Fast Fractionation of Technical Lignins by Organic Cosolvents

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ABSTRACT: A simple and fast fractionation method was developed to obtain carbohydrate-free lignin with well-defined characteristics, such as narrowly distributed molecular weights and a tunable chemical structure for specific applications. In this study, an industrial softwood kraft lignin and a hardwood CELF lignin (coproduct lignin obtained from cosolvent enhanced lignocellulosic fractionation pretreatment) were dissolved in acetone−methanol and tetrahydrofuran−methanol cosolvents, respectively. Hexane was applied as the antisolvent for sequential precipitation of both lignin preparations. A thorough characterization including various NMR techniques (31P, quantitative 13C, and 2D-HSQC), GPC, DSC and TGA was conducted to correlate the molecular weight of obtained lignin fractions with their structural features including distributions of aliphatic and phenolic hydroxyl groups and relative abundance of interunit linkages. It was found that approximately 10% of the softwood kraft lignin was lignin carbohydrate complexes, and the latter one could be removed efficiently by decreasing polarity of the cosolvent.

KEYWORDS: Softwood kraft lignin, CELF poplar lignin, Sequential fractionation, Structural analyses, Molecular weight, Lignin carbohydrate complexes

INTRODUCTION

Lignin, the most abundant aromatic biopolymer, constitutes ~15%−30% of terrestrial plant lignocellulosic materials. It can be composed of up to three primary subunits: guaiacyl (G), syringyl (S), and p-hydroxyphenyl (H). Softwood lignin is primarily composed of guaiacyl subunits, and hardwood lignin is formed by a mixture of guaiacyl and syringyl subunits. Recently, the increasing interest in lignocellulosic biorefining has brought the utilization of lignin back into focus. The annual production of lignin is predicted to be 62 million tons in the United State by 2022.1 Numerous efforts have been devoted to convert lignin into a wide range of value-added products. One approach involves deconstruction of lignin to small molecular weight phenolics or related aromatics through pyrolysis and/or catalytic reforming approaches.2 More frequently, owing to its multifunctional properties, lignin is cross-linked or blended with other polymers or monomers to produce materials such as thermoset resins,3−6 foams,7,8 adhesives,9−13 and thermoplastics.14,15

However, the utilization of technical lignins remains underdeveloped and is most often combusted as a low-cost fuel, and approximately 1%−2% of commercial lignins are used as low-value industrial additives.16 Depending on the reaction conditions, technical lignins differ in structural composition, molecular weight, polydispersity, and functional group distribution. Medium molecular weight Alcell lignin, obtained by sequential organic solvent extraction with increasing hydrogen bonding capacity, generated polyurethane films exhibiting better tensile behavior than those of high and low molecular weight fractions with the same lignin content.17 In addition to their structural complexity, carbohydrate impurities are another obstacle that prevents technical lignins from effective conversion into many commercial polymeric materials.18−20 For example, in the production of lignin-based carbon fiber, ideal technical lignins for melt-spinning should possess...
moderate molecular weights with a narrow distribution, meanwhile the impurities including residual carbohydrates and ash were preferred to be less than 1%.21

The solvent-based fractionation of lignin has been widely investigated in the past decades.22−29 Among the proposed methods, water has been frequently used as a cosolvent to dissolve technical lignins, and then, more water was added to the system as antisolvents to precipitate lignin, which is detrimental to overall energy costs for solvent recovery. The properties of the cosolvent used in the lignin fractionation have been shown to have significant effects on the characteristics of the lignin obtained. An accumulation of carbohydrates was found in low molecular weight kraft lignin obtained from aqueous organic solvent fractionation.27 However, carbohydrates tend to be enriched in high molecular weight fractions when technical lignin fractions were separated by pure organic solvent extraction methodologies.30,31 In this study, methanol was chosen as a low boiling alternative to water as the cosolvent that provides sufficient hydrogen bonding capacity and polarity. Two technical lignin samples, softwood kraft lignin and hardwood CELF lignin, prepared under different reaction conditions were dissolved in low-boiling-point organic cosolvents and then fractionally precipitated with hexane. Finally, the structural evolution across fractions of different molecular weights was thoroughly characterized.

Materials and Methods

Materials. The CELF poplar lignin used in this study is a coproduct of the cosolvent enhance lignocellulosic fractionation (CELF) process applied under mild conditions. During the CELF pretreatment, 1 mm-size poplar wood chips (7.5 wt% loading) underwent one-step delignification and hemicellulose hydrolysis in a tetrahydrofuran (THF)−water (1:1, v/v) cosolvent containing 0.5 wt % H2SO4 at 160 °C with 15 min contact time. CELF lignin was isolated from the resulting liquid product by first neutralization with Ca(OH)2 followed by THF evaporation and vacuum filtration, as mentioned according to literature procedures.32,33 Commercial softwood kraft lignin and hardwood CELF lignin, prepared under different reaction conditions were dissolved in low-boiling-point organic cosolvents and then fractionally precipitated with hexane. Finally, the structural evolution across fractions of different molecular weights was thoroughly characterized.

Figure 1. Flowchart of lignin fractionation.
stirred solution was kept dark for 24 h, whereupon 2 mL of ethanol was added, and the solvents were removed using a rotary evaporator under vacuum at 40 °C. A repeat of the ethanol addition, followed by evaporation, was performed to remove the last trace of pyidine and remaining acetic anhydride. The dried acetylated lignin was dissolved in 2 mL of THF. The solution was filtered through a 0.45 μm PTFE membrane before injecting into an Agilent GPC SECurity 1200 system equipped with several Waters Styragel columns (Water Corporation, Milford, MA), an Agilent UV detector, and a refractive index detector. HPLC-grade THF was used as the eluent at a flow rate of 1.0 mL/min at 30 °C. Standard calibration was performed by using polystyrene standards with weight-average molecular weight ranging from 580–13 000 g/mol. A UV detector signal was collected at λ = 280 nm. Thermal Gravimetric Analysis (TGA). Lignin samples (5 mg) were loaded into ceramic crucibles (PerkinElmer, American Fork, UT). The TGA of lignin was operated on a PerkinElmer Pyris 1 TGA in nitrogen and air atmospheres, respectively. The sample was heated from 25 to 105 °C at 10 °C/min. After incubating at 105 °C for 15 min, it was heated further from 105 to 900 °C at a heating rate of 10 °C/min.

Differential Scanning Calorimetry (DSC). About 5 mg of the powdery sample was encapsulated in an standard aluminum pan and lid (TA Instruments). The DSC measurements were performed in heat–cool–heat mode on a TA Q2000 differential scanning calorimeter (TA Instruments) with a heating/cooling rate of 20 °C/min. RESULTS AND DISCUSSION

The studies of lignin solubility in a single solvent started with the Hildebrand solubility parameter (δ), which is defined as the square root of a substance’s cohesive energy density

\[ \delta = (E/V)^{1/2} \]  
(1)

where \( E \) is the total energy of vaporization at zero pressure, and \( V \) is the molar volume of the pure solvent. As \( E \) is composed of three parts which are contributed by nonpolar/dispersion forces, dipole forces, and hydrogen bonding forces, \( \delta \) can be divided into three corresponding parts as follows:

\[ \delta^2 = \delta_D^2 + \delta_P^2 + \delta_H^2 \]  
(2)

Here, \( \delta_D \), \( \delta_P \), and \( \delta_H \) are the Hansen solubility parameters (HSP) for the dispersion, polar, and hydrogen bonding interactions, respectively. \( R_s \) is the difference between the HSP for a given solvent and polymer. For a good solvent, \( R_s/R_c < 1 \), where \( R_c \) is the interaction radius of polymer.

\[ R_s^2 = 4(\delta_{D2} - \delta_{D1})^2 + (\delta_{P2} - \delta_{P1})^2 + (\delta_{H2} - \delta_{H1})^2 \]  
(3)

It was suggested that solvents with \( \delta \)-values close to 22.5 MPa\(^{1/2}\) were good solvents for lignins. However, technical lignins were found partially soluble in a pure good solvent with high hydrogen bonding capacity such as ethanol or high polarity such as acetone, and full dissolution of lignin in acetone and ethanol can be achieved in the presence of water. During lignin dissolution in aqueous organic solvents, water serves as an highly efficient plasticizer owing to its small molecular size, which facilitates the diffusion of organic solvent molecules into the compact associated lignin complexes.
be fully soluble in dimethyl sulfoxide and dimethylformamide and polar aprotic solvents with high $\delta_P$-values and moderate $\delta_H$-values (Table 1). The HSPs of a solvent mixture are linear functions in relationship to the volume fraction of each solvent; therefore, it is possible to design low-boiling-point cosolvent systems that possess appropriate HSPs within the boundary of lignin dissolution sphere, i.e., $R_a/R_o < 1$.43

Yields and Molecular Weight Characterization of Fractions. Before fractionation, the softwood kraft lignin and the CELF poplar lignin samples were suspended in acetone−methanol (7:3, v/v) and THF−methanol (5:5, v/v), respectively. Methanol was chosen as the secondary solvent owing to its small molecular size and high $\delta_H$-value. It appeared that the HSP theory worked well for lignin dissolution behavior regardless of its sources. As predicted by the $R_a/R_o$ value (Table 1), CELF poplar lignin was found highly soluble in THF−methanol ($R_a/R_o = 0.99$); meanwhile, ~96% softwood kraft lignin was dissolved in acetone−methanol ($R_a/R_o = 1.05$). The $R_a/R_o$ value increases dramatically upon adding hexane, and consequently, lignin macromolecules with different characteristic features were fractionated through sequential precipitation depending on the amount of hexane in the cosolvent. The yield of each fraction for kraft lignin and CELF lignin is listed in Table 2. The volume of hexane added in each fractionation step was influenced by the hydrophilicity of the lignin sample. Kraft lignin which is rich in hydroxyl groups was found quite sensitive to solvent polarity; therefore, 2 mL increments of hexane in each step were sufficient to generate a precipitate with yield >10%, e.g., 19.8% yield for the AM-4H (4 mL of hexane in total solvent mixture) kraft lignin fraction and 16.5% yield for the AM-6H (6 mL of hexane in total solvent mixture) kraft lignin fraction (Table 2). On the other hand, CELF lignin that is less hydrophilic required more hexane for fractionation. In both cases, fractionation stopped at 20 mL of hexane (50% of the total solvent mixture) in the cosolvent systems due to the limited yield.

The changes of weight-average molecular weight ($M_w$) and polydispersity index (PDI = $M_w/M_n$) as a function of hexane volume is depicted in Figure 2. AM-ins, AM-1H, AM-2H, TM-ins, and TM-2H fractions were only partially soluble in pyridine; therefore, their molecular weight characterization results were not representative for the whole sample. The $M_w$ of TM-CELF lignin fractions decreased from 14,500 to 2300 g/mol with polydispersity decreasing from 5.5 to 1.6. The final soluble fraction in THF−methanol containing 50% hexane accounting for 31% of the total CELF lignin was mainly composed of oligomers with $M_w = 1200$ g/mol and PDI = 1.5. The unfractionated kraft lignin exhibited a close $M_w$ (4300 g/mol) but with a narrower distribution (PDI = 2.6), compared to...
with the unfractionated CELF lignin ($M_w = 4400 \text{ g/mol, PDI} = 3.4$). Similar trends were observed for AM-kraft lignin with 16.6% that remained soluble at 50% hexane concentration with a $M_w = 1453 \text{ g/mol and PDI} = 1.9$.

**Functional Group Analysis by $^{31}$P NMR.** As shown in the $^{31}$P NMR spectra (Figure 3), distinct differences between softwood kraft lignin and hardwood CELF lignin were observed in the phenolic region ($\delta 145–136 \text{ ppm}$) in relation to the compositional lignin subunits of different wood species. In addition, phenolic OH (60% of total OH) was predominant in kraft lignin as a result of aryl ether bond cleavage. A reversed order was observed for unfractionated CELF lignin; aliphatic OH content (52% of total OH) was slightly higher than phenolic OH (46% of total OH). Given the fact that the abundant phenolic OH contributes significantly to $\delta H$ and $\delta P$ and enhances hydrophilicity of kraft lignin, fractions with higher phenolic OH content but lower molecular weights were separated in the later stage; i.e., they were more tolerant to increasing hexane percentage in the acetone–methanol cosolvent. These results implied that lignin fractionation by sequential precipitation in pure organic cosolvent was driven by $M_w$ considerations rather than hydrophilicity (Figure 4). In fact, increasing phenolic OH and simultaneous decreasing aliphatic OH with decreasing lignin molecular weight has been reported in different studies of kraft lignin fractionation regardless of the solvent systems applied. Actually, the contents of these hydroxyl groups changed linearly with the molecular weights of kraft lignin fractions as depicted in Figure 4A and B. For CELF lignin, the changes of aliphatic and phenolic (guaiacyl and C5-substituted) OH contents with molecular weight reached plateau when $M_w$ decreased from 14,500 to 6300 g/mol (Figure 4C and D).

**Structural Analyses by HSQC and Quantitative $^{13}$C NMR.** The detailed structure evolution of softwood kraft lignin

![Figure 6. HSQC spectrum of CELF lignin: (A) aliphatic region and (B) aromatic region. Structure: (A) $\beta-O-4'$ linked alkyl aryl ether substructure, (B) $\beta-\beta'$ linked resinol substructure, (C) $\beta-5'$ and $\alpha-O-4'$ linked phenylcoumaran substructure, (D) cinnamaldehyde end group, (G) guaiacyl unit, (S) syringyl unit, (S') $\alpha$-oxidized syringyl unit, and (PB) $p$-hydroxybenzoate substructure.](image-url)
and CELF poplar lignin fractionations were investigated by semiquantitative HSQC and quantitative $^{13}$C NMR. Intensive signals at $\delta_{C}/\delta_{H}$ 101.63/4.32 (xyl1), $\delta_{C}/\delta_{H}$ 72.61/3.09 (xyl2), $\delta_{C}/\delta_{H}$ 73.83/3.30 (xyl3), $\delta_{C}/\delta_{H}$ 75.25/3.56 (xyl4), $\delta_{C}/\delta_{H}$ 63.0/3.22; 3.93 (xyl5), $\delta_{C}/\delta_{H}$ 107.98/4.82 (ara1), $\delta_{C}/\delta_{H}$ 81.61/3.88 (ara2), $\delta_{C}/\delta_{H}$ 77.27/3.70 (ara3), and $\delta_{C}/\delta_{H}$ 86.1/4.03 (ara4) were observed in the first three fractions (AM ins, AM-1H, and AM-2H in Figure 5), which were initially insoluble in acetone−methanol (7:3, v/v) or then precipitated with small amounts of hexane (1 and 2 mL). These cross peaks belong to $\alpha$-L-(1→4) arabinosyl units (ara) and $\beta$-D-(1→4) xylosyl units (xyl) that covalently attach to lignin to form xylan-enriched lignin carbohydrate complexes. The typical interunit linkages of softwood lignin were found in the HSQC spectra of AM-ins, AM-1H, and AM-2H kraft fractions (Figure 5), including $\beta$-O$\rightarrow$4′ alkyl aryl ether (A) and $\beta$-$\beta'$ resinosyl (B), and $\beta$-$\alpha$-phenylcoumaran (C) substructures (at noise level), and their signal intensities were weak compared with those of hemicelluloses. The hemicellulose cross peaks disappeared as fractionation continued: Starting from AM-4H, the HSQC spectra of kraft lignin fractions showed clear aliphatic features of lignin without the interruption from hemicellulose, and no distinguishable hemicelluloses cross peaks were found in the HSQC spectrum of AM-20 sol fraction. It was reported that the xylan-rich lignin carbohydrate complex fraction was slightly soluble in 90% aqueous dioxane. In this study, xylan might contribute to the incomplete dissolution of the AM-ins, AM-1H, and AM-2H in pyridine. The quantitative $^{13}$C NMR results confirmed the presence of xylan with the prominent peak around $\delta$101.8 ppm, and the content of $\beta$-D-(1→4) xylosyl units was 14.9, 14.5, and 11.2 per 100 aromatic rings for AM-ins, AM-1H, and AM-2H kraft lignin fractions, respectively. The peak centered at $\delta$105.4 ppm observed in both $^{13}$C and $^{1}H$ NMR spectra (Figure 7).
HSQC NMR spectra of AM-ins, AM-1H, and AM-2H could represent galactan which was found enriched in high molecular weight residual softwood kraft lignin carbohydrate complexes, and its content was 18.6, 16.9, and 9.2 per 100 aromatic rings for the three fractions, respectively. Neither δ101.8 or δ105.4 ppm was observed in the 31P NMR spectra of AM-4H and AM-6H.

Representative HSQC spectra of CELF poplar lignin are shown in Figure 6. Under acidic conditions, lignin was able to repolymerize at Cα, Cω, and Cα positions after acidolysis of β−O−4′ linkages.48 The cross peak centered at δC/δH 112.7/6.76 (Cωcond) was the C2−H2 correlation of guaiacyl units containing C3 condensed with other lignin side chains.49 On the other hand, condensation of syringyl units with Cα,ω−H2,6 correlations served at δC/δH 106.7/4.69, 105.6/6.43, and 104.9/6.33 (Scond) probably occurred at the Cα due to the steric hindrance arisen from the Cα-substituted methoxyl group.58–60 More insights to the structural evolution of a relationship with lignin molecular weight were provided by semiquantitative HSQC NMR (Figure 7). In general, high molecular weight lignin fractions tend to possess more β−O−4′ linkages, while the subunits of oligomeric lignin molecules prefer to link through C−C bonds. When the molecular weight dropped below 2000 g/mol, the relative abundance of β−O−4′ linkages of lignin dropped to ~12% (Figure 7A); however, CELF lignin preserved more than 50% β−O−4′ as the interunit linkages (Figure 7B). Moreover, the S/G ratio of CELF lignin increased dramatically from 1.1 to 4.0 as Mn decreased from 14,500 to 1200 g/mol. Interestingly, in Figure 7B, the relative abundance of β−O−4′ and β−β′ and β−S′ changed little when the molecular weight of the CELF lignin fraction increased from 6300 to 14 500 g/mol, which was consistent with the findings in the 31P NMR analysis. These results imply that little structural changes occurred on the macromolecules of medium and high molecular weight CELF lignin during CELF pretreatment.

Thermal Analysis. The thermal properties of AM-kraft lignin and TM-CELF lignin fractions were investigated by TGA and DSC. Two-stage thermal degradation was observed in the TGA thermograms of both lignin species (Figure 8). In a nitrogen atmosphere, heterolysis and hemolytic cleavage of β−O−4′ bonds and dehydration of aliphatic hydroxy groups occur around 200 °C, and then, C−C interunit linkages are cleaved accompanying with demethoxylation of aromatic rings between 350 and 400 °C.51,52 When the molecular weight of the lignin fractions decreased, the degradation temperature of the first stage tended to decrease and the second one moved slightly to the opposite direction. This is consistent with the changes in the relative abundance of β−O−4′ and C−C interunit linkages estimated by semiquantitative HSQC NMR. In Figure 8A, the additional peak (~290 °C) appearing on the thermal curves of AM-ins, AM-1H, and AM-2H reflected the degradation of xylan;53 such a peak was not found on the curves of CELF lignin fractions (Figure 8C). When the TGA measurements were performed in air (Figure 8B and D), thermal degradation of the second stage was remarkably detained owing to the condensation reaction between aliphatic side chains and phenolic hydroxyl groups in the presence of oxygen.51 Especially, in the case of CELF lignin (Figure 8D), its fractions were subject to rearrangements as Mn decreased and the ratio between phenolic and aliphatic hydroxyl groups approached 1. The DSC results listed in Table 3 showed that the molecular weight of the lignin fraction played an important role on its glass transition behavior, regardless of the variation in chemical structure of the different lignin fractions. The glass transition temperatures (Tg) of the parent kraft lignin and CELF lignin were significantly lower than their corresponding high and medium molecular weight fractions which account for ~70% of the parent lignin. In the studies of polymeric materials containing very high lignin contents, the presence of low molecular weight lignin was able to reduce the rigidity of lignin-based thermoplastics.15 Therefore, lignin fractionation by organic cosolvents could provide a source of low-cost plasticizers for lignin-based polymeric materials.

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Notes
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The authors declare no competing financial interest.
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