RAMA UNIVERSITY

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DEPARTMENT OF BIOTECHNOLOGY
FACULTY OF ENGINEERING & TECCHNOLOGY

LT.10. Gel Filtration chromatography

Content Outline

1. Gel Filtration: Working & Principle
2. Some GF columns for separation
3. Applications

TOPIC

Gel filtration chromatography

This chromatographic technique is for the separation of molecules on the basis of their molecular size and shape and it exploits the molecular sieve properties of a variety of porous materials. The terms exclusion or permeation chromatography or gel filtration describe all molecular separation processes using molecular sieves.

In a gel filtration chromatography column, the stationary phase is composed of a porous matrix, and the mobile phase is the buffer that flows in between the matrix beads. The beads have a defined pore size range, known as the fractionation range. Molecules and complexes that are too large to enter the pores stay in the mobile phase and move through the column with the flow of the buffer. Smaller molecules and complexes that are able to move into the pores enter the stationary phase and move through the gel filtration column by a longer path through pores of the beads. Bio-Rad offers gel filtration chromatography media and columns with exclusion limits ranging over three orders of magnitude, from 100 daltons to 100,000 daltons (100 kDa).

•Molecules and complexes that can enter the stationary phase will be fractionated according to their sizes. Smaller molecules will migrate deep into the pores and will be retarded more than larger molecules that do not so easily enter the pores, and are thus eluted from the column more quickly. This difference in pore migration leads to fractionation of components by size with the largest eluting first.

➢The partition coefficient Kd values vary between 0 and 1. It is this complete variation of Kd between these two limits that makes it possible to separate analytes within a narrow molecular size range on a given particle type.

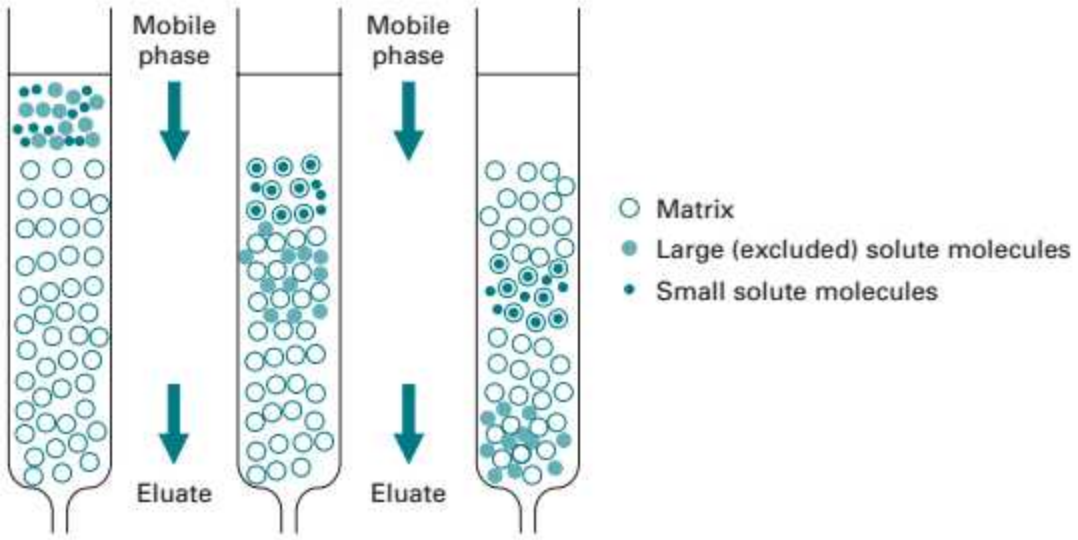

Fig. 11.9 Separation of different size molecules by exclusion chromatography. Large excluded molecules are eluted first in the void volume.

Image source: Wilson & walker

Some GF column used for separation

Hydrophobic / Lipophilic

Hydroxypropyl beaded dextran (Lipohilic Sephadex®), substituted with long chain alkyl ethers. It is used for separation of fatty acids and esters, Fractionation of triglyceride mixtures, Separation of phospholipids, cholesterol and steriods

Sephacryl

Cross-linked co-polymer of allyl dextran and N,N'-methylene bis acrylamide

S-100 HR: peptides, small proteins

S-200 HR: proteins, some small serum proteins

S-300 HR: membrane & serum proteins, monoclonal antibodies

S-400 HR: large proteins & macromolecules with extended structures (e.g. proteoglycans, liposomes).

S-500 HR: large macromolecules, small particles (e.g. plasmids)

Sephadex

Beads are prepared by cross-linking dextan with epichlorohydrin. Widely used in industrial processes and in desalting and buffer exchange.

G-10: very low MW substances; desalting; buffer exchange; peptides

G-15: low MW compounds; desalting; buffer exchange; peptides

G-25: routine desalting; small peptides & proteins; buffer exchange

G-50: desalting; buffer exchange; standard for process scale; very low nonspecific interactions

G-100: very low nonspecific interactions

Sepharose

Beaded agarose for fractionation molecules of high molecular weight. Cross-linked beaded agarose is more resistant to denaturing conditions, and thus offers more versatility in the choice of sample buffer and eluent. The approximate % agarose concentration is indicated by the first number of the Stock Number. Often used as a base matrix for coupling affinity ligands or separation of very large molecules and virus particles.

6B: polio virus purification; proteins; polysaccharides; affinity support

4B: tRNAs; membrane proteins; polysccharides; affinity support

2B: DNA-protein complexes; viruses; asymmetric molecules; affinity support

Superdex

Composite of cross-linked agarose and dextran. High-resolution with short run times and good recovery.

30: peptides; oligosaccharides; small proteins. Selectivity between Sephadex G-25 & G-50

75: high resolution prep separations; wide range of recombinant DNA products. Selectivity similar to Sephadex

G-75

200: monoclonal antibodies; high resolution prep separations; contaminants of low MW; good when protein MW is unknown. Selectivity similar to Sephadex

G-200

Superose

Highly cross-linked beaded agarose. Basically used for preparative work: proteins; DNA fragments; polysaccharides

Bio gel

Made up of polyacrylamide

Applications

Purification

The main application of exclusion chromatography is in the purification of biological macromolecules by facilitating their separation from larger and smaller molecules.Viruses, enzymes, hormones, antibodies, nucleic acids and polysaccharides have all been separated and purified by use of appropriate gels or glass granules.

Relative molecular mass determination

The elution volumes of globular proteins are determined largely by their relative molecular mass (Mr). It has been shown that, over a considerable range of relative molecular masses, the elution volume or Kd is an approximately linear function of the logarithm of Mr. Hence the construction of a calibration curve, with proteins of a similar shape and known Mr, enables the Mr values of other proteins, even in crude preparations, to be estimated

Solution concentration

Solutions of high Mr substances can be concentrated by the addition of dry Sephadex G-25 (coarse). The swelling gel absorbs water and low Mr substances, whereas the high Mr substances remain in solution. After 10 min the gel is removed by centrifugation, leaving the high Mr material in a solution whose concentration has increased but whose pH and ionic strength are unaltered.

Desalting

By use of a column of, for example, Sephadex G-25, solutions of high Mr compounds may be desalted, i.e. removed from contaminants such as salts, detergents, lipids and chaotropic agents. The high Mr compounds move with the void volume, whereas the low Mr compounds are distributed between the mobile and stationary phases and hence move slowly. This method of desalting is faster and more efficient than dialysis. Applications include removal of phenol from nucleic acid preparations, ammonium sulphate from protein preparations and salt from samples eluted from ion-exchange chromatography columns

Test your understanding

Which of the following is/are another term of gel filtration?

i. Desalting
ii. Size exclusion chromatography
iii. Permeation chromatography
iv. Molecular sieve chromatography

a. i and ii only
b. i and iii only
c. ii and iii only
d. ii, iii and iv only

Which of the following is the application of gel filtration?

a. Purification
b. Relative molecular mass determination
c. Protein concentration
d. All of the above

For gel filtration chromatography of proteins, which of the following is True?

a. Large or elongated proteins enter the pores in the beads.
b. Small proteins enter the pores in the beads
c. Large or elongated proteins elute from the bottom of the column later
d. Small proteins elute from the bottom of the column first.

For gel filtration column chromatography, which two of the following has linear relationship?

i. Amount of protein.
ii. Relative elution volume of the protein (avoid volume over the elution volume of the protein).
iii. Logarithm of protein molecular mass.

a. i and iii
b. ii only
c. i only
d. ii and iii

References & Further reading

1. Wilson, K, Walker, J., Principles and Techniques of Practical Biochemistry. 5th Ed. - Cambridge University Press,. Cambridge 1999.
2. Biotechniques, Theory & Practice: Second Edition by SVS Rana, Rustogi Publications.
3. Biochemical Methods of Analysis, Saroj Dua And Neera Garg : Narosa Publishing House, New Delhi.
4. Bioanalytical Techniques, M.L. Srivastava, Narosa Publishing House, New Delhi.