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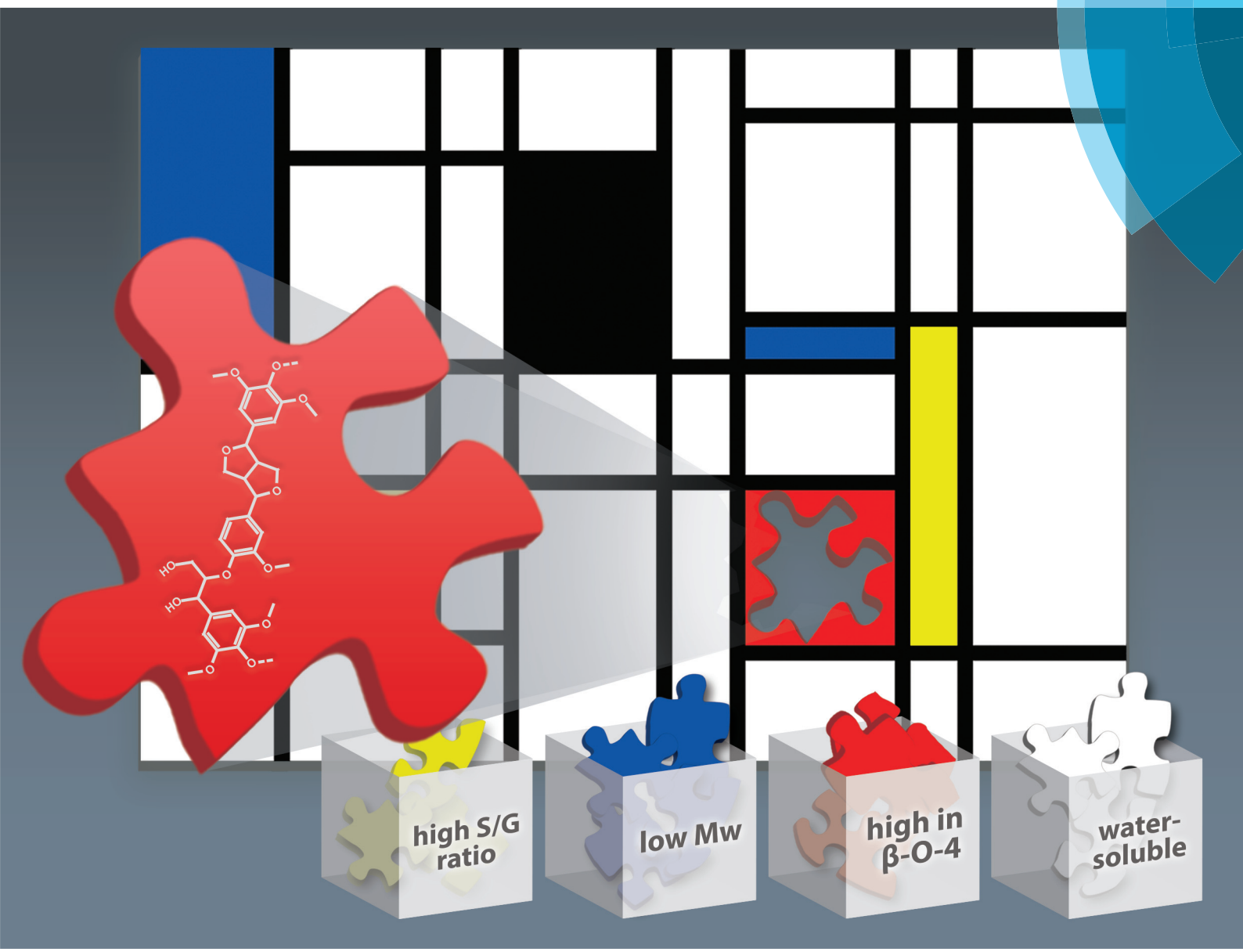

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New insights into the structure and composition of technical lignins: a comparative characterisation study

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New insights into the structure and composition of technical lignins: a comparative characterisation study†

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Detailed insight into the structure and composition of industrial (technical) lignins is needed to devise efficient thermal, bio- or chemocatalytic valorisation strategies. Six such technical lignins covering three main industrial pulping methods (Indulin AT Kraft, Protobind 1000 soda lignin and Alcell, poplar, spruce and wheat straw organosolv lignins) were comprehensively characterised by lignin composition analysis, FT-IR, pyrolysis-GC-MS, quantitative ^{31}P and 2D HSQC NMR analysis and molar mass distribution by Size Exclusion Chromatography (SEC). A comparison of nine SEC methods, including the first analysis of lignins with commercial alkaline SEC columns, showed molar masses to vary considerably, allowing some recommendations to be made. The lignin molar mass decreased in the order: Indulin Kraft > soda P1000 > Alcell > OS-W ~ OS-P ~ OS-S, regardless of the SEC method chosen. Structural identification and quantification of aromatic units and inter-unit linkages indicated that all technical lignins, including the organosolv ones, have considerably been degraded and condensed by the pulping process. Importantly, low amounts of β- ether linkages were found compared to literature values for protolignin and lignins obtained by other, milder isolation processes. Stilbenes and ether furfural units could also be identified in some of the lignins. Taken together, the insights gained in the structure of the technical lignins, in particular, the low β-O-4 contents, carry implications for the design of lignin valorisation strategies including (catalytic) depolymerisation and material applications.

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Introduction

Lignin is attracting much attention due to its potential as a renewable resource for the production of bio-based materials, fuels and chemicals, aromatics in particular. Macromolecular applications of this aromatic polymer include its use in polyurethane foams, phenol-formaldehyde resins, as dispersants or as additives for concrete and rubber.[1] For lignin to serve as a renewable feedstock for chemicals and fuel additives, efficient catalytic depolymerisation is required. Many different approaches and conversion routes have been proposed and are currently being explored.[2] Despite considerable efforts in this direction, the current industrial uses of lignin are still limited and are primarily associated with the (macromolecular) industrial application of lignosulphonates.[3] Although a highly abundant aromatic feedstock, lignin is still largely regarded simply as a source for heat and power for the biorefining or pulping process that liberates the lignin. The lack of established processes that add value to the lignin component can be largely attributed to its chemical recalcitrance and structural complexity. Adding to this complexity, the lignin structure is highly dependent on both the feedstock and lignin isolation process.

Lignin is an aromatic polymer formed in nature by the radical polymerisation of *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol, bearing *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units, respectively. Depending on the biomass source, lignin varies in monomer composition. Herbaceous crops, such as straws, contain all the three monomers and are relatively rich in H units. Gymnosperm lignins, isolated from

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softwoods, lack S units, while angiosperm lignins, isolated from hardwoods, are rich in G and S. The monomer composition affects the molecular structure, for instance in terms of branching of the polymer and the extent of cross-linking with the polysaccharide fraction. Monomer composition thus determines lignin degradability, as well as the processability of the lignocellulosic biomass.[4–6] The radical polymerisation process leads to a variety of inter-unit linkages including the β-O-4, β–β, β-5, 5–5 and 4-O-5 structural motifs.[7] Their relative abundance in native lignin varies from plant to plant but the β-O-4 linkage is typically the most abundant. It is also one of the most labile bonds and is readily cleaved by most depolymerisation (or pretreatment) methods.[2] The β-O-4 content of isolated lignins, therefore, strongly depends on the type of the separation method used and the severity of the applied process conditions. While various (enzymatic) lignin extraction methods that aim at the isolation of lignin with a structure as close to protolignin as possible do exist; such methods have been primarily developed for analytical purposes, *i.e.* for lignin structure determination. Indeed, such mild analytical methods generally do not provide a viable starting point for lignin valorisation schemes. Instead, efficient valorisation routes should rather be found for technical lignins that are isolated as the side-products of the existing or future (industrial) bio-refining processes. Such processes are typically optimised for the valorisation of the carbohydrate part of the biomass and have little regard for the fate of the lignin fraction. As a consequence, lignin is produced in high yield, but with a highly modified structure. Alkaline and organosolv processes yield such technical lignins, for example. Kraft and soda pulping are two alkaline processes, with the former using sodium sulphide in an alkaline aqueous solution as reaction medium,[8] while the latter only uses sodium hydroxide. In both processes, the alkaline conditions result in the breakdown of the protolignin, dissolution of lignin fragments and subsequent recondensation. Not all linkages of the lignin–carbohydrate complex are broken, resulting in a significant amount of residual sugars in the isolated alkaline lignins. In addition, considerable amounts of residual minerals typically remain in the lignin fraction.

Organosolv pulping is a promising alternative in that it yields lignins with considerably lower amounts of both residual carbohydrates and ash. Pulping with a mixture of water and an organic solvent, optionally aided by acid catalysts, allows for an efficient fractionation of biomass. Together with a cellulose pulp, high purity lignin and hemicellulose-derived products are coproduced and easily separated. Many types of organosolv processes have been developed based on different solvents and acids.[9] Most commonly, organosolv processes use aqueous ethanol, typically adding sulphuric acid as a catalyst. However, Alcell lignin, which has been produced at the pilot plant scale, was generated without adding a catalyst.[10] Although organosolv lignins are relatively pure, this pulping process is known to considerably affect the lignin structure, for example by extensive cleavage of the labile β-O-4 linkages.[11–13]

Evidently, further valorisation of technical lignins requires detailed insight into the impact of the isolation method on the lignin structure, as the final molecular structure determines which lignin is best suited for what valorisation strategy. Most lignin structure elucidation efforts, however, have focused on the structure of protolignin rather than technical lignins. A multitechnique approach is required to obtain essential knowledge on the lignin structure, composition, molar mass distribution and thermal behaviour.[14,15] General composition analysis typically distinguishes between the Klason lignin, or Acid Insoluble Lignin (AIL), Acid Soluble Lignin (ASL), residual carbohydrates and ash. The average molar mass (M_w) and polydispersity (PD) of the lignin macromolecule are commonly determined by Size Exclusion Chromatography (SEC) and depend heavily on the experimental setup used, as well as on the calibration and calculation methods applied.[16] Absolute values should therefore be treated with caution and only be used for a relative comparison.[14] Other methods that have contributed to the elucidation of the lignin structure include wet chemical methods, chromatography coupled with mass spectrometry, FT-IR and NMR spectroscopy.

NMR spectroscopy has probably provided detailed insight into the lignin molecular structure, both in terms of inter-unit linkages and functional group decoration of the lignin macromolecules. 2D [^{1}H;^{13}C] Heteronuclear Single Quantum Coherence (HSQC) NMR spectroscopy has proven to be extremely powerful, for example, for further refinement and expansion of the types of lignin linkages,[17] providing particular insight into the different ether linkages and aromatic units in the lignin structure.[18–21] A library of typical lignin structures is now available.[22] In addition to identification, quantification of the linkages is also highly desired. However, this is not straightforward with 2D HSQC. Good signal to noise ratios are essential, demanding long experimentation times or highly sensitive, expensive equipment. The signal response quality is also strongly influenced by experimental parameters, such as relaxation rates, carbon pulse offsets, and coupling constant multiplicity and magnitude.[19,23,24] Although quantitative pulse sequences have been developed,[21,24] the 2D NMR data obtained only allow relative quantification, since the guaiacyl and syringyl aromatic signals are often used as internal standards. ^{31}P NMR is often employed for functional group analysis, as it allows separate quantification of alcohols, (condensed) phenolic, and carboxylic acid groups in lignin. It requires a wet chemical derivatisation step with, for example, 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane prior to NMR analysis.[25]

Despite the multitude of available analytical techniques, the aromatic composition and inter-unit linkages in technical lignins are still not unequivocally established. For example, the S/G/H ratios and the amount of linkages determined by wet-chemical methods or by NMR do not agree with each other.[11,26] In addition, even for the same technique, values can vary significantly depending on the parameters chosen, especially for SEC and NMR studies. Clearly, further standardisation of analysis protocols is needed.

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The objective of this study is to determine and compare the structure of technical lignins obtained by different isolation methods. Six lignins have been selected to cover the main lignin production methods. These include two commercial lignins, *i.e.* softwood Kraft lignin (Indulin AT) and a mixed straw/grass soda lignin (Protobind 1000).[27] In addition, three organosolv lignins have been studied, isolated with an ethanol-based process from wheat straw (OS-W), poplar (OS-P) and spruce (OS-S).[14,28] The widely studied Alcell organosolv lignin was used for benchmarking. The lignins are analysed with respect to composition, by FT-IR, pyrolysis-GCMS, quantitative ^{31}P NMR and 2D HSQC NMR. Additionally, the molar mass distribution has been studied with various SEC systems, including known and new eluents, columns, and calibration standards. The combined data provide valuable information on the structure and composition that will aid future lignin valorisation efforts.

Results and discussion

Lignin composition

The chemical composition of the lignins used is shown in Table 1 and is in accordance with previous studies.[15,16,29] Replicate analyses in two laboratories showed excellent reproducibility (Tables S3 and S4†). As expected, the chemical composition of the lignins depended on the feedstock as well as on the isolation process. The total content of AIL and ASL, ash-free lignin, varied from 90.5 to 96.3. The highest lignin contents were observed for the organosolv lignins, the lowest for the soda lignin. The amount of residual carbohydrates varied between 0.2 and 2.4 wt% depending on the type of lignin. Alkaline lignin contained more residual sugar than organosolv lignin. These carbohydrates may originate from (poly)saccharides still covalently bound to the lignin as in the Lignin–Carbohydrate Complex (LCC)[14] or from carbohydrates that are trapped during the lignin precipitation step and finally end up non-covalently bound in the lignin after drying.[14] The higher purity of organosolv lignins is in line with previous studies.[11,30] Surprisingly, glucomannan, the predominant constituent of softwood hemicellulose, was not found in the Indulin Kraft lignin in contrast to previous studies.[31] Xylan was present in all lignins, contrary to arabinan and mannan, which were exclusively found in wheat straw and spruce lignin, respectively. The relatively high amount of xylan found in the alkaline lignins, soda P1000 in particular, shows that cleavage of the LCC is less complete for alkaline lignin pulping compared to organosolv pulping. Information on residual sugars is important, as these may impact further (catalytic) lignin valorisation. Carbohydrate derivatives have been shown to inhibit hydrodeoxygenation of the lignin model compound guaiacol, for example.[32]

Also, the amount of ash and its composition depended on the feedstock and isolation method. Organosolv lignins showed remarkably low ash contents. Typically, straw and grass contain more minerals per dry weight than wood. In addition, the higher ash content in alkaline lignin may partly originate from salts included after the neutralisation of alkaline liquor during precipitation. Next to sulphur, the most abundant elements in the ash include sodium, potassium and silicon (Table S5†). In addition, significant amounts of aluminium, calcium, magnesium, and iron are detected. It has been reported that the presence of small amounts of alkali and alkaline earth metals can influence cellulose pyrolysis efficiency.[33] While no such data is available for lignin yet, similar effects may be expected. Another possible detrimental effect prohibiting valorisation of lignin may be the accumulation of minerals on a catalyst material, which may affect its activity, as has been shown for the fast pyrolysis of pine wood.[34]

Sulphur is of course a well-known poison for many of the catalysts typically used in hydrogenolysis/hydrodeoxygenation reactions,[35] and its presence in lignins can thus influence reductive lignin depolymerisation methods. The highest amount of sulphur was detected in Kraft lignin, as a result of the Na_2S that was present during pulping. Surprisingly, also in the soda P1000 lignin approximately 1 wt% of sulphur was detected. In this case, the sulphur may originate from sulphuric acid used for the neutralisation step required for lignin precipitation. A very small amount of sulphur (0.1 wt%) was detected in the organosolv wheat straw lignin, which probably emerges from H_2SO_4 that was used as a catalyst in the process.

Chemical structure

Pyro-GC-MS. Pyro-GC-MS has been shown to be a useful tool for lignin structure elucidation.[4,15,36] The aromatic com-

Table 1 Composition of the lignin samples used in this study, expressed in weight percentage and based on dry weight[a]

	Lignin		Carbohydrates[b]								
	AIL[c]	ASL[d]	Arabinan	Xylan	Galactan	Glucan	Mannan	Sum	Ash	Sum	Sulphur
Indulin Kraft	90.3	1.9	0.1	0.6	0.6	0.1	<0.1	1.4	2.6	96.2	1.7
Soda P1000	85.1	5.4	0.2	1.5	0.2	0.5	<0.1	2.4	2.5	95.5	1.1
Alcell	94.3	1.9	<0.1	0.1	<0.1	0.1	<0.1	0.2	<0.1	96.4	0.0
OS-W	94.1	0.9	0.1	0.2	<0.1	0.2	<0.1	0.5	<0.1	95.6	0.1
OS-P	94.3	1.6	<0.1	0.2	<0.1	0.1	<0.1	0.3	<0.1	96.1	0.0
OS-S	95.5	0.8	<0.1	0.2	<0.1	0.3	0.6	1.1	<0.1	97.4	0.0

[a] Error analyses are reported in Table S3. [b] Expressed as polysaccharide. [c] AIL: acid insoluble lignin. [d] ASL: acid soluble lignin.

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ponents identified using Pyro-GC-MS reflect the botanical origin of the lignins, for instance (Fig. S1 and Table S6†). For both softwood lignins, Indulin Kraft and OS-S lignin, the major aromatic components identified are guaiacols, in decreasing order of abundance: 4-methylguaiacol (creosol) > guaiacol > 4-ethylguaiacol > *p*-vinylguaiacol. The relatively high occurrence of 4-ethylguaiacol found in this study is remarkable when compared to previous studies.[15,36] The possible formation of *p*-vinylguaiacol upon pyrolysis from ferulates as reported previously[18] can be excluded for both softwood lignins, given the absence of ferulic acid signals in their 2D NMR spectra (Table 3). In addition to the guaiacols, also demethylated aromatics, such as phenol and catechol, most probably secondary products formed during pyrolysis, were identified.

For hardwood lignins, Alcell and OS-P lignin, the three major components identified were: 4-methylsyringol > syringol > 4-methylguaiacol. A remarkably high amount of phenol was found for OS-P lignin. This might result from the degradation of *p*-hydroxy benzoate units upon pyrolysis (see also the NMR section). Also the pyrograms for both herbaceous lignins, P1000 and OS-W lignin, were similar and in line with previous data for P1000.[15] Key components identified included *p*-vinylguaiacol (possibly partly derived from ferulates,[18] Fig. 3), 4-methylguaiacol, syringol, guaiacol and 4-methylsyringol. Notably, 4-vinylphenol, identified in previous work as a major aromatic component,[15] was not detected here. Acetic acid and furfural were commonly detected for all samples (Table S6†). Herein, furfural may result from pyrolysis of residual carbohydrates as well as from the decomposition of lignin–furan condensation products that were formed during the organosolv pretreatment.[37] Acetic acid may originate from the partial acetylation of the γ-carbons of lignin.[18] In addition, various saturated and unsaturated fatty acids were found for all lignins, except for Kraft lignin (Table S6†). Hexadecanoic acid (palmitic acid) was the most abundant one. Fatty acids are commonly present in plants, especially in herbaceous biomass and the bark of trees and are part of the 'extractives' (Table S1†). Apparently, (derivatives of) fatty acids are resistant to the process conditions applied during organosolv and alkaline pretreatment. Cutin, a fatty acid-based waxy polymer found on aerial surfaces of plants, has been reported previously to persist thermochemical treatments of wheat straw.[38] The (ethyl esters of) fatty acids may have co-precipitated with the lignin or originated from fatty acid-lignin condensation products formed during organosolv pulping.

^{31}P NMR. Aliphatic alcohols, phenolic 5-substituted-OH, guaiacyl-OH, *p*-hydroxy-OH, and carboxylic acid groups present in lignin can be distinguished and quantified by ^{31}P NMR after phosphitylation.[25] Spectral integration limits and assignments are based on recent work by Balakshin *et al.*[39] These authors suggested that combined signal integration of syringyl and condensed 5-substituted phenolic units, prevents the overestimation of S-units and underestimation of G-condensed units. In addition, they claimed that the use of cholesterol and cyclohexanol, the most commonly used internal standards in lignin ^{31}P studies, lead to an underestimation of OH groups of as much as 20%. Therefore, it was suggested to use *endo*-*N*-hydroxy-5-norbornene-2,3-dicarboximide as internal standard instead.[39] However, the latter compound was reported not to be stable in the sample preparation mixture, unlike cholesterol, which is stable under the applied conditions for at least 16 h.[39] We therefore still chose to use cholesterol and cyclohexanol as internal standards. The ^{31}P NMR spectra of the phosphitylated lignins are shown with chemical shift assignments in Fig. 1. Table 2 shows the amount of hydroxyl groups expressed in mmol of functional groups per gram of dry lignin as determined from the ^{31}P NMR spectra. Taking into account the results of Balakshin *et al.*,[39] we only report the total 5-substituted phenolic –OH and do not differentiate between S units and G-condensed units. Measurements in two laboratories showed fair reproducibility, providing results that are in line with previous studies (Tables S7 and S8†).[14,40,41]

The distribution of phenolic OH-groups is directly correlated with the natural abundance of the S/G/H units in the biomass. For example, herbaceous lignins, such as soda P1000 and OS-W, were the richest in *p*-hydroxyphenyl groups. High amounts of 5-substituted OH were observed for the hardwood lignins, Alcell and OS-P, while for the softwood lignins, Indulin Kraft and OS-S, only a weak signal was observed in the 5-substituted OH region. Note that, the protolignins of these softwoods do not contain syringyl units and are exclusively composed of guaiacyl and *p*-hydroxyphenyl units. As evidenced by Balakshin *et al.*, the signals detected for these lignins between 140.5–144.5 ppm correspond only to 5-condensed guaiacyl units.[39] In the literature, similar amounts of 5-substituted OH can be found for softwood Kraft lignin, with values of 1.3 and 1.9 mmol g^{-1} being reported.[42,43] In addition, higher G-OH values were found for the softwood lignins. The higher H-OH amounts found in herbaceous lignins may be related to the high *p*-coumarate contents (see HSQC NMR data below). The total amount of phenolic hydroxyls are similar to the data reported in the literature for organosolv and Kraft lignin.[13,14,43] Lower values were found in lignins from milder pretreatments such as milled wood or enzymatic pulping (0.70–1.2 mmol g^{-1}).[44,45] Heikkinen *et al.* recently identified other covalently-bound phenolic structures in wheat straw lignin, tricin, in particular.[21] Such units were indeed also detected in OS-W.

The broad peak between 145.5 and 149.5 ppm originates from hydroxyl groups on the aliphatic lignin side chains and carbohydrate hydroxyl groups.[46] The carboxylic acid groups revealed by ^{31}P NMR may originate from adventitious oxidation during or after pulping or from associated components such as fatty acids in the lignin.[47] Unfortunately, the differences in chemical shifts of such carboxylates are too small to differentiate. As previously reported,[40,41,48] carboxylic acid groups are present in significant amounts in Indulin Kraft, soda P1000, Alcell and OS-W lignins. It is important to keep in mind the selectivity limitations of the ^{31}P method. For example, the presence of aldehyde groups in the lignin structure may lead to an overestimation of the total amount of hydroxyls.[49,50] In

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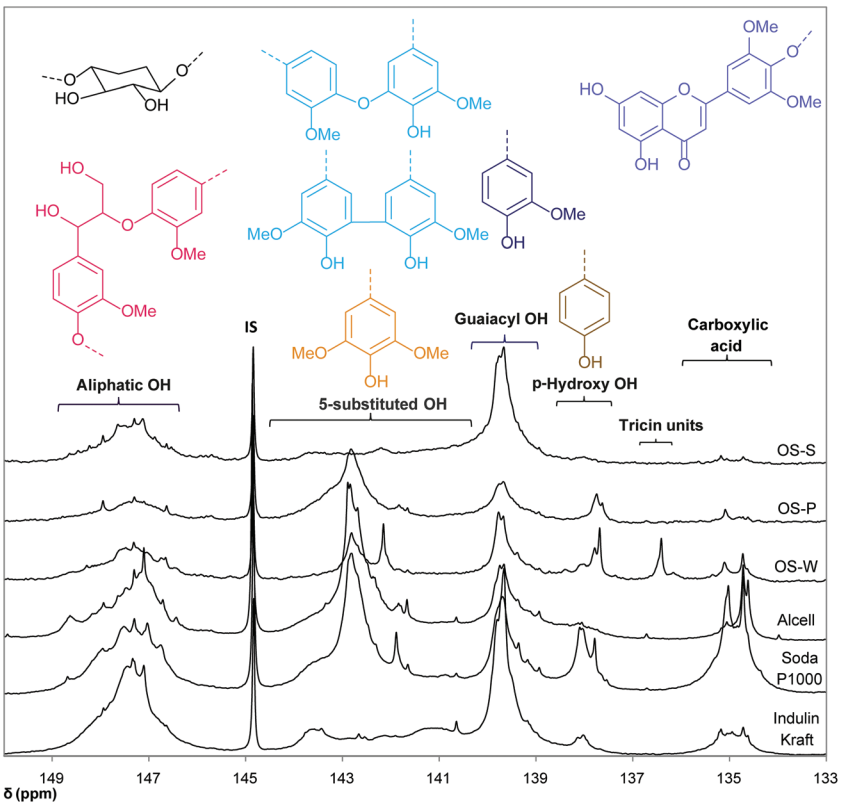

Fig. 1 Functional group analysis of the lignins under study by quantitative ^{31}P NMR measurements after phosphitylation.

Table 2 Content in hydroxyl groups of lignins quantified by ^{31}P NMR using cholesterol as internal standard and expressed in mmol of functional group per g dry lignin[a]

	Aliphatic OH	5-Substituted OH	Guaiacyl OH	*p*-Hydroxyphenyl OH	Total PhOH	COOH	Free COOH/tricin[b]
Indulin Kraft	1.79	1.31	1.30	0.16	2.77	0.33	0.05
Soda P1000	1.26	1.73	0.73	0.40	2.86	0.80	0.14
Alcell	1.04	1.68	0.58	0.11	3.30	0.22	0.00
OS-W	1.27	1.24	0.92	0.38	2.54	0.21	0.20
OS-P	0.80	1.83	0.58	0.18	2.59	0.07	0.00
OS-S	1.43	1.21	1.44	0.08	2.73	0.06	0.00

[a] Equivalent results obtained with cyclohexanol as internal standard and an error analyses are reported in Table S3. [b] Tricin units were quantified by integration of the peak at 136.4 ppm.

addition, the thiol groups present in the alkaline lignins can also react with the phospholane reagent leading to thiol-phospholane compounds;[51] further investigations are needed to assign their chemical shift.

The relatively high phenolic OH to aliphatic OH ratios measured indicate that substantial cleavage of phenolic ether linkages and subsequent recondensation *via* non-classical linkages have occurred during pulping. Indeed, this is also evidenced by the large amount of 5-substituted OH groups that are detected, suggesting recondensation *via* new ether bonds and C–C coupled units. It should finally be noted that, while the ^{31}P NMR spectra provide useful information on the lignin structure, one has to be cautious with drawing strong conclusions from the data, in particular, given the overlap of S and G-condensed units.

2D NMR. The lignins were investigated by quantitative CPMG-improved Q-CAHSQC 2D NMR measurements.[52] The latter method compensates for the influence of $^{1}J_{CH}$ and J_{HH} couplings on signal volumes. The interscan delay was optimised to 6 s, allowing full relaxation of the signals of interest in the aliphatic oxygenated and aromatic regions. To reduce the total experiment time, the number of increments was reduced, compared to the original study.[52] The use of a cryoprobe in combination with high lignin concentrations assured high signal-to-noise ratios. The influence of zero-filling, apodisation and linear prediction parameters was assessed to

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improve spectral resolution without affecting signal intensities. Synthetic model compounds containing β–β and β-O-4 linkages allowed validation of the quantitative nature of the analysis (Table S9†). Reproducibility of quantification, the influence of spectral processing, and standard deviations were determined as discussed in the ESI.† Four regions of interest were considered: (i) the aliphatic side chain region (δ_C/δ_H 5–38/0.5–2.8), (ii) aliphatic oxygenated side chain region (δ_C/δ_H 50–90/2.5–5.8), (iii) aromatic/unsaturated region (δ_C/δ_H 90–150/5–8.5), and (iv) aldehyde region (δ_C/δ_H 170–210/9–10).

(i) In the aliphatic side chain region significant differences were observed for the various lignins (Fig. S2†). The cross peaks that could be identified in this region provided information on the presence of various fatty acids, in line with the pyro-GC-MS data. However, little information could be obtained on the other dominant linkages present. The aromatic and aliphatic oxygenated side chain regions of the recorded spectra are in agreement with previous work and peaks were assigned accordingly.[18,21,22] A common method to quantify lignin HSQC spectra makes use of the aromatic units as internal standard.[19] The number of inter-unit linkages can then be quantified either by using the signal intensity from the entire aromatic region[20] or from selected syringyl and guaiacyl peaks.[19,24,53] The latter method was chosen here to express the abundance of the linkages (A: β-O-4, B: β-5 and C: β–β) and substructures per 100 aromatic units (Ar).

(ii) Aliphatic oxygenated side chain region. The oxygenated side chain part of the HSQC spectra of the six lignins is depicted in Fig. 2. The cross peak assignments and the corresponding abundances of the inter-unit linkages are reported in Table S10† and Table 3, respectively. As expected from the protolignin structure, the presence of methoxy groups is pronounced in all lignin spectra. The relatively high xylan contents in soda P1000 lignin (Table 1) was confirmed by 2D NMR at 3.1/73.1, 3.3/73.9 and 3.6/75.6 ppm (Fig. 2b, black spots). The presence of xylan in other lignins could not be confirmed. The β-aryl-ether (A: β-O-4), phenylcoumaran (B: β-5) and resinol (C: β–β) linkages were omnipresent. However, their relative abundance strongly varied and correlated with the isolation process. The amount of these linkages found in the commercial lignins (Indulin Kraft, soda P1000 and Alcell) is lower than in previous studies (Table S12†).[54,55] For the alkaline lignins, the most abundant linkage, *i.e.* for those that can be detected by HSQC, was the β-O-4 linkage (Table 4). Alcell and OS-W lignin were the richest in β-aryl-ether linkages. The signals for β-O-4 linkages were almost completely absent in the spectra of OS-P and OS-S. Surprising from a chemical point of view, higher amounts of the β-5 linkage were observed in the organosolv lignins. In fact, this linkage was the most abundant in OS-S lignin. It should be noted that the sum of the identified linkages per 100 aromatic units is low, which means that most of the inter-unit linkages in the technical lignins are not identified in or detected by HSQC, which requires C–H fragments with unique correlation patterns. Although linkages involving tertiary and quarternary carbons have been quantified in native lignin (*i.e.*, dibenzodioxocin, 4-O-5), these are not (former) or only limitedly (latter) seen here, suggesting a large increase in other (tertiary or quaternary) C–C bond-based linkages after pretreatment. An important conclusion therefore is that these technical lignins have a low β-O-4 content, as a result of the severe pulping conditions applied. Similar results have been reported for other soda, hydrotropic and organosolv lignins.[11,56]

The values found for OS-P lignin differ significantly from the literature, however, as Bouxin *et al.*[11] reported a substantially higher total amount of inter-unit linkages for an organosolv poplar lignin. The differences may be explained by the milder pulping conditions used by these authors. To put these numbers in perspective, milled wood, cellulase-digested and AFEX lignins contain between 35 to 65 β-O-4 linkages per 100 Ar units.[11,21,53] The acid-catalysed degradation of the inter-unit ether linkages in the organosolv lignin is also evidenced by the presence of a peak corresponding to a Hibbert's ketone structure (Hk). These structures were previously observed in the HSQC NMR spectra of birch and Douglas fir dioxosolv lignins.[57] Given the emphasis put on β-O-4 cleavage in catalytic lignin valorisation studies,[2] the low values clearly put an upper limit on the efficiency of such strategies when applied on such technical lignins. Thus, lignin valorisation or depolymerisation strategies that specifically target the β-O-4 linkage are in need of milder, but still effective, fractionation processes, such as organosolv processes that operate at lower process temperatures[58] or, alternatively, need to be applied to protolignin, *i.e.* use whole biomass as the substrate (also called the 'lignin-first' approach).[59]

(iii) Aromatic/unsaturated region. Fig. 3 shows the aromatic/unsaturated part of the HSQC spectra of the six lignins. The calculated amounts of aromatic units are presented in Table 3. As expected, more guaiacyl components were found in the two hardwood lignins. Syringyl cross peaks were not observed in softwood lignins (Indulin Kraft and OS-S). The softwood lignins also do not show the specific signature of the 4-O-5 linkage.[22] The HSQC NMR data thus aids the assignment of the 5-substituted OH region of the ^{31}P NMR of the all-G softwood lignins, by excluding the presence of any 4-O-5 linkage. The 5–5 condensed units that are expected to contribute to the 5-condensed OH region in the ^{31}P NMR spectra, cannot be distinguished in the HSQC data, as its G2 signal overlaps with the broad G2 signal of the unsubstituted guaiacyls. Higher amounts of H units were detected in the alkaline lignins than in the organosolv ones. This suggests that *p*-substituted units are strongly modified by organosolv pulping, regardless of the biomass. As for G units, condensation of H units in positions 3 or 5 with phenyl or phenoxy groups may occur during pulping. Further NMR investigations on model compounds are needed to determine the chemical shifts of such H-condensed units. In all syringyl-containing lignins, a cross peak is observed at 7.4/107.4 ppm which can be assigned to α-oxidized S units.[17,18] However, the presence of any of the equivalent α-oxidized G units could not be unequivocally established. The signal observed at 7.5/111 ppm might correspond to the G2 cross peak of such an α-oxidized G unit, but the G6 signal that should accompany this signal should then be found at a higher field than observed (7.3 instead of 7.6 ppm).[60,61] These

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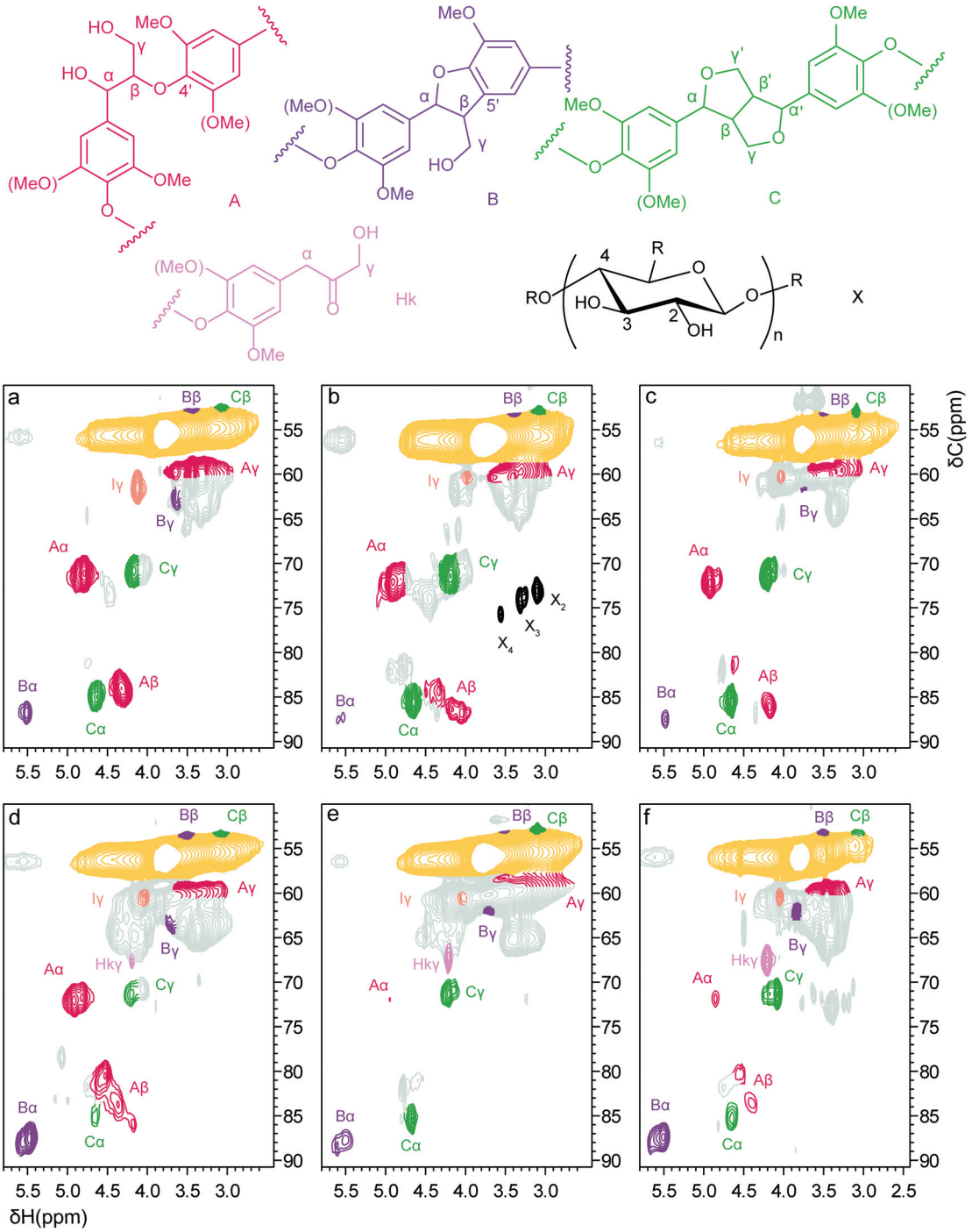

Fig. 2 Top: the main lignin side chain structures identified (A) β-O-4, (B) β-5, (C) β–β, yellow peaks correspond to OMe groups. Hibbert's ketone and carbohydrate signals are also depicted; see Fig. 3 for the structure labelled I; bottom: oxygenated aliphatic side chain (δ_C/δ_H 50–90/2.5–5.8) regions in the 2D HSQC NMR spectra of lignin (a) Indulin Kraft, (b) soda P1000, (c) Alcell, (d) OS-W, (e) OS-P and (f) OS-S.

two peaks may also overlap with ferulate, which casts additional doubt on the presence of α-oxidized G units.

The [^{1}H;^{13}C] NMR data shows that both herbaceous lignins (soda P1000 and OS-W) contain ferulate (Fa) and *p*-coumarate units (Pca, Fig. 3). These units, natively present in grass and cereal lignins, originate from acylation of lignin side chains with *p*-coumaric and ferulic acids and are connected *via* ester and acetal linkages, respectively.[17,62] The *p*-coumarate contents, similar to previous reports,[53,63] were lower than those quantified in enzymatic mild acidolysis lignin.[21] In addition, other end-groups, such as cinnamate units (I), were also found in the lignins (Fig. 2 and 3). Finally, *p*-hydroxy benzoate groups

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Table 3 Quantification of side chains and aromatic units present in lignins[a]

	Indulin Kraft	Soda P1000	Alcell	OS-W	OS-P	OS-S
β-O-4[b]	6.1	3.4	5.3	4.3	0.1	0
β-5[b]	0.3	0	0.8	4.5	1.8	3.3
β–β[b]	1.0	0.7	2.8	0.1	1.1	0.2
Total ether side chains[b]	7.4	4.1	8.9	8.9	3	3.5
Stilbenes (St)[b]	2.3	0	0.4	0.4	0	0.7
p-Coumarate (Pca)[b]	0	3.2	0	2.2	0	0
p-Hydroxy benzoate (Pb)[b]	0	0	0	0	9.4	0
Tricin (T)[b]	0	0	0	3.5	0	0
S[c] (%)	0	50	63	39	53	0
G[c] (%)	97	39	37	58	47	100
H[c] (%)	3	11	0	3	0	0
S/G ratio	0	1.3	1.7	0.7	1.2	0
H/G ratio	0	0.3	0	0.1	0	0

[a] Error analyses are reported in Table S11. [b] Expressed as a number per 100 aromatic units (S + G). [c] Molar percentage (S + G + H = 100).

(PB), typical of angiosperm lignins,[60] were indeed identified in the organosolv lignin from poplar.

The intense peak observed in the aromatic region at 7.0/126 ppm has previously been assigned to stilbene units.[13,64–66] We now confirm this by the 2D HSQC NMR spectra of the model compound 1-methoxy-4-[2-(4-methoxyphenyl)ethenyl] benzene, for which the olefinic cross peaks overlap well with the lignin signal (Fig. 4, S3 and S4†). Stilbenes (St) were observed in most lignins, but not in soda P1000 and OS-P. The large amount seen for Indulin Kraft lignin is remarkable. It has been proposed that stilbenes originate from a β-O-4 linkage, and can be formed by coupling of quinone methide and phenoxy radical intermediates.[67] The cross peaks at 6.2/99.1, 6.6/94.5 and 7.3/104.5 ppm have previously been observed for enzymatic mild acidolysis lignin of wheat straw[18,21] and have been assigned to tricin. We only observed tricin (T) in the OS-W lignin, in significant amounts, where it seems to be covalently bound to the lignin structure. The tricin seems to be partially lost after organosolv pulping. Previously, Heikkinen *et al.* quantified 13 tricin units per 100 aromatic units in EMAL, this value decreased to 2 after a steam explosion treatment[21] and to 3.5 for our OS-W lignin.

(iv) The HSQC NMR analyses also provide insight into aldehyde functions present in the lignin macromolecule, something which has not yet been addressed in the literature. For organosolv lignins an intense signal was observed at δ_H/δ_C 9.6/179 ppm (Fig. 4 and S5†). This signal is assigned to a furfural derivative that is incorporated in the structure, since furfuraldehyde, 5-hydroxymethylfurfural and 5-(methoxymethyl)furfural all resonate at 179 ppm (Fig. S6–S8†). Such cross peaks have not been observed for milled wood lignin.[68] The presence of a furanic aldehyde is further substantiated by the signal seen at 7.5/123 ppm, which can be assigned to a furanic C–H signal, in line with the chemical shift found for 5-substituted furfurals. These furfural-type units arise from the sugar dehydration reactions that occur during the organosolv process; the occurrence of lignin–furfural condensation has been reported extensively for organosolv processes.[28,37]

FT-IR. Analysis of the six lignins by FT-IR spectroscopy confirms the structural observations made above. Indeed, FT-IR allows the lignins to be discriminated in terms of syringyl and guaiacyl composition. The characteristic bands of syringyl units at 1330 and 1117 cm^{-1} are absent in softwood lignins (Fig. S9†), while the relatively high intensity at 1514, 1271 and 1032 cm^{-1} are related to guaiacyl units. Furthermore, two bands in the out-of-plane vibration region of aromatic rings can be found at 860 and 815 cm^{-1}. In the carbonyl region of OS-W a strong, relatively sharp band at 1670 cm^{-1} is observed, that corresponds to the vibration of a conjugated ketone. Additional hydrogen bonding may be responsible for lowering the frequency. This vibration is probably related to the double conjugated carbonyl in the tricin units found for this lignin only. At 965 cm^{-1}, a weak band can be observed only in the Indulin, OS-W and OS-S lignins, which may be assigned to the out-of-plane deformation of *trans* double bonds. Such a *trans* double bond is present in stilbene units, which have been identified only in these lignins by NMR. In the case of soda P1000 lignin two bands were observed at 2917 cm^{-1} and 2850 cm^{-1}, which may be related to the fatty acid derivatives that are contained in this lignin.

Taken together, the multi technique approach used provided important insights into the different (sub)structures of the technical lignins. The distributions in aromatic units (S, G, H) and hydroxyl groups showed the presence of substantial amounts of condensed units. In addition, degradation products, such as stilbenes and an ether-linked furfural, have been identified. Notably, the low total amount of linkages quantified in the present study suggests that most of the lignin linkages can actually not be observed by HSQC NMR, and different approaches need to be developed to elucidate their structure. In any case, the low abundance of cleavable aryl–ether bonds needs to be considered when devising lignin valorisation strategies.

Molar mass distribution

The molar mass distribution of the lignins was determined using Size Exclusion Chromatography (SEC). Without the availability of proper lignin standards, this technique provides relative molar mass values for lignin at best. Previously, a number of studies aimed to address the issue of standardisation and the difficulties associated with lignin molar mass determination using SEC.[16,69] The observed molar mass heavily depends on the experimental set-up used, including the type of columns and eluent, as well as on the calibration and calculation methods applied.[14,16] Commercial and lignin-compatible SEC systems and columns are now available making comparison of data a great deal easier. The objectives of the SEC analyses described here, were to obtain the molar mass of the six lignins under identical conditions and to assess laboratory- and method-dependence of the reproducibility of molar mass determination. For this purpose, a (mini) round robin

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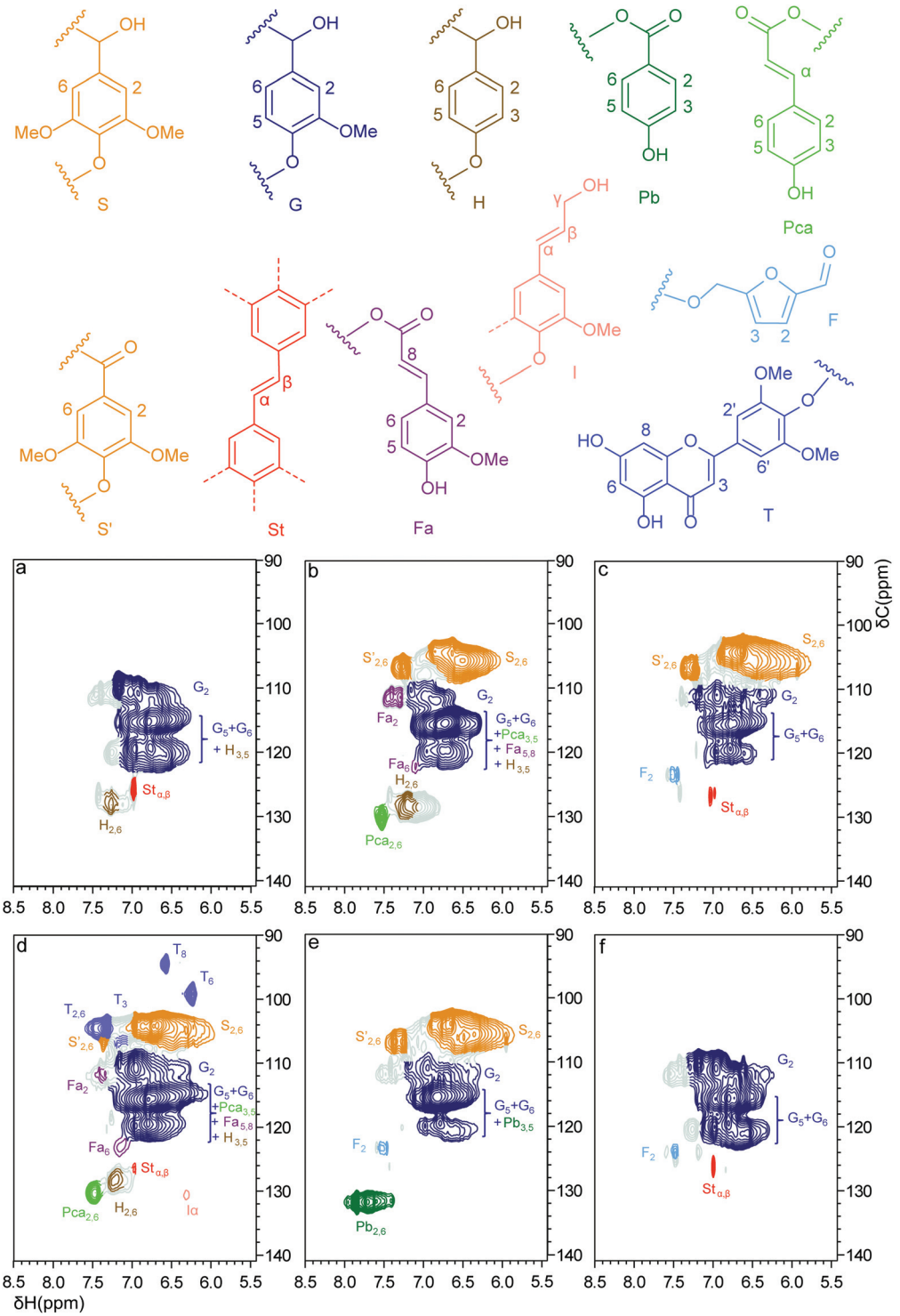

Fig. 3 Top: the main lignin aromatic structures identified; bottom: aromatic/unsaturated regions in the 2D HSQC NMR spectra of lignins (a) Indulin Kraft, (b) soda P1000, (c) Alcell, (d) OS-W, (e) OS-P and (f) OS-S.

within three laboratories has been conducted to compare nine different methods (Tables 4, S13, S14† and Fig. 5). Herein, three different eluents were used, *i.e.* alkaline water, tetrahydrofuran and hexafluoroisopropanol. This latter eluent has been used for the molar mass analysis of polyesters, but not yet for lignin.[70–72] Different systems were compared, such as home-packed *versus* commercial columns and, depending on the system used, different calibration standards (polystyrene, (PS); sodium polystyrene sulphonates, (SPS); or poly(methyl methacrylate), (PMMA)) were applied. Lignin solubility in

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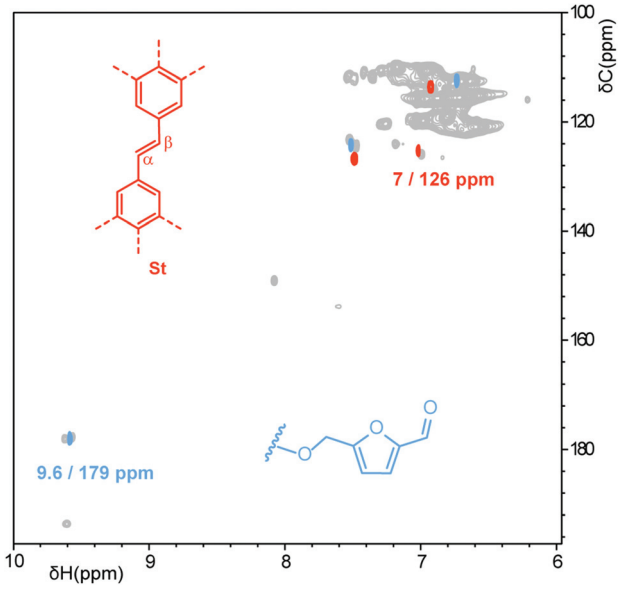

Fig. 4 Partial 2D HSQC NMR spectra of OS-S lignin overlapped with generalized stilbene (red) and oxymethylfurfural structures (blue); the coloured signals correspond to 5-(methoxymethyl)furfural and 1-methoxy-4-[2-(4-methoxyphenyl)ethenyl]benzene.

organic solvents, such as THF, is limited and any data thus obtained will not be representative for the whole sample. Acetylation of lignin solves this problem, since acetylated lignin is fully soluble in THF.[16]

The SEC measurements in alkaline media were performed with four different columns; one of these methods included the use of home-packed columns, prepared in two laboratories (method A and A′). While absolute M_w values found with A and A′ differed, the trend in M_w was the same for both methods. These differences are most likely due to small differences in column packing and data handling. Methods B and C make use of two different commercial columns instead. The differences seen in absolute values show the effect of the stationary phases of the columns, although the general trend is again the same. The addition of a second column resulted in a slightly lower average molar mass (method C *versus* method D). This combined column system expectedly allowed for a better separation and hence should provide more reliable molar mass distribution data. Three different THF-based methods (F–H) were compared to evaluate the influence of column choice and calibration method. Molar masses similar to the alkaline SEC methods were obtained with method F. Rather surprisingly, method G afforded much lower molar mass values (between 600–1000 g mol^{-1}). A comparison of method G and H shows that higher M_w values are obtained with PMMA (method H) than with PS (G), under otherwise identical conditions.

Finally, hexafluoroisopropanol (HFIP) was investigated as a new eluent for lignin molar mass determination, with both lignin and acetylated lignin (method E and E′, respectively). HFIP is expensive, but can be recovered and has the major advantage of easily dissolving (bio)polymers at room temperature.[70–73] Indeed, all six lignins were completely dissolved in HFIP without the need for derivatisation. The molar masses obtained with method E were considerably (1.5–8 times) higher than those recorded with the other methods. This could reflect differences in hydrodynamic volumes, which may be considerably different in HFIP. Interestingly, the use of this solvent allows one to study the effect of acetylation on lignin M_w distribution. As with method E, the molar masses obtained with acetylated lignins (method E′) are again (much) higher than with the other methods. Other than that, no correlation is observed between the values calculated for the acetylated and non-acetylated lignins. Even the order in M_w is different. This might be due to differences in acetylation degree, as the lignins contain different amounts of –OH groups that can be acetylated, as indicated by the ^{31}P NMR results (Table 2). It should be noted here that it is not yet clear if HFIP is completely (chemically) inert towards (acetylated) lignin. Further work is needed to elucidate this.

The above investigations show that the choice of eluent, column type, calibration standard and lignin pretreatment, all expectedly influence the M_w determination. Data handling, including peak integration strategy and choices made in correction for unretained materials also contributes to the differences. Indeed, a comparison of the GPC chromatograms obtained with the different methods, shown for Alcell in Fig. 5c, shows how large the differences can be, with bimodal distributions even being observed in some cases.

Based on the above observations, some recommendations for molar mass determination of technical lignins can be made. Among the alkaline systems studied, the new commercially available columns seem to be a good alternative to home-packed columns. Commercial columns give reproducible results and have found to be stable for at least one year of continuous operation. Independent of the eluent, using a combination of columns enhances separation power. The molar masses obtained by the THF-based methods are more similar to the ones obtained by alkaline SEC for the smaller polymers (such as the organosolv lignins). This method might be preferred, but only when the complete lignin sample is soluble in THF. When derivatisation of the lignin is required for full solubilisation, other solvents are preferred, in particular, alkaline. The use of HFIP as an eluent could bypass such solubility issues, but requires a more detailed study. Given the above, method D (0.5 M NaOH, PSS, 2 commercial columns) is considered as the preferred method for lignin molar mass comparison (see Fig. 5b). The molar mass of lignins obtained with this method ranged from 4290 to 1960 g mol^{-1} (Table 5), with Indulin Kraft lignin exhibiting the highest value and the organosolv lignins showing the lowest. The average molar mass of all organosolv lignins was lower than that for the Alcell lignin standard. No significant effect of the biomass source was found on the average molar mass of the organosolv lignins. However, it does have a significant effect on the M_w distribution, for example OS-P contains a much larger fraction of low M_w components than the others (Fig. 5b). Independent of the SEC method chosen, the average M_w of the lignins

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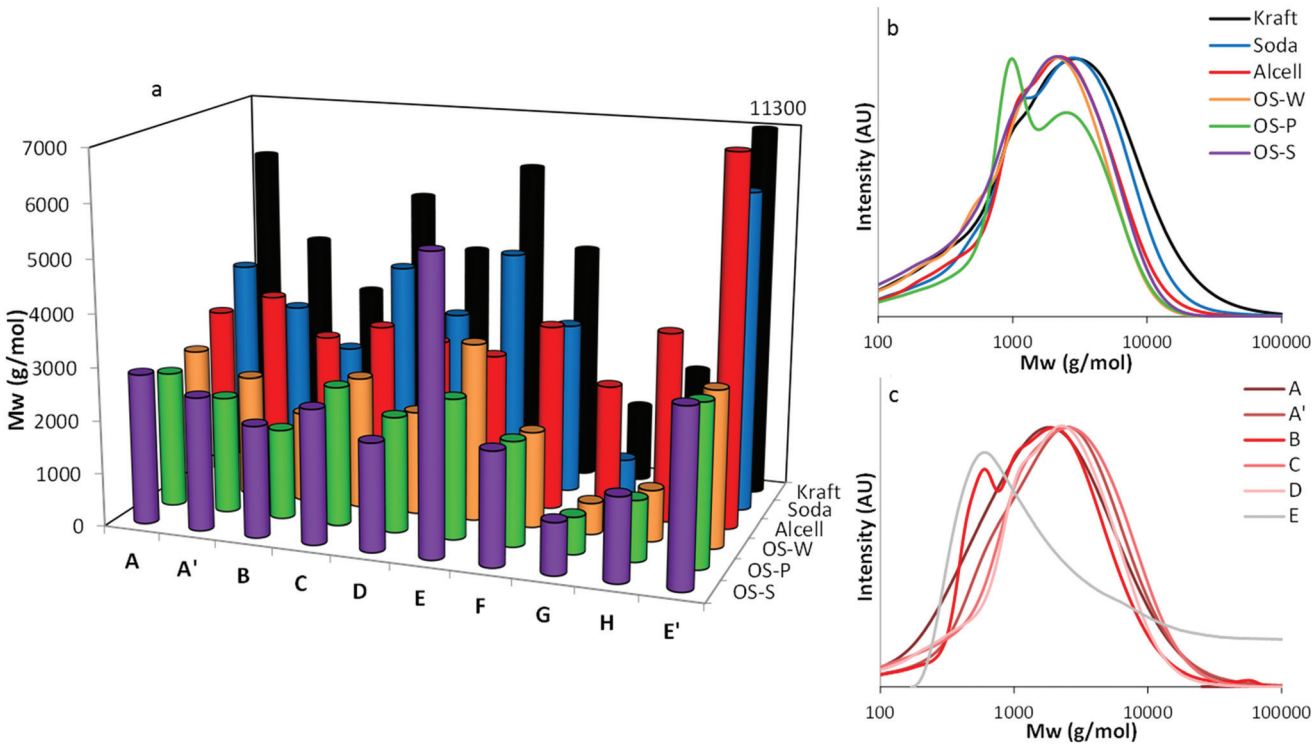

Fig. 5 (a) Molar mass of lignins and acetylated lignins measured with different SEC methods, (b) SEC chromatograms of the six lignins measured with method D, (c) SEC chromatograms of Alcell lignin (non-acetylated) measured with different SEC methods (see Table 4 for method details).

Table 4 Analytical details of the SEC methods employed

Method	Solvent	Column(s)	Column M_w specifications (g mol^{-1})	Standard[b]	Acetylated lignin
A	0.5 M NaOH	Home-packed	1000–700 000	SPS	No
A′[a]	0.5 M NaOH	Home-packed	1000–700 000	SPS	No
B	0.5 M NaOH	PSS MCX	100–35 000	SPS	No
C	0.5 M NaOH	TSKgel GMPWxl	500–8 000 000	SPS	No
D	0.5 M NaOH	2 × TSKgel GMPWxl	500–8 000 000	SPS	No
E/E′	HFIP	PSS PFG	100–1 000 000	PMMA	No/yes
F	THF	3 × PL-gel mixed-E	Up to 25 000	PS	Yes
G	THF	2 × GMHhr-M	100–3 000 000	PS	Yes
H	THF	2 × GMHhr-M	100–3 000 000	PMMA	Yes

[a] Method A′ is identical to method A, but was run at a different laboratory. [b] SPS: sodium polystyrene sulphonate; PMMA: poly(methyl methacrylate); PS: polystyrene.

decreases in the order: Indulin Kraft > soda P1000 > Alcell > OS-W ~ OS-P ~ OS-S. The polydispersity indices (PD) followed the same trend; the highest PD was observed for the Indulin Kraft lignin and the lowest for the organosolv lignins (8.1 and 3.8 respectively). These molar masses are in good agreement with previous studies and can be directly correlated with the severity of the pretreatment. For example, molar masses of alkaline lignin have been reported in a range between 6700 and 7200 g mol^{-1}.[43,74,75] The M_w range found for organosolv lignins, having been more fragmented by the pulping process, is significantly lower (2800–1000 g mol^{-1}).[76,77]

Table 5 Molar masses (M_w, M_n) expressed in g mol^{-1} and polydispersity (PD) of lignins measured with the SEC method D. See Table 4 for method details

	M_w	M_n	PD
Indulin Kraft	4290	530	8.1
Soda P1000	3270	620	5.2
Alcell	2580	600	4.3
OS-W	1960	450	4.4
OS-P	2180	570	3.8
OS-S	2030	420	4.9

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Conclusions

Lignin's structural complexity and its dependence on feedstock and isolation process require comprehensive characterisation in order to assess which (catalytic) depolymerisation method or which materials application is most suited for the particular lignin at hand and *vice versa*. To illustrate this, six lignins have been extensively characterised using a multitechnique approach, covering hardwood, softwood and grass lignins obtained by three common (industrial) isolation methods, Kraft, soda and organosolv pulping. These technical lignins have been analysed in terms of composition, by FT-IR, pyrolysis-GCMS, quantitative ^{31}P NMR and 2D HSQC NMR. Additionally, the molar mass distribution has been studied with various SEC systems, including different eluents, columns, and calibration standards. The most salient features of each of the lignins studied are summarised in Table 6.

Most notably, the very low abundance of cleavable β–ether bonds and of the highly condensed nature of these technical lignins needs to be considered when lignin valorisation strategies are devised. If lignin valorisation to renewable aromatics is targeted, rather harsh depolymerisation conditions are required for all lignins studied above to allow for sufficient C–C bond cleavage. If recondensation can be controlled under such conditions, high yields of a lignin oil of alkylphenolics can be obtained, as was recently shown.[78] The monolignol composition will then contribute to how amenable a lignin is to depolymerisation and will also determine the complexity of the product palette after depolymerisation. With regard to catalyst stability, the high purity of the organosolv lignins may be advantageous, as ash components and carbohydrate derivatives are expected to act as catalyst poisons. The presence of sulphur in the lignin could be detrimental to some, *e.g.* noble-metal based catalysts, but advantageous for others, such as sulphided hydrodeoxygenation catalysts. For material applications, the nature and amount of functional groups and molar mass distribution will be of prime importance. The nature, the relative concentration and accessibility of the hydroxyl groups in the lignin will determine its suitability for specific applications and differs considerably over

Table 6 Summary of compositional and structural characteristics of the six technical lignins studied

		Indulin Kraft	Soda P1000	Alcell	OS-W	OS-P	OS-S
		Softwood	Straw/grass	Mixed hardwood	Wheat straw	Poplar	Spruce
Composition/ elemental analysis	Ash content (wt%)	2.6	2.5	None/very low	None/very low	None/very low	None/very low
	Sulphur content (wt%)	1.7	1.0	None	0.1	None	None
	Other main minerals	Na, K	Na, K	Ca	—	—	—
	Carbohydrate content	Intermediate	High	Low	Low	Low	Intermediate
Pyro-GC-MS			High fatty acid content		High fatty acid contents		
HSQC NMR	General structure	All technical lignins are highly condensed, few ether linkages remaining					
	Total ether linkages (per 100 Ar units)	7.4	4.1	8.9	8.9	3.0	3.5
				β-O-4 > β–β	β-O-4 ≈ β-5	β-5 > β–β	Almost exclusively β-5
		Mainly β-O-4	Mainly β-O-4	Very low in β-5	Very low in β–β	Very low in β-O-4	Very low in β-O-4 and β–β
	Aromatic composition	Exclusively G	S and G	S and G	S and G	S and G	Exclusively G
	S/G/H ratio	0.00/0.97/0.03	0.50/0.39/0.11	0.63/0.37/ 0.00	0.39/0.59/0.03	0.53/0.47/ 0.00	0.00/1.00/0.00
	Other components		Ferulates *p*-Coumarates		Ferulates Tricin *p*-Coumarates	*p*-Hydroxy benzoates	
	Stilbenes	✓	×	✓	✓	×	✓
	Furfural units	×	×	✓	✓	✓	✓
^{31}P NMR	General structure	5-Substituted OH signals suggest a high degree of condensation High ratios of phenolic OH/aliphatic OH supports extensive phenol ether cleavage during pulping					
	Specific OH	Highest ratio of aliphatic to phenolic OH groups	High in carboxylic acids		Tricin units detected		
SEC (method D)	M_w (g mol^{-1})	4290	3270	2580	1960	2180	2030
	PD	8.1	5.2	4.3	4.4	3.8	4.9

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the various samples. Desirable functional groups and M_w properties for use of lignins in, for example, phenol–formaldehyde resins, polyurethane foams, thermoplastics and as antioxidants have been discussed.[14,79] For example, a rigid polyurethane foam has been prepared from lignins with a similar phenolic OH contents to the technical lignins studied here has.[80]

It is important to be aware of the limitations associated with each of the analytical tools. For instance, most of the inter-unit linkages in the technical lignins are actually not detected by [^{1}H;^{13}C]-HSQC NMR spectra, suggesting extensive tertiary or quaternary carbon–carbon bond formation. Different approaches are therefore needed to elucidate their structure. Taken together, the differences in the ash and sulphur content, functional group distribution, degree of condensation, organic impurities and molar mass distribution provide valuable, although yet incomplete, information for an educated selection when matching the choice of lignin feed with materials application or a particular depolymerisation process. In short, the large differences in the molecular structure compared to native lignin, the highly condensed nature of the technical lignins and the plant- and pretreatment-specific contaminations detected, all need to be taken into account.

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