UNIVERSITY OF CHEMISTRY AND TECHNOLOGY, PRAGUE
Faculty of Food and Biochemical Technology
Department of Food Analysis and Nutrition

Gel permeation chromatography

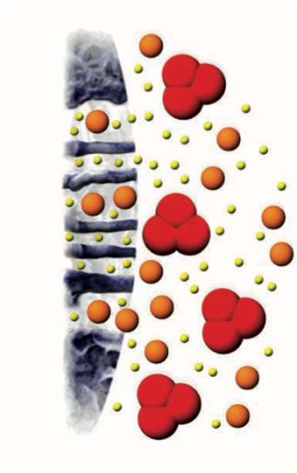
Isolation and separation methods

https://www.gilson.com/system-verity-gpc-cleanup-system.html

1

Gel permeation chromatography (GPC)

- Separation and purification technique
- Allows separation of groups of compounds with similar molecular weight (fractionation)
- Analytes are diluted after separation and must be concentrated
- Requires routine optimization for analyte / matrix combinations
- Fast and easy method, can be automated
- Robust and relatively universally applicable

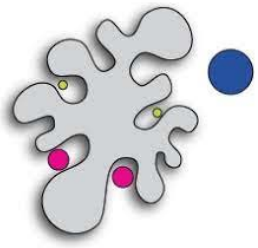


http://wolfson.huji.ac.il/purification/PDF/Gel_Filtration/GE_Size_Exclusion_Chromatography_Handbook.pdf

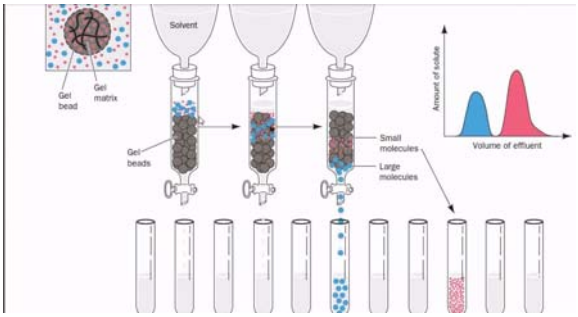
2

GPC - principle

- Method based on differences in molecular weights of individual sample components/fractions
- The difference in molecular weights must be significant. The individual fractions are separated using a porous swollen gel.
- Smaller molecules fit into the pore of the gel and are retained longer.
- Larger molecules do not fit into the pores, or are retained only slightly, and pass through the system without much retention.
- Separation and adsorption mechanisms also apply.

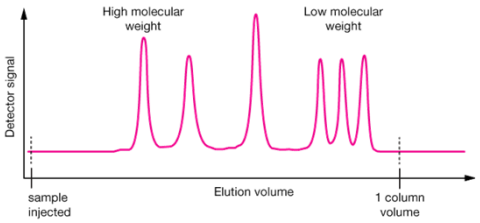


https://xaktly.com/Chromatography.html

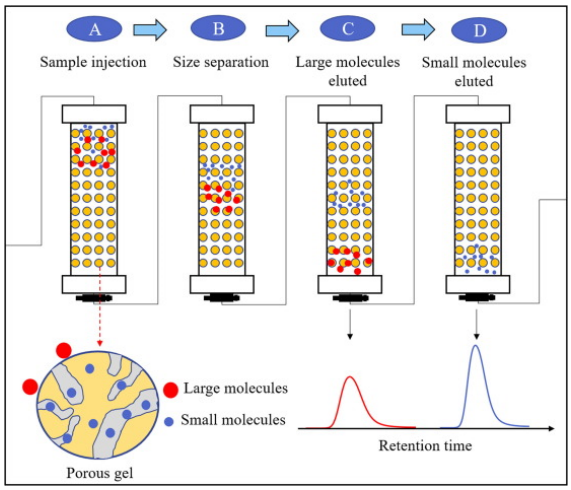


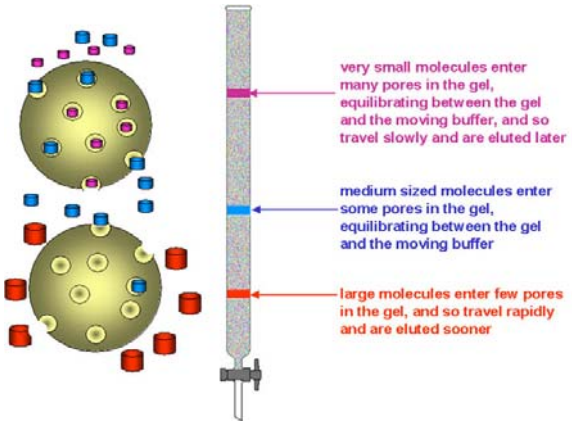
Size Exclusion Chromatography - SEC

- Typically, stationary phases for SEC consist of "beads" made of long polymers of an inert sugar compound called agarose. The material is quite stable, it's easy to manipulate the range of pore sizes, and they are relatively chemically inert.
- Sepharose is a common trade name for SEC columns, which are usually purchased pre-prepared. Sepharose columns can be made with various pore-size ranges, depending on the sizes of molecule that need to be separated.
- Size-exclusion chromatography is often a final step in purification of proteins prior to other experiments in molecular and structural biology.



GPC principle

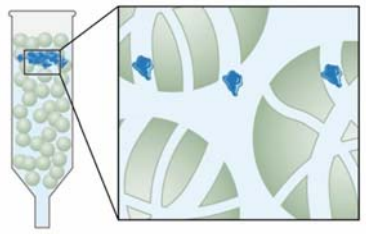


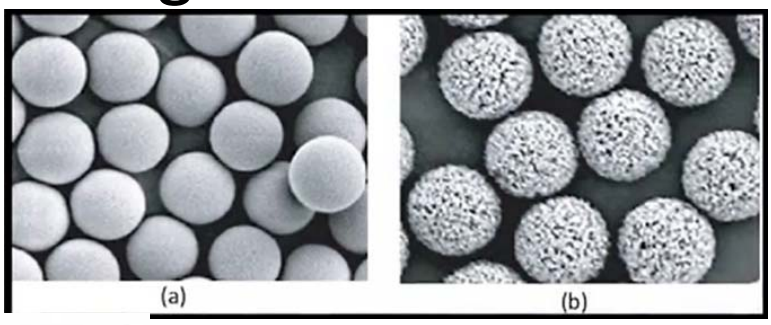


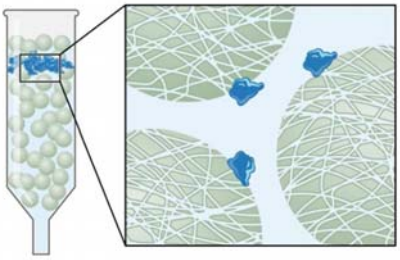
https://www.sciencedirect.com/science/article/abs/pii/S095006182100146X

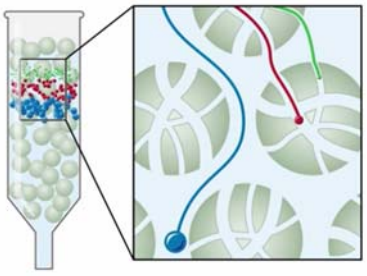
https://www.ucl.ac.uk/~ucbcdab/enzpur/gelexcl.htm

GPC column packings

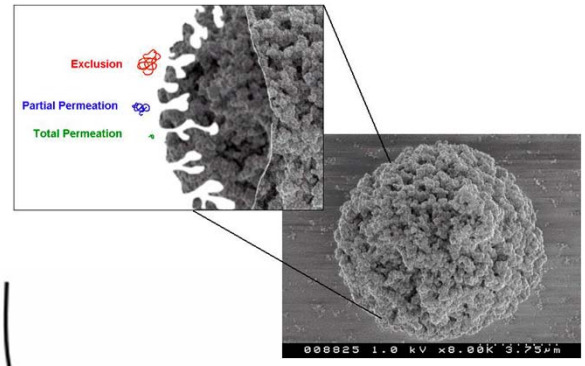


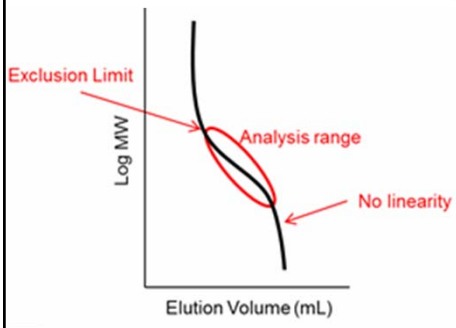


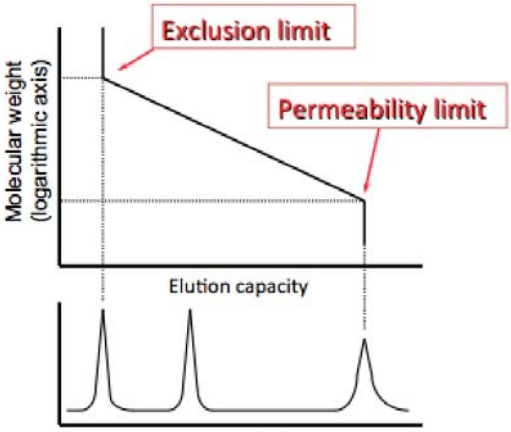




Exclusion and permeation limit

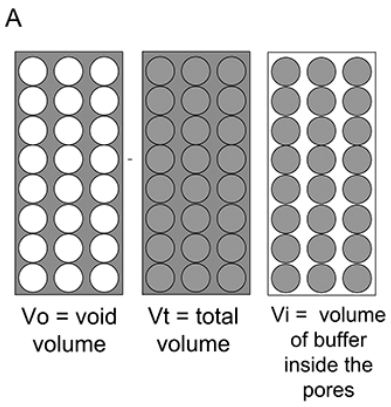


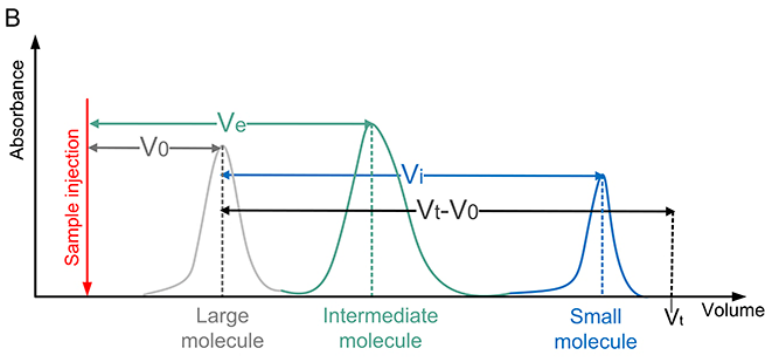




https://www.shodexhplc.com/lessons/lesson-4-sec-column/

GPC - theory





$$V_t = V_i + V_o$$

V_t - total volume of eluent in the column
V_i - eluent trapped in the pores of the gel (stationary phase)
V_o - eluent between gel particles - dead column volume (mobile phase)

https://bio-protocol.org/e2230

GPC - theory

$$V_e = V_0 + K_{SEC}.V_i + K_P.V_i + K_{AD}.V_i$$

$$K_T = K_{SEC} + K_P + K_{AD}$$

$$K_{SEC} = (C_S / C_m)$$

K_P - distribution constant of the distribution mechanism
K_{AD} - distribution constant of the adsorption mechanism

The elution volume of the analyte
C_s - analyte concentration in stationary phase
C_m - analyte concentration in the mobile phase
V_i - eluent trapped in the pores of the gel (stationary phase)
V_o - eluent between gel particles - dead column volume (mobile phase)

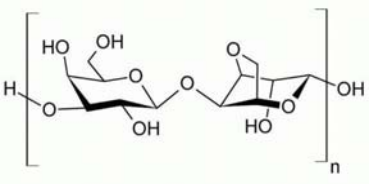
GPC – gel selection

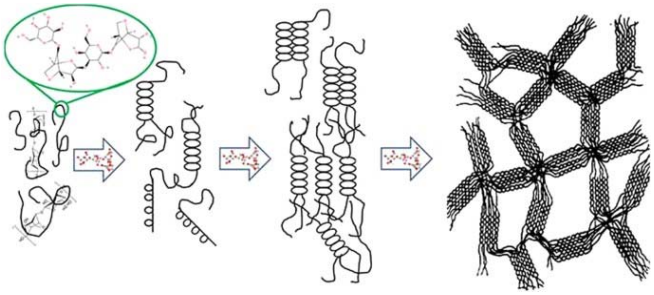
Solubility of analytes (polarity) => choice of solvent gel selection

hydrophilic (polar)… x… hydrophobic (non-polar)

soft (swollen)… x… rigid (fixed, non-blade)

Soft gel - agarose





GPC - gel types

Soft gels (SEC, GPC, GF):

- working range and elimination limit - given by the degree of crosslinking (manufacturer) and swelling (choice m.f. - analyst)
- compressible column => limit usable pressure m.f. (danger of collapse of the gel structure)
- usually, larger capacity
- less robust systems - wide possibilities of optimization

Examples:

- Sephadex: dextran gel, hydrophilic
- Sepharose: agarose gel, hydrophilic
- Bio-Gel P: acrylamide gel, hydrophilic
- Bio-Beads: styrene-divinylbenzene copolymer, hydrophobic

Rigid gels (HPSEC, HPGPC):

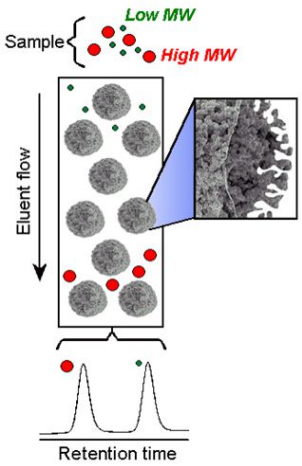
- working range and exclusion limit - practically unchanged
- possibility to change m.f. without overfilling the column
- stability - higher pressure (higher flow) => higher speed, efficiency
- robust systems - limited optimization possibilities

Examples

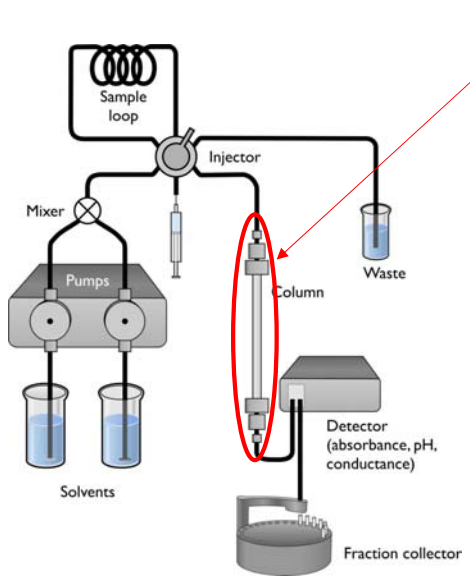
- PL gel: styrene
- BioSepra: ceramic core

GPC – technical parameters

- Columns: steel - stainless steel, titanium, glass, plastic from 10 x 0.5 cm to 60 x 5 cm
- Column: fixed column or adjustable (movable end frit)
- Injection: 0.1 - 10 ml
- Sample capacity: 0.1 - 10 g
- Flow rate: 0.1 - 10 ml / min
- Size of elution volumes (fractions): 20 - 300 ml (0.5 - 50 ml)



Size Exclusion Chromatography (SEC)



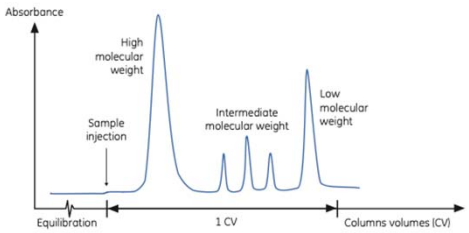


Fig I.3. Typical high-resolution SEC separation.

GPC, SEC - applications

Fractionation - Separation (mostly SEC)

- *separation of compounds into groups:*
  - amino acids
  - peptides
  - proteins
  - nucleic acids
  - carbohydrates

Purification of extracts (GPC)

- *removal of high molecular weight undesirable substances (often in large excess) from low molecular weight analytes*
- trace analysis (pesticides, industrial contaminants)
- Typically, the separation of triacylglycerols from target analytes, especially in high fat matrix extracts.