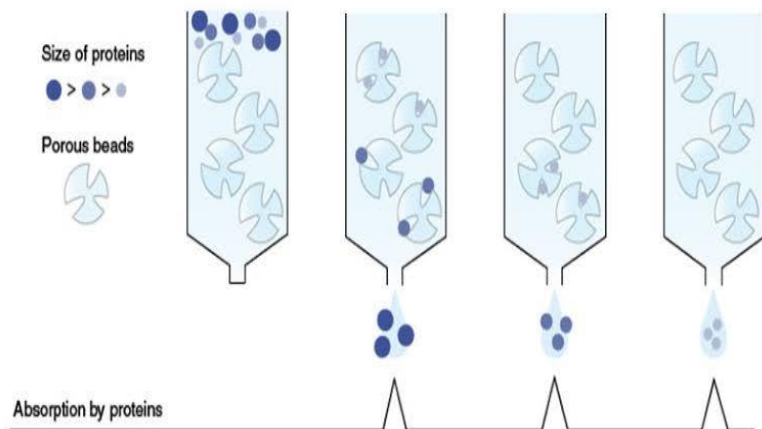
Gel chromatography is a technique for separating chemical substances by exploiting the differences in the rates at which they pass through a bed of porous, semisolid substance.

Other names of gel chromatography:

- 1) Permeation chromatography
- 2) Exclusion chromatography
- 3) Molecular chromatography
- 4) Gel filtration chromatography

Principle:

Gel Filtration Chromatography



- 1) Gel filtration chromatography separates proteins according to their size. And the molecules are “filtered” through the porous beds.

- 2) The gel filtration matrix [stationary phase] contains pores which permit the buffer, small and medium sized molecules to pass through them.
- 3) Large molecules, can't get through any pores in the beads and move more rapidly through the column, emerging (eluting) sooner.
- 4) Medium-sized molecules, can enter the large size pores in the matrix, and so they reach the end of the column later.
- 5) Small molecules can enter through all pores of the beds and they have the largest volume to pass through before emerging from the column last

Theory of separation:

A column is made up of swollen gel particles and solvent used to swell the gel in a suitable tubular container. Analytes that are too large will not be retained; on the other hand, Analytes that are too small will be entirely retained.

An equation is given below:

$$\underline{V_t = V_g + V_i + V_o}$$

Where,

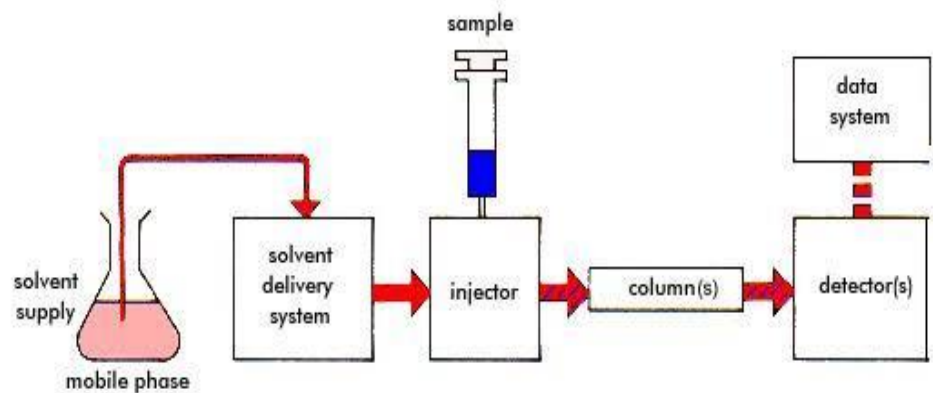
V_m = the volume of gel matrix

v_t = the total volume of the column

v_o = the volume of liquid outside the gel matrix

v_i = the volume of liquid inside the matrix

Instrumentation of gel chromatography:



A. Stationary phase

It has following properties:

1. It is composed of semi-permeable, porous polymer gel beads with a well-defined range of pore sizes
2. Chemically inert
3. Mechanically stable
4. With ideal and homogeneous porous structure (wide pore size give low resolution)
5. A uniform particle and pore size.

Examples of gel:

- 1) Dextran (Sephadex) gel: An α 1-6-polymer of glucose natural gel
- 2) Agarose gel: A 1,3 linked β -D-galactose and 1,4 linked 3,6-anhydro- α , L-galactose natural gel

3) Acrylamide gel: A polymerized acrylamide, a synthetic gel

B. The Mobile Phase

It is composed of a liquid used to dissolve the bio-molecules to make the mobile phase permitting high detection response and wet the packing surface.



C. Columns

Any of the following kinds may be used:

1. Analytical column- 7.5–8mm diameters.
2. Preparative columns-22–25mm
3. Usual column lengths-25, 30, 50, and 60 cm.
4. Narrow-bore columns- 2–3mm diameter have been introduced



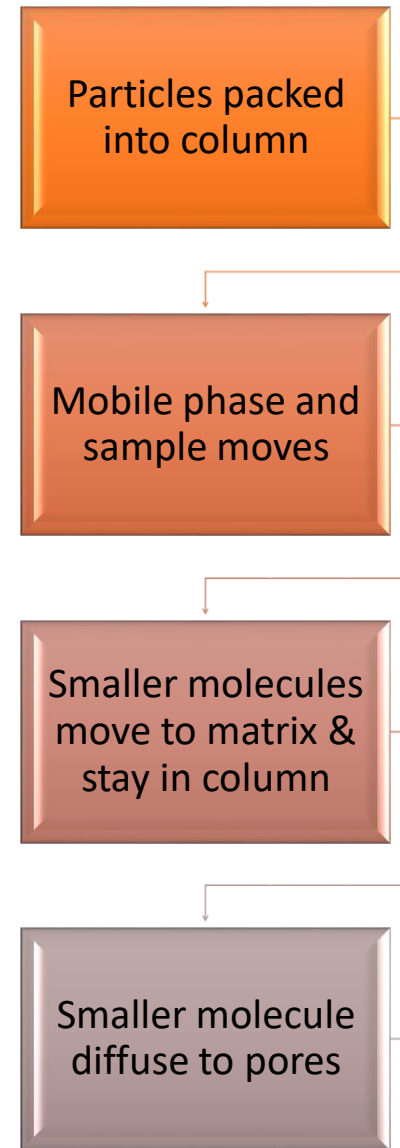
D. Pumps

They are either syringe pumps or reciprocating pumps with a high constant flow rate. It delivers the flow down to column. The pressure delivered by the pump also needs to be smooth so that there are no pulses in the flow.

E. Detectors

The detectors may be concentration sensitive detectors, bulk property detectors, refractive index (RI) detector, etc. Detectors may respond to change in the mobile phase due to the presence of the sample. So it has to be sensitive since the changes they measure in the mobile phase are very small.

Steps involved in Gel Chromatography:

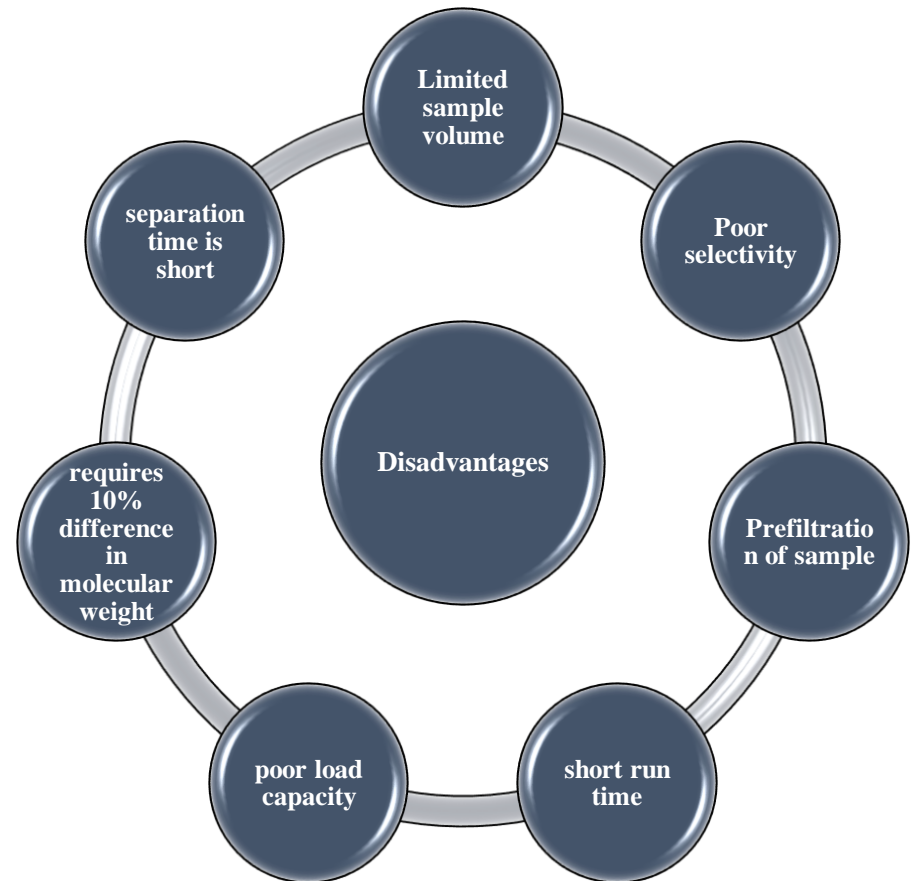


Advantage of gel chromatography:





Disadvantages of Gel Chromatography:



Applications of Gel Chromatography:

1. Gel filtration plays a key role in the purification of enzymes, polysaccharides, nucleic acids, proteins, and other biological macromolecules.
2. Gel filtration can also be used to facilitate the refolding of denatured proteins by careful control of changing buffer conditions.
3. It is used in protein fractionation experiments.
4. Gel filtration technique is also used in molecular weight determination.

5. Separation of sugar, proteins, peptides, rubbers, and others on the basis of their size.
6. Can be used to determine the quaternary structure of purified proteins.

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SUBJECT – Instrumental method of analysis

CLASS - Final Year B.Pharm

ACADEMIC YEAR - 2021-2022