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Fast Fractionation of Technical Lignins by Organic Cosolvents

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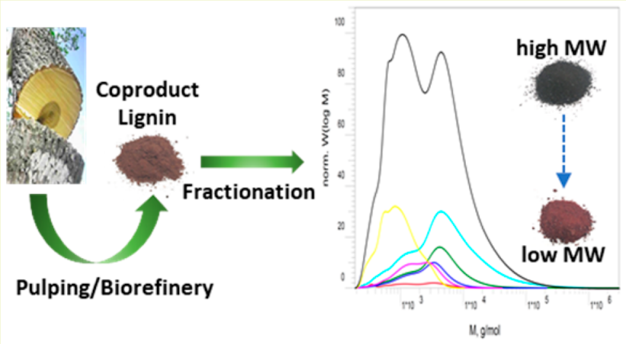
ABSTRACT: A simple and fast fractionation method was developed to obtain carbohydrate-free lignin with well-defined characteristics, such as narrowly distributed molecular weights and a tunable chemical structure for specific applications. In this study, an industrial softwood kraft lignin and a hardwood 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 (^{31}P, quantitative ^{13}C, 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 biorefinering 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

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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-sized 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 % H_2SO_4 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 black liquor lignin was purified according to a published method.[2] In detail, 100 g of dry kraft lignin was suspended in 1000 mL of 0.1 M NaOH with EDTA-2Na^+ (0.5 g/100 mL). After stirring for 1 h, the mixture was filtered through a filter paper (Whatman 1). The filtrate was acidified to pH 6.0 by adding 2.0 M H_2SO_4 dropwise. After stirring for 1 h, the filtrate was gradually acidified to pH 3.0 using 2.0 M H_2SO_4, and the resulting viscous mixture was kept at −20 °C overnight. After thawing, the precipitates were collected by centrifugation and washed thoroughly with distilled water. The air-dried powder was Soxhlet extracted with pentane for 24 h, designated as kraft lignin. The chemicals and solvents used in this work were purchased from Sigma-Aldrich and Fisher Scientific and used as received.

Lignin Fractionation. The kraft lignin and CELF lignin were fractionated according to the scheme presented in Figure 1. Two cosolvent systems were employed in this study: acetone−methanol (7:3, v/v) for kraft lignin and THF−methanol (5:5, v/v) for CELF lignin.

CELF poplar lignin (2.00 g) was suspended in 20 mL a THF−methanol (TM) cosolvent. The suspension was vigorously stirred at RT for 30 min, the undissolved materials (designed as TM-ins) were removed by centrifugation (4000*g*, −4 °C). Hexane (2.00 mL) was added as the antisolvent to the supernatant, and the resulting precipitated fraction (designated as TM-2H) was collected by centrifugation (4000*g*, −4 °C). This procedure was repeated in the foregoing step as demonstrated in Figure 1. Five fractions of CELF lignin were obtained as precipitates after adding hexane sequentially, and they were labeled as TM-2H, TM-6H, TM-10H, TM-14H, and TM-20H. The insoluble fractions were air dried at 40 °C. After adding 20 mL of hexane, the final soluble fraction (designated as TM-20H sol) was dried in a vacuum oven under reduced pressure at 35 °C. After removing the solvent, the isolated fractions were dried at 40 °C for 24 h. The softwood kraft lignin sample was fractionated by acetone−methanol (AM) using hexane as the antisolvent in the same manner as described above. Each fraction was denoted based upon the corresponding cosolvent and the total amount of antisolvent added.

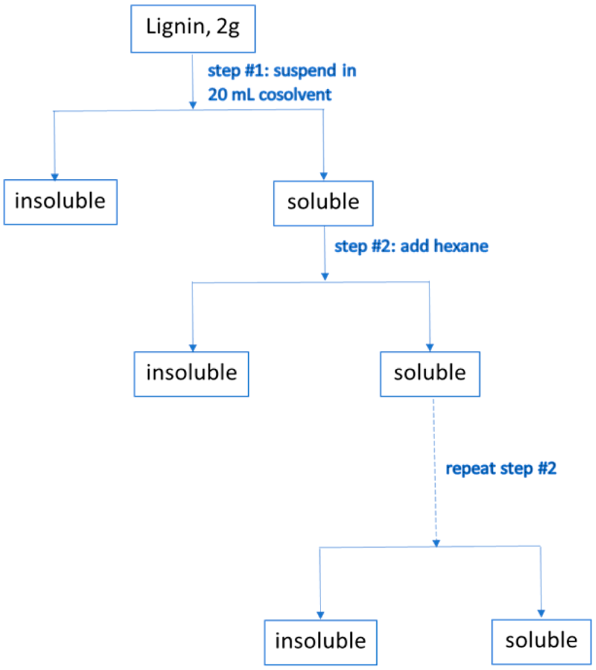

Figure 1. Flowchart of lignin fractionation.

Nuclear Magnetic Resonance (NMR) Analysis. The quantitative ^{31}P NMR spectra were acquired on a 500 MHz Varian VMNRS spectrometer using a 5 mm triple resonance probe. A 90° pulse width, 1.2 s acquisition time, and 25 s pulse delay were used in collecting 64 scans. A 20−30 mg (accurately weighed) lignin sample was phosphitylated with 60 μL of 2-chloro-4,4,5,5 tetramethyl-1,3,2-dixoaphospholane (TMDP) in 700 μL of pyridine/$CDCl_3$ (1.6:1, v/v) containing chromium(III) acetylacetonate (1 mg/mL) as the relaxation agent and *N*-hydroxy-5-norbornene-2,3-dicarboximide (2.5 mg/mL) as the internal standard.[34,35] The TMDP−water phosphitylation product (δ132.2 ppm) was used as the internal reference. The quantitative analysis was carried out using MestReNova software according to a published method.[36]

Heteronuclear single quantum coherence (HSQC) and quantitative ^{13}C NMR spectra were acquired on a Bruker Avance III HD 500-MHz spectrometer. Lignin samples (∼50 mg) were dissolved in 0.5 mL of DMSO-d_6 in a 5 mm-NMR tube. HSQC experiments were carried out using a N_2 cryoprobe (BBO ^{1}H and ^{19}F-5 mm) and a Bruker pulse sequence (hsqcetgpspsi2.2) with the following acquisition parameters: spectra width 12 ppm in the F2 (^{1}H) dimension with 1024 data points (acquisition time 85.2 ms), 166 ppm in the F1 (^{13}C) dimension with 256 increments (acquisition time 6.1 ms), a 1.0 s delay, a J_{C-H} of 145 Hz, and 128 scans. The central DMSO-d_6 solvent peak (δC/δH at 39.5/2.49) was used for a chemical shift calibration. Relative abundance of lignin compositional subunits and interunit linkage were estimated by using the Bruker TopSpin software to integrate the volume of contours in HSQC spectra, and cross peaks were assigned according to the literatures.[11,28,37] ^{13}C NMR acquisition was performed using a 90° pulse with an inverse-gated decoupling pulse sequence, a 2.0 s pulse delay, and 30,000 scans at 30 °C. For the quantitative ^{13}C NMR analysis, 0.01 mg/mL of chromium(III) acetylacetonate was added to decrease the relaxation time.

Gel Permeation Chromatography (GPC). About 2 mg of a dried lignin sample was accurately measured and dissolved in 1 mL anhydrous pyridine. Acetic anhydride (1.00 mL) was added to the mixture soon after complete dissolution of the lignin. The resulting 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 pyridine 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.[38] A UV detector signal was collected at $\lambda = 280$ 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 heating 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[39,40] 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

$$\delta = (E/V)^{1/2} \tag{1}$$

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, δ can be divided into three corresponding parts as follows:

$$\delta^2 = \delta_D^2 + \delta_P^2 + \delta_H^2 \tag{2}$$

Here, δ_D, δ_P, and δ_H are the Hansen solubility parameters (HSP) for the dispersion, polar, and hydrogen bonding interactions, respectively. R_a is the difference between the HSP for a given solvent and polymer. For a good solvent, $R_a/R_o < 1$, where R_o is the interaction radius of polymer.

Table 1. Hansen Solubility Parameters of Solvents and Lignin (R_o = 13.7).[42,43]

	δ_D, MPa$^{1/2}$	δ_P, MPa$^{1/2}$	δ_H, MPa$^{1/2}$	R_a/R_o
Acetone	15.5	10.4	7.0	1.21
Dimethylformamide	17.4	13.7	11.3	0.77
Dimethyl sulfoxide	18.4	16.4	10.2	0.73
Ethanol	15.8	8.8	19.4	0.99
Hexane	14.9	0	0	1.90
Methanol	15.1	12.3	22.3	1.08
Pyridine	19.0	8.8	5.9	0.99
Tetrahydrofuran	16.8	5.7	8.0	1.16
Water	15.5	16.0	42.3	2.08
Acetone−methanol, 7:3 (v/v)[a]	15.4	11.0	11.6	1.05
THF−methanol, 5:5 (v/v)[a]	16.0	9.0	15.2	0.95
Lignin	21.9	14.1	16.9	

[a] HSPs were estimated assuming no volume changing after mixing.

Table 2. Yields of AM-Kraft Lignin and TM-CELF Lignin Fractionated by Hexane

Kraft lignin			CELF lignin		
fraction	hexane, vol %	yield, %	fraction	hexane, vol %	yield, %
AM-ins	0	3.9	TM-ins	0	0.5
AM-1H	4.8	3.4	TM-2H	9.1	0.7
AM-2H	9.1	2.3	TM-6H	23.1	29.5
AM-4H	16.7	19.8	TM-10H	33.3	17.5
AM-6H	23.1	16.5	TM-14H	41.2	9.7
AM-8H	28.6	10.6	TM-20H	50.0	9.8
AM-10H	33.3	8.0	TM-20H sol	50.0	31.1
AM-13H	39.4	8.6			
AM-20H	50.0	10.3			
AM-20H sol	50.0	16.6			

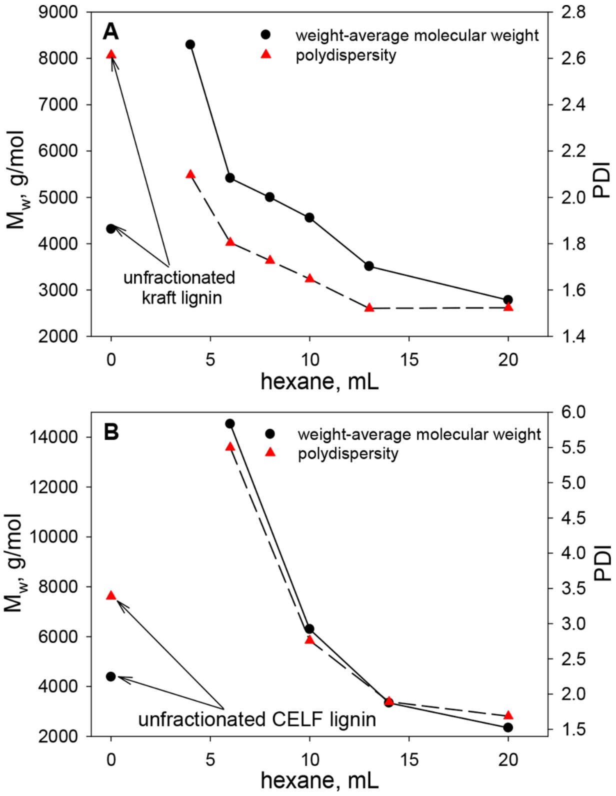

Figure 2. Changes in weight-average molecular weight (M_w) and polydispersity (PDI) of (A) kraft lignin and (B) CELF lignin fractions as a function of hexane volume present in acetone−methanol and THF−methanol, respectively.

$$R_a^2 = 4(\delta_{D2} - \delta_{D1})^2 + (\delta_{P2} - \delta_{P1})^2 + (\delta_{H2} - \delta_{H1})^2 \tag{3}$$

It was suggested that solvents with δ-values close to 22.5 MPa$^{1/2}$ were good solvents for lignins.[39,40] 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,[30] and full dissolution of lignin in acetone and ethanol can be achieved in the presence of water.[26,27,29] 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.[41] Both native and technical lignins are known to

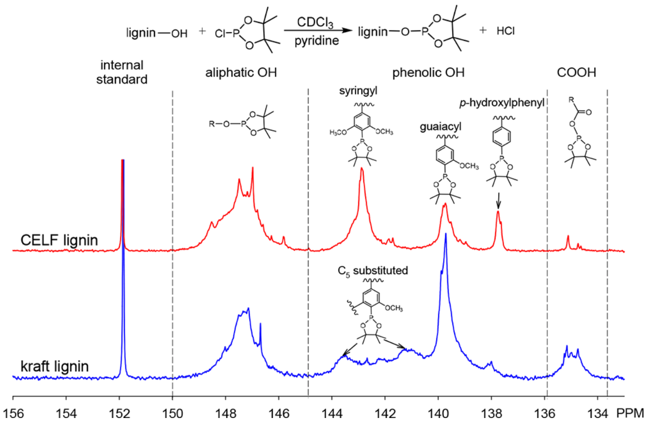

Figure 3. Quantitative ^{31}P NMR spectra of CELF lignin and kraft lignin. Hydroxyl groups were phosphitylated with TMDP.

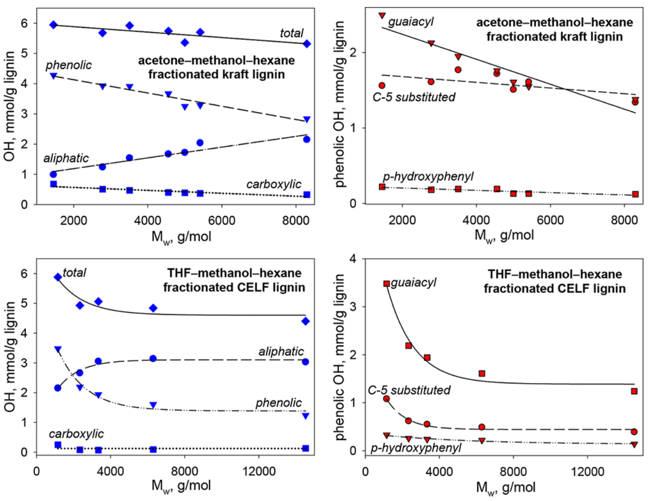

Figure 4. Changes of hydroxyl group contents as a function of M_w for kraft lignin (A, B) and CELF lignin (C, D).

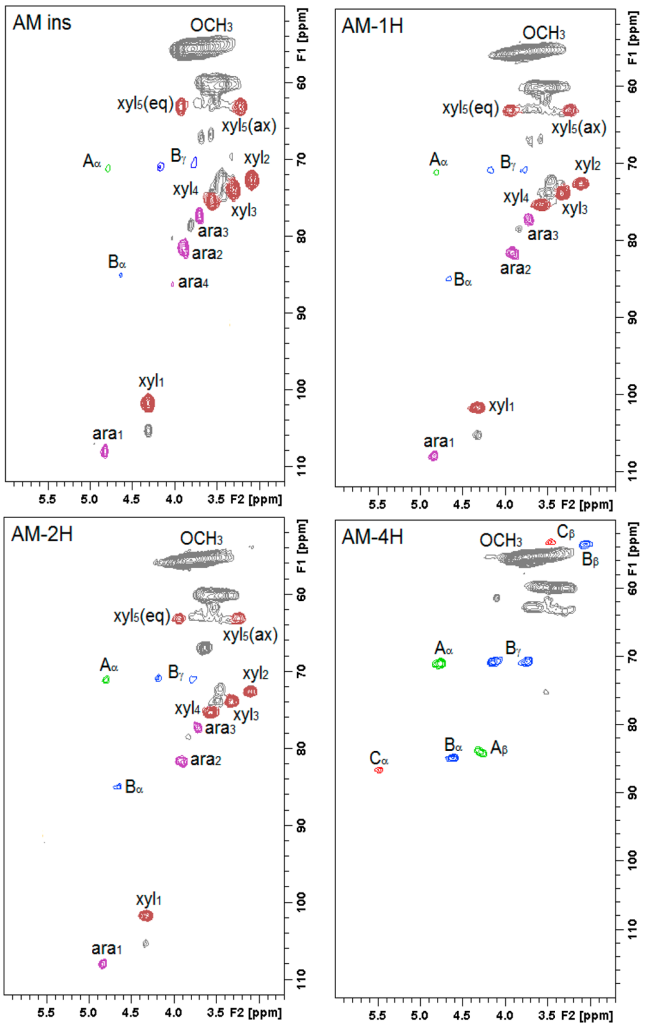

Figure 5. Side chain in the 2D-HSQC spectra of the AM ins, AM-1H, AM-2H, and AM-4H kraft lignin fractions.

be fully soluble in dimethyl sulfoxide and dimethylformamide and polar aprotic solvents with high δ_P-values and moderate δ_H-values (Table 1). The HSPs of a solvent mixture are linear functions in relationship to the volume fraction of each solvent;[42] 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 δ_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

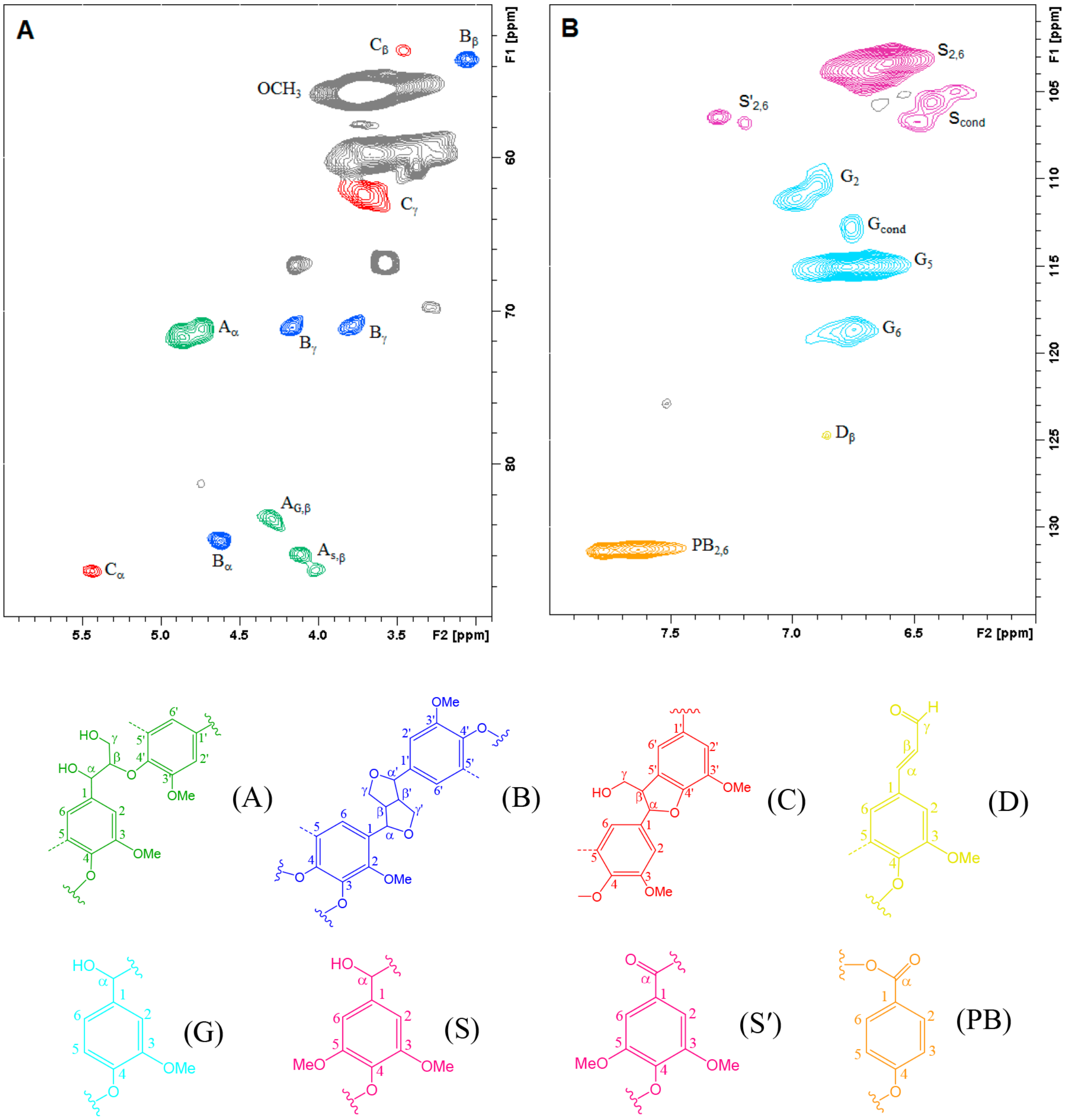

Figure 6. HSQC spectrum of CELF lignin: (A) aliphatic region and (B) aromatic region. Structure: (A) β−O−4′ linked alkyl aryl ether substructure, (B) β−β′ linked resinol substructure, (C) β−5′ and α−O−4′ linked phenylcoumaran substructure, (D) cinnamaldehyde end group, (G) guaiacyl unit, (S) syringyl unit, (S′) α-oxidized syringyl unit, and (PB) *p*-hydroxybenzoate substructure.

with the unfractionated CELF lignin (M_w = 4400 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 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 (δ145−136 ppm) in relation to the compositional lignin subunits of different wood species.[34] In addition, phenolic OH (60% of total OH) was predominant in kraft lignin as a result of aryl ether bond cleavage.[44] 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 δ_H and δ_P and enhances hydrophilicity of kraft lignin,[43] 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.[26−28,45] 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 C_5-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

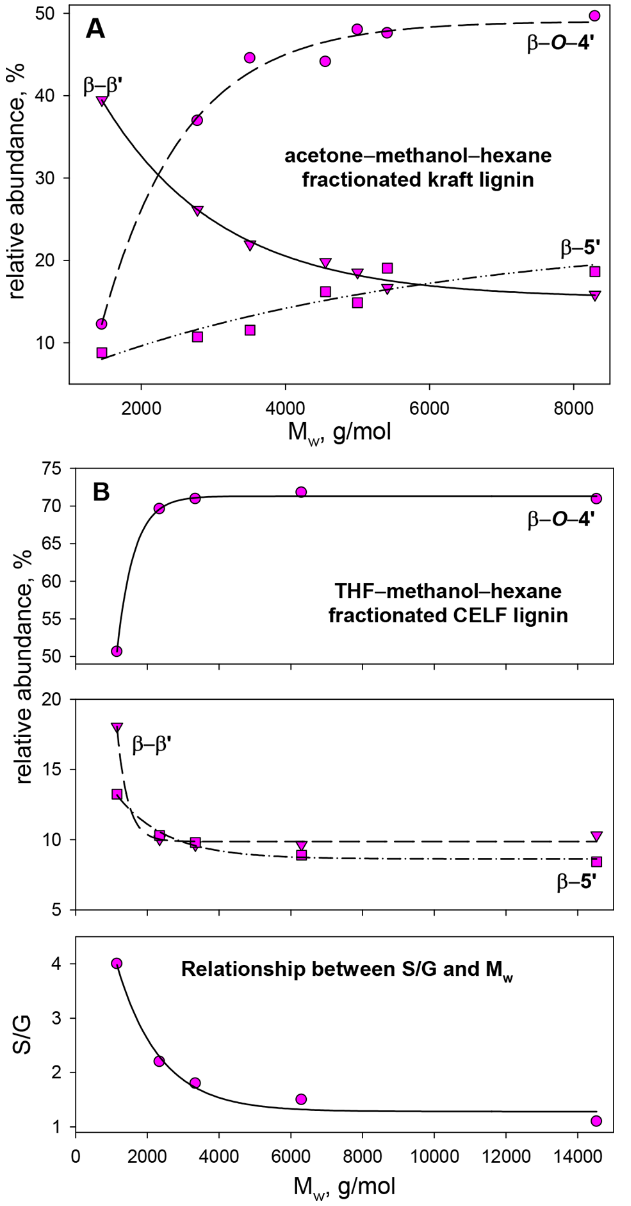

Figure 7. Relationship between the relative abundance of interunit linkages, the S/G ratio, and M_w of (A) acetone−methanol−hexane fractionated kraft lignin and (B) THF−methanol−hexane fractionated CELF lignin.

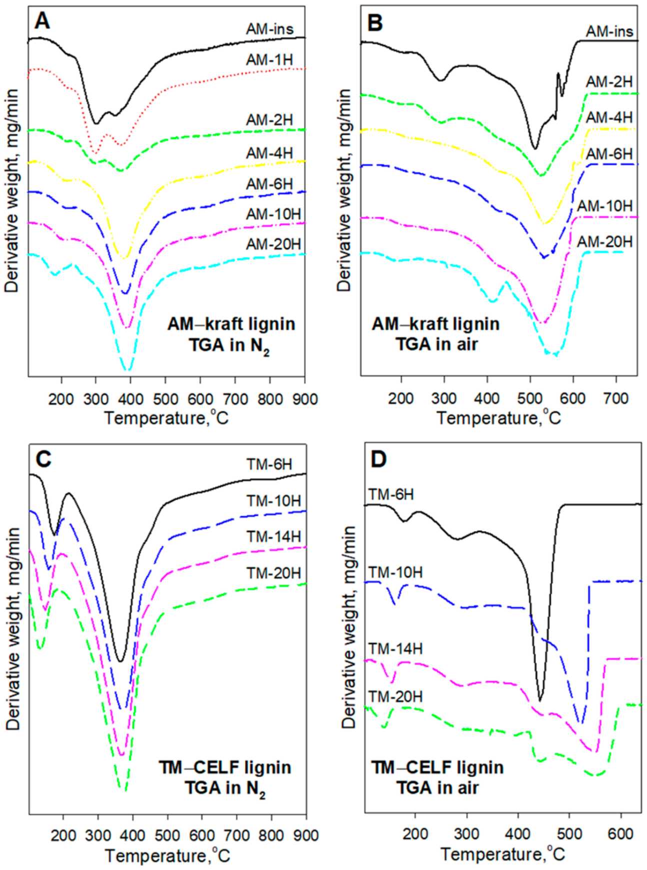

Figure 8. TGA thermograms of the AM-kraft lignin (A, B) and TM-CELF lignin (C, D) fractions.

Table 3. Glass Transition Temperatures of Kraft Lignin and CELF Lignin Fractions

kraft lignin	T_g, °C	CELF lignin	T_g, °C
parent KL	174	parent CELF	121
AM-2H	215	TM-6H	158
AM-4H	213	TM-10H	154
AM-6H	209	TM-14H	141
AM-8H	201	TM-20H	133
AM-10H	194		
AM-13H	180		
AM-20H	160		

and CELF poplar lignin fractionations were investigated by semiquantitative HSQC and quantitative ^{13}C NMR. Intensive signals at δ_C/δ_H 101.63/4.32 (xyl$_1$), δ_C/δ_H 72.61/3.09 (xyl$_2$), δ_C/δ_H 73.83/3.30 (xyl$_3$), δ_C/δ_H 75.25/3.56 (xyl$_4$), δ_C/δ_H 63.0/3.22; 3.93 (xyl$_5$), δ_C/δ_H 107.98/4.82 (ara$_1$), δ_C/δ_H 81.61/3.88 (ara$_2$), δ_C/δ_H 77.27/3.70 (ara$_3$), and δ_C/δ_H 86.1/4.03 (ara$_4$) 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 α-L-(1→4) arabinosyl units (ara) and β-D-(1→4) xylosyl units (xyl) that covalently attach to lignin to form xylan-enriched lignin carbohydrate complexes.[46] 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 β−O−4′ alkyl aryl ether (A) and β−β′ resinol (B), and β−5′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.[46] 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 δ101.8 ppm, and the content of β-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 δ105.4 ppm observed in both ^{13}C and

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,[47] 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 ^{13}C 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 C_5, C_6, and C_α positions after acidolysis of β−O−4′ linkages.[48] The cross peak centered at δ_C/δ_H 112.7/6.76 (G_{cond}) was the C_2−H_2 correlation of guaiacyl units containing C_5 condensed with other lignin side chains.[49] On the other hand, condensation of syringyl units with $C_{2,6}$−$H_{2,6}$ correlations bserved at δ_C/δ_H 106.7/4.69, 105.6/6.43, and 104.9/6.33 (S_{cond}) probably occurred at the C_α due to the steric hindrance arisen from the C_5-substitued methoxyl group.[48−50] 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 kraft lignin dropped to ∼12% (Figure 7 A); however, CELF lignin preserved more than 50% β−O−4′ as the interunit linkages (Figure 7 B). Moreover, the S/G ratio of CELF lignin increased dramatically from 1.1 to 4.0 as M_w decreased from 14,500 to 1200 g/mol. Interestingly, in Figure 7 B, the relative abundance of β−O−4′ and β−β′ and β−5′ 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 ^{31}P 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 atomsphere, heterolysis and hemolytic cleavage of β−O−4′ bonds and dehydration of aliphatic hydroxyl 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 8 D), its fractions were subject to rearrangements as M_w 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 (T_g) 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.

CONCLUSION

Acetone−methanol−hexane and THF−methanol−hexane were employed for fractionating softwood kraft lignin and hardwood CELF lignin, respectively. The volume proportions of acetone−methanol and THF−methanol were carefully chosen based upon the solvent HSPs. The sequential precipitation with hexane as an antisolvent works well with both kraft lignin and CELF lignin fractionation. The sequence of lignin precipitation as a result of gradually adding hexane into the cosolvents was determined by several factors in order: (1) the content of hemicellulose bonding with lignin macromolecules, (2) the molecular weight of lignin itself, and (3) the type and content of OH groups. The ^{31}P NMR analyses demonstrated that high molecular weight lignin possessed a higher frequency of aliphatic OH groups on the macromolecular chains, while the low molecular weight one contained more phenolic OH groups due to the cleavage of alkyl aryl ether bonds. Generally, β−O−4′ alkyl ether linkages tended to be replaced by C−C bonds to some extent in low molecular weight lignin fractions. The chemical structure of CELF lignin macromolecules underwent little modification when its molecular weight was above 6000 g/mol.

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Notes

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