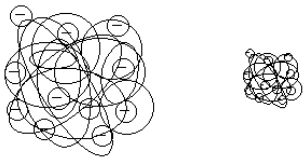
Principles of Size Exclusion Chromatography SEC (GFC, GPC)

Basics

Size Exclusion Chromatography (SEC) separates analytes by their molecular weight (MW) and/or the hydrodynamic radius (size, shape of the molecule).



Hydrodynamic radius = variable size of an analyte with specific MW based on dynamical properties

The packed columns have a stationary phase with defined pore size suitable for a range of molecular weight.

Analytes and stationary phase with defined pore size

The smallest compounds in the sample mixture can enter deep into the pores and have a long elution time. The medium-sized components penetrate the pores in some depth and elute separately according to their size.
Macromolecules, that are bigger than the pore size will not be retained. The maximum molecular weight that can enter the pore is called exclusion limit.

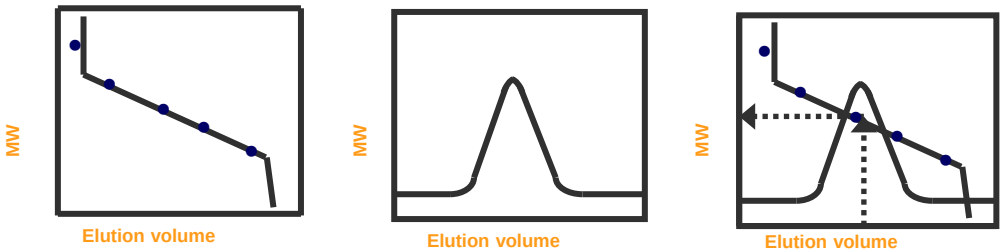
exclusion limit (no interaction with pores, molecules too large to be trapped)

Calibration

Conventional Calibration

- Relative Measurement
- Single concentration detector: RI
- Relationship between MW and elution volume:
    1. Prepare calibration curve with standard samples
    2. Measure elution volume of unknown sample
    3. Calculate MW based on calibration curve

1. 2. 3.



Universal Calibration

- Relative Measurement
- Concentration and MW detector: RI + Viscometer
- Relationship between hydrodynamic volume (MW*intrinsic viscosity) and elution volume

Multiple detection

- Absolute Measurement
- RI + Viscometer + Multi Angle Light Scattering

Detection

Concentration sensitive detectors

- Selective: UV, IR, Fluorescence, Electrochemical
- Universal: RI, Conductivity, Density, ELSD

Molar Mass sensitive detectors

- Viscometer, Multi Angle Light Scattering (MALS)

Applications

Biopolymer applications

- biological activity/native solution
- determination of tertiary and quaternary structures
- no sample loss
- desalination
- purification from small molecules

Synthetic polymer applications

- Size and polydispersity
- MW, end group or branching distribution
- properties like Mn, Mp, Mz
- even for macromolecules over 100.000.000 Da

Columns

Aqueous Solvent SEC

Gel Filtration Chromatography GFC

PROTEIN KW column series

- Material: silica-based, pore sizes 150, 300 or 500 Å
- Sample: **Proteins, peptides, enzymes, antibodies**

OHpak SB column series

- Material: polymer-based (Polyhydroxymethacrylate), higher exclusion limits up to 20.000.000 Da
- Sample: **PEG-proteins, polysaccharides, water-soluble polymers**

Asahipak GS Multimode column series

- Material: polymer-based (Polyvinyl alcohol), additional RP and ion exchange mode
- Sample: **peptides, proteins, nucleic acids, nucleotides/nucleosides**

Organic Solvent SEC

Gel Permeation Chromatography GPC

GPC KF (THF)
GPC K (Chloroform)
GPC KD (DMF)
GPC HFIB
GPC LF (linear calibration)
GPC HT, UT (high temperature)

- Material: polymer-based (Styrene divinylbenzene copolymer), best stability and reproducibility
- Sample: **Plastics like PS, PE, PP, PVC, PA, rubbers, resins, silicones, copolymers**

Asahipak GF Multi-solvent column series

- Material: polymer-based (Polyvinyl alcohol), supports aqueous and organic solvents
- Sample: **proteins, hormones, polyelectrolytes, surfactants**

Shodex™
Phone: +49 (0)89 9399 6237
Email: info@shodex.de
Web: www.shodex.de