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A New Method for Demethylation of Lignin from Woody Biomass using Biophysical Methods

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Abstract

Demethylation of softwood kraft lignin from woody biomass to improve the hydroxyl number in modified demethylated lignin and to produce lignin-based polyols was investigated using several biophysical techniques. Lignin is a tremendously under-developed natural polymer co-generated through papermaking and biomass fractionation. Molecular weights of lignins were analyzed by high-performance size-exclusion chromatography (HPSEC). Molecular weights of softwood kraft lignin (SKL) and demethylated lignin (DL) were determined as 1071 and 891 gmol^{-1}, respectively. For demethylation, iodocyclohexane in dimethyl formamide (DMF) was used under reflux conditions, with a resulting yield of 87%. Fourier transform infrared spectroscopy (FTIR) was used to determine characteristic absorption peaks of softwood kraft lignin and demethylated lignin. Significant spectral differences were noticed between the two types of lignin due to changes in chemical structure. Total hydroxyl numbers were determined by titration. Phosphorus nuclear magnetic resonance spectrometry (^{31}P-NMR) was employed to analyze the structure of lignin and different types of phenolic hydroxyl units. Nevertheless, further chemical and biological modifications within the lignin molecule are needed for various industrial applications to synthesize polyurethane foam by using chemically modified lignin-based polyols.

Keywords: Demethylation; Softwood kraft lignin (SKL); Demethylated lignin (DL); Hydroxyl numbers; FTIR spectroscopy; ^{31}P-NMR

Introduction

Lignin is described as an unsystematic, unstructured, 3-D polymeric complex system that does not possess a regular, precise structure with fixed recurring units. In lignin, the main functional groups are hydroxyl, methoxy, carbonyl, and carboxyl moieties in several amounts, whose components depend on the botanical source and the applied extraction processes [1]. In the plant kingdom, lignin is the most abundant polymeric organic material after cellulose [2]. The lignin biosynthetic pathway utilizes mainly three different types of phenylpropane units to make the lignin molecule. These include a guaiacyl precursor made from coniferyl alcohol and guaiacyl-syringyl precursors derived from coniferyl and sinapyl alcohol. Lignin from softwood (conifers) is mainly composed of guaiacyl units, while hardwood (angiosperms) lignin contains guaiacyl-syringyl units [3].

Both lignins contain mainly glycerol-aryl ether (β-O-4) linkages between short and linear chains of phenylpropane units [4]. The monomeric units of lignin are connected by a number of different C-C and ether linkages, which accounts for the complicated 3-D structure of lignin [2,5].

The different types of functional groups in a complex lignin macromolecule are presented in Figure 1, [3,6]. Lignins are the by-product of pulp and paper industries and are abundant in nature. The uses of lignin in different applications are as a dispersant agent for pesticides, emulsifiers, ion-exchange resins, water treatment agent, pesticide surfactants, heavy metal sequestrant, binders, animal foods, grinding aids, electrolytic refining, and tanning agents, or as a component of composites and copolymers [7-9].

To create value-added applications of lignin, medium- and long-term technologies mainly for the preparation of LMW compounds that can substitute for products produced by the petrochemical industry must be established [10]. Nevertheless, the structure of lignin is not completely recognized yet. Over the last two decades, many studies have been undertaken to understand the industrial applications of lignins [6]. The subjects of study by several research groups are lignin and their advanced applications, including, polyurethanes [10], acrylics [11], epoxies [12], and phenolic resins [13].

For polyurethane synthesis, the utilization of lignin as a macromonomer frequently follows two general methods: direct use of lignin without any major chemical modification, alone or in blends with other polyols [14], or (2) chemical modification, such as depolymerization, esterification and etherification reactions [10,15]. A broad range of lignin-based polyurethane materials (rigid foams, elastomers, sealants) has been produced and the resultant mechanical and thermal properties have been assessed [16].

Due to shared structural similarities, the industrially important organic compound vanillin can be produced from lignin, as has been reported by many scientists [17]. By catalytic degradation of lignin, many other industrially valuable small molecule chemicals can be produced [17]. However, to control for serious practical problems related to the enormous energy costs and basic purification processes, additional research is required to produce small molecule chemicals from this complex natural biopolymer. The second approach for the utilization of lignin is as a primary material in a diversity of green polymer products [18]. Nevertheless, the synthesis of commercial based

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sustainable polymers requires high-purity initial materials having great potential and reproducible reactivity.

Lewis acids have been comprehensively studied for the demethylation of methoxyl groups in small molecule organic chemistry [19]. The methoxyl group in lignin can be converted to a more reactive hydroxyl group via demethylation [18,20]. In this study, we used iodocyclohexane as a hydriodic acid (HI) source in DMF. By increasing lignin hydroxyl-group contents and its reactivity, this treatment was found to be valuable, compared with other Lewis acids such as boron tribromide (BBr_3) [21], and trimethylsilyl iodide (TMSI) [22] which were not.

The aim of this study was to explore the demethylation of the lignin molecule and to characterize different hydroxyl groups present in commercially available technical and demethylated lignins using different biophysical methods. The hydroxyl contents are needed to characterize commercial polyols for the synthesis of bio-based polyurethane foam used for several applications as shown in Figure 3 and this concept was earlier presented by da Silva et al. [23].

Materials and Methods

Softwood kraft lignin (SKL) was provided by Krugar Wayagamack Inc. Quebec, Canada, contains 1.50% ± 0.02ash. The ash contents were determined by gravimetric method whilst sample ignition heating up to 525°C using Sybron-Thermolyne® bench top muffle furnace. Polystyrene standards were purchased from American Polymer Standards Corporation, Mentor, OH.

Analytical HPSEC

Polystyrene standards were used to determine the molecular weight of lignin. The calibration curve standards were used for several fractions of very low poly dispersity close to unity in HPSEC analysis. Polystyrene standards are commonly used for lignin molecular weight determination. Lignin fractions and polystyrene standards exhibited very comparable behavior throughout a large range of molecular weights in a regular HPSEC analysis of lignin [24]. The experimental conditions for HPSEC were: flow-rate 1.0ml/min, solvent DMF, sample concentration of 2mg/ml of DMF, volume injected variable from 100 µl to 200 µl, column temperature 80°C, RI detector temperature 40°C, UV-vis scanning range at 270-350 nm wavelength, Refractive Index scanning range at 400nm wavelength, Refractive Index detector-Waters 410 (Differential Refractometer), UV-vis detector-Waters M-490,GPC software- EMPOWER, HPLC system by Millipore-Waters and Styragel HR1 column 104A. Calibration curves were constructed with polystyrene standards.

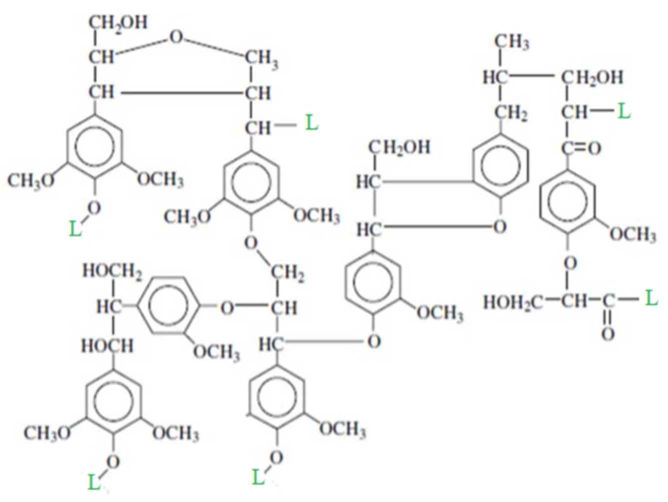

Figure 1: Partial lignin structure proposed by Adler [3,6].

Optimized Reaction Conditions for Demethylation

Reaction mixtures were prepared in a three neck round bottom flask and included 25mg of softwood kraft lignin (SKL) dissolved in 2.0 ml of dimethyl formamide DMF and 1.3 ml of iodocyclohexane [25]. The reaction mixture was purged with nitrogen to remove oxygen and setup with a reflux condenser. The reaction system was refluxed for 3h at 145°C, cooled, poured into water (20ml) and extracted with tetrahydro furan (THF) and toluene (20 ml x 3). The organic layer was washed with saturated aqueous sodium bisulphite ($NaHSO_3$) and brine solution and the samples were dried over oven-dry sodium sulphate (Na_2SO_4) and then filtered. The filtrate was concentrated at 82°C in a rotary evaporator to remove all organic solvents, including iodocyclohexane, and afterward dried in a vacuum desiccator to obtain demethylated lignin (13.7mg, 87% ± 0.51 yield). The process is illustrated by the general equation as shown in Figure 2.

FTIR Spectroscopy

FTIR spectra of softwood kraft lignin (SKL) and demethylated lignin (DL) were measured using a Bruker Tensor 27 FTIR spectrometer equipped with an IR-microscope and MCT detector (Karlsruhe, Germany). For spectral analysis, 2.0 mg of each lignin

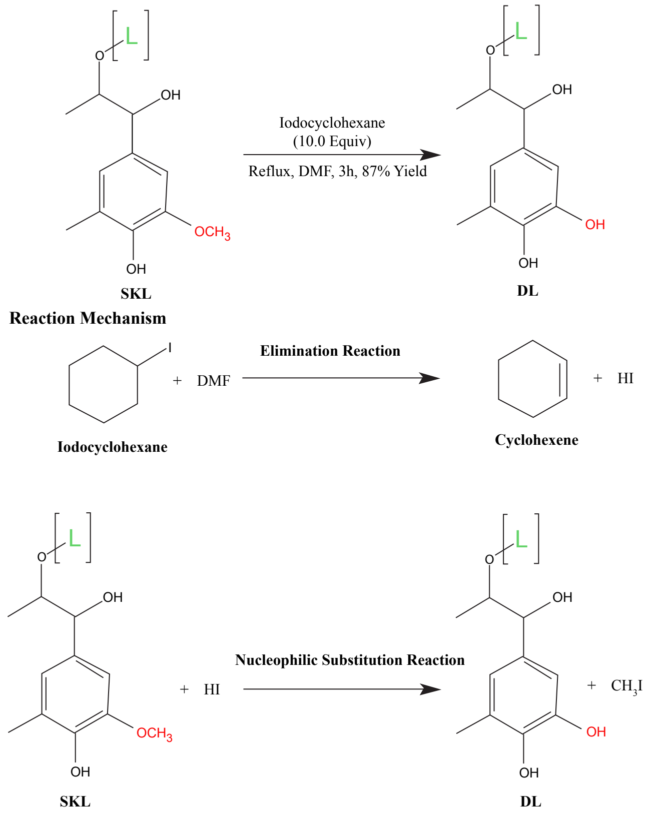

Figure 2: Reaction mechanism for demethylation process illustrating the replacement of methoxyl to hydroxyl group under optimized reaction conditions, where L indicates lignin molecule.

sample (SKL and DL) was mixed with200 mg of KBr. The transmission spectra (3500 to1000cm^{-1}) of SKL and DL were measured at room temperature. To prevent moisture contamination of the pellet, the following two steps were performed: SKL, DL and KBr were left at 40°C for 24 h under reduced pressure before pellet preparation, and (2) pellets were maintained under the same conditions for 12 h before FTIR analysis. By using Opus software, the baseline was corrected in the collected transmission spectra and stabilized according to the highest band.

Hydroxyl Number Determination by Esterification

Phthalation is one of the most common wet chemical methods for lignin hydroxyl group determination. The total hydroxyl content can be measured by a standard procedure with a phthalic anhydride, imidazole/dioxane mixture, followed by back-titration with a NaOH solution. This provides a simple method for determining total hydroxyl content. Using the esterification approach as reported by Kurimoto et al. [26,27] aided in the determination of the total OH value.

A mixture of 0.2-0.5 g of each lignin (SKL and DL) sample and 25 ml of phthalation reagent was separately heated at 110°C for 20 min. After cooling, 50ml of a dioxane/water solution (80:20 v/v) was added to the mixture and back titrated with 1N NaOH solution to get the equivalence point using a pH meter. The phthalation reagent is the combination of three components: 30g phthalic anhydride, 4.84 g imidazol, and 200 ml dioxane. The OH value was calculated based on esterification as follows:

Hydroxyl Value (mmol/g) = (B - A)N/W + acid value

where B is the volume of 1N NaOH solution used to titrate the blank solution (ml); A is the volume of 1N NaOH solution used to titrate the sample solution (ml); N is the normality of the NaOH solution; and W is the weight of the sample (g).

The acid values were also determined by titration with 1N NaOH. lg of each lignin (SKL and DL) was dissolved separately into 100ml of a dioxane/water solution (80:20 v/v) and titrated with 1N NaOH to determine the equivalence point. The acid value was calculated as follows:

Acid value=(C - B)N/W

where C is the volume of 1N NaOH solution used to titrate the sample solution(ml); and B is the volume of 1N NaOH solution used to titrate the blank solution (ml) (Tomoko et al. [28]).

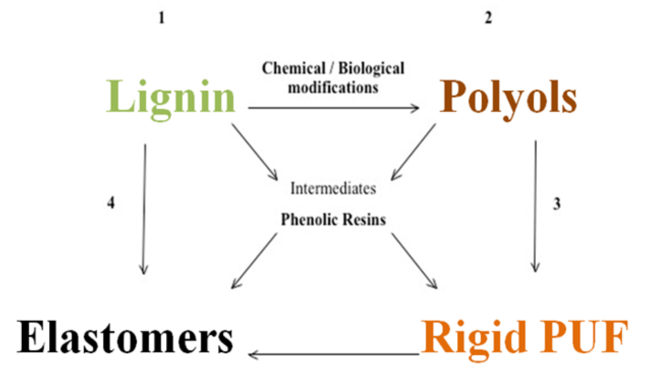

Figure 3: A general view for the synthetic routes of lignin mixing expended for different applications (1) direct use, (2) after chemical/biological modifications convert into phenolic resins (intermediates) and liquid polyol, (3) synthesis of rigid polyurethane foam (PUF) using polyols, (4) synthesis of rigid polyurethane elastomers using lignin based polyols. Previously, this concept was proposed by B.d Silva et al. [23].

Sample Preparation for ^{31}P-NMR Analysis

The lignin samples were phosphitylated as described by Hoareau et al. [29]. A combination of pyridine and $CDCl_3$ (1.6/1, v/v) was prepared and dried over molecular sieves. The above solution was further used for the preparation of relaxation reagent with 5 mg/ml of chromium (III) acetylacetonate $[Cr(C_5H_7O_2)_3]$ solution and 5 mg/ml of cholesterol solution, which served as an internal standard.

Phosphitylation of lignin samples was carried out with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane. 20mg of each lignin were dissolved in 0.4 ml of DMF in a sealed vial with a Teflon-faced septum. Next, 0.25 ml of solvent solution was added, followed by the addition of 0.1 ml of internal standard and relaxation solution. The (0.1 ml) phosphitylation reagent was then added, and the sample was shaken to ensure homogeneity. After derivatization, the resulting solution was transferred to a 5-mm tube, and the ^{31}P-NMR spectrum was recorded by a Bruker Ultrashield Plus-400 MHz spectrometer.

Results and Discussion

The lignin macromolecule has been noted by many authors for its structural uniqueness and limited industrial usage [30-33]. Its complex structure and mass dispersion have also impeded the identification of its polymer properties.

Molecular Weight Determination by HPSEC

Polystyrene standards were used for the calibration of HPSEC columns and a calibration curve was obtained by applying a 3rd order polynomial equation to the polystyrene data. The calibration curve was then used to determine the molecular weights of lignins. The results demonstrate that the average molecular weight values (M_n) and the distributions of molecular weight (M_w) values obtained for both lignins were very similar, as shown in Table 1.

FTIR Spectra of SKL and DL

FTIR is one of the effective tools to determine the functional groups [4] and the structural properties of the lignin molecule. The peak assignments of IR bands and their relative transmittance in SKL and DL are listed in Table 2. The characteristic IR transmission peaks of SKL and DL are shown in Figure 4. In the fingerprint region, spectral differences were observed between SKL and DL in the range between (1600 to 1000 cm^{-1}). Due to aromatic skeletal vibration and guaiacyl ring breathing with carbonyl stretching, the dominating spectra were observed at band positions of 1603 and 1095 cm^{-1} in the SKL lignin.

Standards/ Lignins	Identification	Retention time (tr) minutes	M_w(Da)	M_n(Da)	(M_w/ Mn)
Polystyrene Standards	ps1	9.814	500	490	1.0
	ps2	8.682	610	600	1.0
	ps3	8.640	892	870	1.0
	ps4	8.086	1000	998	1.0
	ps5	6.682	3000	2980	1.0
	ps6	6.1	4020	4000	1.0
	ps7	6.065	5590	5570	1.0
	ps8	5.562	9145	9100	1.0
Lignins	SKL	7.916	1071	986	1.08
	DL	8.211	891	744	1.19

Table 1: HPSEC retention times of calibration curve standards.

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As contrasted to SKL, the primary peak of DL was observed at bands 1664, 1389, and 1248 cm^{-1}. Large spectral differences were monitored in SKL and DL and we observed significant changes in lignin structure due to the displacement of methoxy to hydroxyl groups. These results are consistent with those reported by Kubo et al. [4] and Pandey et al. [34]. The different types of hydroxyl groups in SKL and DL were further characterized by ^{31}P-NMR.

Demethylation

The basicity of the solvent during demethylation plays a critical role in reaction efficiency. The production of hydriodic acid (HI), which is essential for demethylation, from iodoalkanes requires a strongly basic solvent. However, the solvent must not be so strongly basic as to neutralize HI. In situ, an elimination (E1) reaction produces HI, which afterward reacts with methoxyl groups of lignin by nucleophilic substitution (SN^2) reaction to produce methyl iodide (CH_3I) and demethylated lignin (DL). The optimized reaction conditions are shown in (Figure 2); the methoxyl groups were basically removed with 10.0 equivalents of iodocyclohexane to give the desired product, with a 87%±0.51 yield, without further purification. The method described here is more efficient than that which Canonica et al. [35] reported.

NMR Studies

The different types of hydroxyl groups in SKL and DL were investigated by ^{31}P-NMR. The spectra of SKL and DL are shown in Table 3 and Figure 5. For condensed phenolic units, the two sharp signals were observed at 146-145 ppm. Table 4 shows signal assignments for both lignins. The following spectral regions were integrated to acquire the different types of hydroxyl groups: aliphatic hydroxyl signals at 150 ppm, syringyl phenolic hydroxyls from 140-141ppm, guaiacyl phenolic hydroxyl signals at 137.5ppm, *p*-hydroxyphenyl phenolic hydroxyl at 132.5ppm, and carboxylic acid units from 131-130 ppm. The proportion of different hydroxyls can be computed by assimilating various signals of ^{31}P-NMR spectra, whereas the arrangement of different types of phenolic hydroxyl units in lignin can be seen in Figure 5. The phenolic peak of DL is higher than the peak of SKL due to the presence of different condensed phenolic hydroxyl units, as was previously reported by Carolina et al. [36]. By using the esterification method, the total hydroxyl values were determined containing phenolic or sterically hindered hydroxyl groups; the values were 3.06 (±0.50) and 5.01 (±0.57) mmol/g for SKL and DL, respectively. In each lignin, the quantity of different hydroxyl groups was determined by ^{31}P-NMR method presented in (Table 4). The most abundant groups found in both lignins were carboxylic acid and guaiacyl-OH as compared to other hydroxyl units reported by Diao et al. [37].

Serial No.	SKL Band position (cm^{-1})	DL Band position (cm^{-1})	Assignment
1	3424	3454	O-H stretching
2	2960	2937	C-H stretching
3	2920	2870	C-H stretching
4	2652	-	C-H stretching
5	-	1664	C=O stretching (unconjugated)
6	1603	-	Aromatic skeletal vibration+C=O stretching
7	1511	1501	Aromatic skeletal vibration
8	1455	1437	C-H deformation (CH_3 and CH_2)
9	-	1409	C-H in-plane deformation with aromatic ring stretching
10	-	1389	C-O of syringyl (S) ring
11	1264	1248	C-O of guaiacyl (G) ring
12	1206	-	C-C + C-O stretch
13	1095	1092	Aromatic C-H in-plane deformation in the guaiacyl ring

Table 2: Results of the IR Bands obtained in SKL and DL.

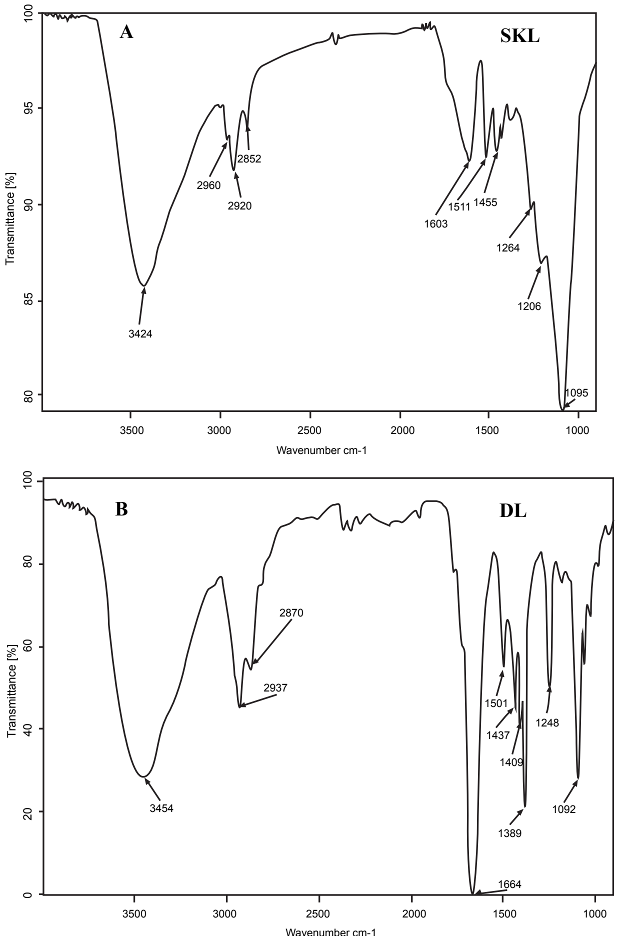

Figure 4: FTIR spectra of (A) softwood kraft lignin (SKL), and (B) demethylated lignin (DL).

Signal (ppm)	Assignment
150	Aliphatic-OH
146-145	Condensed phenolic units
140-141	Syringyl phenolic-OH
137.5	Guaiacyl phenolic-OH
132.5	p-Hydroxyphenyl-OH
131-130	Carboxylic acid units

Table 3: Signal Assignments of SKL and DL by ^{31}P-NMR spectrometry.

Conclusions

The industrial production of goods mainly depends on sustainable growth, which involves the use of safe raw materials resources. Kraft lignin, as a large available biomass resource (by-product of pulp mills), is a very appealing and favourable raw material to produce biopolymers and biofuels for use in process unit operations. Economical affordability of lignin-based processes and advances in this field are likely to play a key role in the industrial development

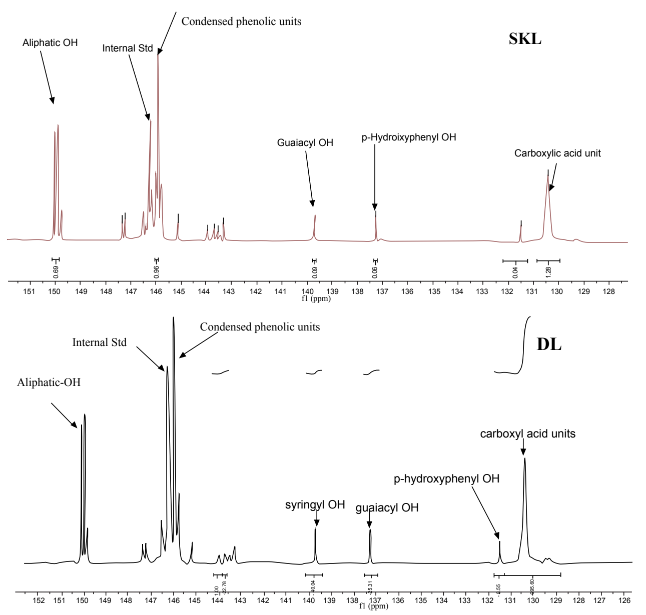

Figure 5: ^{31}P-NMR spectra of SKL and DL shows qualitative and quantitative characterization of different types of hydroxyl groups.

Hydroxyl Groups	SKL(mmol/g)	DL (mmol/g)
Carboxylic Acid	1.28	2.44
Guaiacyl-OH	0.96	1.32
Syringyl-OH	0.01	0.03
Aliphatic-OH	0.69	0.82
p-Hydroxyphenyl-OH	0.12	0.4

Table 4: Quantification of different hydroxyl groups of SKL and DL by ^{31}P-NMR spectrometry.

of lignin. HPSEC results showed that it is possible to use polystyrene standards for lignin molecular mass analyses. The fractions of different molecular masses have different chemical compositions and functional groups as indicated by FTIR analysis. Notable spectral differences were observed in SKL and DL due to changes in lignin structure and displacement of methoxyl to hydroxyl groups. The different types of phenolic hydroxyl units were investigated by ^{31}P-NMR. In summary, demethylation of the lignin molecule using iodocyclohexane as HI source in DMF has been developed. It was demonstrated that the total hydroxyl numbers (1.6-fold increase in DL as compared to SKL) indicate demethoxyl groups were removed successfully in high yields under optimized reaction conditions. The strategy offers a new alternative approach for protecting phenolic groups, which is helpful in regulating the optimum processing condition during the development of lignin based polyols and its application for the synthesis of bio-based polyurethane foams.

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