Lignin Structure and Aggregation Behavior in a Two-Component Ionic Liquid Solvent System

Susanne Bylin,[a,b] Tyrone Wells,[a,†] Qining Sun,[c] Art Ragauskas,[d] and Hans Theliander [a,b,∗]

Ionic liquids are of potential interest in the processing of lignocellulosic biomass. In this study, the ionic liquid co-solvent system of 1-methylimidazole (MIM) and 1-ethyl-3-methyl-imidazolium acetate (EMIMAc) was used to solvate LignoBoost lignin fractionated from black liquor obtained from a kraft paper mill. Lignin ethanol-precipitated (LEP) and ethanol-soluble (LES) fractions were characterized *via* gel permeation chromatography (GPC) and ^{13}C- and ^{31}P-nuclear magnetic resonance spectroscopy (NMR) to determine structural characteristics and their relationship to polymer solubility in the system. Polymer integrity and solubility were optimal at ~20% lignin loading (w/w). Results showed that LEPs were generally of higher apparent molecular weight (M_w) and enriched with condensed/aliphatic ether linkages and aliphatic hydroxyls. The LESs had a lower apparent M_w and were enriched with carboxylic and phenolic groups. This newly gained knowledge on lignin fractionation and aggregation in the present solvent system provides future opportunities for tuning fractionation/extraction to suit a specific biomass-derived product, *e.g.*, carbon fibers.

INTRODUCTION

Ionic liquids (ILs) can be used to solubilize the prominent components of lignocellulosic biomass, including cellulose, lignin, and hemicellulose, the latter of which is chiefly composed of galactoglucomannans and xylans (Kilpelainen *et al*. 2007; Huang *et al*. 2008; Tan *et al*. 2010; Froschauer *et al*. 2013). The extent of degradation and solubilization of these wood biopolymers varies among ILs (Mäki-Arvela *et al*. 2010; Pinkert *et al*. 2011). Benefits of using ILs in biomass processing include energy efficiency with respect to fast solubilization at low temperatures and materials production with respect to high material efficiency and unique properties of IL-treated samples. Moreover, ILs are a highly versatile class of solvents that can be used either neat or with co-solvents to improve certain properties. Development of biorefinery processes that include an IL extraction step can either be aimed at selective component extraction or at a total biomass solubilization process with IL solvation as a pre-treatment step. However, the development

bioresources.com

of an optimized process for either aim requires an advanced understanding of the mechanisms of solvation to improve solvent system design while maintaining polymer integrity.

Previously, we have demonstrated that 1-methylimidazole (MIM) and 1-ethyl-3-methyl-imidazolium acetate (EMIMAc, Fig. 1) can be used to solvate both cellulose and xylan with high efficacy and minimal polymer degradation (Bylin *et al*. 2014). These results were obtained with a low volume of the more expensive ionic liquid component (EMIMAc) and at relatively low temperatures (30 to 70 °C). Furthermore, the lower viscosity of the MIM component will generally improve important factors for biomass solvation such as polymer mass transport and particle dispersion in solution, compared to using IL alone. Hence, there are several potential economic and process-design benefits for the use of this two-component system, and improved understanding of the chemical structure and dispersion/aggregation effects caused by MIM/EMIMAc can further augment its utility.
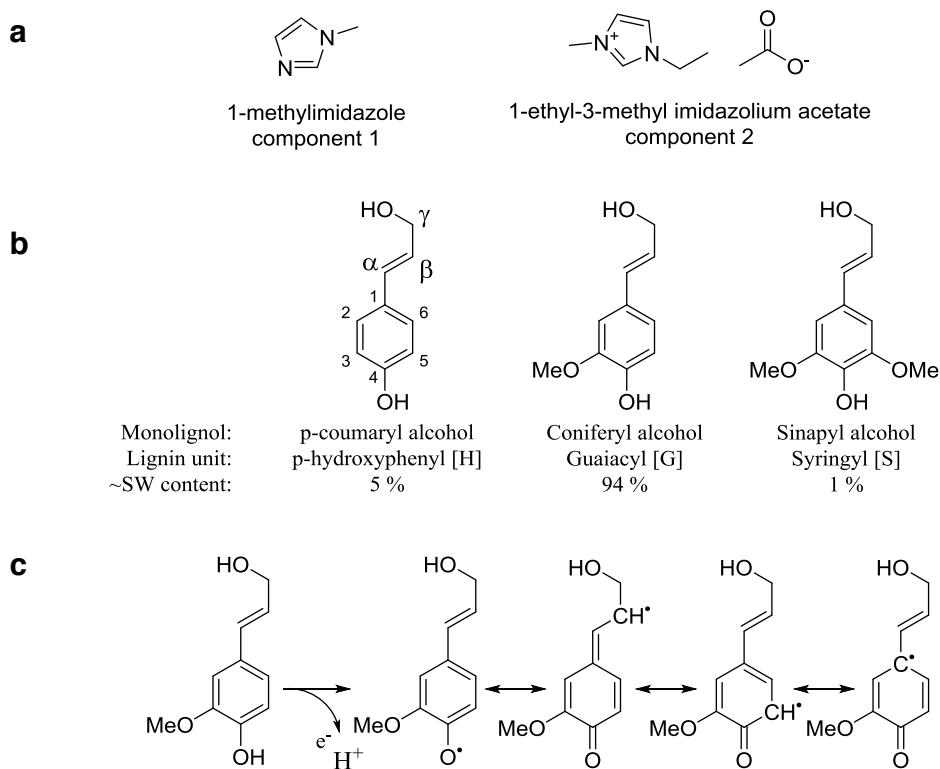

Fig. 1. (a) Molecular structures of 1-ethyl-3-methyl imidazolium acetate (EMIMAc) and the co-solvent of the present study, 1-methylimidazole (MIM); (b) content of the monolignols p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol in SW lignin. When incorporated into the lignin structure, these monolignols are referred to as the lignin units p-hydroxyphenyl [H], guaiacyl [G], and syringyl [S], respectively; (c) resonance-stabilized radical coniferyl alcohol structures

The current study is aimed at the solvation of lignin primarily from Scandinavian spruce softwood (SW), in which lignin is the second most abundant polymer component after cellulose. Experiments were performed using LignoBoost lignin fractionated from the

black liquor of the kraft process by a sequence involving acid precipitation. This is a high-purity lignin currently produced for large-scale commercial application at the Domtar Plymouth mill in the US; in 2015, production is due to start at the Sunila mill in Finland (Ollila 2013). Therefore, the study of the behavior of LignoBoost lignin is not only of interest as a model SW lignin compound, but also for its potential future commercial applications as a key component in renewable materials (Hu 2002; Stewart 2008; Paterson 2011). Specifically, LignoBoost lignin was herein solvated in the two-component ionic liquid solvent system MIM/EMIMAc with the aim of studying the stability of the biopolymer in the system and its fractionation and/or aggregation behavior upon regeneration.

General Structure and Reactivity of Softwood Lignins

Lignin is a three-dimensional polyaromatic macromolecule composed mostly of three types of phenylpropane (C9) units: p-hydroxyphenyl [H], guaiacyl [G], and syringyl [S] (Fengel and Wegener 1984; Dimmel 2010) (Fig. 1b). Cross-coupling of monolignols during biosynthesis is believed to occur through enzymatic dehydrogenation and a radical coupling mechanism (Freudenberg and Neish 1968; Sarkanen and Ludwig 1971). In fact, the ability of the monolignol structures to form resonance stabilized structures (*e.g.*, radical coniferyl alcohol, Fig. 1c) governs most of lignin's reactivity, both in formation and degradation of the lignin structures (*e.g.*, during pulping). The C9 units are present in varying amounts, depending on wood type and species. Their relative abundance in SW is presented in Fig. 1b, which shows that SW lignin is almost entirely composed of [G]-type lignin. Figure 2 presents the most common bonds formed in native SW lignin.
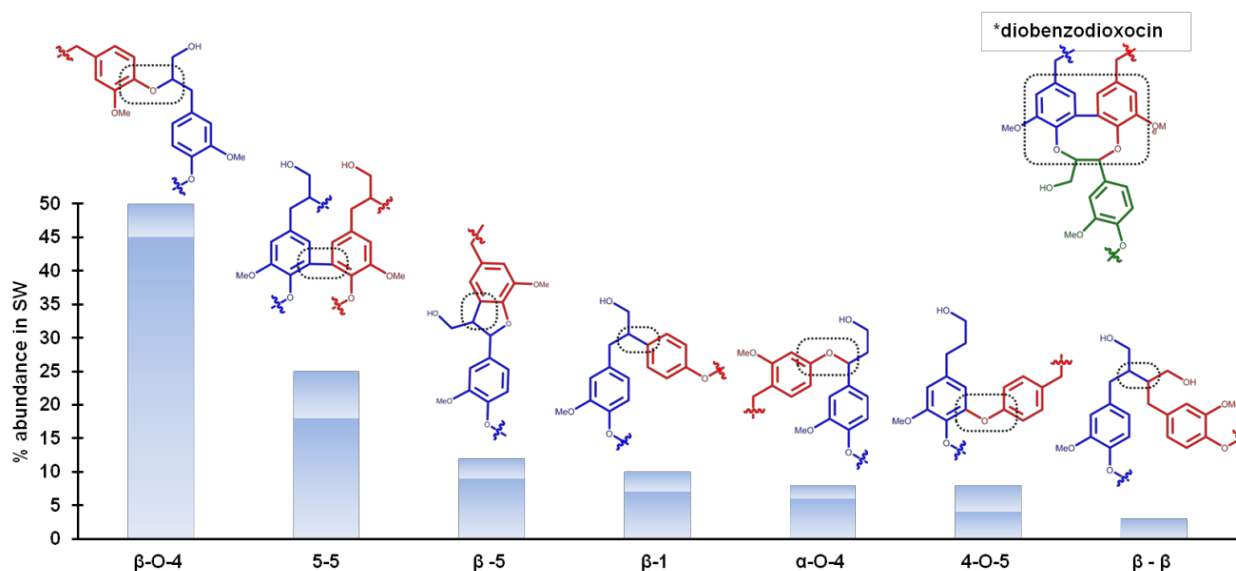
Fig. 2. Prevalence of the most common lignin linkages found in softwood lignin. The moieties responsible for the bond-names are marked inside the dotted lines. Structures are drawn based on the most commonly occurring elements in softwood only, *e.g.*, as guaiacyl units and without free phenolic groups. Intervals for percent abundance in different softwoods are marked at the top of each column (*the dibenzodioxocin structure is incorporated in the 5-5-linkage)

The high percentage of β-O-4-bonds is revealing as to the stability and/or reactivity of the different resonance forms of the guaiacyl unit. Moreover, the abundance of types of covalent linkages found in different lignins is also dependent on the harshness of the isolation method for that particular lignin (Björkman 1956; Crestini and Argyropoulos 1997; Crestini *et al*. 2011). For instance, many β-O-4-bonds are degraded in the kraft process, whereas some carbon-carbon bonds are concomitantly formed (Öhman *et al*. 2007).

Lignin Solubility and Aggregation Behavior

Regarding the solubility of lignin, Björkman first proposed that physicochemical parameters, rather than covalent bonding, were of greater importance than commonly thought at the time (Björkman 1956). He observed that lignins isolated in different manners, even though soluble as a single component in a specific system, were not necessarily possible to extract from wood using that same system. Regardless of the nature of the difficulty in extracting lignin from wood, studying the behavior of lignins in various solvent systems will give important clues as to how to optimize any extraction method and what possible issues might exist in that system. To date, studies have been conducted for various types of lignin isolates and model compounds in both aqueous and organic media (Ni and Hu 1995; Cathala and Monties 2001; Lawoko *et al*. 2006). Analysis of lignin by gel permeation chromatography (GPC) can result in either bimodal or unimodal elution profiles, depending on the type of lignin and the set conditions (Connors *et al*. 1980; Cathala *et al*. 2003; Guerra *et al*. 2007). Early on, it was proposed that lignin associative effects were governed by HOMO-LUMO interactions (highest occupied molecular orbital and lowest unoccupied molecular orbital, respectively), resulting in π-π-stacking of aromatic structures (Sarkanen *et al*. 1984). Cathala *et al*. confirmed these findings and further stated that the elution bimodality was not due to a reversible association-dissociation mechanism between aggregated and molecularly dispersed species. Rather, it was the result of association between different structural elements and how these interact, covalently or not, resulting in organized macromolecular clusters of different densities. The relative importance of the π-π-stacking interactions on the clustering of lignin was demonstrated by other researchers through the addition of iodine to a solution of acetobrominated enzymatic mild acidolysis lignin (EMAL) in THF. The complex-forming ability of iodine to the π-face of aromatic moieties overcame the lignin to lignin π-π-stacking and resulted in a unimodal elution profile (Guerra *et al*. 2007).

EXPERIMENTAL

To fulfill the aims of the study, lignin fractions were analyzed using GPC to provide information on the association behavior of the lignin and any changes in that behavior caused by prior treatments. Molecular weight distribution curves were determined in relation to the molecular structural elements found in the treated and untreated lignins using quantitative carbon and phosphorous nuclear magnetic resonance spectroscopy (NMR). NMR is an important and expanding field in lignin research that has provided much information on the structural composition and covalent linkages of lignin macromolecules

(Argyropoulos 2010; Ralph and Landucci 2010). To separate the effects potentially induced by regeneration from those induced by treatment in the solvent system, a reference LignoBoost lignin was treated with the regeneration media in parallel. Comparisons were also made to the original untreated LignoBoost lignin.

Finally, to examine the potential effects of derivatization on data obtained using GPC, and therefore also on the agglomeration behavior of lignin, the MIM/EMIMAc-treated samples were acetylated prior to a second set of GPC analyses.

Materials

LignoBoost lignin from the Bäckhammar mill in Sweden was dried in an oven overnight (40 °C), increasing the dry content from 36% to 96%. Bäckhammar is a softwood kraft paper mill that produces unbleached pulp. The IL 1-ethyl-3-methylimidazolium acetate ($\geq$ 90% purity) and the co-solvent 1-methylimidazole ($\geq$ 99% purity) were obtained from BASF and Sigma-Aldrich, respectively. Both solvents were kept under inert atmospheres and were used without further processing. Ethanol (99.7% purity) used for precipitation was obtained from Solveco.

Methods

Solvation

Lignin (3 g; 21.4% w/w in the system) was separately added to eight glass vials equipped with magnetic stir bars. IL and co-solvent were added to each vial according to Table 1. The samples were treated in the two-component solvent system at 70 °C with constant stirring, and after 4 h, they were each precipitated/regenerated in ethanol (300 mL). Centrifugation was performed (3200 rpm, 35 min), and the supernatants containing ethanol-soluble materials were decanted. The solid materials (pellets) were then re-slurried in a clean batch of ethanol, and centrifugation/decantation was repeated once more. Upon fractionation, precipitated samples were left to dry and were then collected as the lignin ethanol-precipitated materials LEP0-LEP14.

Ethanol-soluble fractions from the two centrifugation/decantation cycles were combined for each sample treatment, and the ethanol was subsequently removed by evaporation at 40 °C. The residues were re-precipitated in water at pH 2, and precipitates were collected through filtration (Glass Microfibre, MGA, d: 100 mm), resulting in lignin the ethanol-soluble materials LES0-LES14. A reference sample of LignoBoost lignin (not treated in the two-component solvent system) was treated with the precipitation agent (ethanol) and separated into two fractions according to the same procedure used for IL-treated samples. This gave ethanol-precipitated and ethanol-soluble reference samples LEPref and LESref. Furthermore, a reference sample of completely un-treated and un-fractionated LignoBoost lignin (Luntreated) was also included in the sample series. A schematic representation of the process is depicted in Fig. 3.

Gel permeation chromatography (GPC)

Lignin degradation and aggregation/elution behavior was assessed using molecular weight determinations by means of GPC. Analyses were performed using a PL-GPC 50 Plus integrated instrument system coupled with RI and UV (280 nm) detectors and equipped with a PL-AS RT auto-sampler (Polymer Laboratories, Varian Inc.).
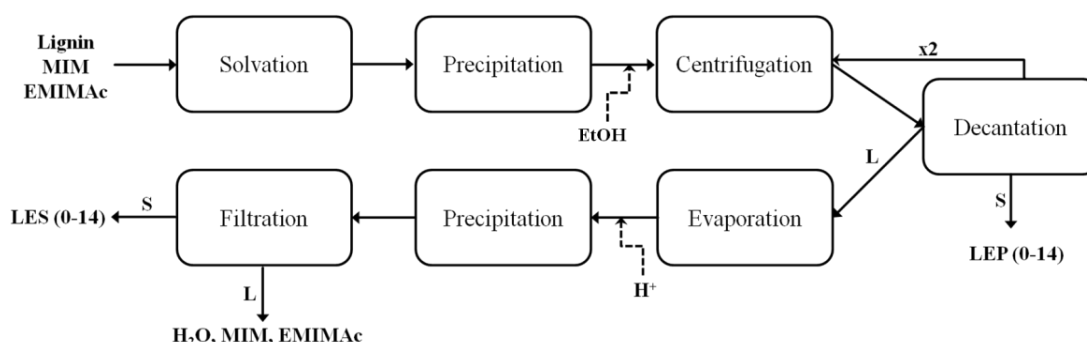
Fig. 3. Process for solvation and precipitation/fractionation of model LignoBoost lignin in MIM/EMIMAc. L denotes liquid phase and S denotes solid phase in the solid/liquid separation steps.

Chromatographic separations were achieved using two PolarGel-M columns and a guard column (300 x 7.5 mm, 50 x 7.5 mm). Samples were pre-dissolved in the mobile phase (DMSO/LiBr, 10 mM) and filtered through a syringe filter (GHP Acrodisc, d = 13 mm, 0.2-μm GHP membrane) prior to analysis.

Isocratic separation was performed with a flow rate of 0.5 mL/min. Data analyses were performed using Cirrus GPC software version 3.2. A 10-point calibration curve with Pullulan standards was used to determine molecular weights (M_w) and polydispersity (PD) (708, 375, 200, 107, 47.1, 21.1, 11.1, 5.9, 0.667, and 0.180 kDa, Polysaccharide Calibrations Kit, PL2090-0100, Varian).

Lignin acetylation

Lignin samples LEP0-14 and LES0-14 (300 mg) were acetylated using acetic anhydride in pyridine according to procedures from the literature (Monties 2012). Following acetylation, samples were pre-dissolved, filtrated, and analyzed as previously discussed.

^{13}C-NMR

Quantitative ^{13}C-NMR experiments were performed using a Bruker 800 MHz spectrometer operating at room temperature. The spectrometer was further equipped with a 5 mm CryoProbe. Samples (100 mg) were dissolved in deuterated dimethylsulfoxide (DMSO-d_6, 600 μL). Spectra were obtained using a 180° pulse angle, 12-s pulse delay, 2048 scans, spectrum width of 248.6, and an acquisition time of 1.3 s. The pulse sequence used was as detailed in the literature (Xia *et al*. 2008). Spectral integrations were conducted by calibrating the aromatic region (154 to 106 ppm) to a reference value of 6.00, representing six aromatic carbons. Consequently, all regions were recorded as equivalences *per* aromatic ring (equiv/Ar). Chemical shift assignments for both ^{13}C-NMR and ^{31}P-NMR were based on previous reports of pretreated lignin (Wells *et al*. 2013).

^{31}P-NMR

Experiments were performed using a Bruker AMX-400 MHz spectrometer operating at room temperature. Quantitative ^{31}P-NMR spectra were acquired after *in situ* derivatization of lignin samples (15.0 mg) with 2-chloro-4,4,5,5-tetramethyl-1,3,2-

dioxapholane (TMDP, 100 µL) in a solution of pyridine/CDCl3 (450 µL; 1.6:1 v/v), chromium acetylacetonate (relaxation agent), endo-N-hydroxy-5-norbornene-2,3-dicarboximide (NHND, internal standard), and N,N-dimethylformamide (DMF, 200 µL) as co-solvent. The spectrum was acquired using an inverse-gated decoupling sequence, 90° pulse angle, 25-s pulse delay, 128 scans, and an acquisition time of 1.29 s.

RESULTS AND DISCUSSION

Solvation Experiments

Complete solvation of lignin in MIM occurred within minutes. The time required for this solvation slightly increased with increasing IL in the system; however, all samples were solvated well before the end of the 4-h treatment time. Longer solvation times were likely caused by the slight increase in viscosity as a consequence of the increasing amount of IL; a higher viscosity leads to particles not being fully dispersed as well as a lower velocity of particles and a slower mass transport of polymers in the liquid. Upon regeneration in ethanol, a majority of the material was precipitated out of solution (53 to 88% for LEP0-LEP14 and LEPref, Table 1). The material remaining in solution (ethanol-soluble material) produced a translucent yellow liquid. This color was removed upon acid precipitation, indicating that virtually no material was left in solution at this point.

Table 1. GPC Results for Lignin Ethanol-Precipitated (LEP) and Ethanol-Soluble (LES) LignoBoost Lignin Treated in the System

Sample name $(L^a)^b$	%IL n/n_{tot}	%IL w/w_{tot}	%IL V/V_{tot}	M_w LEP	PD LEP	M_w LEP[c]	PD LEP[c]	M_w LES[d]	M_w LES[c]	%precip. LEP[e]
L untreated	-	-	-	16.5[d]	2.9[d]	-	-	-	-	-
L(EP/ES)ref	-	-	-	23.4	2.5	-	-	8.2	-	73
L(EP/ES)0	0	0	0	26.1	2.2	27.3	3.8	6.2	3.8	53
L(EP/ES)2	7	14	10	23.1	2.4	21.4	3.1	5.3	3.4	68
L(EP/ES)4	16	29	21	23.0	2.5	22.2	3.2	5.0	2.6	73
L(EP/ES)6	26	43	33	22.2	2.5	24.4	3.4	5.3	2.7	74
L(EP/ES)8	38	57	46	23.2	2.5	22.4	3.2	5.7	2.7	78
L(EP/ES)10	53	70	61	24.0	2.5	21.6	3.2	5.2	3.4	81
L(EP/ES)12	71	84	78	23.4	2.5	23.6	3.2	6.0	3.5	88
L(EP/ES)14	100	100	100	22.1	2.4	24.3	3.3	6.1	3.0	83

[a] Sample numbers reflect absolute weight [g] of IL used during solvation; [b] All samples were analyzed on a DMSO/LiBr GPC-system; [c] Acetylated samples; [d] Higher M_w peak of bimodal elution profile for untreated, LES, and acetylated samples; [e] Amount of ethanol-precipitated material

GPC

Data from the GPC analysis of all lignin samples are presented in Table 1. The original LignoBoost lignin exhibited a bimodal elution curve in the DMSO/LiBr system, with a weight-average molecular weight (M_w) of 16.5 kDa for the peak of highest apparent M_w (Fig. 4). As exemplified by the LES0 sample in Fig. 4, LES fractions still showed a bimodal pattern after precipitation in ethanol and subsequent procedures. However, these fractions contained much less of the higher-molecular weight aggregates in comparison to the untreated sample.

The highest molecular weight peaks for the LESs ranged from 5.0 to 6.2 kDa. In contrast, the LEP fractions showed a unimodal elution profile, with the LEP0 sample (only treated with MIM) displaying the highest M_w value of the sample set at 26.1 kDa. This sample had the lowest amount of precipitated material in the LEP series at 53%. Furthermore, the LEP0 sample displayed a lower polydispersity (PD=M_w/M_n) than the rest of the LEP sample set and the untreated lignin alike (2.2, ~2.5, and 2.9, respectively). Seemingly, the procedure fractionates some lower- and higher-molecular weight species, resulting in more uniformly aggregated structures in the LEPs in DMSO/LiBr.
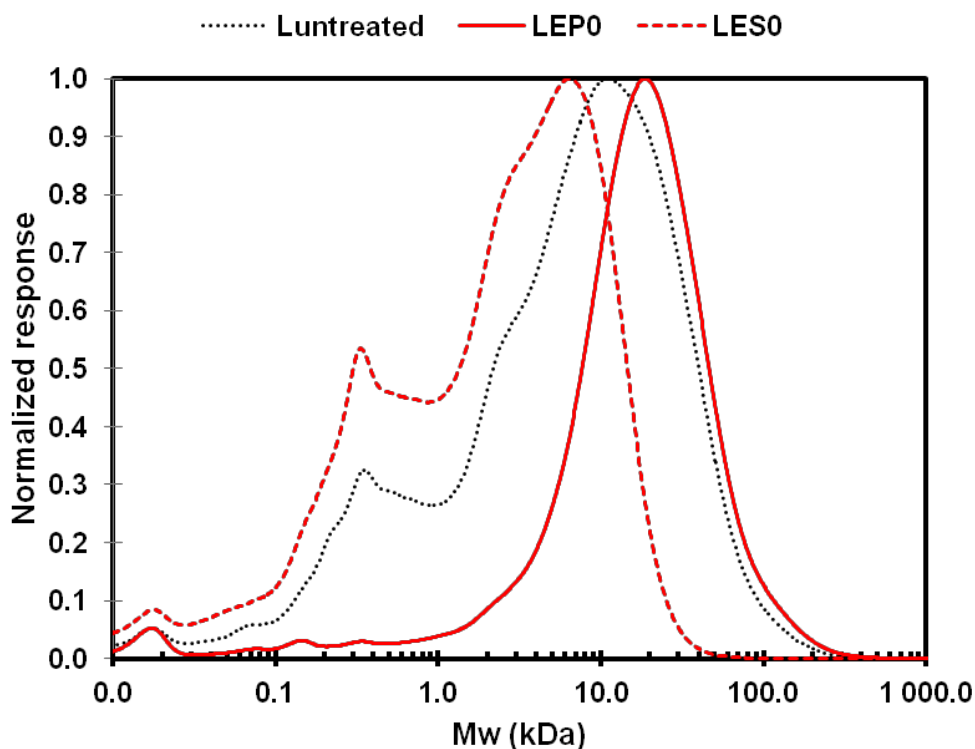

Fig. 4. Elution profiles of the MIM-treated lignin ethanol-precipitated (LEP0) and ethanol-soluble (LES0) samples, as well as the original lignin (Luntreated) control sample

When compared to the other LEPs, it is possible that the lower PD of the LEP0 sample reflects a separation of structural elements leading to solubility differences of the

supramolecular lignin structures. This explanation would result in a smaller amount of precipitated material, a lower PD, and possibly a higher apparent M_w, which agrees with results presented. Regarding this solubility effect, it should be noted that in the present system, the ratio of ethanol to MIM upon precipitation might influence the degree of fractionation and therefore also the aggregation of LEPs and LESs. Further investigations into the partitioning-behavior of the lignins in the system are needed to determine if, and how much, the solubility and aggregation equilibriums could be altered. The data in Table 1 further show that addition of IL, regardless of the amount, does not change the elution profile and thus does not change the aggregation behavior of the fractionated lignin in DMSO/LiBr to a noticeable extent. Importantly, this does not exclude the possibility of variations in the amounts and types of structural moieties included in the aggregates. Furthermore, there is no evidence of degradation of the lignin polymer based on the GPC data. Even though results reflect the *apparent* M_w of the samples, no new M_w peaks, increases in the relative ratios, or broadening of existing peaks were detected.

A recent study presents an apparent M_w of 6.4 kDa for acetylated LignoBoost lignin (Norberg *et al*. 2013). Other SW lignin isolates such as benzoylated MWL and EMAL have been documented at 36.8 and 6.1 kDa, respectively (Zoia *et al*. 2011). The chromatographic solvent employed by these authors was THF and not DMSO, as in the present study. As noted for the LignoBoost comparison, this is of some consequence for the overall supramolecular aggregation of the lignin and therefore also the measured M_w. Moreover, sample derivatization through acetylation and benzoylation to achieve adequate solubility further makes direct comparisons to an un-derivatized LignoBoost lignin unsuitable. To evaluate the difference in apparent M_w that such a derivatization has in the DMSO/LiBr system, samples LEP0-14 and LES0-14 were acetylated and re-analyzed. The data for the acetylation/GPC-analysis are presented in Table 1.

Regarding apparent M_w, no clear trends were found in the data obtained from the acetylated LEP samples. However, the PD of all acetylated LEPs increased from ~2.5 to ~3.3, indicating either polymer degradation and/or supramolecular re-organization into less uniformly shaped agglomerates, which could possibly also indicated an increased ability of the derivatized structure to swell in the system. On the other hand, the LESs showed a direct general decrease in apparent M_w compared to the un-acetylated samples. Derivatization through acetylation clearly has a higher influence on the apparent M_w of the lower M_w samples and primarily influences the PD of LEPs. The addition of Li-salts, as in the case of DMSO/LiBr, is known to counteract aggregation caused by ionization effects (Connors *et al*. 1980). Such effects are therefore not likely to be of any greater significance to these results.

It is evident that derivatization has an effect on the supramolecular agglomeration of lignin. In combination with the potential risk of sample degradation during derivatization, it should therefore be more suitable to use a system that permits analysis of un-derivatized samples such as DMSO/LiBr.

^{13}C- and ^{31}P-NMR

Nuclear magnetic resonance spectroscopy is a powerful elucidation tool commonly implemented in the characterization of lignin. To characterize and quantitate the structural moieties found in the different fractions and to determine the possible enrichment of these

structures in any of the samples, quantitative ^{13}C- and ^{31}P-NMR were performed. Structural details regarding the aromatic and aliphatic functional groups present within the LES and LEP lignin fractions were elucidated.

Chemical shift assignments are summarized in Table 2. Overall, samples exhibited predominant signals attributable to [G]-type lignin units. In detail, these encompassed ^{13}C-NMR (DMSO-d$_6$, 800 MHz) δ135.2 (C1 etherified [G]-β-O-4 units), δ111.2 (C2 [G]-units), δ149.4 (C3 etherified [G]-units), δ147.5 (C4 etherified [G]-unit), δ115.4 (C5 in [G] etherified and non-etherified unit), and δ119.5 (C6 in [G] etherified and non-etherified unit). Prominent linkages were determined at 71.1, 63.3, and 60.5 ppm, which correlated to the Cγ in β-β, β-O-4 with α-carbonyl and β-O-4 linkages, respectively ([G] units). Additionally, peaks found at 134.3 ppm and 132.5 ppm were representative of C5/C5' in 5-5 linkages and C5/C5' in non-etherified units, respectively. No significant peaks were present from 102 to 90 ppm, which is indicative of low carbohydrate content.

Comparison of LES and LEP spectra provided clear distinctions between the two sample batches. Integrals corresponding to aliphatic functional groups were more prominent in LEPs than LESs, as evidenced by δ140.0 to 127.0 (C1-aromatic C-C bond), δ65.0 to 61.5 (aliphatic C-OR), and δ61.5 to 57.5 (aliphatic C-O and Cγ in β-O-4). Condensed linkages were also greater for LEPs based on higher signals at δ54.0 to 52.0 (Cβ in β-β and Cβ in β-5) and δ127.0 to 123.0 (C5 in non-etherified, aromatic C-C bond). This result is in agreement with the lower integral value at δ117.0 to 114.0 (C5 in aromatic C-H bond) for LEPs. Lastly, LEPs generally exhibited greater extent of methoxylation based on δ57.5 to 54.0. The higher proportion of condensed and aliphatic linkages in LEPs suggests a higher degree of polymerization of these lignin fractions, which is in agreement with data obtained *via* GPC. Furthermore, carboxylation was expectedly greater for LESs based on the generally greater integral values for δ178.0 to 167.5 (un-conjugated –COOH) and δ167.5 to 162.5 ppm (conjugated –COOH).

Despite the distinct correlations observed with ^{13}C-NMR, an increasing presence of residual EMIMAc was detected in the LEP series, as evidenced by sharp peaks at δ136.9, δ123.9, δ122.3, δ44.5, δ36.0, and δ15.46 belonging to C2, C4, and C5 carbons of the imidazole ring, the two carbons of the N1 ethyl group, and the methyl on N3, respectively. Such residues were also found in the LESs; however, they were retained to a lower extent, as seen in Fig. 5.

Accordingly, the spectra for LEP0 and LES0 did not contain peaks from EMIMAc or peaks from residuals of the MIM component present. Thus, the current procedure successfully separated MIM from the lignin materials, whereas the EMIMAc was retained to some extent. An extra washing step or higher ethanol-to-solvent ratios during precipitation might improve the procedure.

^{31}P-NMR was performed to cross-reference results obtained with ^{13}C-NMR. An advantage of ^{31}P-NMR analysis is that it is based on the specific derivatization of lignin phenolic hydroxyls with TMDP, which provides a basis to calculate phenolic content based on a NHND internal standard. The chemical shift assignments for the ^{31}P-NMR are summarized in Table 3.

Table 2. Quantitative Comparison among LEP and LES Fractions Based on ^{13}C-NMR

Assign. Sample name	Unconj. -COOH	Conj. -COOH	C3/4 Ar. C-C ether -OH	C1 Ar. C-C	C5 non-ether C-C	C6 Ar. C-H	C5 Ar. C-H	C2 Ar. C-H	Aliph. C-O, Cα in β-5, β-O-4, β-β	Aliph. C-O, Cα in β-O-4	Aliph. C-OR	Aliph. C-O, Cγ in β-O-4	-OMe	Cβ in β-β, Cβ in β-5
Shifts (ppm)	(178.0-167.5)	(167.5-162.5)	(154.0-140.0)	(140.0-127.0)	(127.0-123.0)	(123.0-117.0)	(117.0-114.0)	(114.0-106.0)	(90.0-78.0)	(79.0-67.0)	(65.0-61.5)	(61.5-57.5)	(57.5-54.0)	(54.0-52.0)
L untr.	0.09	0.01	1.89	1.33	0.53	0.81	0.60	0.84	0.30	0.44	0.14	0.26	1.57	0.09
LEP0	0.05	0.03	1.90	1.42	0.51	0.84	0.52	0.81	0.33	0.45	0.15	0.28	1.86	0.08
LEP2	0.10	0.01	1.75	1.45	0.57	0.99	0.49	0.75	0.42	0.57	0.20	0.39	3.93	0.10
LEP4	0.08	0.00	1.74	1.42	0.60	1.03	0.50	0.71	0.38	0.52	0.20	0.35	3.83	0.10
LEP6	0.10	0.00	1.75	1.43	0.61	1.01	0.49	0.71	0.37	0.51	0.20	0.35	3.12	0.10
LEP8	0.11	0.00	1.77	1.42	0.62	1.01	0.48	0.70	0.39	0.53	0.20	0.35	3.24	0.12
LEP10	0.13	0.00	1.76	1.40	0.64	1.04	0.47	0.69	0.43	0.57	0.21	0.38	3.88	0.13
LEP12	0.11	0.00	1.69	1.43	0.65	1.02	0.49	0.72	0.46	0.66	0.28	0.55	N/A	0.11
LEP14	0.09	0.01	1.79	1.37	0.66	1.00	0.48	0.70	0.37	0.49	0.17	0.31	2.38	0.11
LEPref	0.07	0.02	2.00	1.33	0.53	0.78	0.54	0.82	0.34	0.48	0.15	0.28	1.96	0.10
LES0	0.11	0.01	1.74	1.33	0.63	0.83	0.65	0.82	0.26	0.32	0.14	0.22	1.36	0.07
LES2	0.12	0.01	1.72	1.35	0.59	0.85	0.66	0.83	0.27	0.32	0.15	0.23	1.37	0.07
LES4	0.09	0.01	1.70	1.35	0.59	0.85	0.67	0.84	0.22	0.24	0.11	0.17	1.35	0.05
LES6	0.10	0.01	1.70	1.33	0.58	0.87	0.67	0.85	0.21	0.24	0.11	0.18	1.36	0.04
LES8	0.15	0.02	1.69	1.37	0.60	0.87	0.65	0.82	0.27	0.33	0.15	0.22	1.33	0.07
LES10	0.14	0.01	1.74	1.39	0.56	0.83	0.65	0.83	0.28	0.32	0.16	0.24	1.36	0.08
LES12	0.17	0.02	1.71	1.37	0.60	0.88	0.63	0.81	0.26	0.32	0.15	0.24	1.34	0.07
LES14	0.15	0.02	1.73	1.37	0.58	0.87	0.63	0.82	0.28	0.33	0.15	0.23	1.34	0.07
LESref	0.12	0.00	1.77	1.33	0.53	0.85	0.67	0.85	0.20	0.32	0.11	0.21	1.30	0.08

Abbreviations: Assign. – NMR chemical shift assignments; Unconj. – unconjugated; Conj. – conjugated; Ar. – aromatic; Aliph. – aliphatic; untr. – untreated. Integrals reflect equivalent *per aromatic ring*.
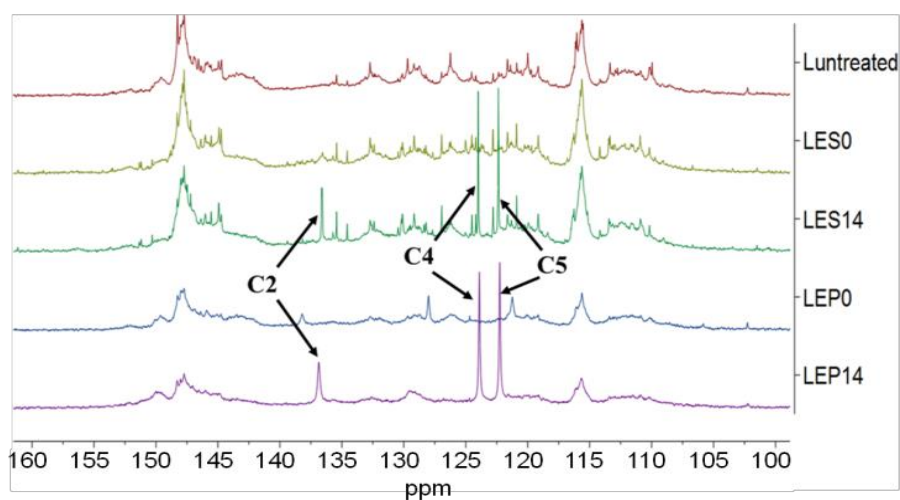
Fig. 5. ^{13}C-nuclear magnetic resonance (NMR) spectra for an untreated LignoBoost sample (Luntreated), lignin ethanol solubles LES0 and LES14, and lignin ethanol precipitates LEP0 and LES14 treated with 100% MIM and 100% EMIMAc. Sharp peaks at 136.9, 123.9, and 122.3 ppm belonging to C2, C4, and C5 carbons of the imidazole ring are clearly visible in samples LES14 and LEP14. Untreated lignin LES0 and LEP0 show no imidazole peaks.

Table 3. Quantitative Comparison among LEP and LES Fractions based on ^{31}P-NMR

Chemical shift assignments	Aliphatic -OH	Phenolic -OH	Condensed Phenolic	Guaiacyl -OH	Carboxylic acid -OH
Shift (ppm)	(148.5-	(144.0-	(144.5-	(139.5-	(135.0-133.5)
Sample name					
L untr.	1.78	2.01	0.37	1.42	0.36
LEP0	1.95	1.40	0.31	0.91	0.30
LEP2	1.79	1.19	0.16	0.87	0.31
LEP4	1.59	1.44	0.32	0.93	0.30
LEP6	1.79	1.43	0.33	0.96	0.30
LEP8	1.47	1.44	0.35	0.88	0.27
LEP10	1.67	1.71	0.48	1.03	0.33
LEP12	1.67	1.52	0.34	0.98	0.36
LEP14	1.84	1.31	0.22	0.90	0.40
LEPref	2.44	1.62	0.37	1.09	0.28
LES0	1.29	2.19	0.24	1.78	0.42
LES2	1.20	2.17	0.27	1.75	0.39
LES4	1.17	1.96	0.07	1.72	0.48
LES6	1.17	1.75	0.08	1.60	0.44
LES8	1.08	1.72	0.03	1.57	0.51
LES10	0.99	1.60	0.02	1.52	0.42
LES12	0.98	1.75	0.03	1.61	0.57
LES14	1.05	1.84	0.05	1.64	0.55
LESref	1.52	2.75	0.37	2.07	0.58

Integral values reflect mmol/g

As expected, these results also showed predominantly [G]-type lignin and that the LEPs exhibited higher aliphatic hydroxyl and condensed phenolic content compared to the LES fractions based on integrals ^{31}P-NMR (pyridine/CDCl$_3$, 400 MHz) δ150.0 to 145.5 (aliphatic -OH) and δ144.5 to 140.2 (condensed phenolic). Likewise, the LESs exhibited greater carboxylic group content, as indicated from δ133.5 to 135.1 (carboxylic acid -OH). Regarding phenolic content, ^{31}P-NMR showed a greater content of free phenolic groups in the LESs, as indicated by the signals at δ144.7 to 137.6 (phenolic -OH) and δ140.0 to 139.0 ([G] -OH), than in the LEPs. Likewise, ^{13}C-NMR showed a greater amount of total C3/C4 aromatic ethers and/or hydroxyls in the LEPs than in the LESs. Consequently, the LEPs contained a lower amount of free hydroxyls than the LESs, verifying the ^{31}P-NMR data. Based on known structural elements in softwood lignins, the greater amount of aromatic ethers could encompass moieties such as α-O-4 ethers (non-benzylic, *i.e.*, LCC), the α-ether linkage of a β-5-structure, and to some extent residual dibenzodioxocin structures and β-O-4 ethers (Argyropoulos *et al.* 2002; Froass *et al.* 1998).

Overall, the results from ^{31}P-NMR agreed with data obtained from ^{13}C-NMR, with both analyses supporting the conclusion that the LEPs were generally enriched with condensed and aliphatic ether linkages and also aliphatic hydroxyls. The LESs, on the other hand, were enriched with carboxylic and phenolic groups.

In summary, the NMR results in combination with data obtained *via* GPC in this study proved in strong agreement with results from Cathala *et al.* (2003) regarding association of certain structural moieties of the lignin macromolecule and also with Guerra *et al.* (2007) showing the importance of the π-π-stacking on the aggregation behavior of higher-M_w SW lignins. Such π-π-stacking is likely of lesser importance for interactions between lower-M_w lignins.

Generally, and consistent with results herein, a lignin that is of lower M_w is also more oxidized, *e.g.*, LignoBoost lignin compared to MWL or EMAL, and therefore has a higher occurrence of oxygen-containing moieties such as carboxylic groups and phenolic hydroxyls. These are available for hydrogen-bonding (n→σ*) and specific carboxyl carbon (n→π*) interactions, where n is the lone pair bonding orbital of the hydrogen bond acceptor (*e.g.*, oxygen), σ* is the anti-bonding orbital of the hydrogen bond donor (*e.g.*, hydrogen of phenolic hydroxyl), and π* is the anti-bonding orbital of the carbonyl carbon (*e.g.*, carboxyl carbon). Such interactions are known to play a significant role in the folding of proteins when hydrogen bonds are formed and carbonyl moieties aggregate (Bartlett *et al.*, 2013). Hence, it is likely that aggregation of lower-M_w lignins is governed not only by π-π-stacking but also by n→σ* and n→π* interactions to a significant extent, whereas aggregation of higher-M_w lignins is less controlled by the latter two.

In addition, current findings are in line with theoretical calculations of interaction energies of aromatic compounds (Grimme 2008). These calculations further support the importance of π-orbitals on the net interaction energies of larger aromatic systems, *i.e.* systems >10-15 carbon atoms. This size range is comparable to the aromatic carbons of a lignin dimer with an approximate total M_w of 400 Da (depending on the exact structure). Moreover, these interactions are shown to be strongly enhanced as the system grows larger, which is not accounted for by exchange repulsion, electrostatics, or polarization. The apparent molecular weights of the current study varied from 5 to 26 kDa, *i.e.* approximately 12.5 to 65 interlinked C9 units, suggesting π-π interactions to be of relative high importance in all samples, although as suggested increasing with the size of the macromolecule. Thus, NMR verifies the existence of certain structural elements (*e.g.*, carbonyls) in the lower-M_w

LESs and higher-M_w LEPs (*e.g.*, condensed linkages) that are known to aggregate amongst each other through different bonding mechanisms.

Interestingly, as noted in the GPC-results section, the acetylation of the lignin fractions resulted in a broadening of the higher-M_w peaks of the LEPs. Although this might reflect polymer degradation to some extent, the other possibility of re-organization of the supramolecular structure could partly be induced by increasing opportunities for n→σ* and n→π* interactions.

CONCLUSIONS

In this work, the solvation/precipitation in terms of structure and aggregation behavior of Lignoboost lignin using a MIM/EMIMAc solvent system was investigated using ^{13}C-NMR, ^{31}P-NMR, and GPC, which generated the following conclusions:

1. Lignin first solvated in the MIM/EMIMAc solvent system was found to fractionate into low- and high-molecular weight fractions during the regeneration procedure.

2. ^{13}C-NMR and ^{31}P-NMR showed that these fractions displayed separate structural characteristics: the higher-M_w lignins treated in the IL system were enriched with condensed and/or aliphatic ether linkages and aliphatic hydroxyls, and the lower-M_w lignins were enriched with carboxylic and phenolic groups.

3. Acetylation of the materials clearly changed the supramolecular structure of the lignin fractions, high-lighting the importance of non-covalent interactions on lignin aggregation behavior.

These results, in combination with process-related benefits described herein, are of great potential importance in further development of the present IL solvent system for the purpose of either fractionation and/or extraction to suit a specific biomass process or biomass-derived product, such as carbon fibers.

ACKNOWLEDGMENTS

REFERENCES CITED

Argyropoulos, D. S. (2010). "Heteronuclear NMR spectroscopy of lignins," in: *Lignin and Lignans*, C. Heitner, D. R. Dimmel, and J. A. Schmidt (eds.), CRC Press, Boca Raton, FL, pp. 245-265. DOI: 10.1201/EBK1574444865-c6

Argyropoulos, D. S., Jurasek, L., Krištofová, L., Xia, Z., Sun, Y., and Paluš, E. (2002). "Abundance and reactivity of dibenzodioxocins in softwood lignin," *Journal of Agricultural and Food Chemistry* 50(4), 658-666. DOI: 10.1021/jf010909g

Björkman, A. (1956). "Studies on finely divided wood. Part 1: Extraction of lignin with neutral solvents," *Svensk Papperstidning* 59(13), 477-485.

Bylin, S., Olsson, C., Westman, G., and Theliander, H. (2014). "Solvation behavior of cellulose and xylan in the MIM/EMIMAc ionic liquid solvent system: Parameters for small-scale solvation," *BioResources* 9(1), 1038-1054.

Cathala, B., and Monties, B. (2001). "Influence of pectins on the solubility and the molar mass distribution of dehydrogenative polymers (DHPs, lignin model compounds)," *International Journal of Biological Macromolecules* 29(1), 45-51. DOI: 10.1016/S0141-8130(01)00145-3

Cathala, B., Saake, B., Faix, O., and Monties, B. (2003). "Association behaviour of lignins and lignin model compounds studied by multidetector size-exclusion chromatography," *Journal of Chromatography A* 1020(2), 229-239. DOI: 10.1016/j.chroma.2003.08.046

Connors, W., J., Sarkanen, S., and McCarthy, J. L. (1980). "Gel chromatography and association complexes of lignin," *Holzforschung* 34(3), 80-85. DOI: 10.1515/hfsg.1980.34.3.80

Crestini, C., and Argyropoulos, D. S. (1997). "Structural analysis of wheat straw lignin by quantitative 31P and 2D NMR spectroscopy. The occurrence of ester bonds and α-O-4 substructures," *Journal of Agricultural and Food Chemistry* 45(4), 1212-1219. DOI: 10.1021/jf960568k

Crestini, C., Melone, F., Sette, M., and Saladino, R. (2011). "Milled wood lignin: A linear oligomer," *Biomacromolecules* 12(11), 3928-3935. DOI: 10.1021/bm200948r

Dimmel, D. R. (2010). "Overview," in: *Lignin and Lignans*, C. Heitner, D. R. Dimmel, and J. A. Schmidt (eds.), CRC Press, Boca Raton, FL, pp. 1-10. DOI: 10.1201/EBK1574444865-c1

Fengel, D., and Wegener, G. (1984). *Wood: Chemistry, Ultrastructure, Reactions*, Walter de Gruyter, Berlin.

Freudenberg, K., and Neish, A. C. (1968). *Constitution and Biosynthesis of Lignin*, Springer-Verlag, New York. DOI: 10.1007/978-3-642-85981-6

Froass, P. M., Ragauskas, A. J., and Jiang, J.-E. (1998). "Nuclear magnetic resonance studies. 4. Analysis of residual lignin after kraft pulping," *Industrial & Engineering Chemistry Research* 37(8), 3388-3394. DOI: 10.1021/ie970812c

Froschauer, C., Hummel, M., Iakovlev, M., Roselli, A., Schottenberger, H., and Sixta, H. (2013). "Separation of hemicellulose and cellulose from wood pulp by means of ionic liquid/cosolvent systems," *Biomacromolecules* 14(6), 1741-1750. DOI: 10.1021/bm400106h

Grimme, S. (2008). "Do special noncovalent π–π stacking interactions really exist?" *Angewandte Chemie International Edition* 47(18), 3430-3434. DOI: 10.1002/anie.200705157

Guerra, A., Gaspar, A. R., Contreras, S., Lucia, L. A., Crestini, C., and Argyropoulos, D. S. (2007). "On the propensity of lignin to associate: A size exclusion chromatography study with lignin derivatives isolated from different plant species," *Phytochemistry* 68(20), 2570-2583. DOI: 10.1016/j.phytochem.2007.05.026

Hu, T. Q. (2002). *Chemical Modification, Properties, and Usage of Lignin*, Springer US, New York. DOI: 10.1007/978-1-4615-0643-0

Huang, H.-J., Ramaswamy, S., Tschirner, U. W., and Ramarao, B. V. (2008). "A review of separation technologies in current and future biorefineries," *Separation and Purification Technology* 62(1), 1-21. DOI: 10.1016/j.seppur.2007.12.011

Kilpelainen, I., Xie, H., King, A., Granstrom, M., Heikkinen, S., and Argyropoulos, D. S. (2007). "Dissolution of wood in ionic liquids," *Journal of Agricultural and Food Chemistry* 55(22), 9142-9148. DOI: 10.1021/jf071692e

Lawoko, M., Henriksson, G., and Gellerstedt, G. (2006). "Characterisation of lignin-carbohydrate complexes (LCCs) of spruce wood (*Picea abies* L.) isolated with two methods," *Holzforschung* 60(2), 156-161. DOI: 10.1515/hf.2006.025

Monties, B. (2012). "Lignins," in: *Methods in Plant Biochemistry*, P. M. Dey (ed.), Elsevier, Amsterdam, pp. 113-158.

Mäki-Arvela, P., Anugwom, I., Virtanen, P., Sjöholm, R., and Mikkola, J. P. (2010). "Dissolution of lignocellulosic materials and its constituents using ionic liquids - A review," *Industrial Crops and Products* 32(3), 175-201. DOI: 10.1016/j.indcrop.2010.04.005

Ni, Y., and Hu, Q. (1995). "Alcell® lignin solubility in ethanol–water mixtures," *Journal of Applied Polymer Science* 57(12), 1441-1446. DOI: 10.1002/app.1995.070571203

Norberg, I., Nordström, Y., Drougge, R., Gellerstedt, G., and Sjöholm, E. (2013). "A new method for stabilizing softwood kraft lignin fibers for carbon fiber production," *Journal of Applied Polymer Science* 128(6), 3824-3830. DOI: 10.1002/app.38588

Öhman, F., Wallmo, H., and Theliander, H. (2007). "A novel method for washing lignin precipitated from kraft black liquor – Laboratory trials," *Nordic Pulp & Paper Research Journal* 22(1), 9-16. DOI: 10.3183/NPPRJ-2007-22-01-p009-016

Ollila, J. (2013). "Future Valmet to supply a LignoBoost plant for the new biorefinery at the Stora Enso Sunila mill in Finland," Metso, Press release.

Paterson, R. J. (2011). *Lignin: Properties and Applications in Biotechnology and Bioenergy*, Nova Science Publishers, Hauppauge, NY.

Pinkert, A., Goeke, D. F., Marsh, K. N., and Pang, S. (2011). "Extracting wood lignin without dissolving or degrading cellulose: Investigations on the use of food additive-derived ionic liquids," *Green Chemistry* 13(11), 3124-3136. DOI: 10.1039/c1gc15671c

Ralph, J., and Landucci, L., L. (2010). "NMR of lignins," in: *Lignin and Lignans*, C. Heitner, D. R. Dimmel, and J. A. Schmidt (eds.), CRC Press, Boca Raton, FL, pp. 137-243. DOI: 10.1201/EBK1574444865-c5

Sarkanen, K. V., and Ludwig, C. H. (1971). *Lignins: Occurrence, Formation, Structure and Reactions*, Wiley-Interscience, New York.

Sarkanen, S., Teller, D. C., Stevens, C. R., and McCarthy, J. L. (1984). "Lignin. 20. Associative interactions between kraft lignin components," *Macromolecules* 17(12), 2588-2597. DOI: 10.1021/ma00142a022

Stewart, D. (2008). "Lignin as a base material for materials applications: Chemistry, application and economics," *Industrial Crops and Products* 27(2), 202-207. DOI: 10.1016/j.indcrop.2007.07.008

Tan, S. S. Y., MacFarlane, D. R., Upfal, J., Edye, L. A., Doherty, W. O. S., Patti, A. F., Pringle, J. M., and Scott, J. L. (2009). "Extraction of lignin from lignocellulose at atmospheric pressure using alkylbenzenesulfonate ionic liquid," *Green Chemistry* 11(3), 339-345

Wells, Jr., T., Kosa, M., and Ragauskas, A. J. (2013). "Polymerization of Kraft lignin via ultrasonication for high-molecular-weight applications," *Ultrasonics Sonochemistry* 20(6), 1463-1469. DOI: 10.1016/j.ultsonch.2013.05.001

Xia, Y., Moran, S., Nikonowicz, E. P., and Gao, X. (2008). "Z-restored spin-echo 13C 1D spectrum of straight baseline free of hump, dip and roll," *Magnetic Resonance in Chemistry* 46(5), 432-435. DOI: 10.1002/mrc.2195

Zoia, L., King, A. W. T., and Argyropoulos, D. S. (2011). "Molecular weight distributions and linkages in lignocellulosic materials derivatized from ionic liquid media," *Journal of Agricultural and Food Chemistry* 59(3), 829-838. DOI: 10.1021/jf103615e