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

Magdolna R. Mihályi, József Valyon, Gyula Novodárszki
Research Centre for Natural Sciences

Project meeting
„Joint chemical laboratory for the service of bioeconomy in the Slovak-Hungarian border region”
Interreg, SKHU/1902/4.1/001/Bioeconomy

Faculty of Chemical and Food Technology STU in Bratislava
Radlinského 9, 812 37 Bratislava, Slovak Republic
28 September, 2022

ttk

Interreg Slovakia-Hungary EUROPEAN UNION

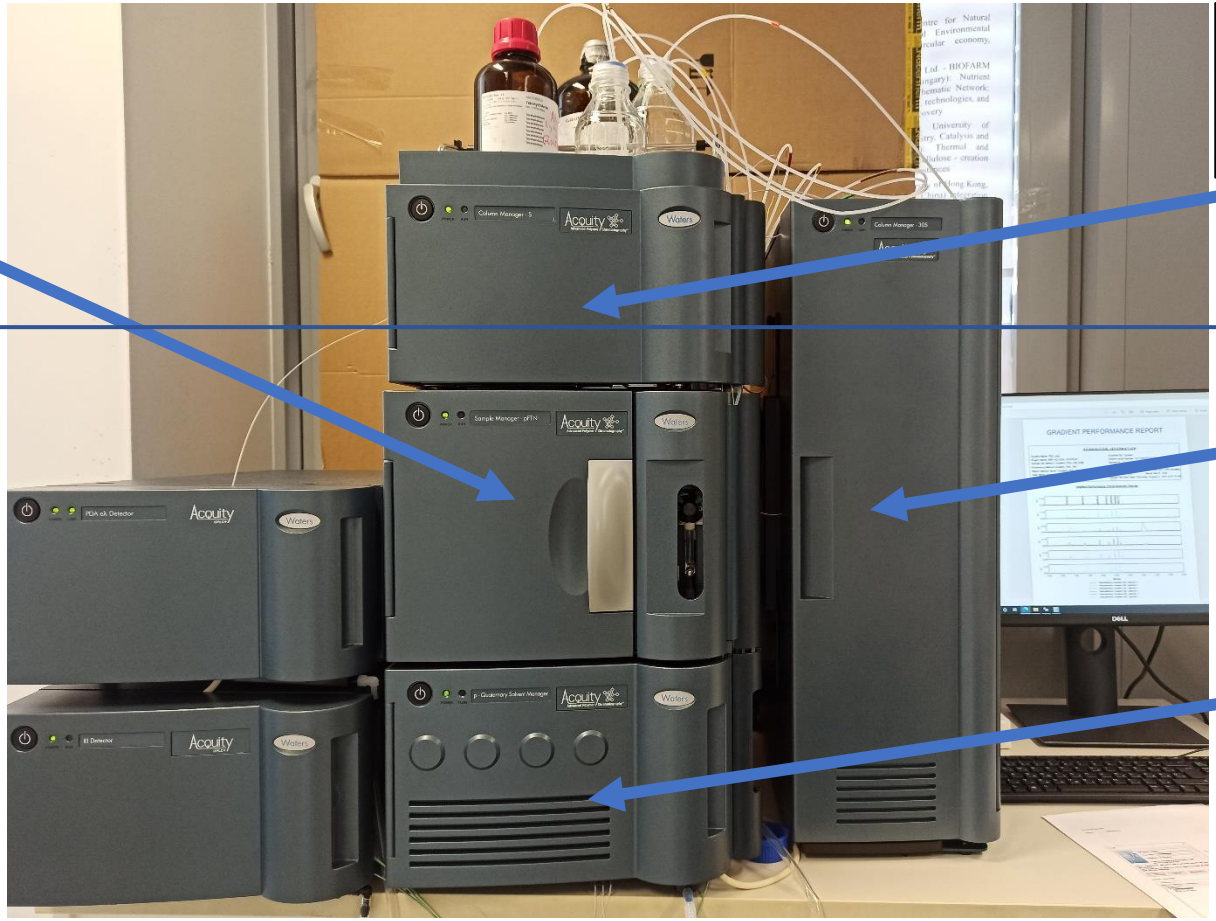
Building Partnership

STU SLOVAK UNIVERSITY OF TECHNOLOGY IN BRATISLAVA

www.ttk.hu/palyazatok/bioeconomy

Advanced Polymer Chromatograph (APC) / Ultra High Performance Liquid Chromatograph (UHPLC)

1000 bar



Thermostat I
for small columns
(4.6 x150 mm)

Thermostat II
for large columns
(7.8 x 300 mm)

Sample
Manager

Diode-array
UV-Vis detector
(0.5 µl)

Refractive
Index
detector
(1.3 µl)

Quaternary
Solvent
Manager

Waters
Empower 3
software

SEC/GPC/APC

SEC: Size Exclusion Chromatography (1959, Porath and Flodin)
GPC: Gel-Permeation Chromatography (1974, Down Chemical. Co.)
stacionary phase: synthetic polymer, e.g. PS
APC: Advanced Polymer Chromatography (2004, Waters Co., UPLC)
stacionary phase: rigid, 2.5 μm-size modified silica particles with pore size of 45Å - 900Å.

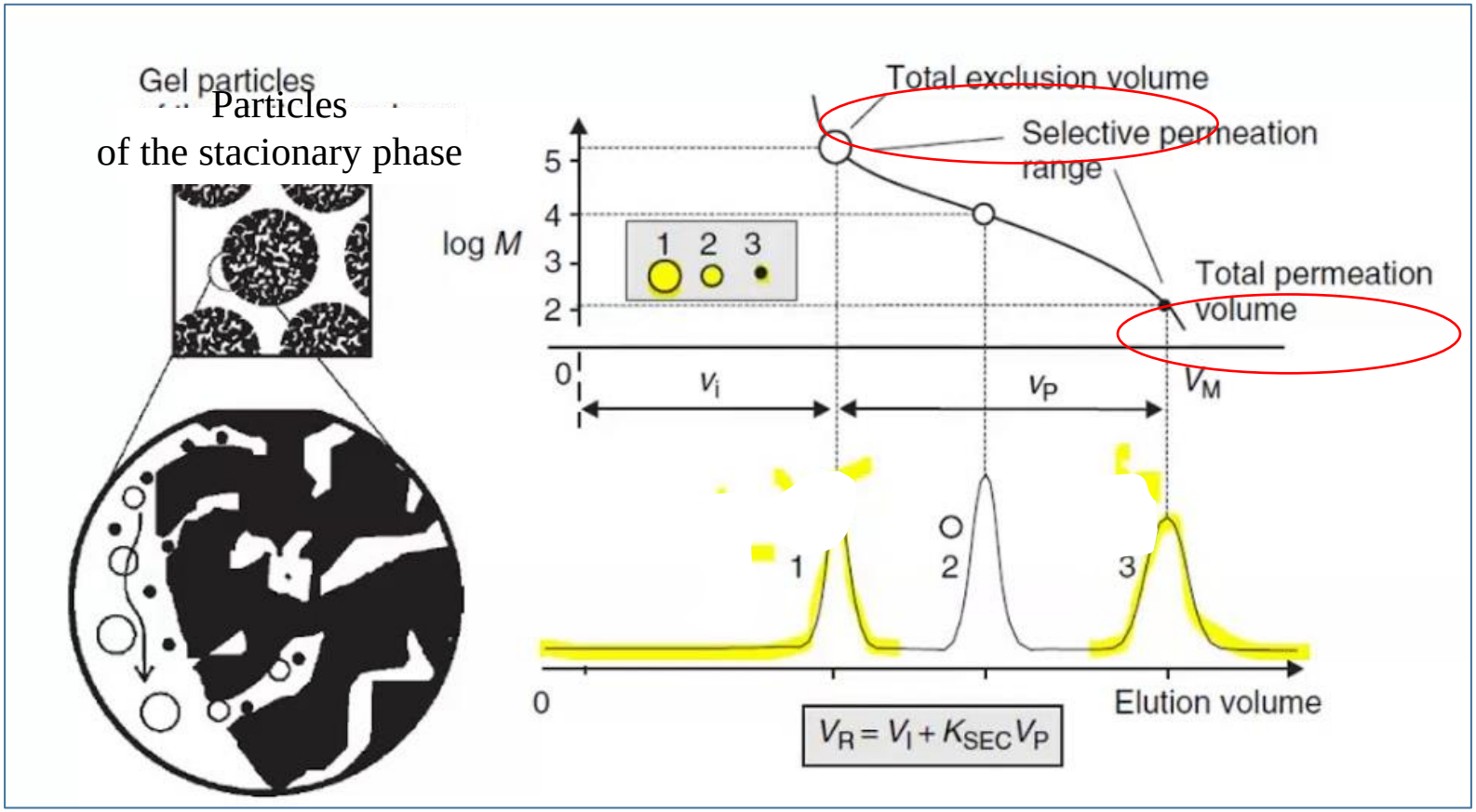
SEC/GPC/APC

- molar mass averages,
- molar mass distribution of synthetic and biopolymers

number average molecular weight	$M_n = \Sigma N_i M_i / \Sigma N_i$,
weight average molecular weight	$M_w = \Sigma N_i M_i^2 / \Sigma N_i M_i$
polydispersity index	$D = M_w / M_n$

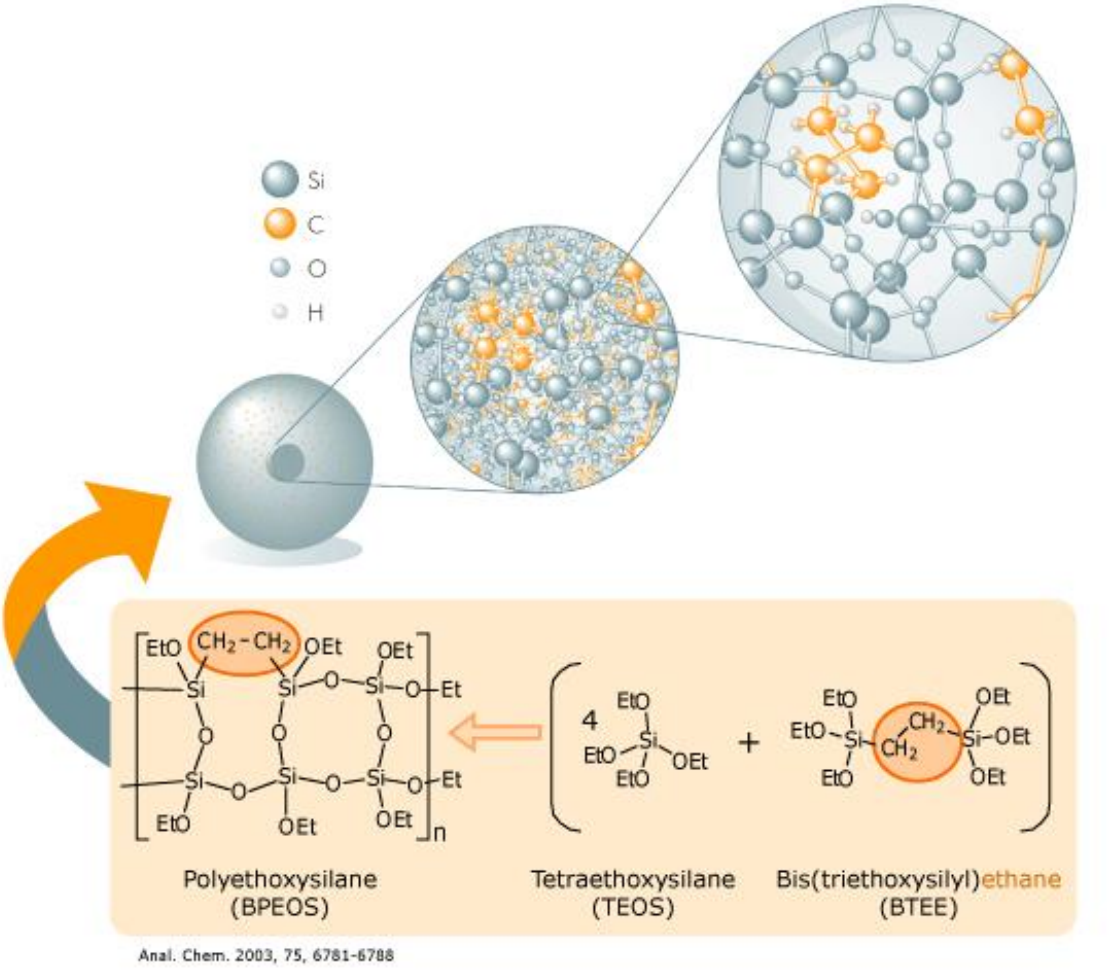
SEC principle

- 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



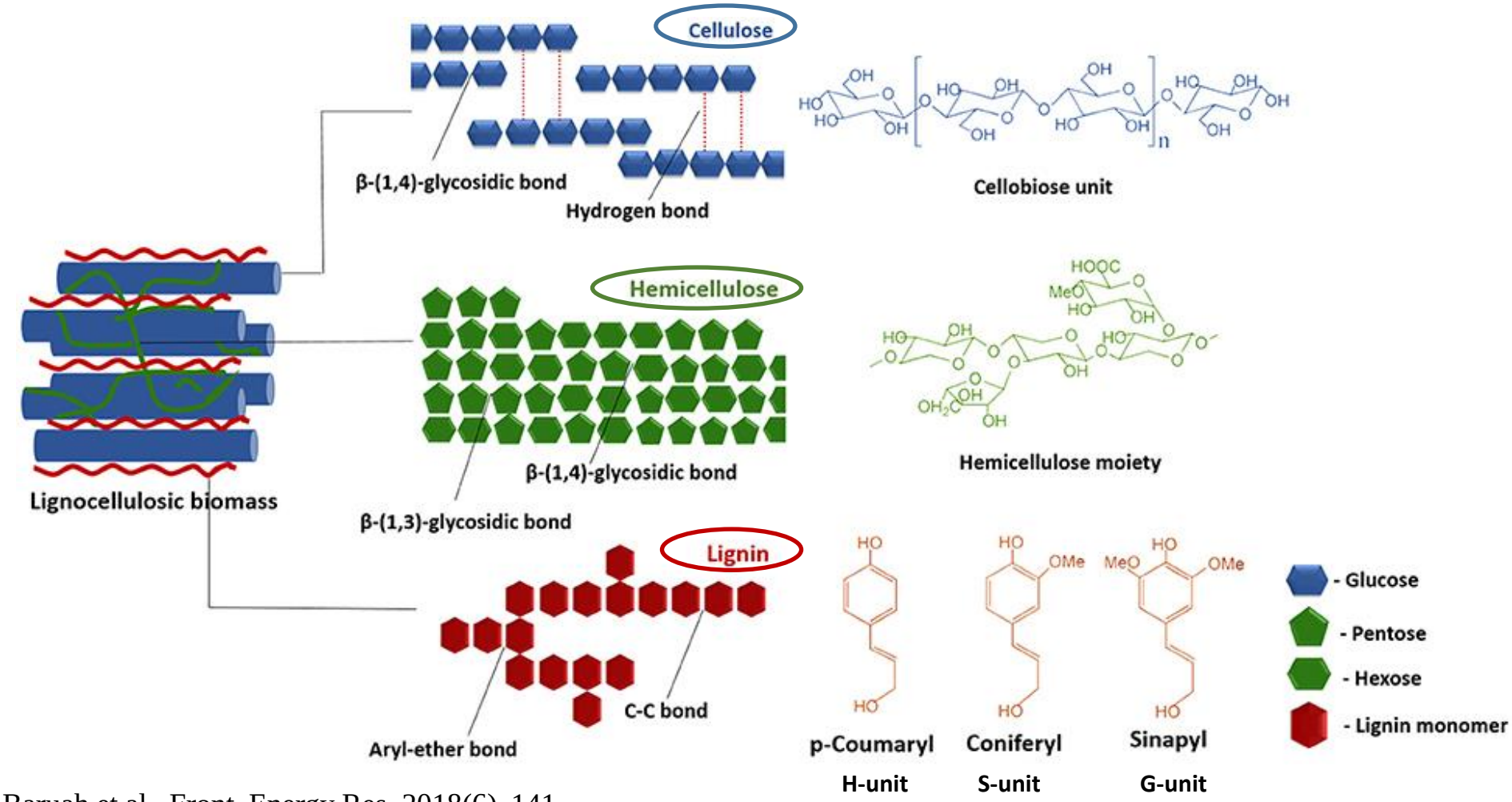
- strong and rigid particles
- particle size: 1.7 and 2.5 μm
- resist shrinking, swelling
- easy solvent swithing
- high reproducibility

APC columns

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

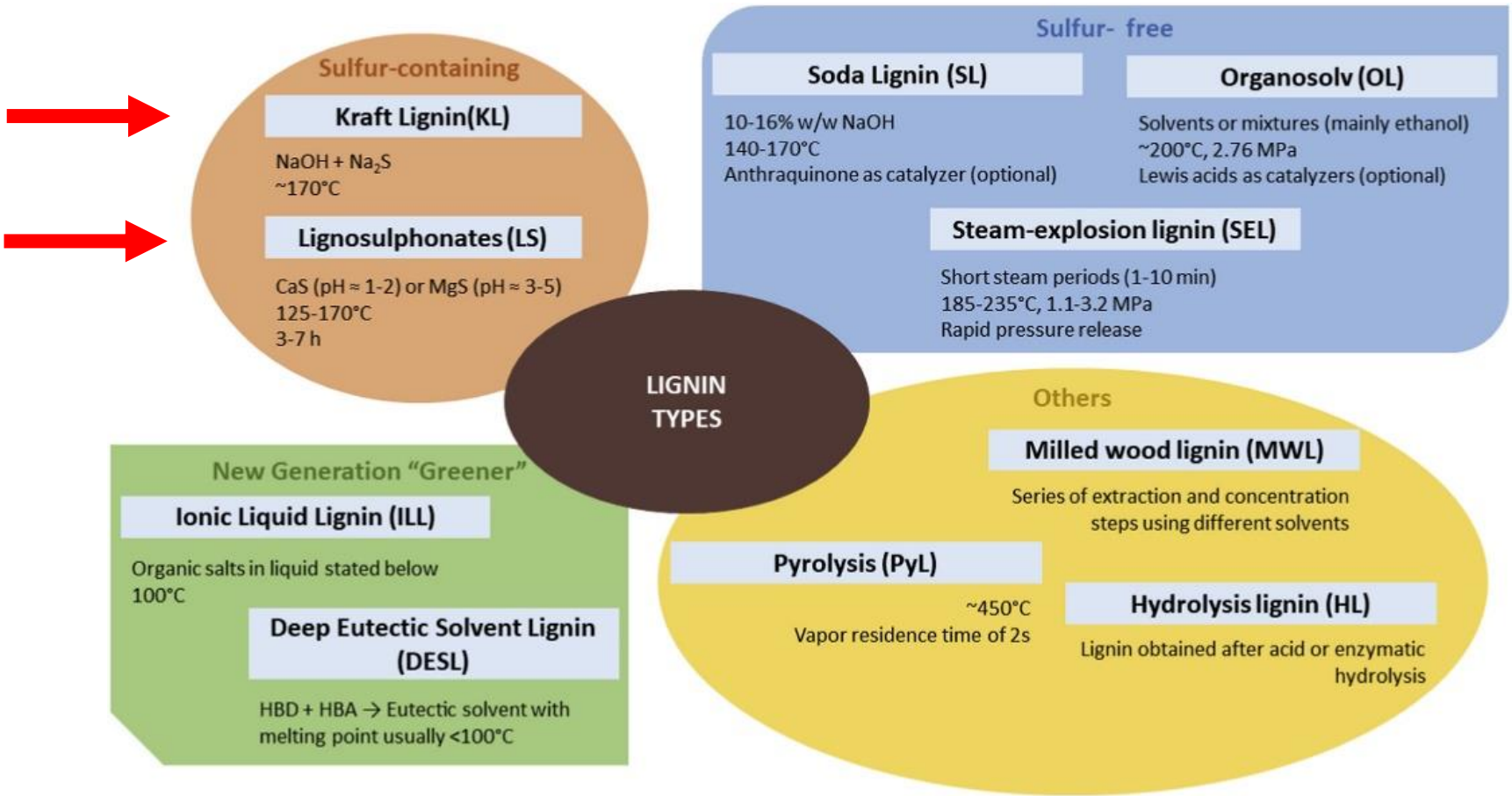
	Solvent	Temp. limit (°C)	pH	Pore size (Å)	Particle size (µm)	Molar mass range (g/mole)
ACQUITY APC XT 45	organic	90	1-11	45	1.7	200 - 5 000
ACQUITY APC XT 125	organic	90	1-11	125	2.5	1 000 - 30 000
ACQUITY APC XT 200	organic	90	1-11	200	2.5	3 000 – 70 000
ACQUITY APC XT 450	organic	90	1-11	450	2.5	20 000 – 400 000
ACQUITY APC XT 900	organic	90	1-11	900	2.5	300 000 - 2 000 000
ACQUITY APC AQ 45	aqueous	45	1-9	45	1.7	200 - 5 000
ACQUITY APC AQ 125	aqueous	45	1-9	125	2.5	1 000 - 30 000
ACQUITY APC AQ 200	aqueous	45	1-9	200	2.5	3 000 – 70 000
ACQUITY APC AQ 450	aqueous	45	1-9	450	2.5	20 000 – 400 000
ACQUITY APC AQ 900	aqueous	45	1-9	900	2.5	300 000 - 2 000 000

Lignocellulose structure



Baruah et al., Front. Energy Res. 2018(6), 141.

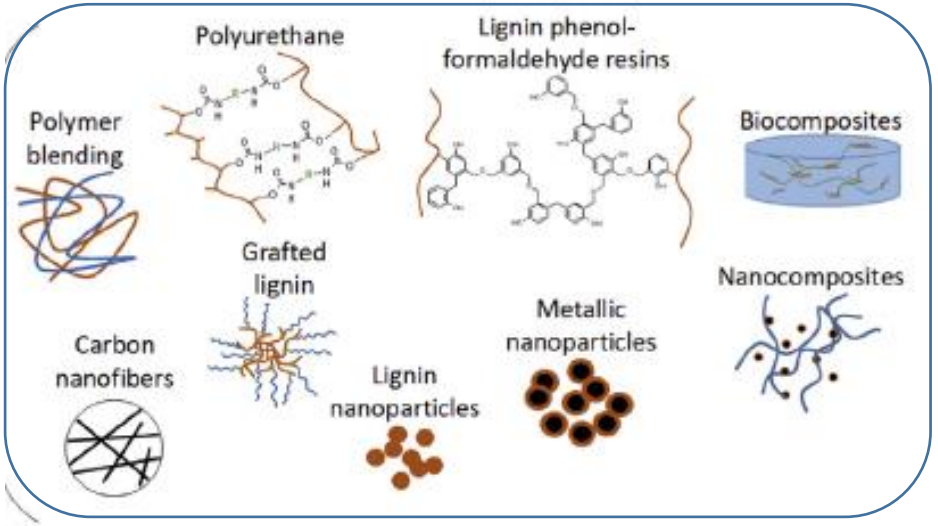
Lignin types and conditions of extraction / production



Torres L. A. Z. et al., Journal of Cleaner Production, 263 (2020) 121499.

Lignin

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

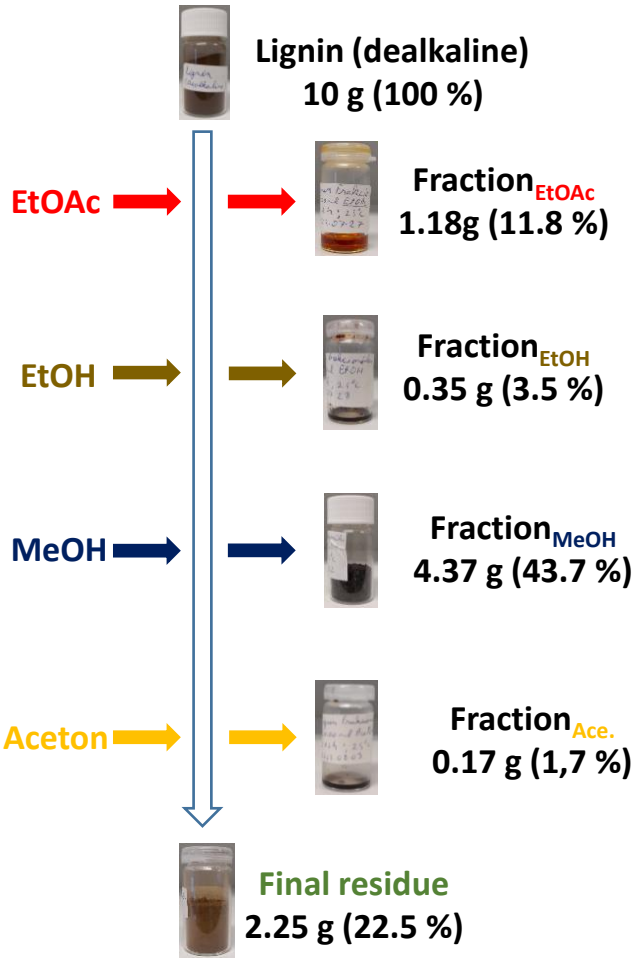


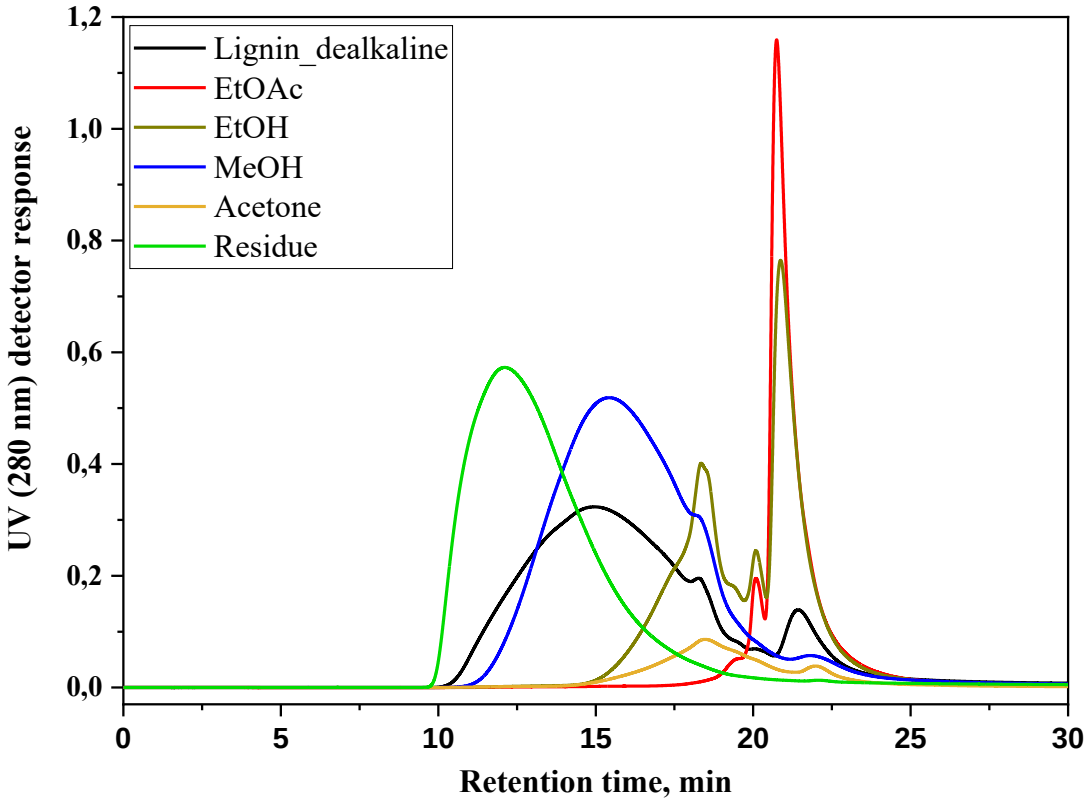
- Polydispersity of Kraft lignin limits its application in polymer-based materials

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

Giummerrala et al., *ACS Sustainable Chem. Eng.* 2020, 8, 1112−1120.

Solvent fractionation of LIGNIN

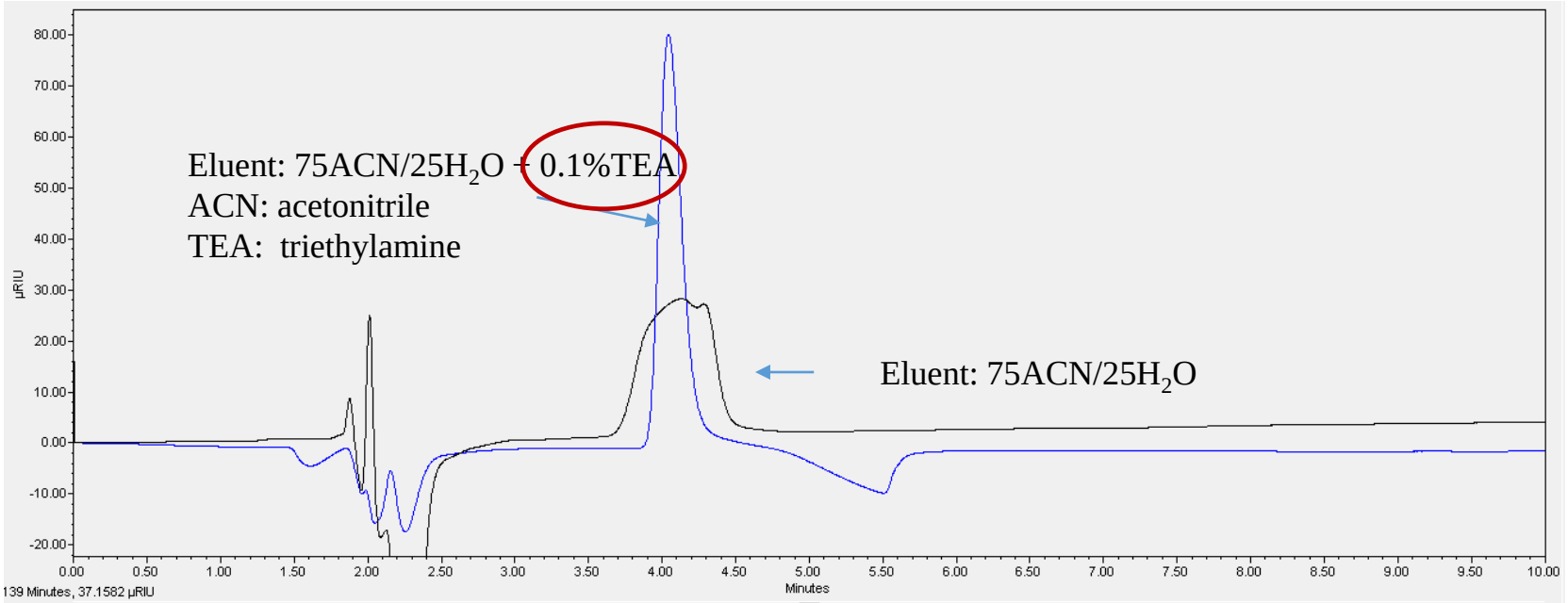




Sample: 1 mg/ml; Injection volume: 30 uL
Columns: Waters Acquity APC XT 200 Å, 125 Å, 45 Å, 80 °C
Eluent: 0.25 ml/min, DMSO + 0.5 % LiBr

- **EtOAc lignin fraction has low dispersity (M_n and M_w values of 350 and 750 g/mol, resp.)**

UHPLC chromatograms of D-fructose



Visible	Time Offset (Minutes)	Smoothing Type	Smoothing Level	Vial	Injection	SampleName	Sample Type	Date Acquired	Acq Method Set	Injection Volume (uL)	Channel	Channel Description	Injection Status	Chann Status
☑	0.000	None	None	2:A,2	1	1mg/ml D-fructose	Unknown	Monday, April 25, 2022 5:04:01 PM CEST	Cukor proba 50C 100A	15.00	RI	RI	Complete	Acquisition Fin
☑	0.000	None	None	2:A,2	1	1mg/ml D-fructose	Unknown	Wednesday, April 27, 2022 4:26:07 PM CEST	Cukor proba 50C 100A	15.00	RI	RI	Complete	Acquisition Fin

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**

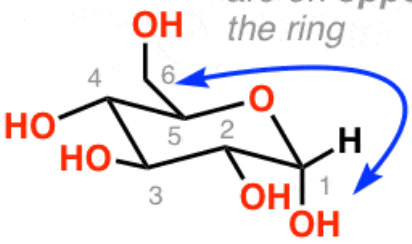
Mutarotation of sugars

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:

*C_5-CH_2OH (up) and C_1-OH (down) are on **opposite** faces of the ring*



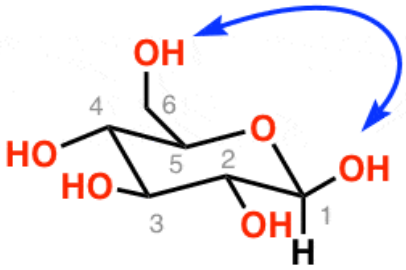
α-D-Glucose

drawn as "chair"

Specific rotation: $[\alpha]_D^{20}$ + 112°

"beta" (β) isomer:

*C_5-CH_2OH (up) and C_1-OH (up) are on the **same** face of the ring*



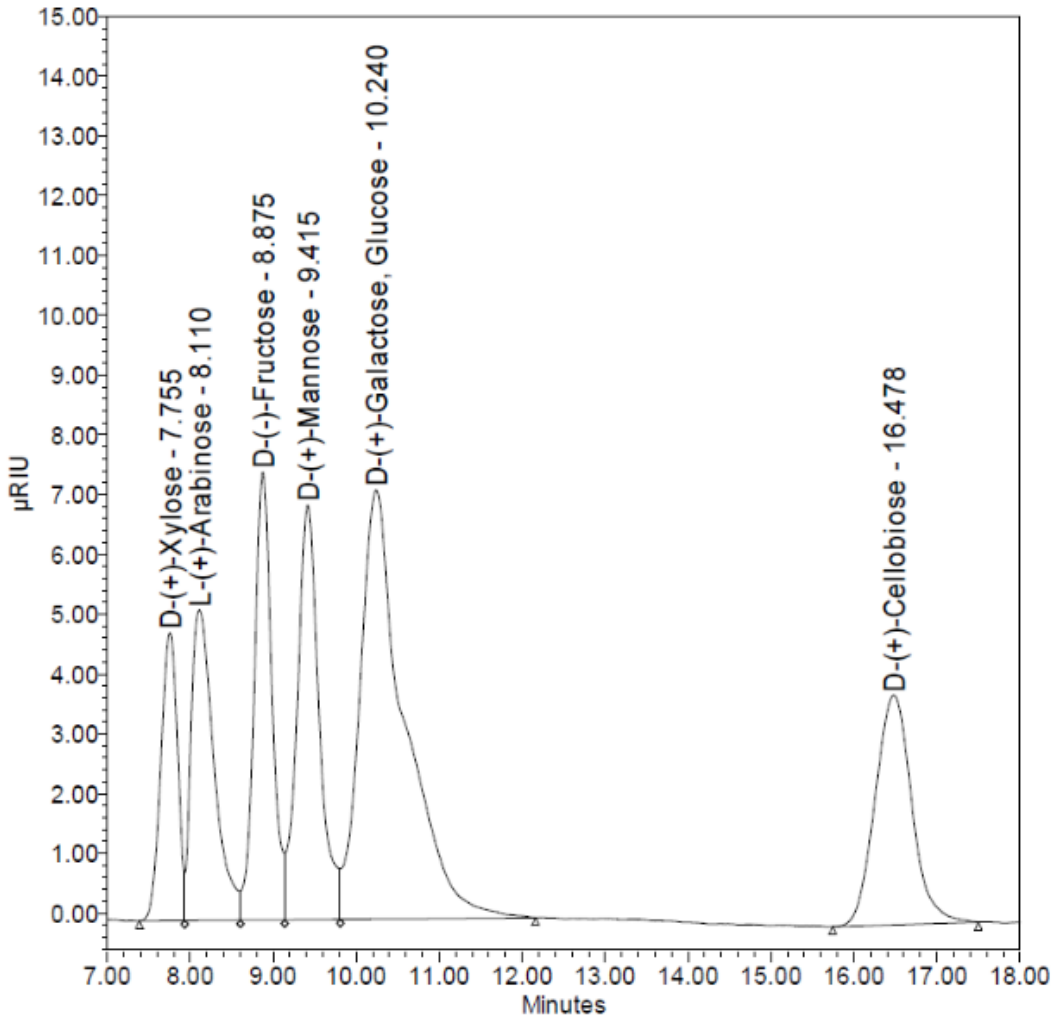
β-D-Glucose

drawn as "chair"

Specific rotation: $[\alpha]_D^{20}$ + 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/25H_2O+ 0.1%TEA, 0.13 ml/min
Sample: 15 µl, 1mg/ml sugars
RI detector (40 °C)

Thank for

Barthos Róbert
Lónyi Ferenc
Solt Hanna
Szabó Blanka
Szegedi Ágnes
Vikár Anna
Valyon József
Kaszonyi Alexander
Wellischné Farkas Ágnes
Fekete Miklós
Izsák Lívia
Horváth Blazej

Thank you for your kind attention!

Acknowledgement

European Regional Development Fund (Interreg, SKHU/1902/4.1/001/Bioeconomy)
www.skhu.eu

www.ttk.hu/palyazatok/bioeconomy