APC and UHPLC characterization of products obtained by lignocellulose extraction and/or depolymerization

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Project meeting
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Interreg, SKHU/1902/4.1/001/Bioeconomy

Faculty of Chemical and Food Technology STU in Bratislava
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www.ttk.hu/palyazatok/bioeconomy
Advanced Polymer Chromatograph (APC) / Ultra High Performance Liquid Chromatograph (UHPLC)

- Diode-array UV-Vis detector (0.5 μl)
- Refractive Index detector (1.3 μl)
- Waters Empower 3 software
- Thermostat I for small columns (4.6 x 150 mm)
- Thermostat II for large columns (7.8 x 300 mm)
- Sample Manager
- Quaternary Solvent Manager

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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

\[
M_n = \frac{\Sigma N_i M_i}{\Sigma N_i}, \\
M_w = \frac{\Sigma N_i M_i^2}{\Sigma N_i M_i}, \\
D = \frac{M_w}{M_n}
\]
- Polymers are separated by hydrodinamic 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>Molar mass range (g/mole)</th>
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<td>90</td>
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<td>1.7</td>
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</table>
Lignocellulose structure

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

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

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

Lignin

➢ The second most abundant biopolymer on Earth
➢ Technical lignin from Kraft paper pulp process: \(7 \times 10^7\) t/year
➢ Commercially available Kraft lignin: \(10^5\) t/year
➢ Lignin valorization: as a macromolecule for polymer blending

➢ Polydispersity of Kraft lignin limits its application in polymer-based materials

➢ Solvent fractionation is a method to get well-defined Kraft lignin fractions with low dispersity

Solvent fractionation of LIGNIN

Sample: 1 mg/ml; Injection volume: 30 uL
Columns: Waters Acquity APC XT 200 Å, 125 Å, 45 Å, 80 °C
Eluent: 0.25 ml/min, DMSO + 0.5 % LiBr

➢ EtOAc lignin fraction has low dispersity ($M_n$ and $M_w$ values of 350 and 750 g/mol, resp.)
Eluent: 75ACN/25H₂O + 0.1% TEA
ACN: acetonitrile
TEA: triethylamine

Eluent: 75ACN/25H₂O

Column: 100 mm, XBridge BEH Amide XP column, T: 50°C,
Eluent: 0.13 ml/min 75ACN/25H₂O,
Sample: 15 µl, 1mg/ml D-fructose,
RI detector (40°C)

➢ No mutarotation in presence of TEA

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Mutarotation of sugars

Alpha (α) and beta (β) isomers ("anomers") differ in the orientation of the OH at the C-1 hemiacetal carbon

Example: D-glucose

"alpha" (α) isomer:

C5-CH2OH (up) and C1-OH (down) are on opposite faces of the ring

α-D-Glucose
drawn as "chair"
Specific rotation: [α]D 20 + 112°

"beta" (β) isomer:

C5-CH2OH (up) and C1-OH (up) are on the same face of the ring

β-D-Glucose
drawn as "chair"
Specific rotation: [α]D 20 + 18.7°

Note different specific rotations!
UHPLC chromatograms of C5 and C6 sugars

Column: 100 mm, XBridge BEH Amide XP column, T: 50 °C,
Eluent: 75ACN/25H₂O+ 0.1%TEA, 0.13 ml/min
Sample: 15 µl, 1mg/ml sugars
RI detector (40 °C)
Thank for

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Szabó Blanka  

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Vikár Anna  
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