Characterization of biopolymers by advanced polymer chromatography (APC)

Magdolna R. Mihályi
Research Centre for Natural Sciences

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Interreg, SKHU/1902/4.1/001/Bioeconomy

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Magyar tudósok körútja 2, Budapest H-1117, Hungary

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www.ttk.hu/palyazatok/bioeconomy
SEC/GPC/APC

SEC: Size Exclusion Chromatography (1959, Porath and Flodin)

GPC: Gel-Permeation Chromatography (1974, Down Chemical. Co.)
stationary phase: synthetic polymer, e.g. PS

APC: Advanced Polymer Chromatography (2004, Waters Co., UPLC)
stationary phase: rigid, 2.5 μm-size modified silica particles with pore size of 45Å - 900Å.

SEC/GPC/APC
• molar mass averages,
• molar mass distribution of synthetic and biopolymers

number average molecular weight
\[ M_n = \frac{\Sigma N_i M_i}{\Sigma N_i} \]

weight average molecular weight
\[ M_w = \frac{\Sigma N_i M_i^2}{\Sigma N_i M_i} \]

polydispersity index
\[ D = \frac{M_w}{M_n} \]
SEC principle

- Polymers are separated by hydrodynamic volume
- Big One Comes Out First (BOCOF) followed by the smaller molecules
APC columns for aqueous and organic polymer separation

Ethylene Bridged Hybrid (BEH) technology, Waters

- strong and rigid particles
- particle size: 1.7 and 2.5 µm
- resist shrinking, swelling
- easy solvent switching
- high reproducibility
## APC columns

10 small columns, diameter: 4.6 mm; length: 150 mm

<table>
<thead>
<tr>
<th></th>
<th>Solvent</th>
<th>Temp. limit (°C)</th>
<th>pH</th>
<th>Pore size (Å)</th>
<th>Particle size (µm)</th>
<th>Linear range (g/mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACQUITY APC XT 45</td>
<td>organic</td>
<td>90</td>
<td>1-11</td>
<td>45</td>
<td>1.7</td>
<td>200 - 5 000</td>
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<tr>
<td>ACQUITY APC XT 125</td>
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<td>90</td>
<td>1-11</td>
<td>125</td>
<td>2.5</td>
<td>1 000 - 30 000</td>
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<tr>
<td>ACQUITY APC XT 200</td>
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<td>1-11</td>
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<td>2.5</td>
<td>3 000 – 70 000</td>
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<tr>
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<td>900</td>
<td>2.5</td>
<td>300 000 - 2 000 000</td>
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<tr>
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<td>45</td>
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Advanced Polymer Chromatograph (APC) / Ultra High Performance Liquid Chromatograph (UHPLC)

1000 bar

**Sample Manager**

**Diode-array UV-Vis detector** (0.5 μl)

**Refractive Index detector** (1.3 μl)

**Thermostat I** for small columns (4.6 x 150 mm)

**Thermostat II** for large columns (7.8 x 300 mm)

**Quaternary Solvent Manager**

Waters Empower 3 software
Behind doors…

Quaternary Solvent Manager

1. Vacuum degasser, four chambers
2. Propotioning valve
3. Pump I
4. Pump II
5. Mixer
Behind doors…

Sample Manager with flow-through needle

Two plates for 96 samples in 2-mL vial holders
Temperature range: 4-40 °C
Thermostat I for small columns (4.6 x 150 mm)

Temperature range: 4 – 90 °C

1. column: 450 Å
2. column: 200 Å
3. column: 125 Å

0.2 μm Filter
Lignocellulose structure

Lignin structure, most common bonds (β-O-4, β-β) and three types of monomer groups (S, G, H)

4-hydroxyphenyl (H) group
Guaiacyl (G) group
Syringyl (S) group

Lignin valorization

Non-catalytic thermochemical depolymerization

- **Pyrolysis**
  - Bio-oil
  - Phenols, Ketones, CAs, Ethers, Aldehydes, Alcohols
  - Bio-char

- **Gasification**
  - Syngas, $\text{H}_2$

Catalytic depolymerization

- **Cracking/hydrolysis**
  - Bio-oil
  - Phenols, Ketones, CAs, Ethers, Aldehydes, Alcohols
  - Bio-char

- **Reduction**
  - Phenol, BTX

- **Oxidation**
  - Vanillin, DMSO

Lignin types and conditions of extraction / production

- **Sulfur-containing**
  - **Kraft Lignin (KL)**
    - NaOH + Na₂S
    - ~170°C
  - **Lignosulphonates (LS)**
    - CaS (pH ≈ 1-2) or MgS (pH ≈ 3-5)
    - 125-170°C
    - 3-7 h

- **Sulfur-free**
  - **Soda Lignin (SL)**
    - 10-16% w/w NaOH
    - 140-170°C
    - Anthraquinone as catalyst (optional)
  - **Organosolv (OL)**
    - Solvents or mixtures (mainly ethanol)
    - ~200°C, 2.76 MPa
    - Lewis acids as catalysts (optional)
  - **Steam-explosion lignin (SEL)**
    - Short steam periods (1-10 min)
    - 185-235°C, 1.1-3.2 MPa
    - Rapid pressure release

- **New Generation “Greener”**
  - **Ionic Liquid Lignin (ILL)**
    - Organic salts in liquid stated below 100°C
  - **Deep Eutectic Solvent Lignin (DESL)**
    - HBD + HBA → Eutectic solvent with melting point usually <100°C

- **Others**
  - **Milled wood lignin (MWL)**
    - Series of extraction and concentration steps using different solvents
  - **Pyrolysis (PyL)**
    - ~450°C
    - Vapor residence time of 2 s
  - **Hydrolysis lignin (HL)**
    - Lignin obtained after acid or enzymatic hydrolysis

APC chromatogram and UV spectrum of Sodium lignosulfonate (Aldrich)

- Sample: 1000 µg/mL lignosulphonate; at 280 nm, Sampling rate: 20 pt/s (Hz); Injection volume: 50 µL;
- 3 APC QT columns: 450Å + 200Å + 125Å
- T = 30°C;
- 0.5 ml/min
- 0.3M NaNO₃/MeOH (80:20)

UV detector response

Match Plot

Auto-Scaled Chromatogram

at 280 nm
Molecular weight distribution of LB lignin and sequential solvent fractions determined by APC

APC elution patterns of guaiacol, β-O-4 dimer and guaiacol-based oligomer model

APC XT columns: 125 Å and 45 Å; 85 °C; 0.5 ml/min; DMSO + 0.5 % LiBr; UV at 280 nm

Thank for

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