Permeability of Microcapsules by Inverse Size Exclusion Chromatography

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Outline

• INTRODUCTION
  • Permeability of microcapsules
  • Experimental techniques

• INVERSE SIZE EXCLUSION CHROMATOGRAPHY
  • Principle
  • Evaluation
  • Representative results
    • Case 1: PMCG microcapsules
    • Case 2: “COST865” microcapsules

• OPTIONAL TECHNIQUES

• CONCLUSIONS

P = D x K

P  permeability
D  diffusion coefficient
K  partition coefficient

\( P = D \times K \)

\( P \) permeability
\( D \) diffusion coefficient
\( K \) partition coefficient

\( \dot{E} \) driving force to move molecules
- obstruction from matrix
- hydrodynamic drag
- heterogeneity of matrix
- interactions

\( \dot{E} \) equilibrium distribution

\( \dot{E} \) pore size and pore size distribution

Introduction: permeability of solutes via hydrogel matrix

Introduction: microcapsule for islet transplantation

\( \dot{E} \) equilibrium distribution

\( \dot{E} \) pore size and pore size distribution

\( \dot{E} \) pore size and pore size distribution

Introduc on: permeability of solutes via hydrogel matrix


Immunoisolating device
Introduction: microcapsule for islet transplantation

**Membrane role:**
1. Provide immunoprotection
2. Ensure cell viability

- Exclude immune cells
- Exclude soluble components
- Allow for permeation of nutrients, O₂, insulin

**Nanometer range:** molecular weight vs size of some proteins

<table>
<thead>
<tr>
<th>Sample</th>
<th>MW (Da)</th>
<th>Rc (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroglobulin</td>
<td>370,000</td>
<td>8.60</td>
</tr>
<tr>
<td>o-Calprotectine</td>
<td>518,000</td>
<td>0.86</td>
</tr>
<tr>
<td>Apo-A-II</td>
<td>483,000</td>
<td>6.06</td>
</tr>
<tr>
<td>Catalase</td>
<td>232,000</td>
<td>5.23</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>67,000</td>
<td>1.90</td>
</tr>
<tr>
<td>γ-Globulin</td>
<td>158,000</td>
<td>5.93</td>
</tr>
<tr>
<td>Alcohol dehydrogenase</td>
<td>150,000</td>
<td>4.55</td>
</tr>
<tr>
<td>Albumin (human)</td>
<td>125,000</td>
<td>4.90</td>
</tr>
<tr>
<td>Albumin (porcine)</td>
<td>86,000</td>
<td>3.30</td>
</tr>
<tr>
<td>Transferrin</td>
<td>77,000</td>
<td>3.02</td>
</tr>
<tr>
<td>Alumina</td>
<td>66,000</td>
<td>3.62</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>44,000</td>
<td>2.83</td>
</tr>
<tr>
<td>α-Lactalbumin</td>
<td>35,000</td>
<td>2.70</td>
</tr>
<tr>
<td>Hemoglobin (human)</td>
<td>32,000</td>
<td>2.40</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>28,000</td>
<td>2.01</td>
</tr>
<tr>
<td>Cyanocystein</td>
<td>25,780</td>
<td>2.50</td>
</tr>
<tr>
<td>Oxygenase</td>
<td>22,000</td>
<td>2.75</td>
</tr>
<tr>
<td>Megalin</td>
<td>17,000</td>
<td>1.91</td>
</tr>
<tr>
<td>α-Lactalbumin</td>
<td>15,380</td>
<td>2.02</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>14,000</td>
<td>1.85</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>13,790</td>
<td>1.75</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>13,790</td>
<td>1.75</td>
</tr>
<tr>
<td>Complement component C3</td>
<td>12,000</td>
<td>1.67</td>
</tr>
<tr>
<td>Aggregated fibrinogen</td>
<td>6,780</td>
<td>1.50</td>
</tr>
</tbody>
</table>

Q1: Can the hydrogel be designed to have such "nm" control over the pore size?

Q2: What exactly is the proper "nm range"? The transplantation results should tell.

Introduction: target in microcapsule characterization

**Molecular weight cut-off (MWCO)**

q the lowest size (nm) and/or the lowest molecular weight (Da) of a solute which can permeate through the membrane

...in addition, the functional semipermeable membrane has to exhibit proper diffusion properties (a “YES” is often “automatically” assumed)

Introduction: methods for permeability characterization

<table>
<thead>
<tr>
<th>Category</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solute type</td>
<td>Proteins – unlabeled, radiolabeled</td>
</tr>
<tr>
<td></td>
<td>Dextran</td>
</tr>
<tr>
<td></td>
<td>Pullulans</td>
</tr>
<tr>
<td>Analytical method</td>
<td>Protein assay kit</td>
</tr>
<tr>
<td></td>
<td>UV-VIS spectrometry</td>
</tr>
<tr>
<td></td>
<td>Radioactivity</td>
</tr>
<tr>
<td></td>
<td>SEC or inverse SEC</td>
</tr>
<tr>
<td></td>
<td>Fluorescence microscopy</td>
</tr>
<tr>
<td>Static/dynamic methods</td>
<td>Inverse SEC</td>
</tr>
<tr>
<td></td>
<td>Incubation</td>
</tr>
<tr>
<td>Direction of diffusion</td>
<td>Into the capsule (ingress)</td>
</tr>
<tr>
<td></td>
<td>Out the capsule (egress)</td>
</tr>
<tr>
<td>Parameter</td>
<td>Molecular weight cut-off</td>
</tr>
<tr>
<td></td>
<td>Release or binding protein</td>
</tr>
<tr>
<td></td>
<td>Pore size distribution</td>
</tr>
</tbody>
</table>


U. Schuldt, D. Hunkeler, Minerva Biotecnologica 2000, 12, 249.
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Inverse size-exclusion chromatography: principle

Size-exclusion chromatography (SEC)

Column separation technique based on enthalpy-free partitioning of analyzed polymer chains of different length (size) between mobile and stationary phases.

\[ V_e = V_0 + K_{SEC}(V_t - V_0) \]

- \( V_e \) - elution volume for given size
- \( V_0 \) - free (interstitial) volume between particles of column packing
- \( V_t \) - total (interstitial plus pore) volume

COLUMN PARAMETERS
- Length (30 – 60 cm)
- Diameter (~ 1 cm)
- Particle size (5-20 \( \mu \)m)
- Pore size / exclusion limit (100 – 10 000 Å)
- Pore size distributions (narrow)

Inverse size-exclusion chromatography

Injection of polymer of unknown molecular weight characteristics

Commercial columns of defined characteristics for column packing:
- Diameter, exclusion limit, pore-size distribution

Detector

Calibration curve with standards: elution volume = f(MW)

Molecular weight characteristics (MWG, Mn, Mw) of polymer

创业板

Column separation technique based on enthalpy-free partitioning of analyzed polymer chains of different length (size) between mobile and stationary phases.

Inclusion of polymer of known molecular weight characteristics

Detector

Calibration curve for a given column: elution volume = f(MW)

Molecular weight distribution

COLUMN PARAMETERS
- Length (10 - 20 cm)
- Diameter (~ 1 cm)
- Particle size (300 - 1000 \( \mu \)m)
- Pore size / exclusion limit UNKNOWN
- Pore size distribution UNKNOWN
Inverse size-exclusion chromatography: principle

**Step 1: Measurement of elution curves**

- Standards (e.g., pullulan) between 700,000 and 180 Da injected onto the column formed by capsules
- Determination of $K_{SEC}$ at $V_e = 50\%$ peak area

Inverse size-exclusion chromatography: evaluation

**Step 2: Evaluation of elution curves**

- Calibration curve for the column (made of capsules)

**Step 3: Further processing of calibration curve**

- MWCO and PSD based on MW of pullulans
- MWCO and PSD based on SIZE of pullulans

MWCO based on SIZE of proteins

<table>
<thead>
<tr>
<th>Pullulan/kDa</th>
<th>$R_g$/nm</th>
<th>Protein/kDa</th>
<th>$M_w$/kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>6.7</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>4.6</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>3.5</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>2.2</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.4</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

MWCO to proteins

Molecular weight cut-off: to remember…

1. **MWCO is a size-related parameter**

2. **MWCO when expressed by “molecular weight”, it is a solute-type related parameter** (polysaccharide ≠ protein)

![Molecular weight cut-off graph](image)

Inverse size-exclusion chromatography: expt. conditions

**COLUMN PARAMETERS**
- Omnifit glass column with adjustable plungers
- Length: 10 - 30 cm
- Diameter: 1 cm
- Microcapsule volume: 10 - 20 ml
- Microcapsule size: tested up to ~1.5 mm

**ELUENT**
- Saline solution or any culture media (with NaN₃)
- Flow rate: 0.1 – 0.2 ml/min

**TESTING SOLUTE TYPE**
- Pullulan narrow distributed standards, PDI ~ 1.1 (note: dextran ~ 1.5)
- Proteins (may interact enthalpic separation?)

**HARDWARE (~ 30 k€)**
- Degasser - HPLC Pump – Injector – RI Detector – (Software)

**TIME OF ANALYSIS AND EVALUATION** ~ 3 days

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**Inverse size-exclusion chromatography: Case study #1**

**“PMCG” microcapsule**
- Coating by cellulose sulfate (MW dependence)

![Inverse size-exclusion chromatography diagram](image)

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**Inverse size-exclusion chromatography: Case study #1**

![Inverse size-exclusion chromatography diagram](image)

---
Inverse size-exclusion chromatography: Case study #1

**“PMCG” microcapsule**

Coating by cellulose sulfate (time dependence)

 tô tuning the MWCO values

![Graph showing MWCO values with time](image)

MWCO \(\approx 60\) kDa

Inverse size-exclusion chromatography: Case study #2

**COST865: Alginate / PLL microcapsule (Paul)**

MWCO (pullulans) \(\sim 15 – 25\) kDa \(\sim 6 – 8\) nm \ MWCO (protein) \(40 – 100\) kDa

![Graph showing MWCO values with time](image)

q broad pore size distribution, similar to chitosan microcapsules
q MWCO \(\sim 100\) kDa (pullulans) \(\sim 16\) nm \(\sim 800\) kDa (proteins)
Inverse size exclusion chromatography: final remarks

molecular weight cut-off (MWCO) and effective pore size distribution

MWCO value
- 
- 3.5 nm corresponds to:
- ~ 30 kDa for pullulans
- ~ 70 kDa for a protein

Extremely valuable tool for (1) comparison among the batches and (2) optimization process, (3) indirectly, stability studies (eluent can be saline solution as well as media / artificial body fluids)

We are convinced this is true; to my knowledge, currently no other groups use it

It should not be the “cost” issue...

It may underestimate the MWCO compared to the direct (long-term) ingress measurements

Usually MWCO is determined as $K_{SEC}$ = 0.9 – 1.0

MWCO: search for the first solute which concentration starts to decrease (injector + RI detector)

Partition coefficient: Can be correlated to I-SEC

Static incubation: ingress of dextrans or pullulans from supernatant

(Note: dextrans are polydisperse - pullulans preferred)

EPFL: incubation in a cocktail of standards, quantity analyzed on SEC columns

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Optimal techniques

A number of techniques have been available and are in use

Polymer Institute in Bratislava in cooperation with International laser centre

CLSM: ingress of fluorescently labeled dextrans (?) and proteins (IgG)


Static incubation:

Ingress of dextrans or pullulans from supernatant

(Note: dextrans are polydisperse - pullulans preferred)

EPFL: incubation in a cocktail of standards, quantity analyzed on SEC columns


**Optimal techniques**

1. Immobilization of Protein-A Sepharose particles, which bind IgG
2. Measurement of bound radiolabeled IgG in the capsule

**MWCO of PMCG capsule (dextran)**

<table>
<thead>
<tr>
<th>MWCO (kDa)</th>
<th>IgG retained (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>230 kDa</td>
<td>0</td>
</tr>
<tr>
<td>120 kDa</td>
<td>10</td>
</tr>
<tr>
<td>44 kDa</td>
<td>20</td>
</tr>
</tbody>
</table>


- CLSM and RIA of alginate beads
- Permeable to IgG

**Conclusions**

1. Permeability properties represent an important material characteristics and, therefore, have to accompany any microcapsule development

2. Different experimental approaches and/or the same experimental approaches performed at different laboratories may lead to discrepancies
   - COST865 tries to find a solution
   - Precise description
   - To compare various microcapsules, the analysis in one laboratory is recommended as the first “practical” step

3. The permeability (and other) properties of microcapsules after application, i.e. after explantation, are almost completely missing
   - The inverse SEC is not suitable because of a limited amount of capsules
   - The egress/ingress methods needed only a few capsules are recommended and should be regularly used

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COST 865