On the implications of calibration techniques and detector systems on GPC-based analyses of lignin

COST Action FP 0901
“Biorefinery analytics – Outcomes from COST Action FP0901”

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Agenda

Introduction

GPC set-ups

Calibration & analysis issues

Sample preparation

Practical measurements and theoretical considerations

Consequences

Introduction

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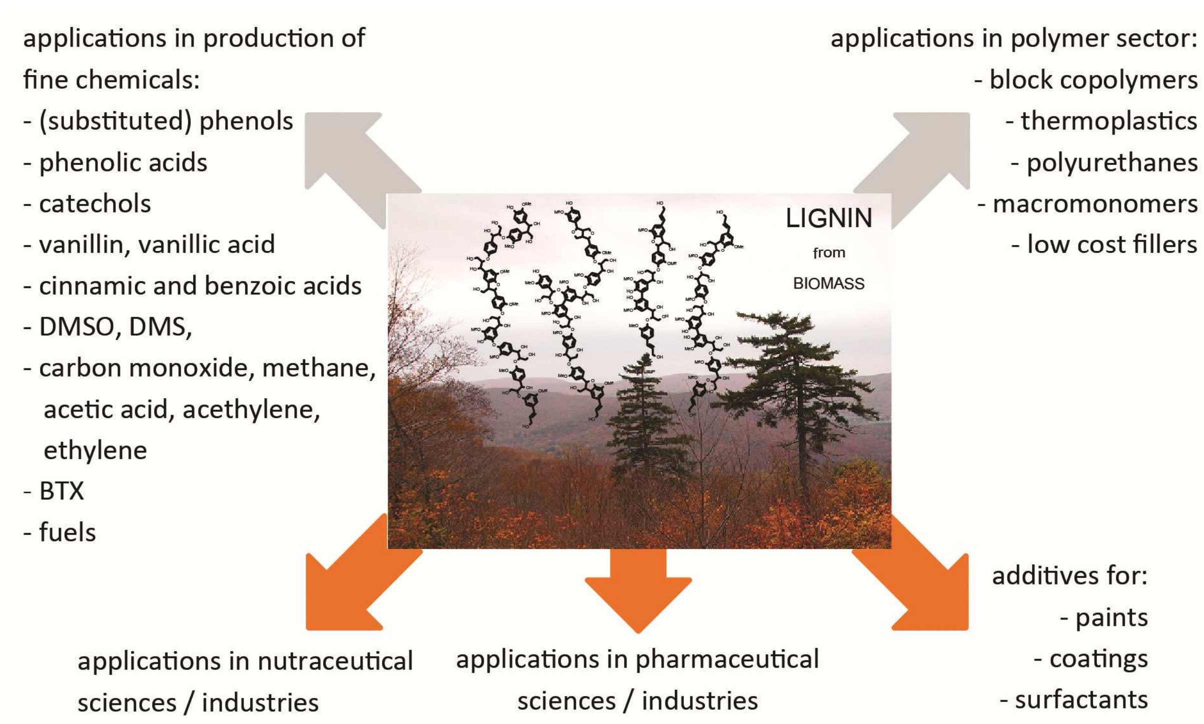
Introduction

Lignin – the under-utilized biomass component

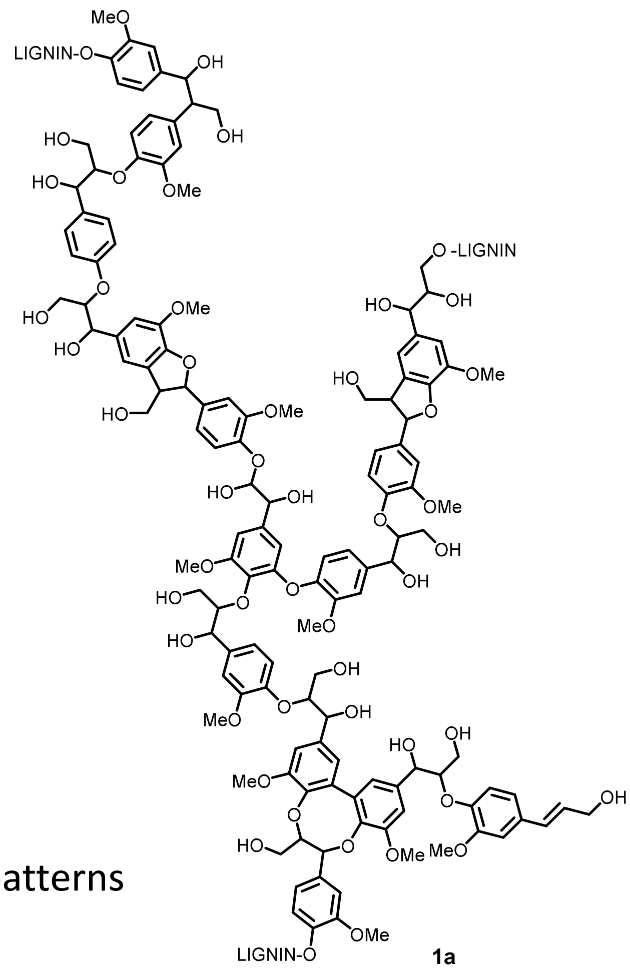
- Lignin holds the potential to be the renewable resource for fine chemicals, block co-polymers,etc.



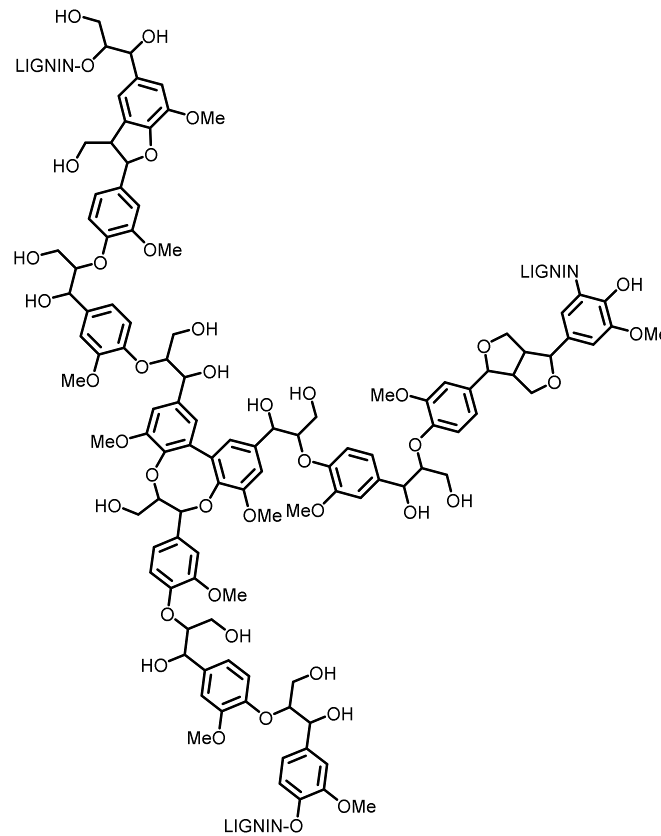
[1] M. Aresta, A. Dibenedetto, F. Dumeignil (editors); Biorefinery: From biomass to chemicals and fuels. de Gruyter (Berlin/Boston) **2012**.

Introduction

Lignin – structurally divers…..



1a



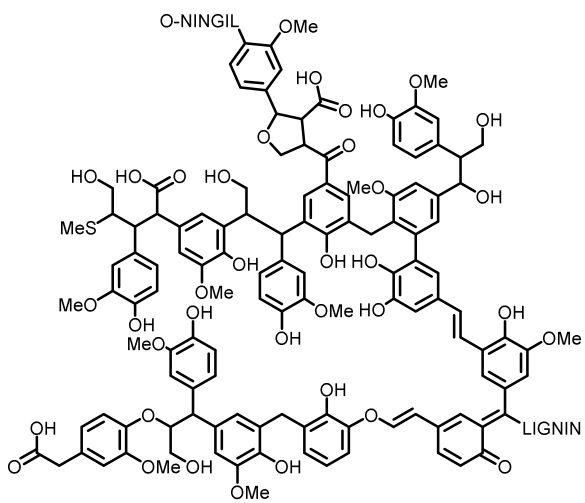
1b

- Random 3D network
- No repetitive units
- No repetitive bonding patterns

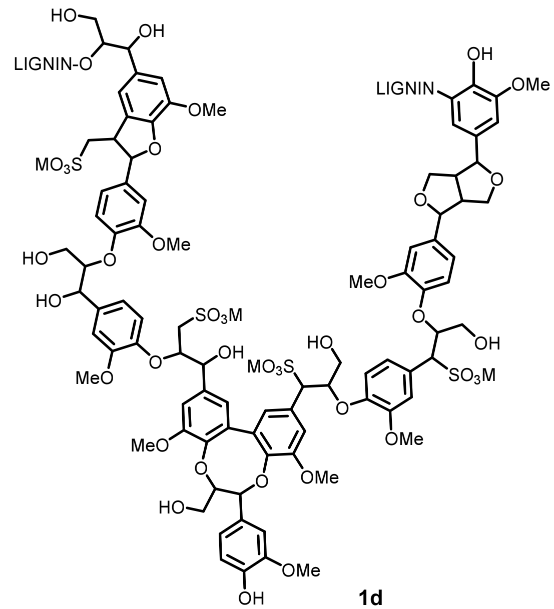
[2] S.E.Lebo Jr., J.D. Gargulak, T.J. McNally (editors); Lignin. Kirk-Othmer Encyclopedia of Chemical Technology, 4th Edition. John Wiley & Sons, Inc. (New York) **2001**.

Introduction

Lignin – Characteristics of isolated lignin samples differ depending on the isolation process



1c



1d

kraft lignin

lignosulfonate

[3] J. Gierer; Chemical aspects of kraft pulping. Wood Sci. Tech. **1980**, *14*, 241.

[4] F.S. Chakar, A.J. Ragauskas; Review of current and future softwood kraft lignin process chemistry. ***Industrial Crops Prod*** **2004**, *20*, 131.

Introduction

Lignin – Characteristics of isolated lignin samples differ depending on the isolation process

Lignin type	C9 Molecular formula	Monomer molecular weight [u]	Number-average molecular weight (M_n)	Poly-dispersity (M_w/M_n)
Milled wood lignin [a]	$C_9H_{7.80}O_{2.41}(OCH_3)_{0.95}$	198	2800-14200	3.7-12.9
Cellulolytic enzyme lignin [b]	$C_9H_{8.02}O_{2.82}(OCH_3)_{0.90}$	187	~1900	5.7-6.7
Enzymatic mild acidolysis lignin (EMAL) [b]	$C_9H_{8.02}O_{2.82}(OCH_3)_{0.90}$	187	~2000	~3
Kraft lignin [c]	$C_9H_{8.5}O_{2.1}S_{0.1}(OCH_3)_{0.8}(CO_2H)_{0.2}$	180	1000-3000	2-4
Lignosulfonated lignin (softwood) [d]	$C_9H_{8.5}O_{2.5}(OCH_3)_{0.85}(SO_3H)_{0.4}$	215-254	5000-20000	4-9
Lignosulfonated lignin (hardwood) [d]	$C_9H_{7.5}O_{2.5}(OCH_3)_{0.39}(SO_3H)_{0.6}$	188	5000-20000	4-9
Organosolv lignin [e]	$C_9H_{8.53}O_{2.45}(OCH_3)_{1.04}$	188	>1000	2.4-6.4
Pyrolysis lignin [f]	$C_9H_{6.3-7.3}O_{0.6-1.4}(OCH_3)_{0.3-0.8}(OH)_{1-1.2}$	n.d.	300-600	2.0-2.2
Steam explosion lignin [g]	$C_9H_{8.53}O_{2.45}(OCH_3)_{1.04}$	188	1100-2300	1.5-2.8

[5] H. Lange, S. Decina, C. Crestini; Oxidtive upgrade of lignin – recent routes reviewed. *Eur. Polym. J.* **2013**, *49*, 1151-1173.

Introduction

Lignin – how to quickly analyse?

- **Problem**: Lignin is becoming a famous starting material, but tricky to analyse…

  => Different laboratories obtain different results with the same analyses methods.

- **Problem**: No properly standardised analysis protocols (DIN-type)

- **Real problem**: How to get to comparable results, reflecting the true MW situation of a lignin sample?

  => Implications for **synthetic applications**

  => Implications for **material science-type application**

  => Implications on ***in silico* studies** on lignin

Introduction

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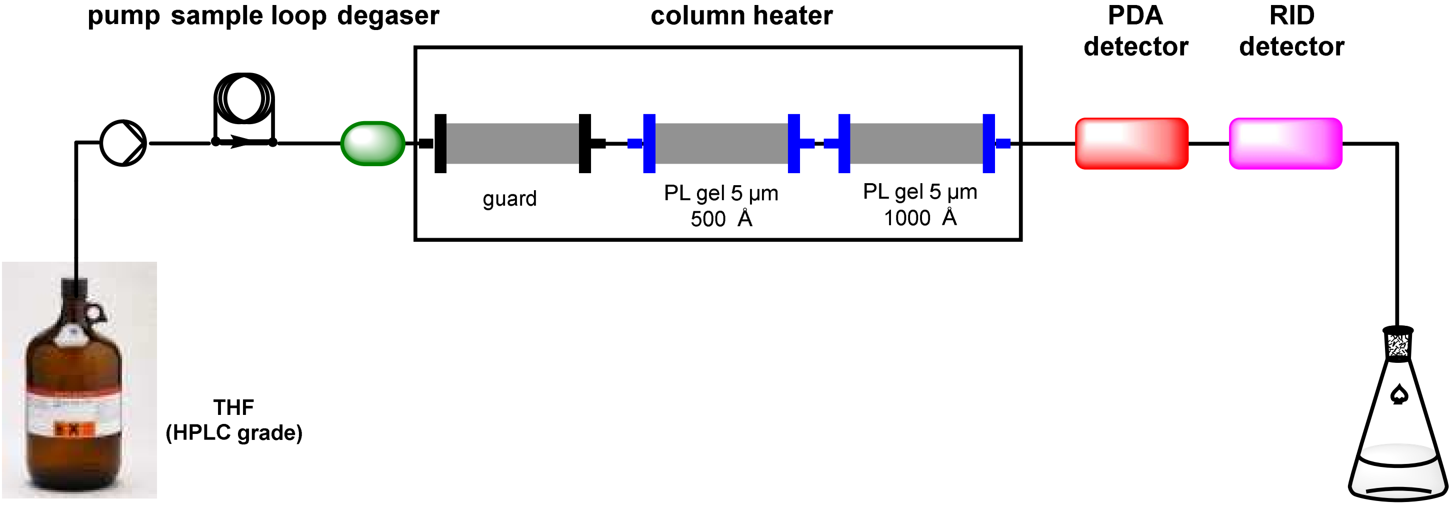
[5] H. Lange, S. Decina, C. Crestini; Oxidtive upgrade of lignin – recent routes reviewed. *Eur. Polym. J.* **2013**, *49*, 1151-1173.

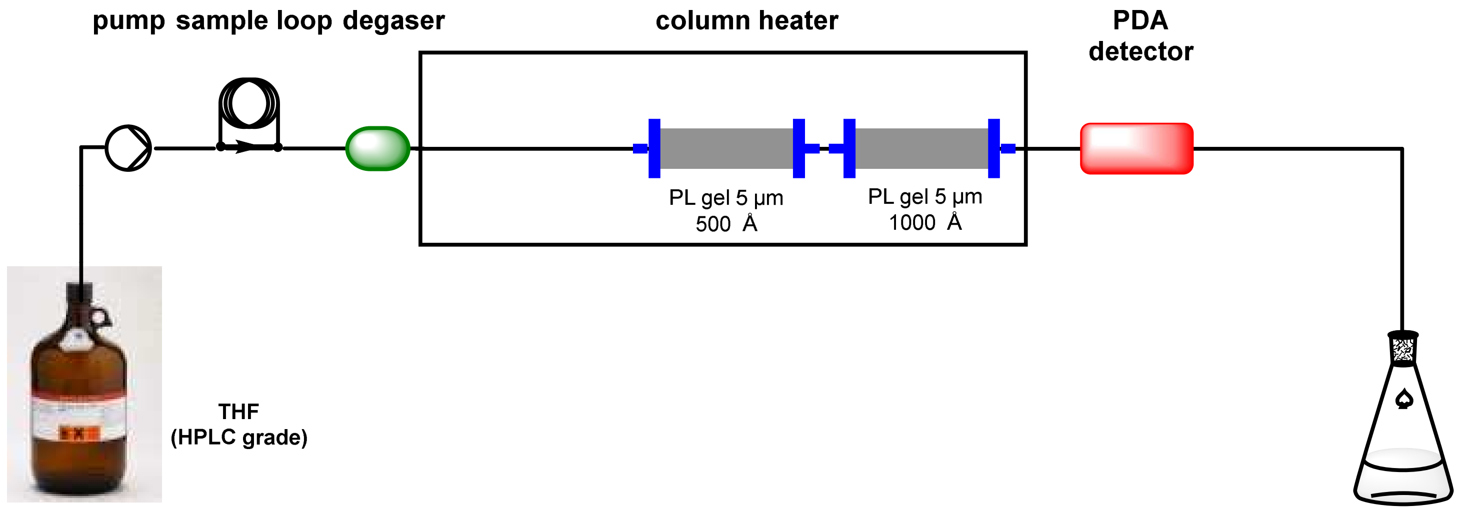
GPC set-ups

GPC – general (standard?) set-up



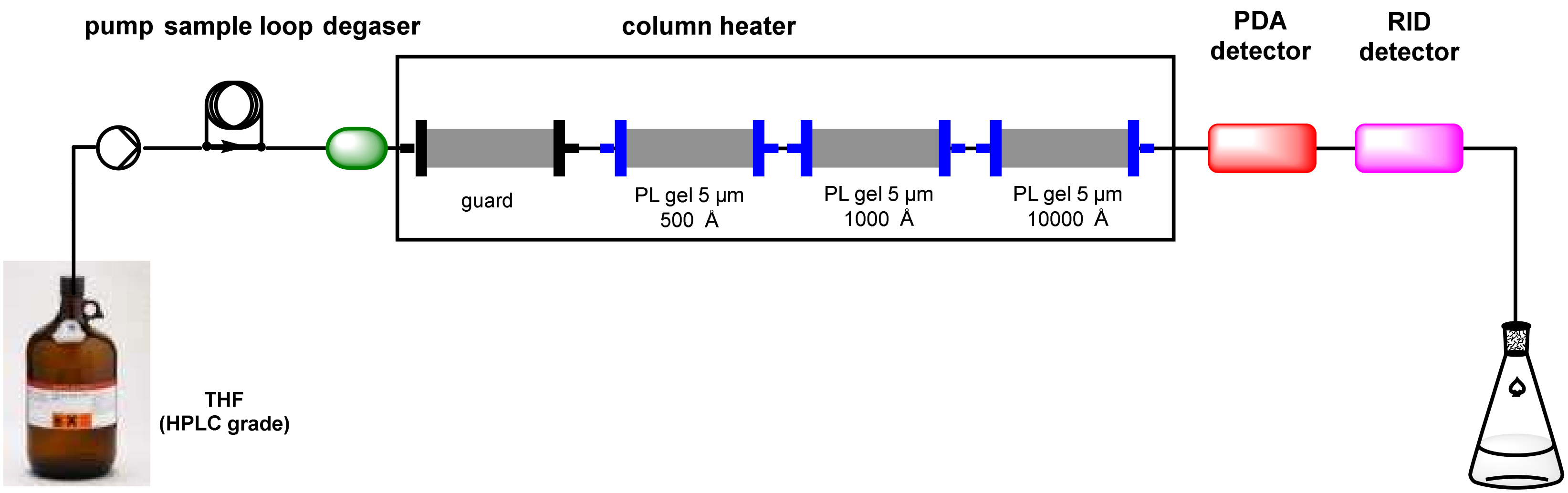
- ***Problem***: What is necessary, and what might be inadequate?

GPC – the minimum set-up



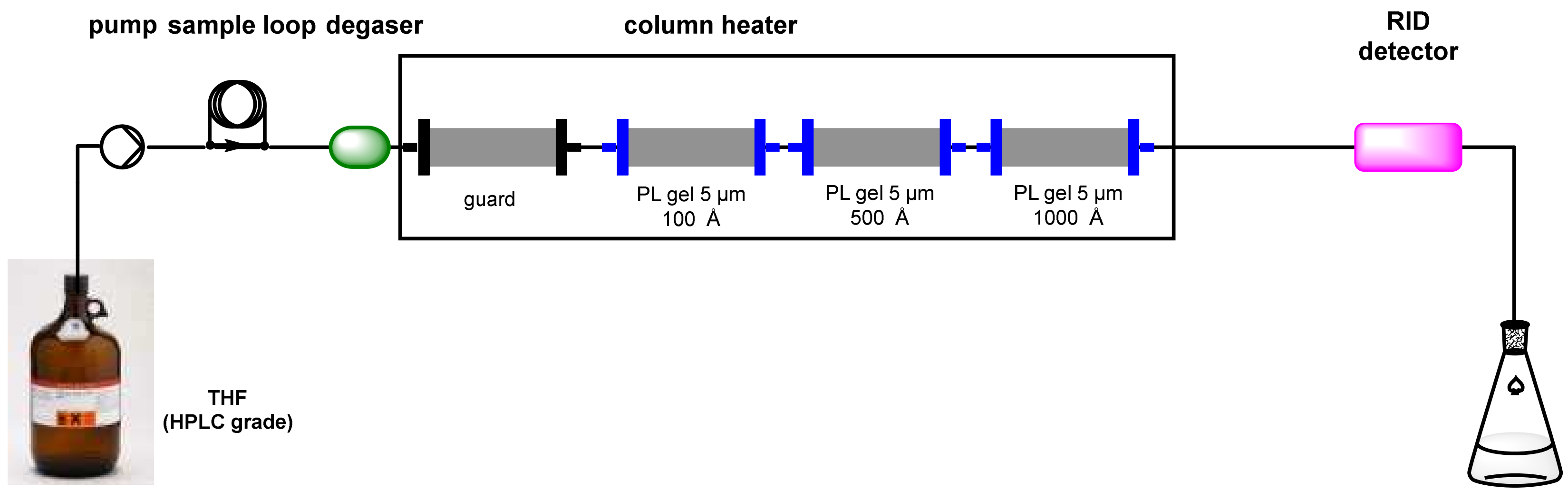
GPC set-ups

GPC – our proposed standard set-up



GPC set-ups

GPC – potentially inadequate set-up

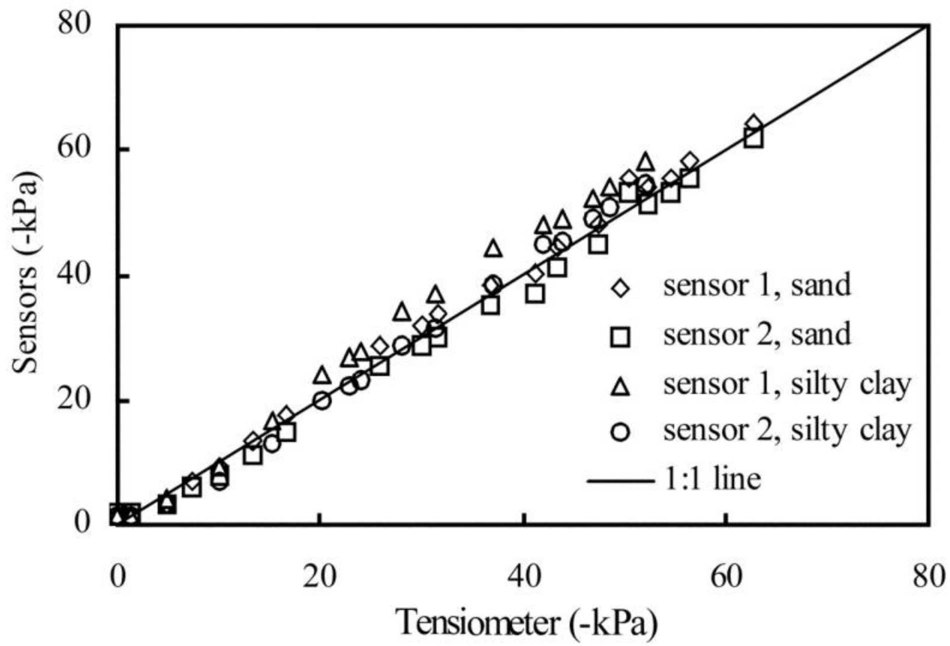


Calibration & calibration issues

Calibration & calibration issues

GPC – calibration issues

- ***Main problem***: significant structural differences between standard standards and potential samples (*e.g.*: polystyrene *vs.* polyphenol)
- ***Best solution***: universal calibration ('gold standard' of calibration methods)



- ***Drawbacks***: time consuming

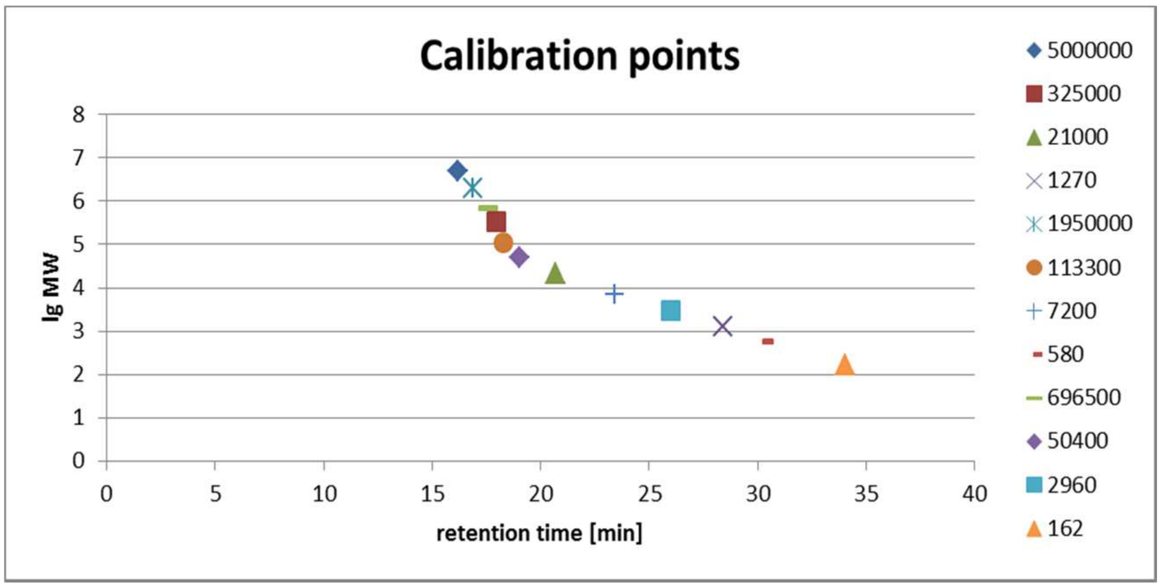
  improvement does not necessarily correlate with additional efforts….

[6] See for example: M. E. Himmel, K. Tatsumoto, K. K. Oh, K. Grohmann, D. K. Johnson, H. L. Chum. In: W. G. Glaser, S. Sarkanen, editors; Lignin – Materials and properties and materials. ACS Symposium Series 397. Washington, DC: American Chemical Society, **1988**, p82.
[7] Joint efforts: H. Lange, L. Zoia, C. Crestini, M. Orlandi; University of Rome 'Tor Vergata' & University of Milan

Calibration & calibration issues

GPC – calibration issues

- ***Practical solution***: commercially available polystyrene standards
- ***New practical challenge***: representative MW range?



Calibration & calibration issues

GPC – calibration issues

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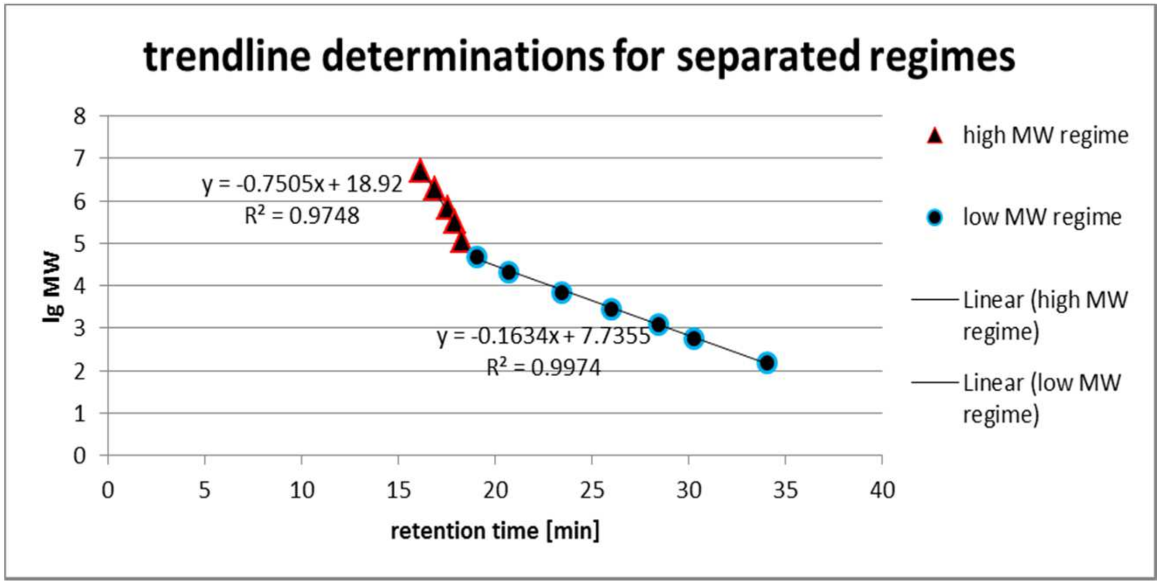
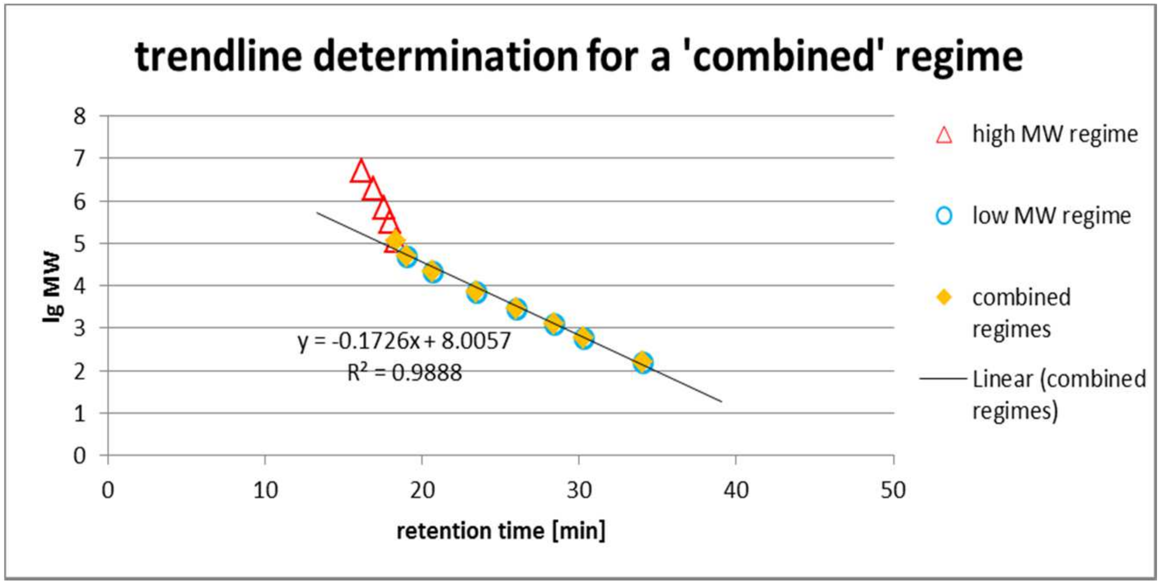
- ***New practical challenge***: representative MW range?



Calibration & calibration issues

GPC – calibration issues

- ***Practical solution***: commercially available polystyrene standards
- ***New practical challenge***: representative MW range?

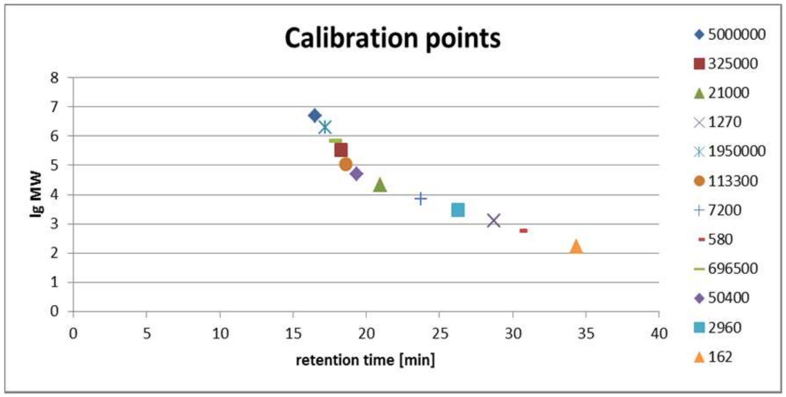


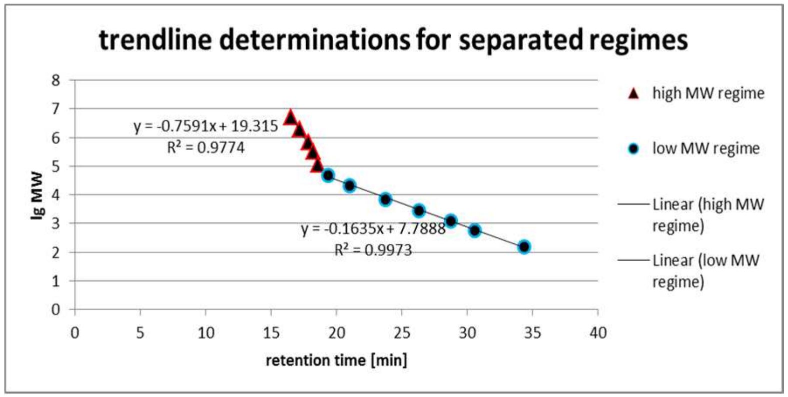
- Combined regime (113300 – 162 Da) = representative regime for most lignins

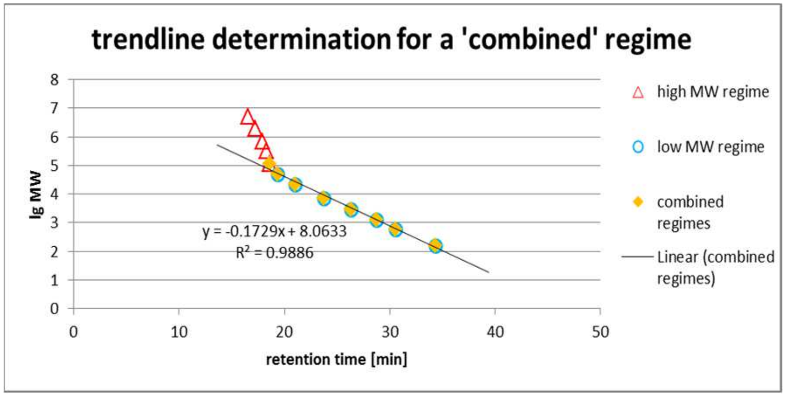
Calibration & calibration issues

GPC – calibration issues

- ***Additional issue***: Which detector to use? New calibration for different detectors?







- As such, no difference between detectors apart from intensity…

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Sample preparation

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Sample preparation

GPC – analysis programs

- ***Issue***: Sample preparation => overcome solubility issues
  => overcome 'detector-blindness'
  => avoid concentration-dependent supramolecular aggregation

- HQ MWL / OSL sample: - low solubility
  - well detectable in UV detectors (preferentially at 280 nm)
  - hardly detectable using RID detectors
- Carbohydrates: - good solubility
  - no UV trace
  - weakly detectable using RID detectors
- 'normal' sample: - **mix** of above mentioned facts

Sample preparation

GPC – analysis programs

- ***Issue***: Sample preparation => overcome solubility issues

  => overcome 'detector-blindness'

- 'normal' sample:

  - **acetobromination** ensures decent solubility at up to 5 mg per ml THF

  - benzoylation ensures detection of LCC-complexes[8]

  - Artifact peaks caused by THF peroxides in varying amounts

  [- possibility: use of solvent mixtures (e.g., THF with 5 % dioxane) to further improve selectivity]

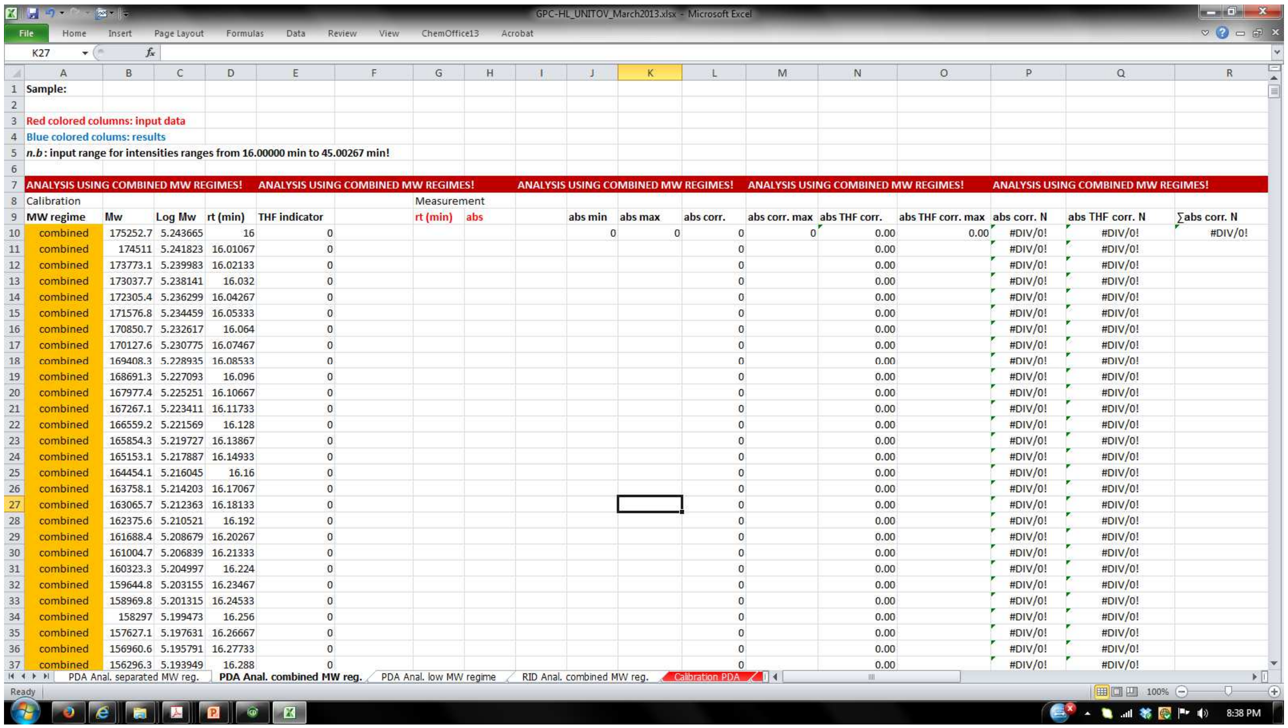
[8] A. Salanti, L. Zoia, E.-L. Tolppa, M. Orlandi; Chromatographic Detection of Lignin–Carbohydrate Complexes in Annual Plants by Derivatization in Ionic Liquid. *Biomacromolecules* **2012**, *13*, 445–454.

Practical measurements and theoretical considerations

Practical measurements and theoretical considerations

GPC – analysis programs

- ***Issue***: Specialized analyses software *vs*. generic table-calculations



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	Sample:																	
2																		
3	Red colored columns: input data																	
4	Blue colored colums: results																	
5	*n.b*: input range for intensities ranges from 16.00000 min to 45.00267 min!																	
6																		
7	ANALYSIS USING COMBINED MW REGIMES!				ANALYSIS USING COMBINED MW REGIMES!				ANALYSIS USING COMBINED MW REGIMES!				ANALYSIS USING COMBINED MW REGIMES!			ANALYSIS USING COMBINED MW REGIMES!		
8	Calibration						Measurement											
9	MW regime	Mw	Log Mw	rt (min)	THF indicator		rt (min)	abs		abs min	abs max	abs corr.	abs corr. max	abs THF corr.	abs THF corr. max	abs corr. N	abs THF corr. N	∑abs corr. N
10	combined	175252.7	5.243665	16	0					0	0	0	0	0.00	0.00	#DIV/0!	#DIV/0!	#DIV/0!
11	combined	174511	5.241823	16.01067	0							0		0.00		#DIV/0!	#DIV/0!	
12	combined	173773.1	5.239983	16.02133	0							0		0.00		#DIV/0!	#DIV/0!	
13	combined	173037.7	5.238141	16.032	0							0		0.00		#DIV/0!	#DIV/0!	
14	combined	172305.4	5.236299	16.04267	0							0		0.00		#DIV/0!	#DIV/0!	
15	combined	171576.8	5.234459	16.05333	0							0		0.00		#DIV/0!	#DIV/0!	
16	combined	170850.7	5.232617	16.064	0							0		0.00		#DIV/0!	#DIV/0!	
17	combined	170127.6	5.230775	16.07467	0							0		0.00		#DIV/0!	#DIV/0!	
18	combined	169408.3	5.228935	16.08533	0							0		0.00		#DIV/0!	#DIV/0!	
19	combined	168691.3	5.227093	16.096	0							0		0.00		#DIV/0!	#DIV/0!	
20	combined	167977.4	5.225251	16.10667	0							0		0.00		#DIV/0!	#DIV/0!	
21	combined	167267.1	5.223411	16.11733	0							0		0.00		#DIV/0!	#DIV/0!	
22	combined	166559.2	5.221569	16.128	0							0		0.00		#DIV/0!	#DIV/0!	
23	combined	165854.3	5.219727	16.13867	0							0		0.00		#DIV/0!	#DIV/0!	
24	combined	165153.1	5.217887	16.14933	0							0		0.00		#DIV/0!	#DIV/0!	
25	combined	164454.1	5.216045	16.16	0							0		0.00		#DIV/0!	#DIV/0!	
26	combined	163758.1	5.214203	16.17067	0							0		0.00		#DIV/0!	#DIV/0!	
27	combined	163065.7	5.212363	16.18133	0							0		0.00		#DIV/0!	#DIV/0!	
28	combined	162375.6	5.210521	16.192	0							0		0.00		#DIV/0!	#DIV/0!	
29	combined	161688.4	5.208679	16.20267	0							0		0.00		#DIV/0!	#DIV/0!	
30	combined	161004.7	5.206839	16.21333	0							0		0.00		#DIV/0!	#DIV/0!	
31	combined	160323.3	5.204997	16.224	0							0		0.00		#DIV/0!	#DIV/0!	
32	combined	159644.8	5.203155	16.23467	0							0		0.00		#DIV/0!	#DIV/0!	
33	combined	158969.8	5.201315	16.24533	0							0		0.00		#DIV/0!	#DIV/0!	
34	combined	158297	5.199473	16.256	0							0		0.00		#DIV/0!	#DIV/0!	
35	combined	157627.1	5.197631	16.26667	0							0		0.00		#DIV/0!	#DIV/0!	
36	combined	156960.6	5.195791	16.27733	0							0		0.00		#DIV/0!	#DIV/0!	
37	combined	156296.3	5.193949	16.288	0							0		0.00		#DIV/0!	#DIV/0!	

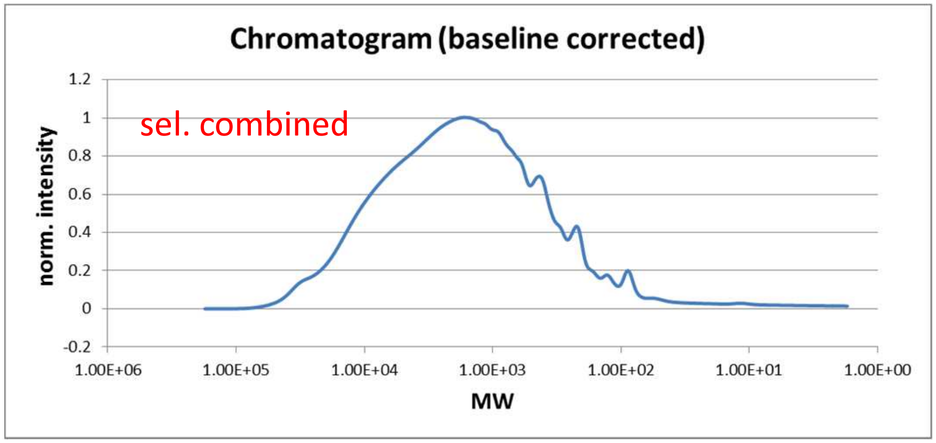
- Table calculations: No decision on peak area necessary in case UV-detectors are used

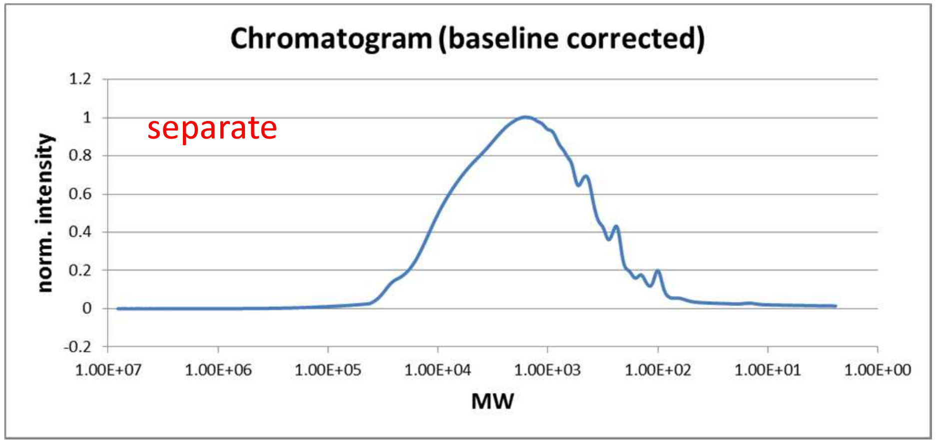
=> reduction of manual input error

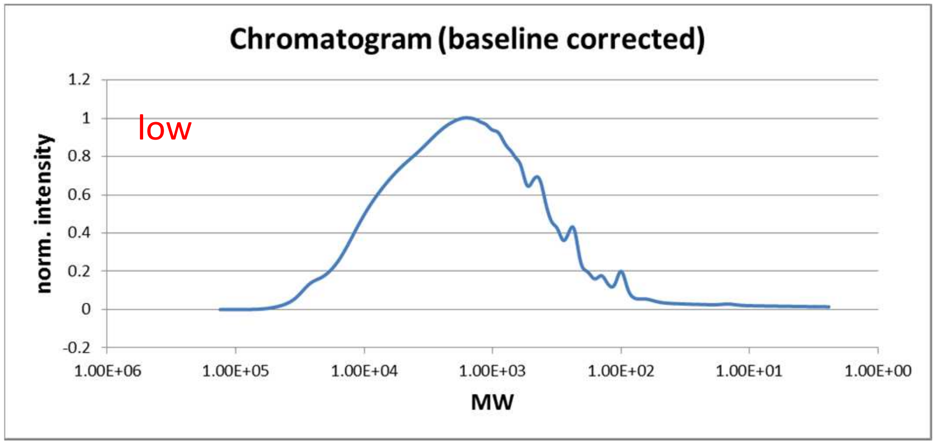
Practical measurements and theoretical considerations

GPC – analysis of real life sample

- PDA-traces obtained for acetobrominated sample for different calibration shown

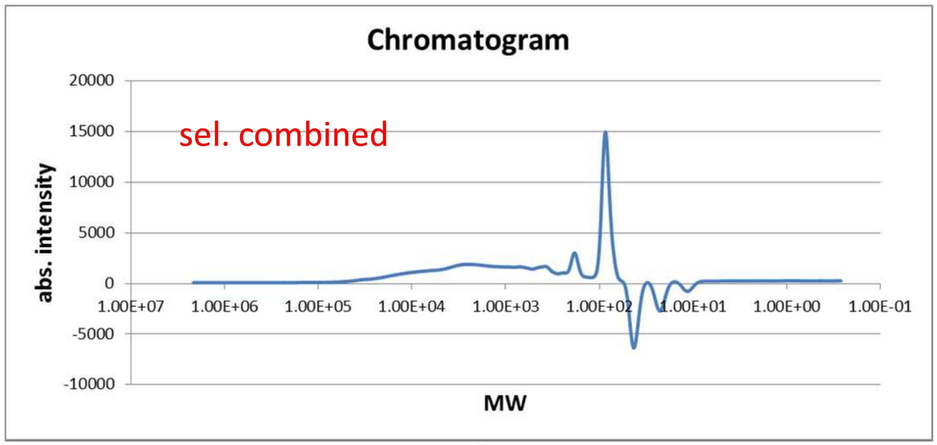


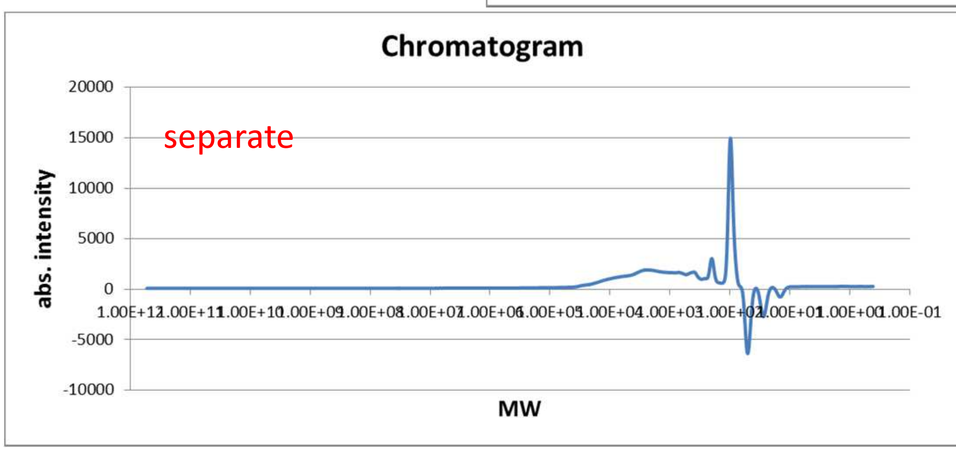


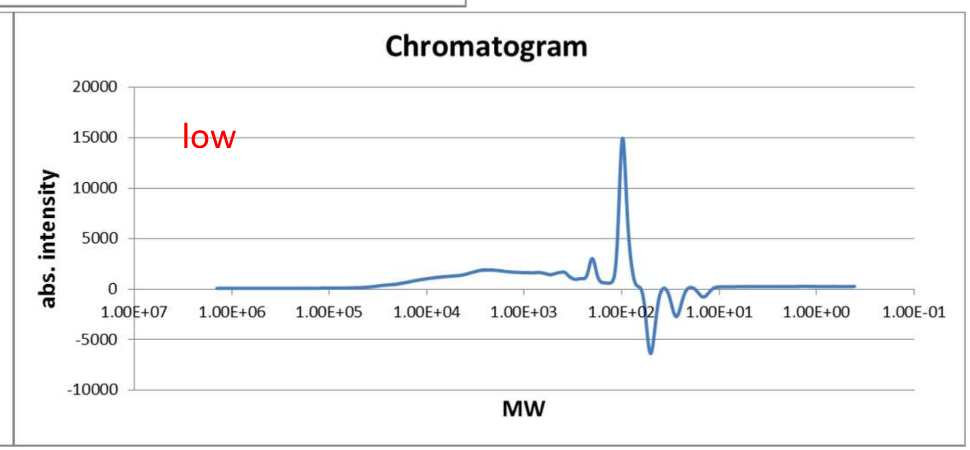


GPC – analysis of real life sample

- RID-traces obtained for acetobrominated sample for different calibration shown







Practical measurements and theoretical considerations

GPC – analysis of real life sample

- Delineated data (PDA)

Calibration	Mn corr.	Mn THF corr.	Mw corr.	Mw THF corr.	Mw/Mn corr.	Mw/Mn THF corr.
all	8394	8399	54337	54337	6.47	6.47
separated	3802	3804	68979	68979	18.14	18.13
only high	414	414	545857	545857	1318.61	1317.85
only low	3651	3653	11735	11735	3.21	3.21
sel.combined	4113	4115	14558	14558	3.54	3.54

Practical measurements and theoretical considerations

GPC – analysis of real life sample

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sel.combined	4113	4115	14558	14558	3.54	3.54

=> Depending on what is needed, the calibration is chosen?

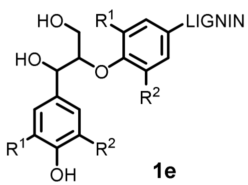
=> Misguided choices in calibration step suggests uselessness of a lignin sample?

=> What do these data represent anyway when lignin was derivatised?

=> GPC only useful for comparative studies using relative changes?

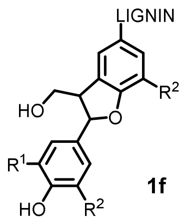
Lignin – structural motifs

β-coupling structures - monolignol coupling modes



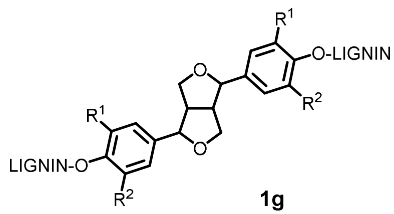
1e

β-O-4'
(β-ether)
G,S,H



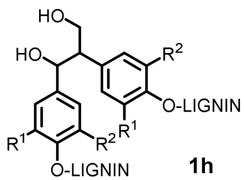
1f

β-5'
(phenylcoumaran)
G,H



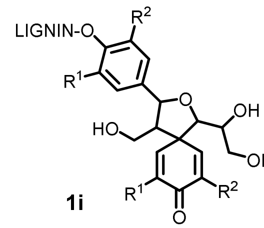
1g

β-β'
(pinoresinol)
G,S,H



1h

β-1'
(diphenyl ethane)
G,S,H



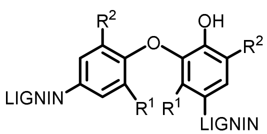
1i

spirodienone
G,S,H

5-coupling structures - oligomer coupling modes

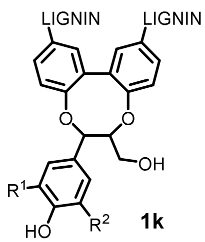
G-type: R^1 = H, R^2 = OMe
S-type: R^1 = R^2 = OMe
H-type: R^1 = R^2 = H

coupling motif emerging from pulping processes



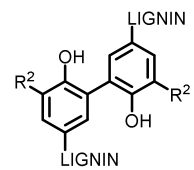
1j

4-O-5'
(diphenyl ether)
G,H



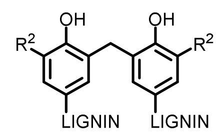
1k

5-5'-α,β-O-4'
(dibenzodioxocine)
G,H



1l

5-5'
(biphenyl)
G,H



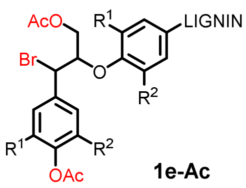
1m

diphenylmethane

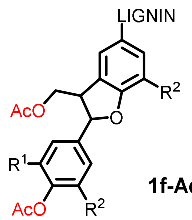
Practical measurements and theoretical considerations

Lignin – structural motifs

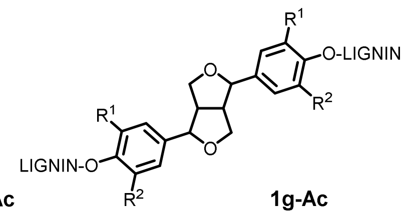
β-coupling structures - monolignol coupling modes



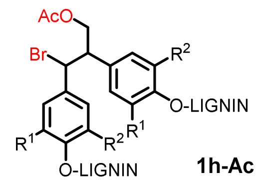
1e-Ac



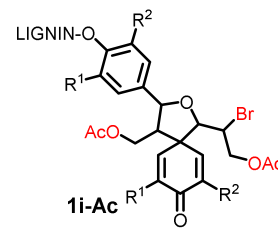
1f-Ac



1g-Ac



1h-Ac



1i-Ac

β-O-4'
(β-ether)
G,S,H

β-5'
(phenylcoumaran)
G,H

β-β'
(pinoresinol)
G,S,H

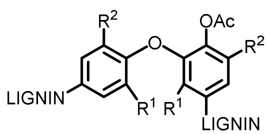
β-1'
(diphenyl ethane)
G,S,H

spirodienone
G,S,H

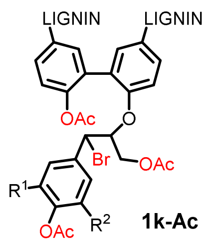
5-coupling structures - oligomer coupling modes

G-type: R^1 = H, R^2 = OMe
S-type: R^1 = R^2 = OMe
H-type: R^1 = R^2 = H

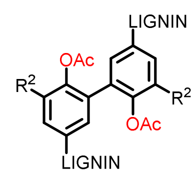
coupling motif emerging from pulping processes



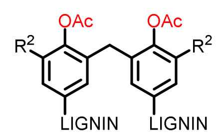
1j-Ac



1k-Ac



1l-Ac



1m-Ac

4-O-5'
(diphenyl ether)
G,H

5-5'-α,β-O-4'
(dibenzodioxocine)
G,H

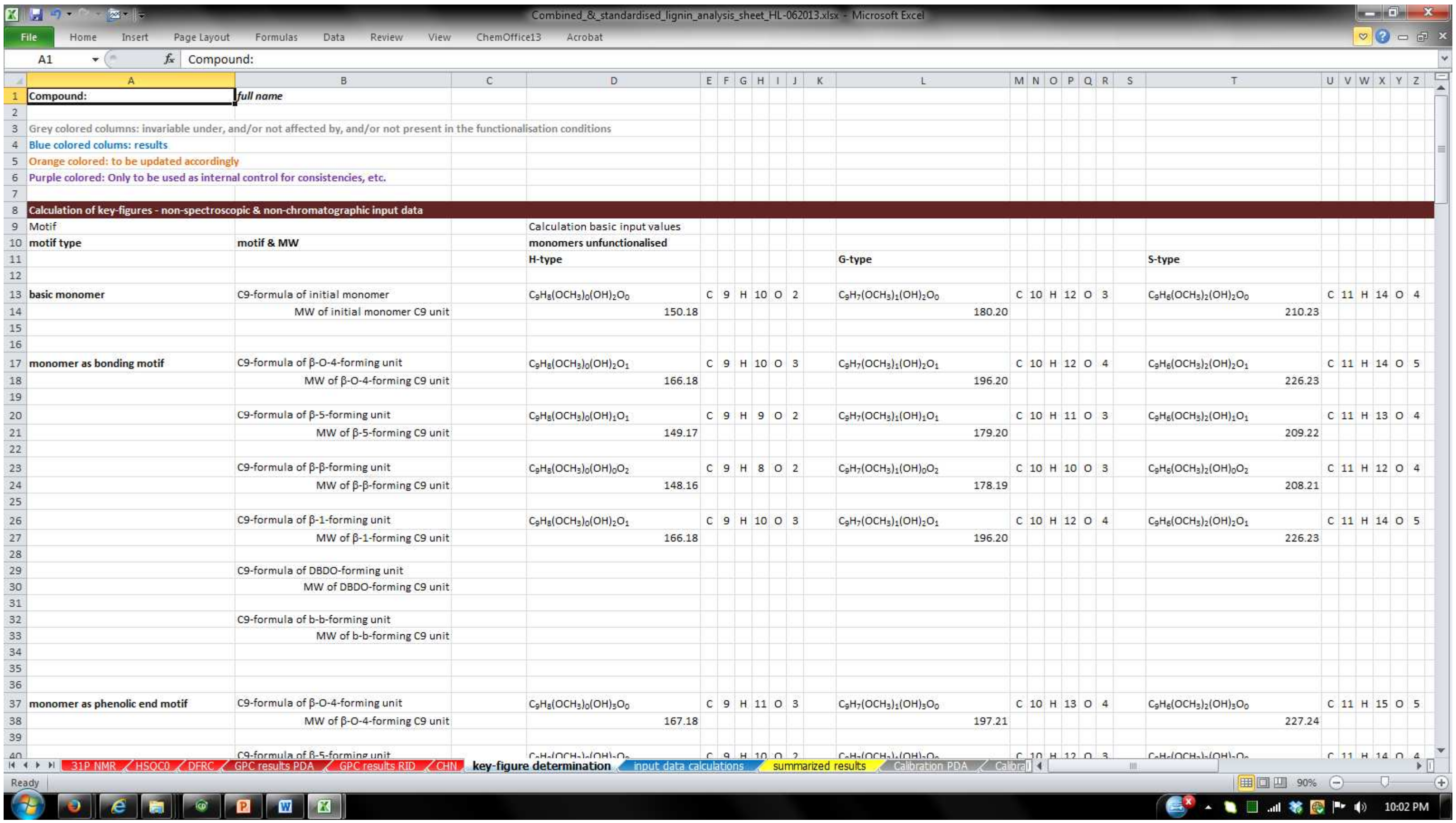
5-5'
(biphenyl)
G,H

diphenylmethane

Practical measurements and theoretical considerations

GPC – analysis of real life sample

- Universal data survey and processing



	A	B	D	E	F	G	H	I	J	L	M	N	O	P	Q	R	T	U	V	W	X	Y	Z
1	**Compound:**	*full name*																					
2																							
3	Grey colored columns: invariable under, and/or not affected by, and/or not present in the functionalisation conditions																						
4	Blue colored colums: results																						
5	Orange colored: to be updated accordingly																						
6	Purple colored: Only to be used as internal control for consistencies, etc.																						
7																							
8	Calculation of key-figures - non-spectroscopic & non-chromatographic input data																						
9	Motif		Calculation basic input values																				
10	**motif type**	**motif & MW**	**monomers unfunctionalised**																				
11			**H-type**							**G-type**							**S-type**						
12																							
13	**basic monomer**	C9-formula of initial monomer	$C_9H_8(OCH_3)_0(OH)_2O_0$	C	9	H	10	O	2	$C_9H_7(OCH_3)_1(OH)_2O_0$	C	10	H	12	O	3	$C_9H_6(OCH_3)_2(OH)_2O_0$	C	11	H	14	O	4
14		MW of initial monomer C9 unit	150.18							180.20							210.23						
15																							
16																							
17	**monomer as bonding motif**	C9-formula of β-O-4-forming unit	$C_9H_8(OCH_3)_0(OH)_2O_1$	C	9	H	10	O	3	$C_9H_7(OCH_3)_1(OH)_2O_1$	C	10	H	12	O	4	$C_9H_6(OCH_3)_2(OH)_2O_1$	C	11	H	14	O	5
18		MW of β-O-4-forming C9 unit	166.18							196.20							226.23						
19																							
20		C9-formula of β-5-forming unit	$C_9H_8(OCH_3)_0(OH)_1O_1$	C	9	H	9	O	2	$C_9H_7(OCH_3)_1(OH)_1O_1$	C	10	H	11	O	3	$C_9H_6(OCH_3)_2(OH)_1O_1$	C	11	H	13	O	4
21		MW of β-5-forming C9 unit	149.17							179.20							209.22						
22																							
23		C9-formula of β-β-forming unit	$C_9H_8(OCH_3)_0(OH)_0O_2$	C	9	H	8	O	2	$C_9H_7(OCH_3)_1(OH)_0O_2$	C	10	H	10	O	3	$C_9H_6(OCH_3)_2(OH)_0O_2$	C	11	H	12	O	4
24		MW of β-β-forming C9 unit	148.16							178.19							208.21						
25																							
26		C9-formula of β-1-forming unit	$C_9H_8(OCH_3)_0(OH)_2O_1$	C	9	H	10	O	3	$C_9H_7(OCH_3)_1(OH)_2O_1$	C	10	H	12	O	4	$C_9H_6(OCH_3)_2(OH)_2O_1$	C	11	H	14	O	5
27		MW of β-1-forming C9 unit	166.18							196.20							226.23						
28																							
29		C9-formula of DBDO-forming unit																					
30		MW of DBDO-forming C9 unit																					
31																							
32		C9-formula of b-b-forming unit																					
33		MW of b-b-forming C9 unit																					
34																							
35																							
36																							
37	**monomer as phenolic end motif**	C9-formula of β-O-4-forming unit	$C_9H_8(OCH_3)_0(OH)_3O_0$	C	9	H	11	O	3	$C_9H_7(OCH_3)_1(OH)_3O_0$	C	10	H	13	O	4	$C_9H_6(OCH_3)_2(OH)_3O_0$	C	11	H	15	O	5
38		MW of β-O-4-forming C9 unit	167.18							197.21							227.24						
39																							

Practical measurements and theoretical considerations

GPC – analysis of real life sample

- 'Functionalisation-corrected' data (PDA)

  => functionalisation accounted for in a simple factor that reflects sample characteristics

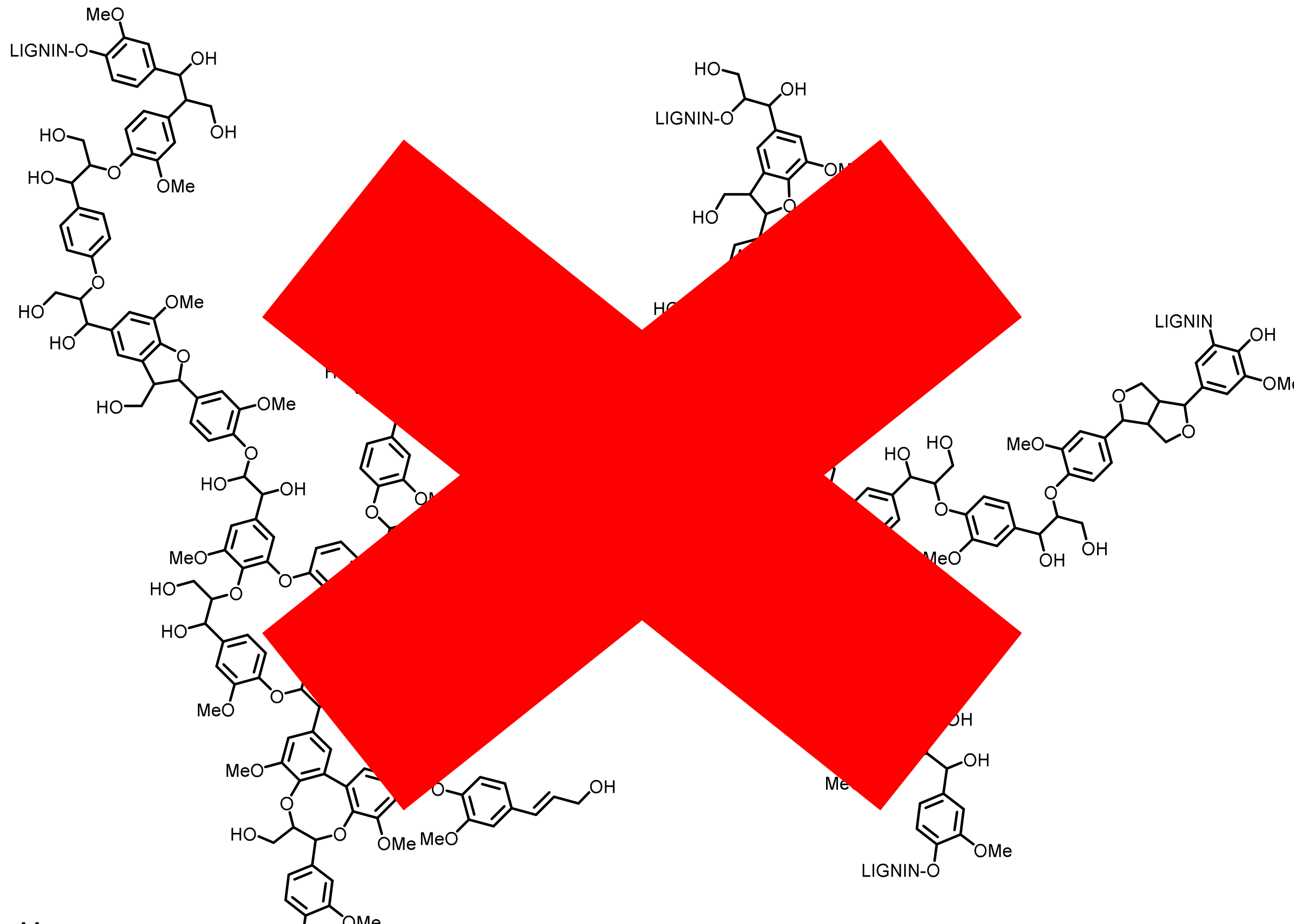
  => MW (WS-OSL monomer, acetobrominated): 261 Da
  MW (WS-OSL monomer, natural): 198 Da
  "Conversion factor" GPC: 1.32

  => **Mn (natural) = 2900,   Mw (natural) = 10100**

Consequences

Consequences

Consequences with respect to lignin structures



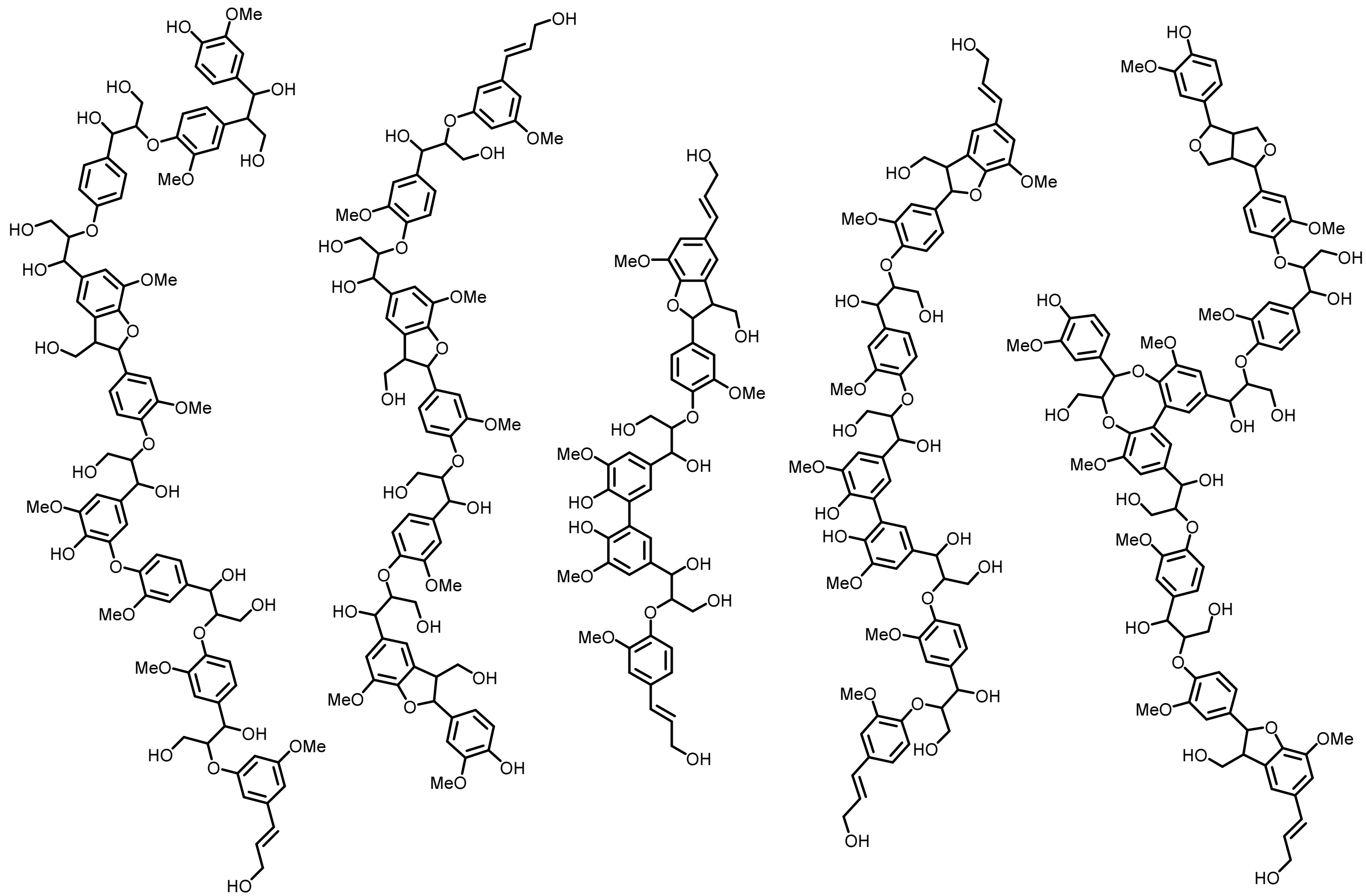
1a 1b

- Random ~~3D~~ network
- No repetitive units
- No repetitive bonding patterns

[4] S.E.Lebo Jr., J.D. Gargulak, T.J. McNally (editors); Lignin. Kirk-Othmer Encyclopedia of Chemical Technology, 4th Edition. John Wiley & Sons, Inc. (New York) **2001**.
[5] C. Crestini , F. Melone, M. Sette, R. Saladino; Milled wood lignin: A linear oligomer. *Biomacromolecules* **2011**, *12,* 3928.

Consequences

Consequences with respect to lignin structures



1n 1o 1p 1q 1r

- Linear, oligomeric chains, rather small….

[9] C. Crestini , F. Melone, M. Sette, R. Saladino; Milled wood lignin: A linear oligomer. *Biomacromolecules* **2011**, *12,* 3928.

Consequences

Consequences for future and ongoing work

- GPC set-ups need to be more uniform to ensure comparability of results
- Calibrations must be done more thoughtful with respect to calibration ranges
- Sample preparation needs to be standardised
- And: for more than relative comparisons, data obtained need to interpreted in light of all other data available for the sample

=> With lignin becoming a more famous starting material,

powerful standardised analysis protocols should be reinforced for data reporting

Acknowledgements

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