Characterization of biopolymers by advanced polymer chromatography (APC)

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

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ttk

Interreg Slovakia-Hungary EUROPEAN UNION

Building Partnership

STU SLOVAK UNIVERSITY OF TECHNOLOGY IN BRATISLAVA

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.

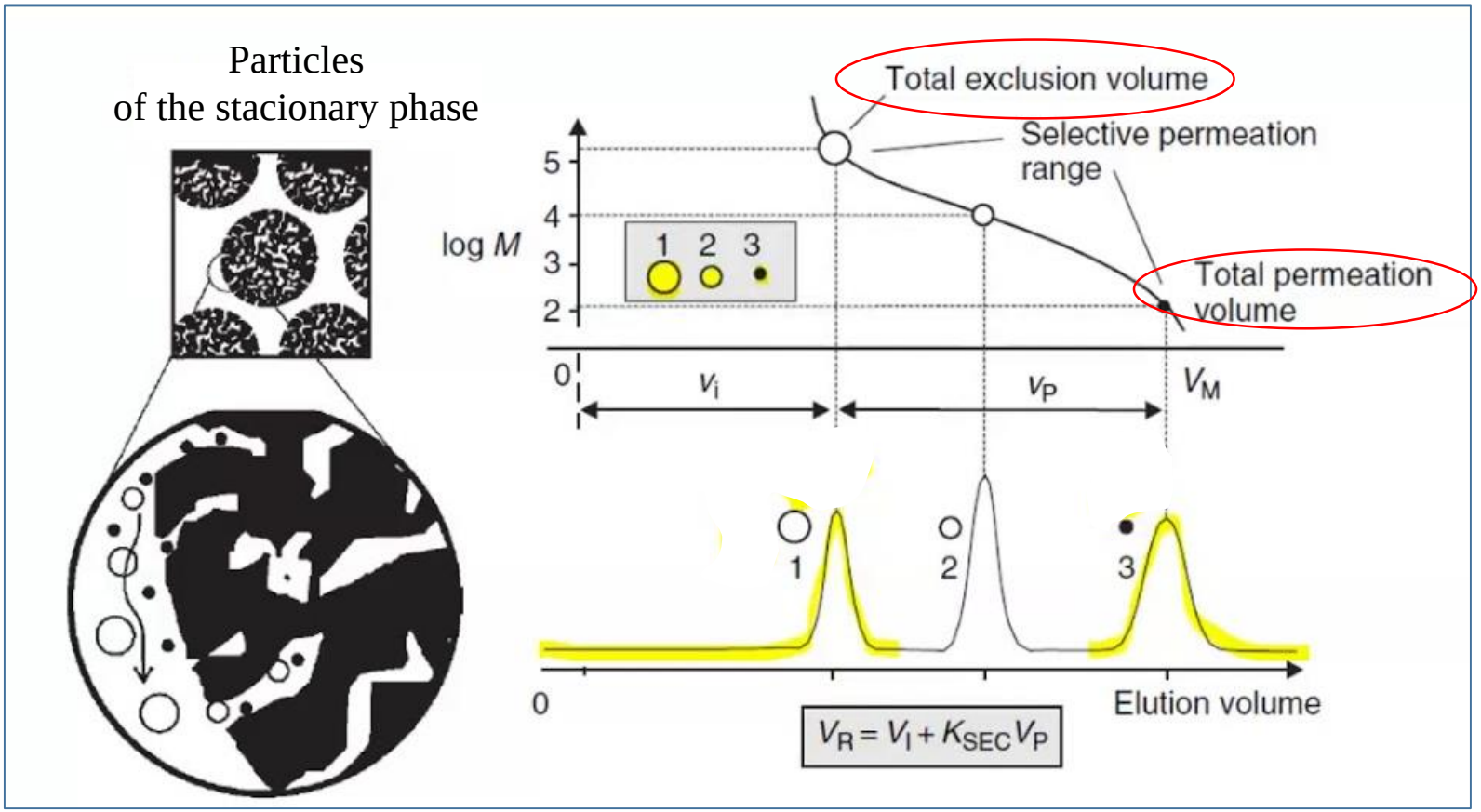
SEC/GPC/APC

- 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	$D = M_w M_n$

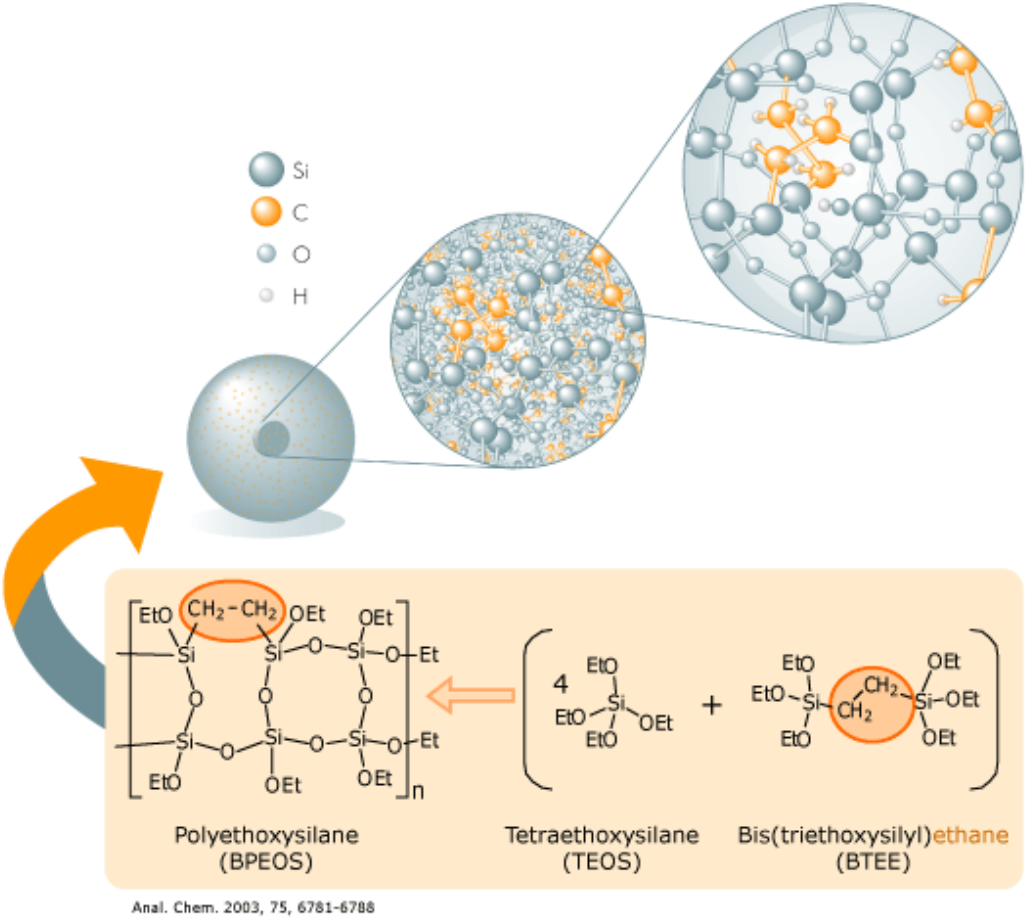
SEC principle

- 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



Anal. Chem. 2003, 75, 6781-6788

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

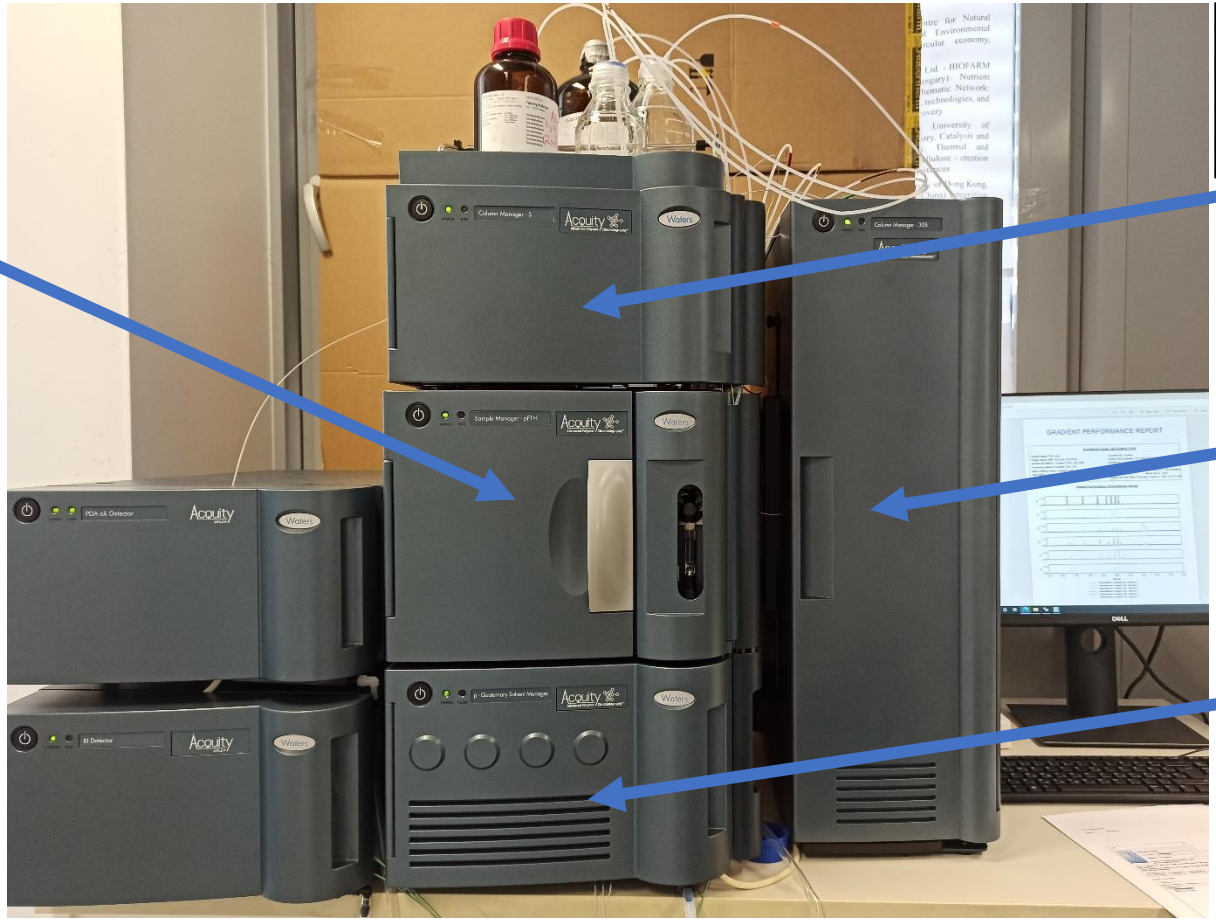
APC columns

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

	Solvent	Temp. limit (°C)	pH	Pore size (Å)	Particle size (µm)	Linear 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

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

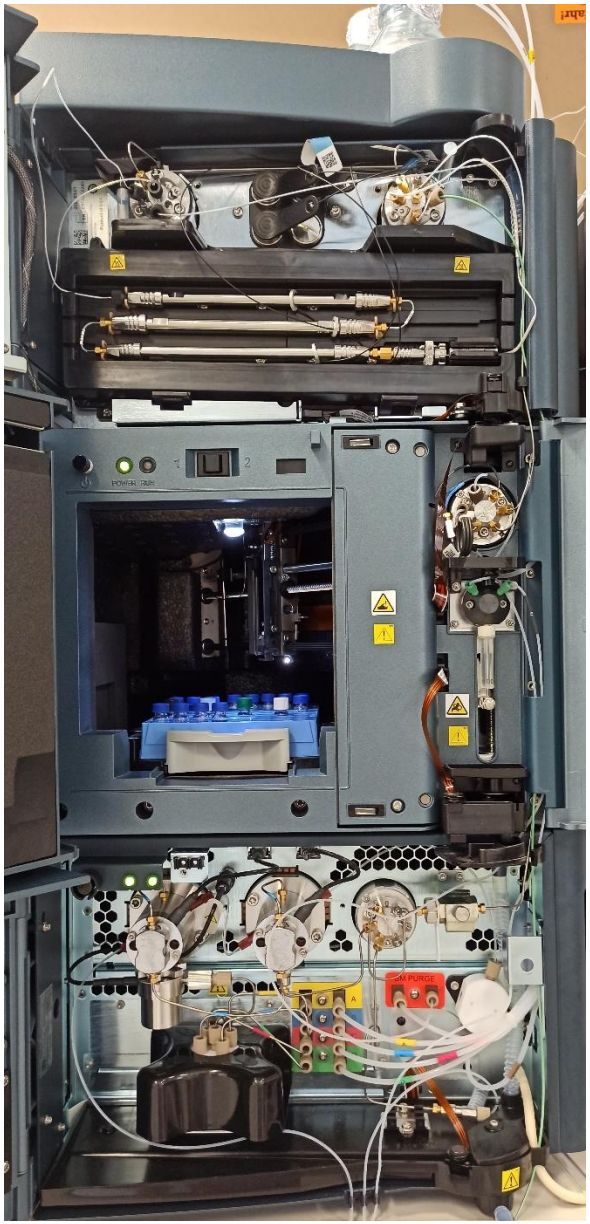
Diode-array
UV-Vis detector
(0.5 µl)

Refractive
Index
detector
(1.3 µl)

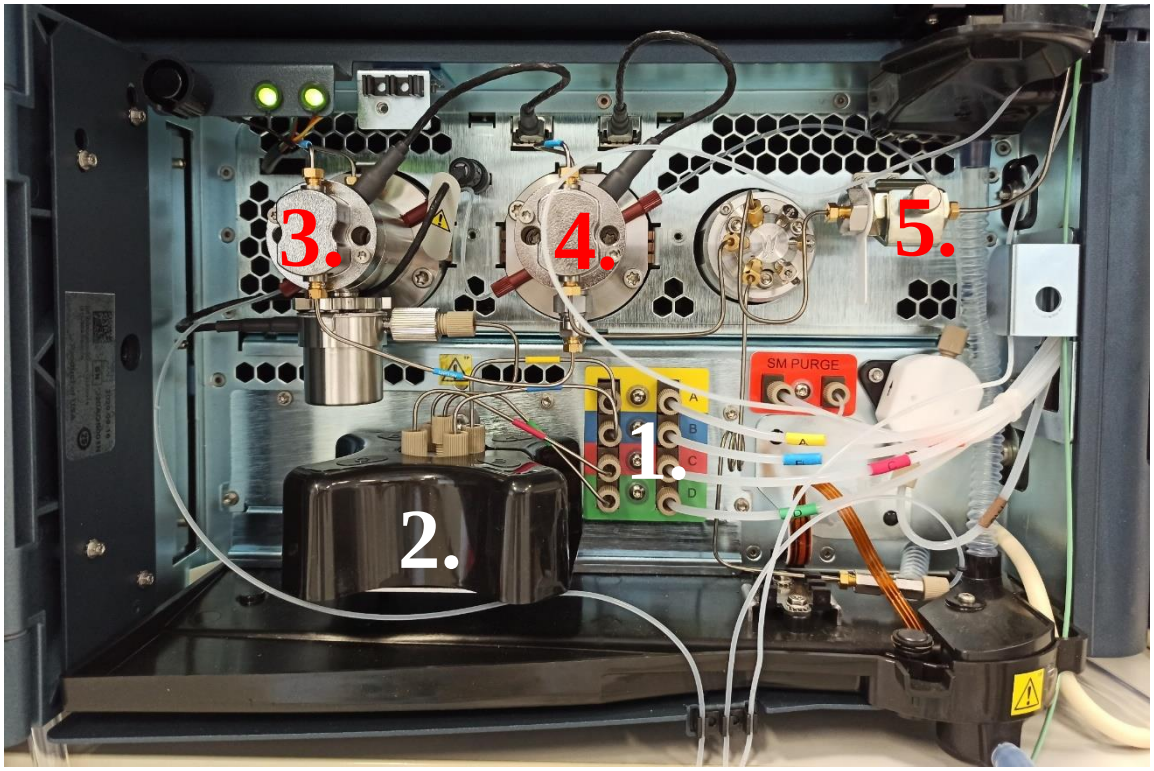
Quaternary
Solvent
Manager

Waters Empower 3 software

Behind doors…

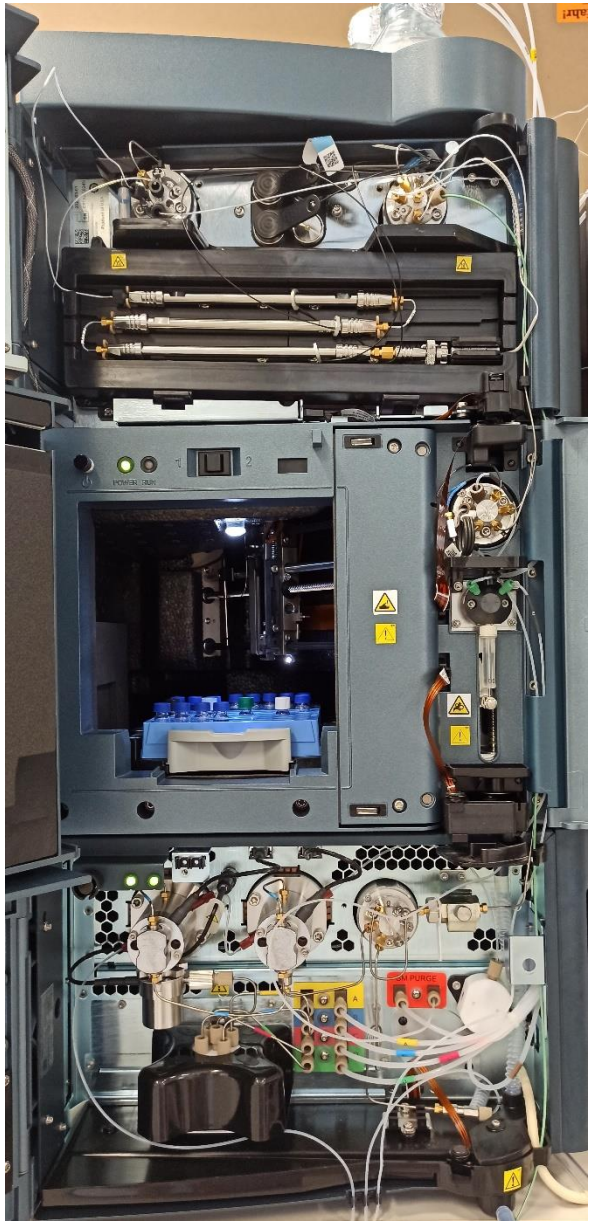


Quaternary Solvent Manager

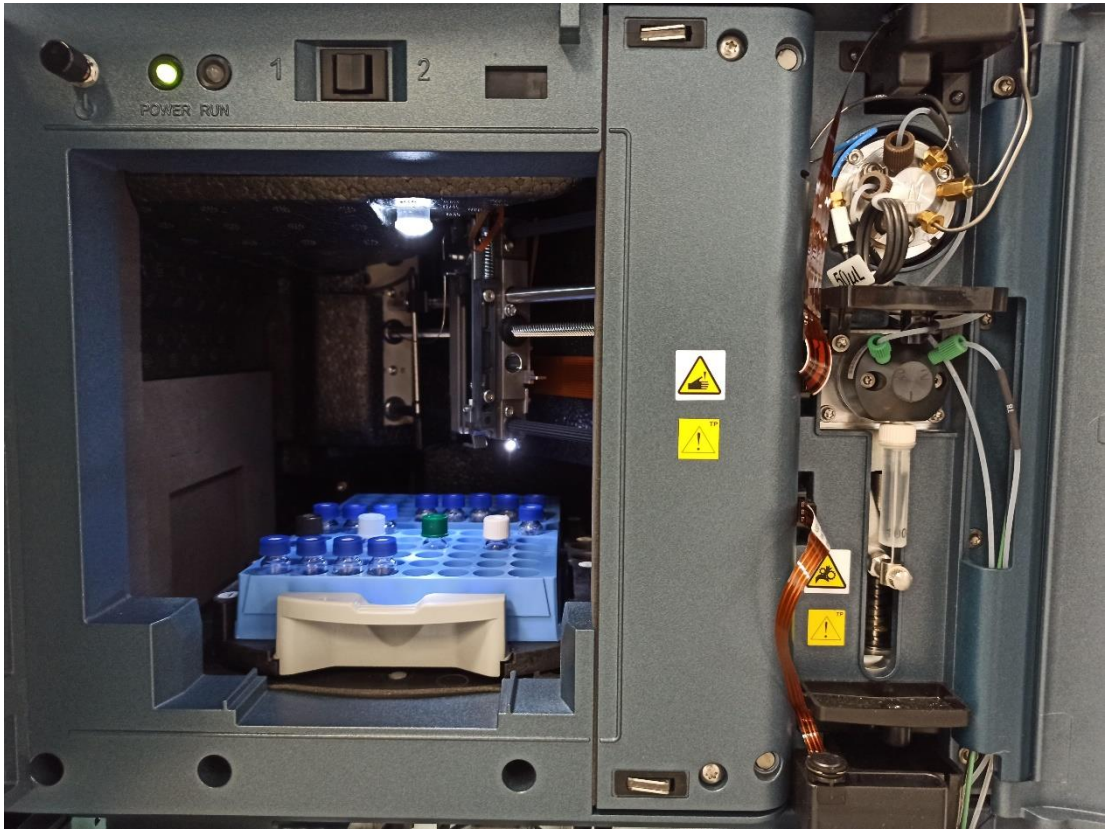


1. Vacuum degasser, four chambers
2. Propotioning valve
3. Pump I
4. Pump II
5. Mixer

Behind doors…

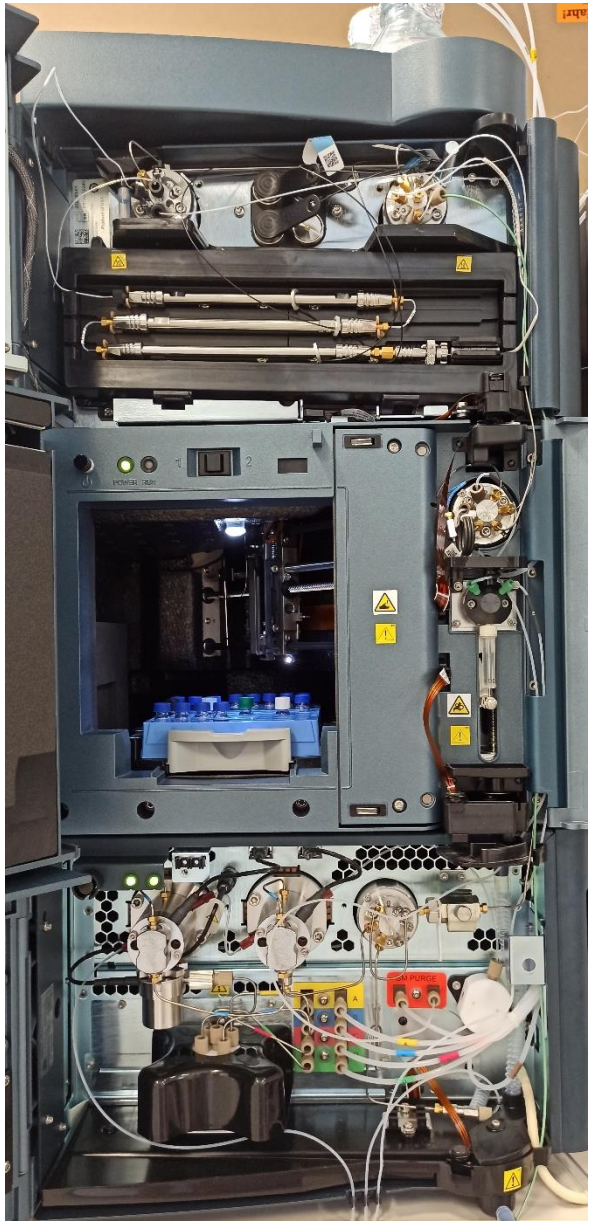


Sample Manager with flow-through needle

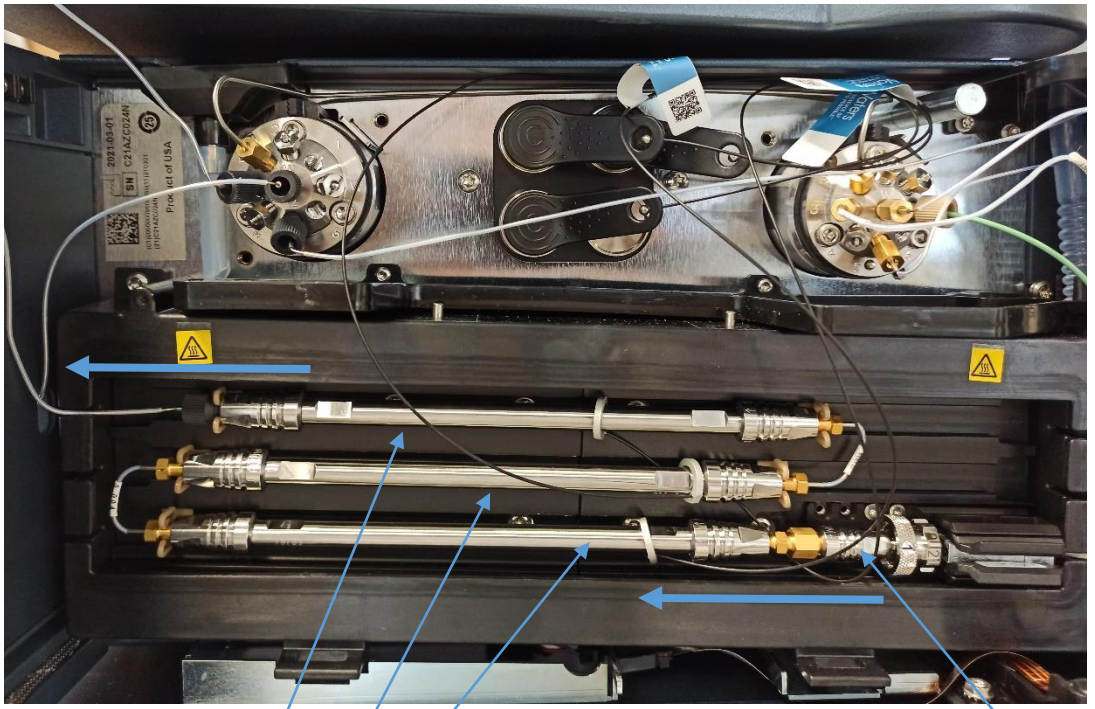


Two plates for 96 samples in 2-mL vial holders
Temperature range: 4-40 °C

Behind doors…



Thermostat I for small columns (4.6 x 150 mm)

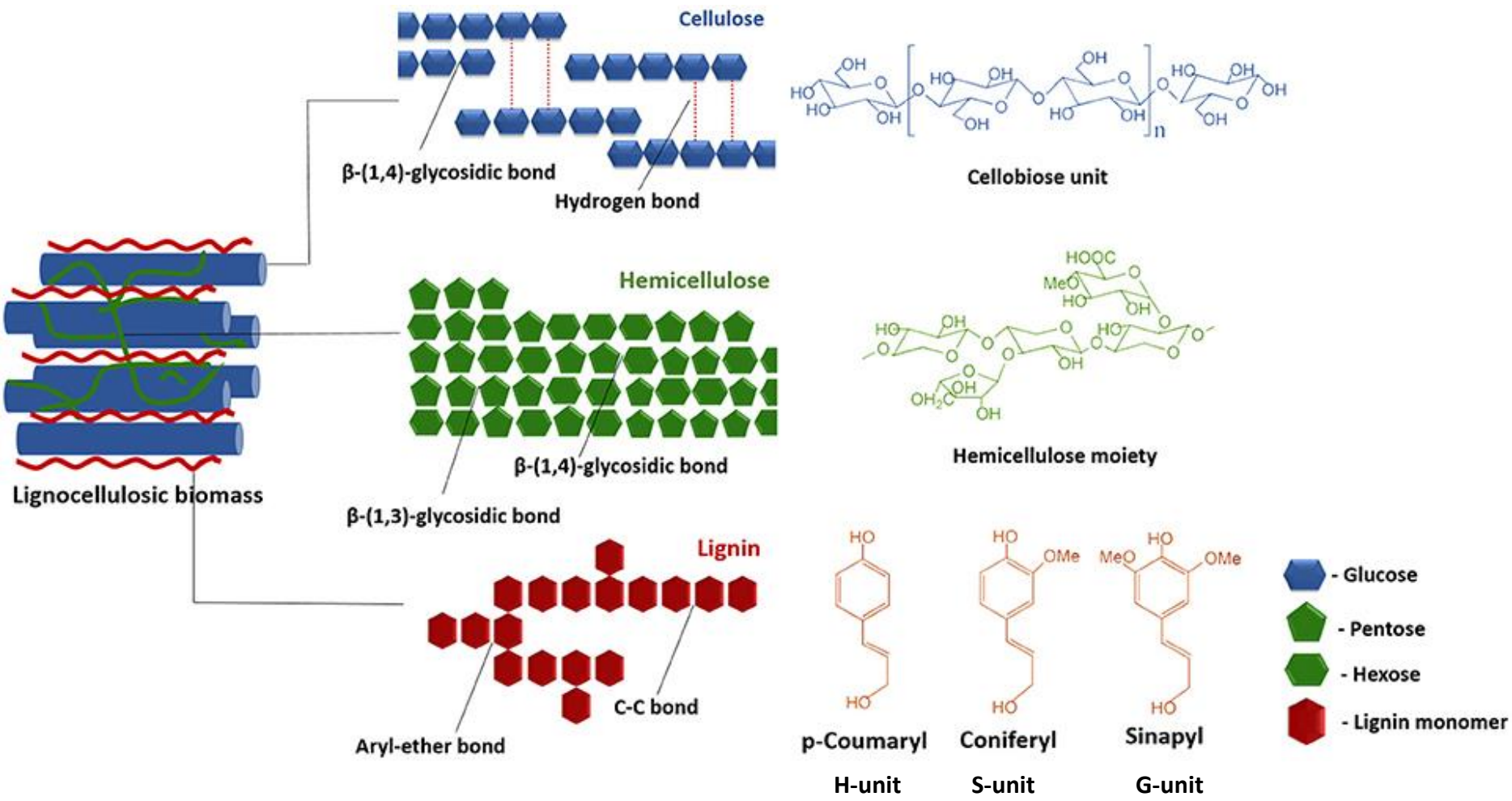


1. column: 450 Å
2. column: 200 Å
3. column: 125 Å

0.2 µm Filter

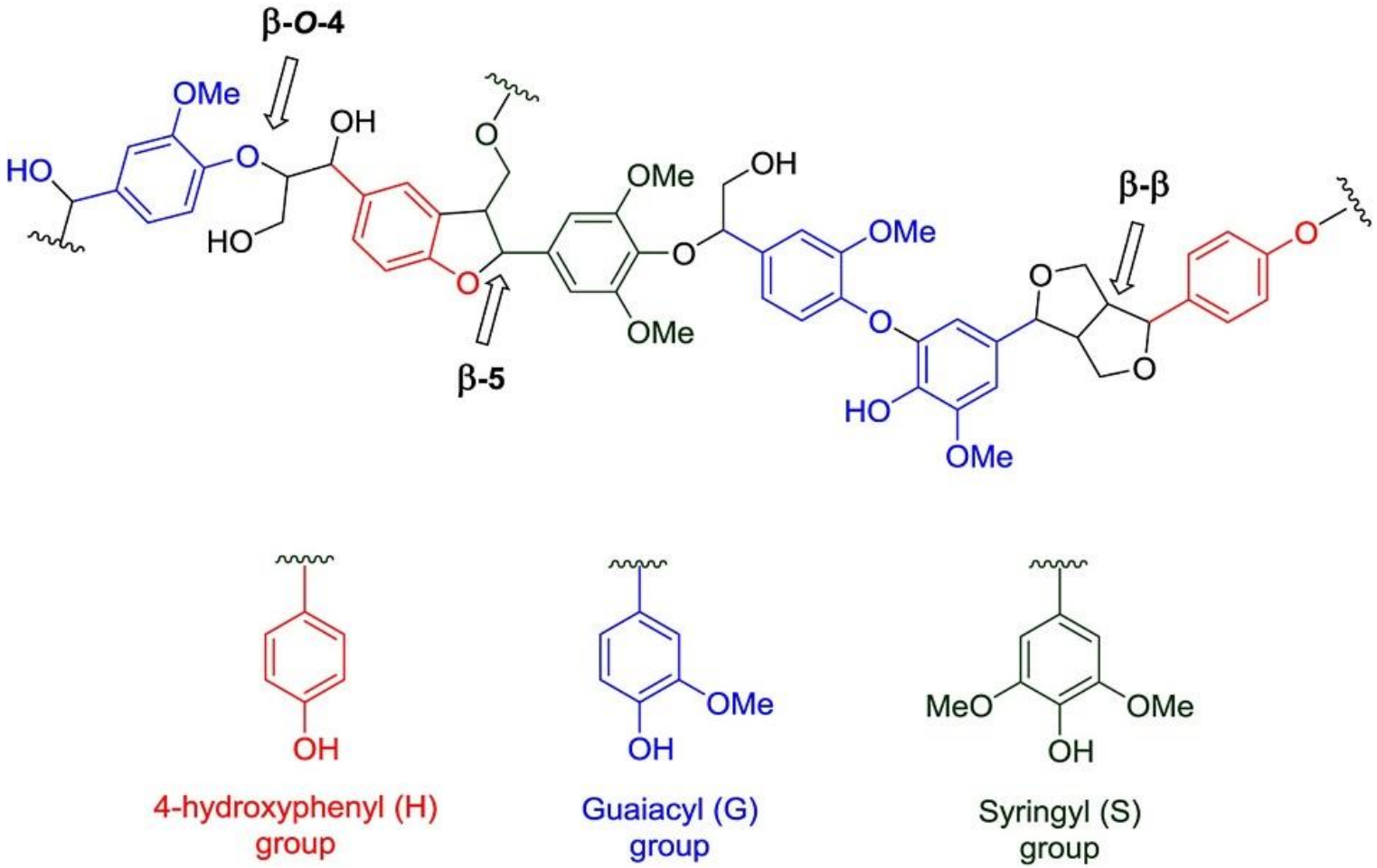
Temperature range: 4 – 90 °C

Lignocellulose structure



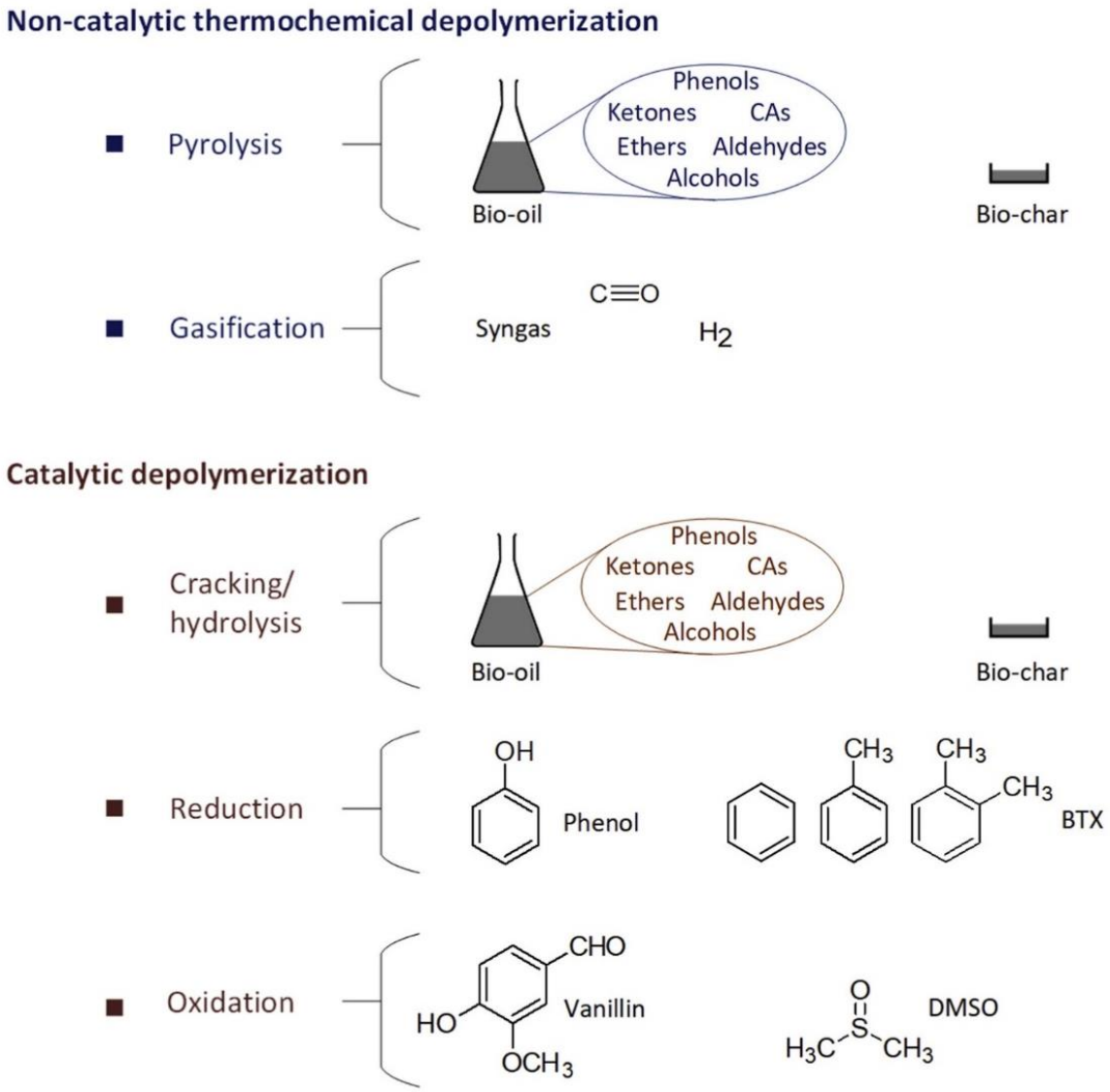
Baruah et al., Front. Energy Res. 2018(6), 141.

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



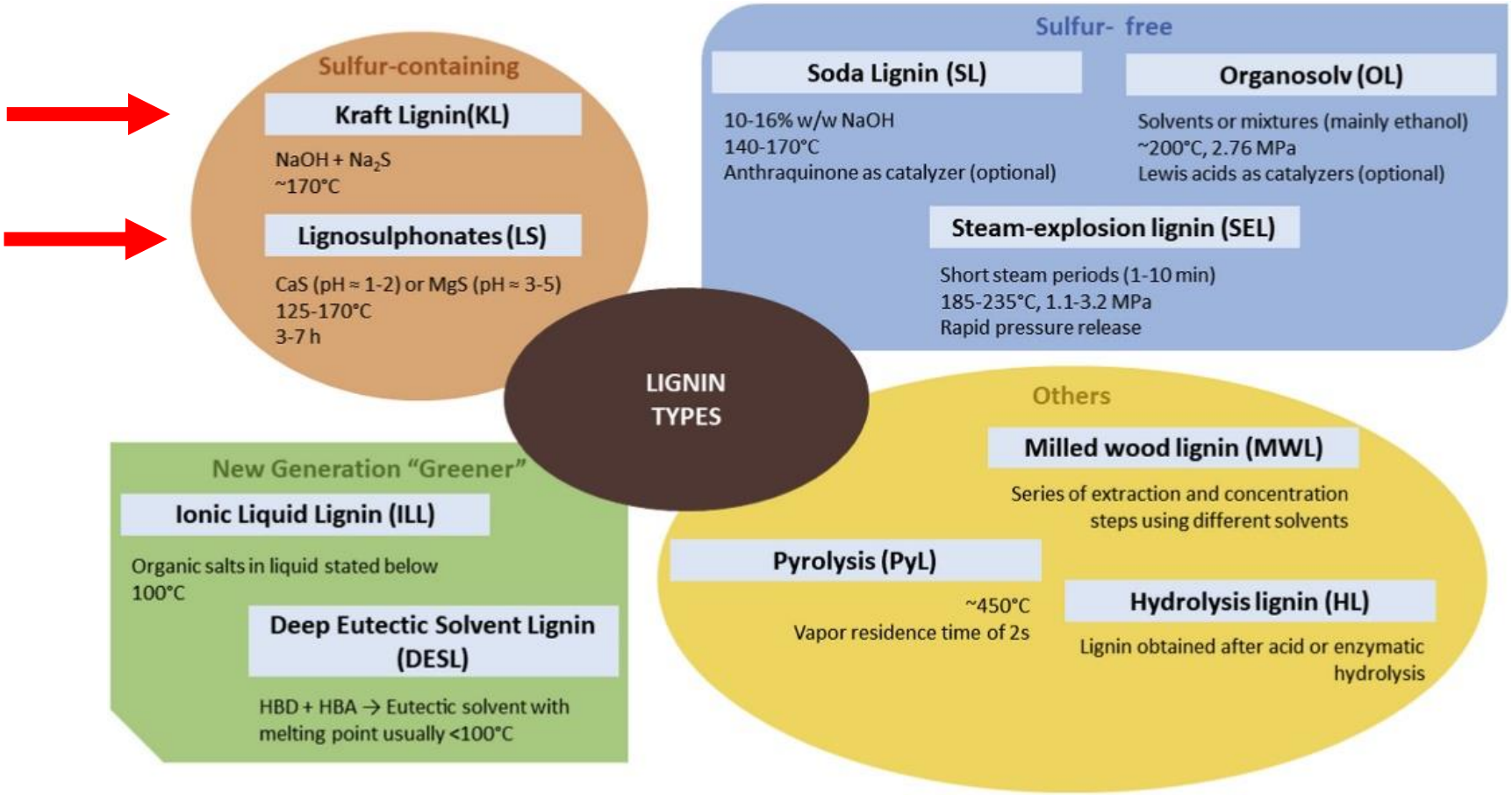
Gonzalo G.et al. / Journal of Biotechnology 236 (2016) 110–119.

Lignin valorization



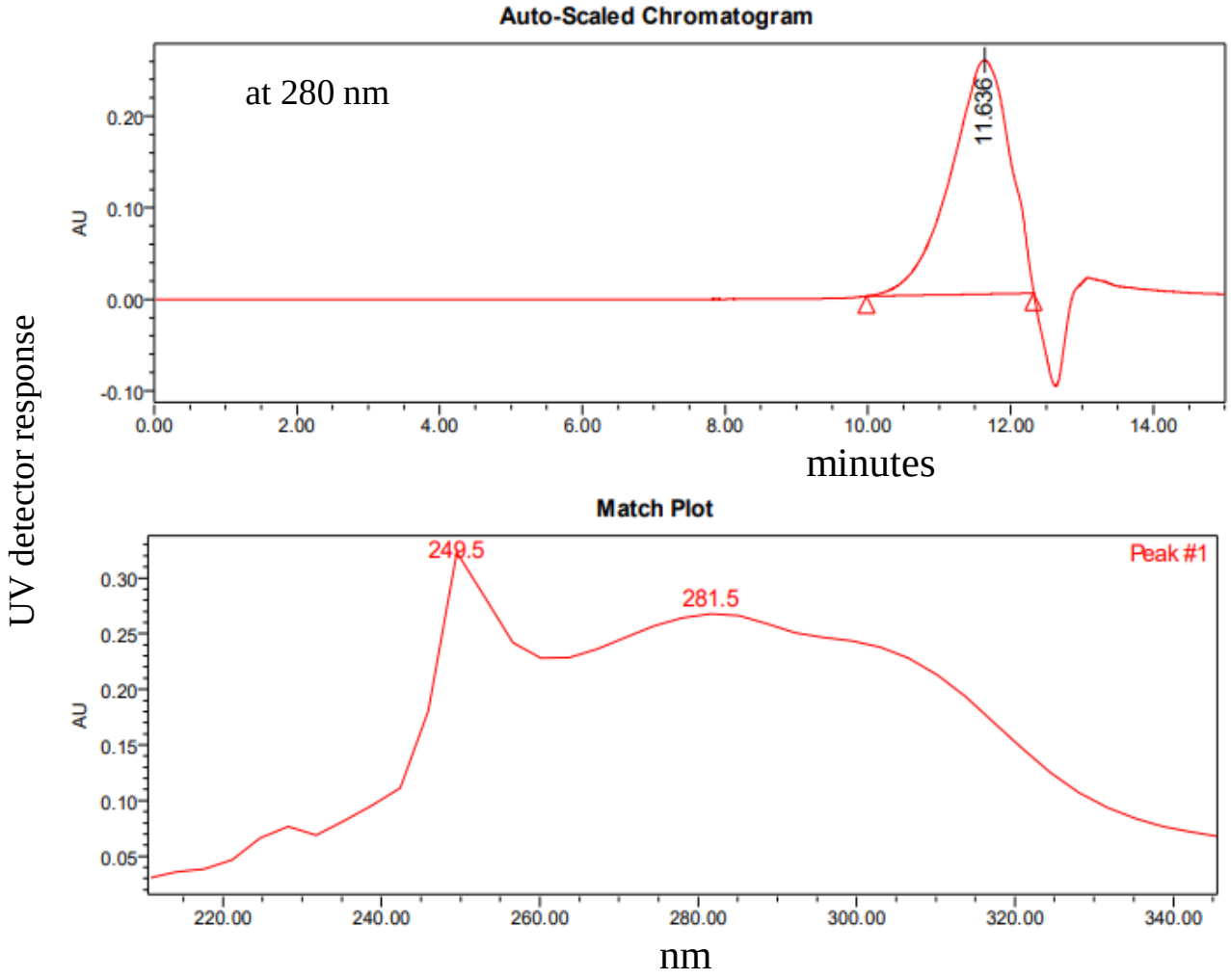
Torres L. A. Z. et al., Journal of Cleaner Production, 263 (2020) 121499.

Lignin types and conditions of extraction / production



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

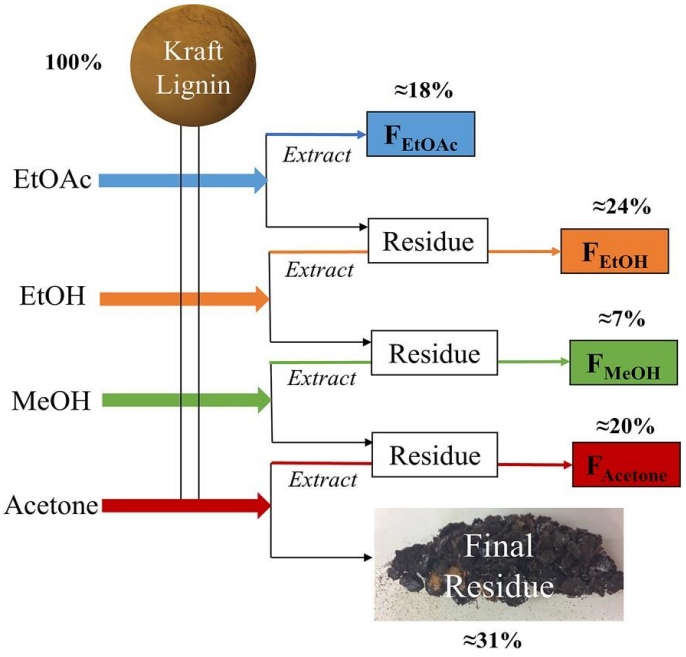
APC chromatogram and UV spectrum of Sodium lignosufonate (Aldrich)

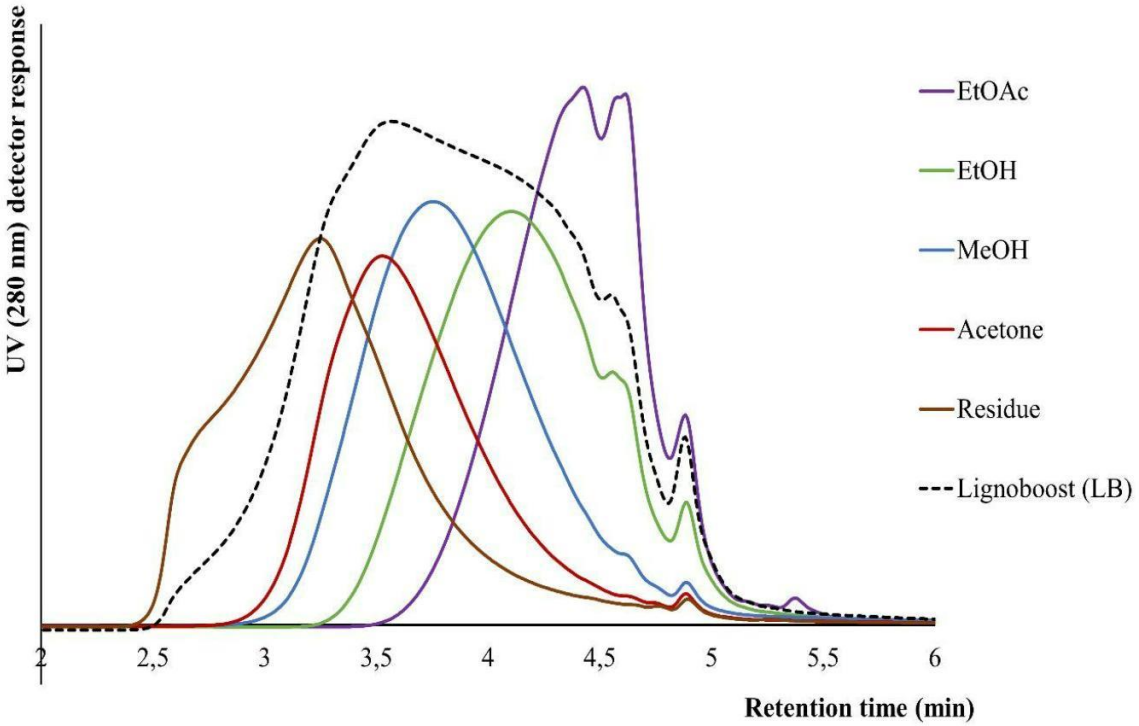


3 APC QT columns:
450Å + 200Å + 125Å
T = 30°C;
0.5 ml/min
0.3M $NaNO_3$/MeOH (80:20)

Sample: 1000 µg/mL lignosulphonate; at 280 nm, Sampling rate: 20 pt/s (Hz); Injection volume: 50 µL;

Molecular weight distribution of LB lignin and sequential solvent fractions detemined by APC

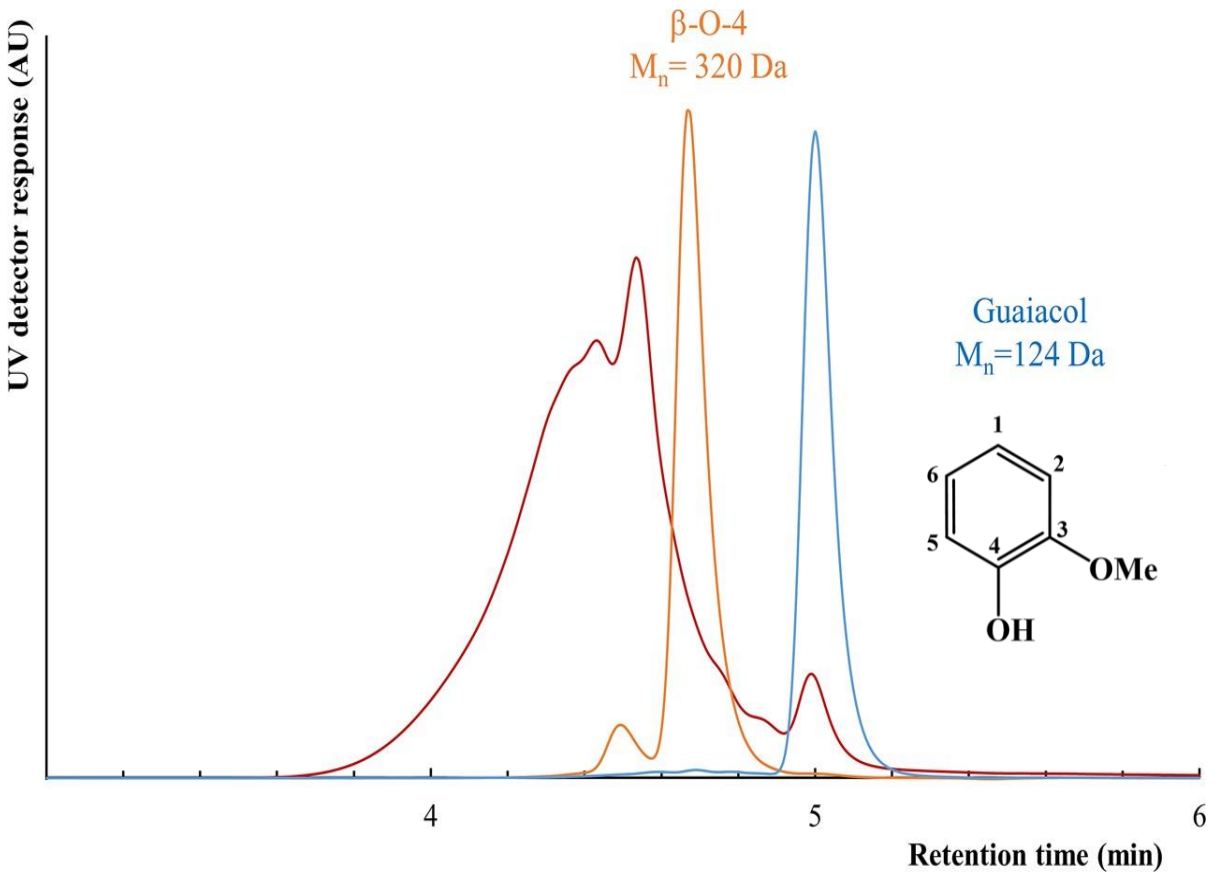




	LB	EtOAc	EtOH	MeOH	Acetone	Residue
Mn:	700	300	500	800	1300	2600
Mw:	5400	400	900	2500	4300	20500
Mz:	34500	700	2200	6100	8800	62100
D:	7.85	1.29	1.97	2.94	3.38	7.97

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

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

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

Thank for

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www.skhu.eu

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