Size-Exclusion Chromatography

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Introduction

Size-exclusion chromatography (SEC) is a chromatographic method in which molecules in solution are separated by their size, and in some cases molecular weight

It is usually applied to large molecules or macromolecular complexes such as proteins and industrial polymers

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Theory

Gel filtration medium is packed into a column to form a packed bed

The medium is a porous matrix in the form of spherical particles that have been chosen for their chemical and physical stability, and inertness

The liquid inside the pores is the stationary phase and this liquid is in equilibrium with the liquid outside the particles called to as the mobile phase

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Group separation

Group separation mode to remove small molecules from a group of larger molecules and as a fast, simple solution for buffer exchange

Small molecules such as excess salt (desalting) or free labels are easily separated

is often used in protein purification schemes for desalting and buffer exchange

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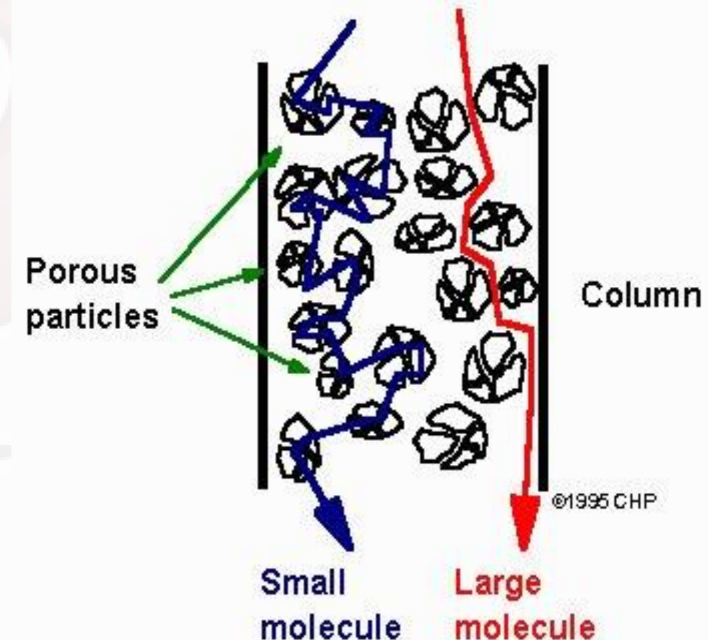
High resolution fractionation

- Gel filtration is used in fractionation mode, uses porous particles to separate multiple components in a sample on the basis of differences
  - Molecules that are smaller than the pore size can enter the particles and therefore have a longer path and longer transit time than larger molecules that cannot enter the particles



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Mechanism of action

- Samples that contain few components or partially purified by other chromatography techniques will give the best result

- Single buffer system, packed bed(chemically and physically stable and inert), pore size in stationary phase separates proteins according to their molecular weight

- Elution of Proteins: one buffer used for both loading and elution of the sample

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- Molecules larger than the pore size can not enter the pores and elute together as the first peak in the chromatogram

- Molecules that can enter the pores will have an average residence time in the particles that depends on the molecules size and shape

- Different molecules therefore have different total transit times through the column

- Molecules that are smaller than the pore size can enter all pores, and have the longest residence time on the column and elute together as the last peak in the chromatogram

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Principle

- One requirement for SEC is that the analyte does not interact with the surface of the stationary phases

- Differences in elution time are based solely on the volume the analyte

- A small molecule that can penetrate every corner of the pore system of the stationary phase (where the entire pore volume and the interparticle volume ~80% of the column volume) and will elute late

- A very large molecule that cannot penetrate the pore system only the interparticle volume (~35% of the column volume) and will elute earlier when this volume of mobile phase has passed through the column

- The underlying principle of SEC is that particles of different sizes will elute (filter) through a stationary phase at different rates. Particles of the same size should elute together

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Analysis

The collected fractions are often examined by spectroscopic techniques to determine the concentration of the particles eluted

Common spectroscopy detection techniques are refractive index (RI) and ultraviolet (UV)

For molecules, which can enter the beads, there is an inverse logarithmic relationship between the size of the molecule and the volume eluted from the column. Finally, can use a standard curve to estimate the molecular weight

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Commercially avaiable columns

The typical column diameters are 7.5–8mm for analytical columns and 22–25mm for (semi)preparative columns; usual column lengths are 25, 30, 50, and 60 cm

The packings are based on either porous silica or semirigid (highly crosslinked) organic gels, in most cases copolymers of styrene and divinylbenzene

For example: TSKgel GFC columns for protein analysis (TSKgel SW-type columns are silica-based)

125Å pore size for analysis of small proteins and peptides
250Å pore size for most protein samples
450Å pore size for very large proteins and nucleic acids

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Advantages

- Unlike ion exchange or affinity chrom. molecules do not bind to the medium so buffer composition does not directly affect resolution
- is well suited for biomolecules that may be sensitive to changes in pH, conc. of metal ions or co-factors and harsh environmental conditions
- conditions can be varied to suit the type of sample or the requirements for further purification, analysis or storage without altering the separation
- Can be used after any chrom. tech. bcz components of any elution buffer will not affect the final separation

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