Gel Permeation Chromatography - GPC

Separation and clean-up method

Group separation of compounds with similar molecular weight – fractionation

Analytes are diluted in eluate
  ⇒ necessary to concentrate (RE)

Optimisation for given combinations analyte / matrix

Quick, rugged, easily automatable, almost universal
GPC principle

Porous particles

Small molecule

Large molecule

Column

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Fractionation according to MW

Diagram showing the process of fractionation according to molecular weight (MW) with a chromatogram, sample mixture, and concentration detector.
GPC column characterisation

Exclusion limit

Working range

Diol-120

Diol-60

Fractionation

No retention

Penetration

MW
1. Pullulan (P-800) 853,000
2. Pullulan (P-400) 380,000
3. Pullulan (P-200) 186,000
4. Pullulan (P-100) 100,000
5. Pullulan (P-50) 48,000
6. Pullulan (P-20) 23,700
7. Pullulan (P-10) 12,200
8. Pullulan (P-5) 5,800
9. Maltopentadecase (G_{15}) 2,448
10. Maltoundecaose (G_{11}) 1,800
11. Maltoheptacose (G_{7}) 1,152
12. Maltopentaoease (G_{5}) 824
13. Maltotriose (G_{3}) 504
14. Maltose (G_{2}) 342
15. Glucose (G_{1}) 180
\[ V_t = V_i + V_0 \]

- \( V_t \) – total volume of eluent in column
- \( V_i \) – volume of eluent held in pores of gel (stationary phase)
- \( V_0 \) – volume of eluent outside of gel particles – dead volume (mobile phase)
1. Size Exclusion Chromatography (SEC)

Separation based just on MW differences

\[ K_{SEC} = \left( \frac{C_s}{C_m} \right) \]

\[ V_e = V_0 + K_{SEC} V_i \]

\( V_e \) – analyte elution volume

\( C_s \) – analyte concentration in stationary phase

\( C_m \) – analyte concentration in mobile phase

\( K_{SEC} = 0 \Rightarrow \text{compound is excluded in dead volume} \)

\( K_{SEC} = 1 \Rightarrow \text{compound is totally retained} \)
2. Gel Permeation Chromatography (GPC)

Separation based on MW differences and also on other mechanisms – e.g. partition and adsorption

\[ K_T = K_{SEC} + K_P + K_{AD} \]

\[ V_e = V_0 + K_{SEC} V_i + K_P V_i + K_{AD} V_i \]

- \( K_P \) – distribution constant of partition mechanism
- \( K_{AD} \) – distribution constant of adsorption mechanism
Solubility Parameters (Cohesive Energy Density) (according to Hildebrand)

*Cohesive energy density* – \( c (\Delta E) \)

\[ \Delta H - \text{vaporisation heat} \]

\[ R - \text{universal gas constant} \]

\[ T - \text{temperature} \]

\[ V_m - \text{molar volume} \]

\[ c = \frac{\Delta H - RT}{V_m} \]

*Solubility parameter* - \( \delta \)

Units: \( \text{cal}^{1/2}\text{cm}^{-3/2} = 0.48888 \text{ MPa}^{1/2} \)

\( \text{MPa}^{1/2} = \text{SI} = 2.0455 \text{ cal}^{1/2}\text{cm}^{-3/2} \)

\[ \delta = \sqrt{c} \]
Non polar compounds – only dispersion forces

Polar compounds – other types of molecular interactions

Total solubility parameter: $\delta_t$

$$\delta_t^2 = \delta_d^2 + \delta_o^2 + 2\delta_{\text{ind}}\delta_o + 2\delta_a\delta_b$$

$\delta_d$ – dispersion interaction

$\delta_o$ - dipole interaction

$\delta_{\text{ind}}$ – induced dipole interaction

$\delta_a$ - proton donor character

$\delta_b$ - proton acceptor character
## Selected solubility parameters (\(\text{cal}^{1/2}\text{cm}^{-3/2}\))

<table>
<thead>
<tr>
<th>Liquid / gel</th>
<th>(\delta_d)</th>
<th>(\delta_o)</th>
<th>(\delta_{\text{ind}})</th>
<th>(\delta_a)</th>
<th>(\delta_b)</th>
<th>(\delta_t^a)</th>
<th>(\delta_t^b)</th>
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<tbody>
<tr>
<td>Hexane</td>
<td>7,3</td>
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<td>-</td>
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<td>-</td>
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<td>4,0</td>
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<td>2,7</td>
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<td>-</td>
<td>0,6</td>
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<td>9,1</td>
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<td>3,0</td>
<td>9,4</td>
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<td>&lt; 3,9 &gt;</td>
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<td>-</td>
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<td>9,7</td>
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<td>Polystyrene</td>
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<td>-</td>
<td>-</td>
<td>9,1-9,4(^c)</td>
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<tr>
<td>Polyacrylamide</td>
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<td>-</td>
<td>15(^c)</td>
<td>-</td>
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</tbody>
</table>

* \(a\) – value calculated using equation for \(\delta_t\) ; \(b\) – value determined by evaporation ; \(c\) – estimation
Application of solubility parameters for GPC optimisation

Estimation of $\delta_{MX}$ for solvent mixtures:

$$\delta_{MX} = \sum \Phi_i \delta_i$$

$\Phi_i = \text{solvent fraction in mixture}$

The same $\delta$ values ...X... different molecular interactions

**Equation for capacity ratio: - $K_i$**

$$\ln K_i = \frac{V_{m,i}}{RT} \left( \delta_m + \delta_s - 2\delta_i \right) \cdot \left( \delta_m - \delta_s \right) + \ln \frac{n_s}{n_m}$$

$K_i = \frac{n_s}{n_m}$

$\delta_m$ – mobile phase solubility parameter

$\delta_s$ – stationary phase solubility parameter

$n_m$ - number of moles in mobile phase

$n_s$ – number of moles in stationary phase
Mobile and stationary phase selection

Analyte solubility (polarity)

⇒ solvent selection

⇒ gel selection

**GELS**

HYDROPHILIC (POLAR) ...X... HYDROPHOBIC (NONPOLAR)

SOFT (SWELLING) ...X... RIGID (NONSWELLING)
**Soft gels (SEC, GPC, GF)**

*Working range and exclusion limit*
– corresponds to gel cross linking (producer) and swelling (mobile phase selection – analyst)

*Compressible column ⇒ limited applicable pressure (risk of gel structure collapse)*

*Usually higher capacity*

*Less rugged systems – many possibilities for optimisations*

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 – practical invariable*

*Possibility of mobile phase changes – without column refilling*

*Stability – higher pressure (higher flow rate)*

⇒ *higher speed, efficiency*

*Rugged systems – limited optimisation*

PL gel: styrene based

BioSeptra – ceramic core
Soft gel - agarose

Rigid gel - BioSepra
Simple system: hand injection and collection of fractions

Automatic system: programmable injection and collection of fractions

Diagram:
- Mobile phase
- Pump
- 6 port valve
- GPC column
- Injection loop
- Eluate collection
Technical parameters:

Columns: steel - stainless, titanium, glass, plastic
from 10 x 0.5 cm to 60 x 5 cm

Gel bed: firm – defined by column size
variable – e.g. movable frits

Injection: 0.1 - 10 ml

Sample capacity: 0.1 - 10 g

Flow rate: 0.1 - 10 ml/min

Elution volumes (fractions): 20 - 300 ml (0.5 - 50 ml)
Model example: Graphic output Interpretation

**Fractions range:**
FROM – TO
(in ml or in min)

Alternatively:
Retention volume (time)
GPC applicability

1. Fractionation – separation
- amino acids, peptides, proteins, nucleic acids, carbohydrates

2. Clean-up
- removal of higher molecular weight undesirable compounds (often in over-abundance = waste)
  from lower molecular weight compounds (collected fraction)
- trace analysis (pesticides, industrial contaminants)