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Lignin behaviour during wood liquefaction—Characterization by quantitative ^{31}P, ^{13}C NMR and size-exclusion chromatography

Edita Jasiukaitytė [a,*], Matjaž Kunaver [a], Claudia Crestini [b,*]

[a] National Institute of Chemistry, Hajdrihova 19, SI-1000, Ljubljana, Slovenia
[b] Dipartimento di Scienze e Tecnologie Chimiche, Tor Vergata University, Via della Ricerca Scientifica 1, 00133 Rome, Italy

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ABSTRACT

A blend of spruce (*Picea* spp.) and fir (*Abies* spp.) sawdust was liquefied by an optimized technique in the presence of multifunctional alcohols and *p*-toluene sulfonic acid monohydrate as a catalyst. The lignin behaviour during wood liquefaction was studied at different reaction times. Samples prepared by the new separation procedure were characterized by quantitative ^{31}P, ^{13}C NMR and by size-exclusion chromatography. The final products showed increased molar masses indicating the condensation reactions between the lignin and the multifunctional alcohols. Aliphatic chains were found to be incorporated into the lignin network after liquefaction. The resulting polymer showed an increased content of aliphatic hydroxyl groups with respect to the starting material and a high degree of solubility in multifunctional alcohols.

1. Introduction

Renewable plant biomass sources, such as lignocellulosics and other polysaccharides, are gaining importance as a suitable replacement for fossil-fuel resources. Biomass, especially that obtained from industrial residues and not effectively used, has received particular attention.

Wood is one of the most important renewable natural products. The depolymerization of macromolecular wood components in a liquefaction process, followed by reaction with specific organic reagents, enables the conversion of wood into a potential feedstock for the synthesis of new, environmentally friendly polymers [1,2]. Wood liquefaction can be achieved with phenol or polyhydric alcohols, under acid-catalyzed conditions [3–6]. Despite the high efficiency of wood liquefaction using phenol as liquefying agent, the removal of not reacted phenol from the reaction mixture is very difficult in addition to its high recovery costs and environmental pollution. Thus, there were many efforts to replace phenol with multifunctional alcohols as (poly)ethylene glycol, glycerol and diethylene glycol [7–9].

The obtained liquefied wood is a material containing a number of highly reactive aromatic and aliphatic hydroxyl groups which can be used as reaction sites in the preparation of adhesives, foams or other moldings, opening a new application field in the utilization of waste wood materials [10,11]. The use of liquid wood in the preparation of new polymers is not only meaningful in the efficient utilization of renewable resources, but also gives a way to replace the raw materials otherwise produced from crude oil.

On the other hand, because of the diversity in both the chemical composition and the reactivity of wood components, the molecular structures of liquefied wood are quite complex. Therefore, the comprehensive understanding of wood liquefaction pathway is essential.

Up to date, wood liquefaction has been studied using only model compounds. The liquefaction of lignin with phenol has been studied on models, such as guaiacylglycerol-β-guiacyl ether [12,13]. Recently, a study of liquefaction of cellulose with ethylene glycol has been published where authors proved the formation of ethylene glycol–glucoside in the early stage of liquefaction, which later decomposes into the 2-hydroxyethyl levulinate [14]. The cellulose degradation that is considered to be the rate-limiting step in wood liquefaction was studied in the presence of ethylene glycol under the catalysis of sulfuric acid (H_2SO_4) and the *p*-toluene sulfonic acid monohydrate (PTSA) [15]. The cellulose was degraded to the similar extent using both catalysts—H_2SO_4 or PTSA. However, in order to achieve the lowest sulfur content in the final product, the use of PTSA is recommended [16].

The combination of glycerol and diethylene glycol was used in our recent experiments. They were carried out under the PTSA catalysis, thus allowed to liquefy wood in a 3:1 (by mass) ratio, respectively [17]. Due to the high wood content in the final product the removal of unreacted glycol is not required and the reaction slurry can be directly used in the further polymer synthesis without additional isolation steps. Additionally, the use of glycerol

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* Corresponding authors. Tel.: +39 0672594734.
E-mail address: crestini@uniroma2.it (C. Crestini).

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in combination with relatively low amounts of diethylene glycol opens a possibility of effective utilization of the glycerol obtained as a side product in the biodiesel production [18].

In the present work we report on the structural characterization of lignin-derived products in liquefied wood samples obtained under optimized conditions in the presence of a glycerol and diethylene glycol combination and *p*-toluene sulfonic acid monohydrate as a catalyst. A specific isolation protocol for liquefied wood was developed. The chemical characterization of isolated samples was carried out by quantitative ^{31}P, ^{13}C NMR analysis and by monitoring the change of molar masses using size-exclusion chromatography. Wood liquefaction pathway, especially lignin behaviour during the wood liquefaction, was studied by evaluating the dynamic change of aromatic and aliphatic hydroxyl group content with time as determined by ^{31}P NMR and the liquefying agent incorporation into the lignin molecule as confirmed by quantitative ^{13}C NMR. Additionally, the increase of molar masses with time is discussed.

2. Experimental

2.1. Materials

Milled-wood lignin (MWL) was isolated from the blend of spruce (*Picea* spp.) and fir (*Abies* spp.) according to the previously described procedures [19–21]. The spruce (*Picea* spp.) and fir (*Abies* spp.) sawdust were dried at 105 °C for 24 h. Glycerol (Merck), diethylene glycol (Merck), *p*-toluene sulfonic acid monohydrate (PTSA, Acros Organics), *N*,*N*-dimethylacetamide for HPLC (DMAc, Fluka), lithium bromide (LiBr, Acros Organics, dried before use and kept in desiccator), *N*-hydroxynaphthalimide (Acros Organics), chromium(III) acetylacetanoate (Acros Organics), 2-chloro-1,3,2-dioxaphospholane (Fluka), 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (Aldrich), 1,3,5-trioxane (Aldrich), deuterated chloroform ($CDCl_3$-*d*, Merck), deuterated dimethylsulfoxide (DMSO-d_6, Merck) were of reagent grade and were used without further purification.

2.2. Wood liquefaction

The respective polyhydroxy alcohol mixture of glycerol (144 g) and diethylene glycol (DEG) (36 g), dried spruce (*Picea* spp.) and fir (*Abies* spp.) sawdust (60 g) were placed into the 500 cm^3 glass reactor (three necks), equipped with a mechanical stirrer (IKA Labortechnik) and condenser. To catalyze the reaction 3% (w/w, based on the glycols) of *p*-toluene sulfonic acid monohydrate (PTSA) was added to the mixture. The particular glycerol and DEG combination (4:1) used as a liquefying agent was chosen after the initial experiments, with a goal to maximize the glycerol content as a by-product of biodiesel production. A certain proportion of DEG was added to provide a degree of viscosity reduction for the final product. The liquefaction mixture was heated for 4 h at 150 °C in silicon oil bath while being constantly stirred at 300 rpm. Samples from the reaction mixture were taken at different time intervals and immediately cooled in an ice-bath to quench the reaction.

2.3. Measurement of residual wood content

The yield of wood liquefaction was evaluated by determining the wood residue. Each sample was diluted with excess amount of acetone and was filtered using filter paper in order to isolate the wood residue. The filtered residue was rinsed repeatedly with acetone, 1,4-dioxane, water and pyridine in order to completely remove the soluble products. The samples were dried at 60 °C under reduced pressure for 24 h. The residue content was determined as the mass of the obtained solids relative to the starting amount of wood (Eq. (1)).

$$\mathrm{WR}\ (\%) = \frac{W^{t}_{\mathrm{WR}}}{W^{0}_{\mathrm{WR}}} \times 100 \tag{1}$$

Here, W^{0}_{WR} is the mass of starting wood and W^{t}_{WR} is the mass of wood residue in sample taken after *t* min of the liquefaction.

The soluble products were obtained by removing the acetone, 1,4-dioxane, water and pyridine from the filtrate under reduced pressure at 35–40 °C.

2.4. Liquefied wood separation

Fig. 1 illustrates the separation procedure of the soluble products in liquefied wood. A part of soluble products was precipitated from the excess of acidified distilled water. The precipitate was collected by centrifugation for 10 min at 4500 rpm, freeze-dried and assigned as a ‘lignin-based polymer’ (LBP). The supernatant was collected, neutralized with 0.1 N NaOH solution and concentrated under reduced pressure at 35–40 °C. The product was then added drop-wise into 96% ethanol. Obtained white precipitate was filtered using a filter paper and was identified as sodium chloride using infrared spectroscopy. The ethanol was removed by rotary evaporation and the sample was assigned as a ‘glycol sample’ (GS).

LBP content formed during the wood liquefaction was determined as the mass of the obtained solid powder relative to the soluble product amount taken for precipitation and was defined as follows (Eq. (2), Table 1):

$$\mathrm{LBP}\ (\%) = \frac{W^{t}_{\mathrm{LBP}}}{W^{t}_{\mathrm{SP}}} \times 100 \tag{2}$$

Here, W^{t}_{SP} is the mass of liquefied wood soluble products that was precipitated into acidified water to yield LBP assigned as W^{t}_{LBP} in Eq. (2). Thus, only freeze-dried LBP samples were used for the elucidation of structural changes in lignin.

2.5. Acetylation

Precipitated LBP samples were acetylated with pyridine/acetic anhydride (2:1, v/v) mixture for 8 h at 50 °C. After 8 h, pH of the solutions was adjusted to ∼3 with 0.1 N HCl and left at room temperature for 12 h with continuous stirring. The reaction mixtures were evaporated under reduced pressure, sequentially coevaporated with ethanol, toluene and chloroform until dryness.

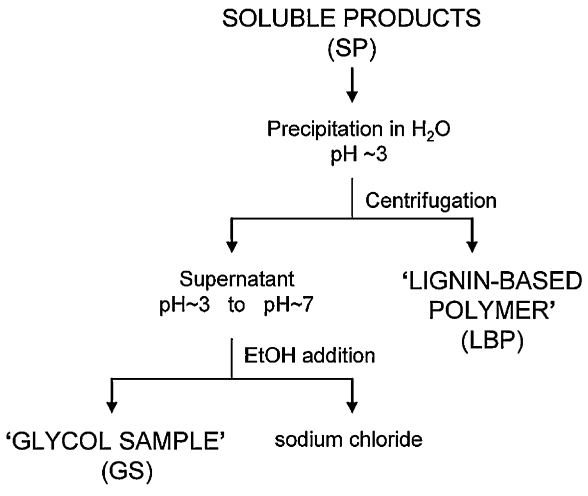


Fig. 1. Separation procedure for sample preparation.

Table 1
Mass (g) of fractions obtained during liquefied wood sample preparation scheme with time.

Time, min	LW, g	WR, g	SP, g[a]	LBP, g
30	39.11	3.74	10.02	0.60
60	30.43	1.91	10.36	0.97
90	29.40	1.53	10.18	1.17
120	27.76	1.14	10.11	1.25
150	26.49	0.80	12.10	1.51
180	31.13	0.76	10.04	1.56
210	29.96	0.79	11.15	1.94
240	23.27	0.29	10.00	1.87

[a] Amount of the sample precipitated into acidified water to yield LBP.

2.6. Quantitative ^{31}P NMR

Approximately 30 mg of LBP were transferred into a sample vial, dissolved in 400 μL of pyridine and deuterated chloroform (1.6:1, v/v). *N*-Hydroxynaphthalimide and chromium (III) acetylacetanoate were used as the internal standard and relaxation agent, respectively. 100 μL of the solution prepared from 0.1 mmol/mL of internal standard and 0.0143 mmol/mL of relaxation agent in the solvent system above, were added to sample vial. Finally, 100 μL of phosphitylating reagent I (2-chloro-1,3,2,-dioxaphospholane) or reagent II (2-chloro-4,4,5,5-tetramethyl-1,3,2-dixaphospholane) was added and the mixture was left at room temperature for 1.5 h with continuous stirring. The prepared sample solution was transferred into a 5 mm NMR tube. The spectra were acquired using a Bruker 300 MHz spectrometer equipped with a Quad probe dedicated to ^{31}P, ^{13}C, ^{19}F and ^{1}H acquisition. A sweep width of 10 000 Hz was observed, and spectra were accumulated with time delay of 25 s between pulses. A pulse width causing 90° flip angle was used. Line broadening of 4 Hz was used in processing spectra. All chemical shifts reported in this paper are relative to the reaction product of water with phosphitylating reagents (I or II) which have been observed to give sharp signals in pyridine/$CDCl_3$ at 121.1 and 132.2 ppm, respectively [22,23].

The ^{31}P NMR data reported in this effort are averages of three experiments. The maximum standard deviation of our results was 2×10^{-2} mmol/g, while the maximum standard error was 1×10^{-2} mmol/g.

2.7. Quantitative ^{13}C NMR

Approximately 80 mg of acetylated LBP and 0.8 mg of 1,3,5-trioxane used as internal standard were dissolved in 600 μL of deuterated chloroform ($CDCl_3$-*d*). Quantitative ^{13}C NMR spectra of acetylated LBP samples were recorded with a Bruker 300 MHz spectrometer equipped with a Quad probe dedicated to ^{31}P, ^{13}C, ^{19}F and ^{1}H acquisition at 308 K using an inverse gated decoupling pulse sequence. A sweep width of 10 000 Hz was observed, and spectra were accumulated with time delay of 12 s between pulses. A pulse width causing 90° flip angle was used. The chemical shifts were referred to the internal standard signal at 93.4 ppm. Line broadening of 4 Hz was applied to FIDs before Fourier transform. For each spectrum, typically about 12 000 scans were accumulated.

The quantitative ^{13}C NMR data reported in this work is a result of a single experiment, while the maximum standard error was calculated for each analysis separately and made ~10% of estimated value.

2.8. Size-exclusion chromatography

SEC of liquid wood samples was performed on a size-exclusion chromatographic system (HP-AGILENT system) equipped with a UV detector set at 280 nm. Analyses were carried out at 40 °C using 0.01 M DMAc/LiBr as eluent at a flow rate of 0.7 cm^3/min. Aliquots (100 μL) of each LBP sample dissolved in 0.01 M DMAc/LiBr (1 mg/mL) and GS (18 mg/mL), were injected into PLgel 5 μm MIXED D 7.5 mm × 300 mm and Polar Gel L 8 μm 7.5 mm× 300 mm, respectively. The SEC system was calibrated with polystyrene standards in the molecular weight range of 500–3.9×10^5 g/mol for LBP samples and in the range of 500–3.0×10^4 g/mol for GS. The chromatographic data were processed with PSS (Polymer Standards Service) WinGPC Unity software. The SEC data reported in this effort are averages of three experiments. Chromatograms depicted in Figs. 7a,b and 8a,b are normalized to the sample weight.

SEC characterization has been done on the basis of previously developed methods for lignin molecular weight determination [24]. The obtained SEC results were analogous to the ones reported elsewhere [19,25,26].

3. Results and discussion

3.1. Wood liquefaction

The initial decrease of residual wood content in liquefaction mixture with time, presented in Fig. 2, implies the conversion of approximately 60% of starting wood into the soluble products. Wood liquefaction in the presence of glycerol and diethylene glycol as liquefying agents and PTSA as a catalyst proceeds by the degradation of cellulose micro-fibrils and synchronous dissolution of accessible lignin. This process is followed by the reaction between lignin and glycols resulting the formation of condensed polymeric material assigned in this study as LBP.

The obtained amount of the precipitated material at the end of the wood liquefaction was approximately 20% of all liquefied wood sample after insoluble residue removal. This supported the hypothesis of a nearly complete lignin recovery. According to the literature [27], wood contains about a similar amount of lignin that could be isolated after the cellulose micro-fibrils have been degraded. The rapid amorphous cellulose degradation during the wood liquefaction [15] allowed dissolving accessible lignin already at the beginning of the liquefaction. Therefore, the highest amount of isolated lignin that during wood liquefaction reacts with the glycols present was obtained at the end of the treatment followed by the least amount of residual not solubilized wood.

It is estimated that the glycerol–DEG liquefied wood contains saccharides derived from cellulose, highly decomposed nonsaccharide substances, lignin derivatives and lignin degradation products,

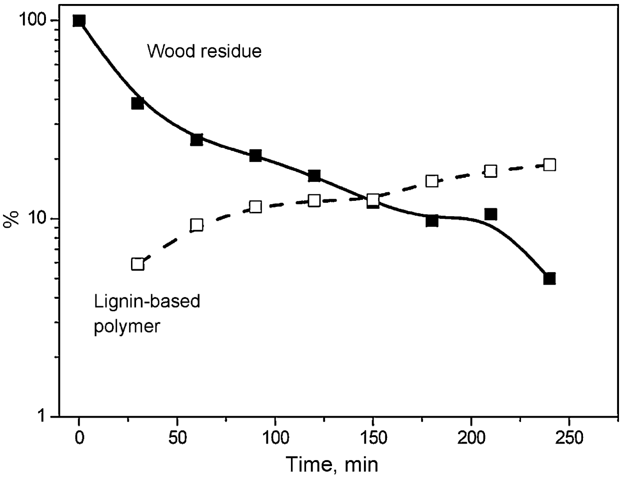


Fig. 2. The decrease of wood residue and the increase of 'lignin-based polymer' (LBP) amount in % with time.

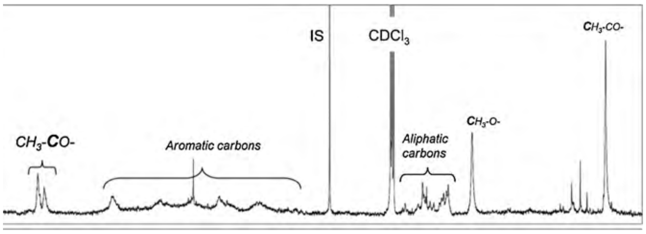


Fig. 3. Quantitative ^{13}C NMR spectrum of LBP sample taken after 30 min of wood liquefaction.

glycerol, DEG, polymerized glycerol and DEG, PTSA catalyst. Therefore, soluble products were fractionated according to scheme depicted in Fig. 1 in order to isolate and characterize lignin derivative (LBP) structure in liquefied wood. The saccharide rich fraction together with low-molecular weight lignin degradation products dissolved in glycerol and DEG mixture was assigned as 'Glycol Sample' (GS). Efforts on the characterization of cellulose liquefaction in acidified ethylene glycol as well as to study the formation of reducing sugars and their derivatives such as 2-hydroxyethyl-D-glucopyranoside were already published [14,15]. Thus, in this paper we report on the structural changes that undergo lignin during glycerol–DEG wood liquefaction.

3.2. NMR spectroscopy

The initial assumption about presumable isolation and modification of lignin was confirmed by ^{31}P and ^{13}C NMR analysis. The structural changes of precipitated lignin and possible incorporation of liquefying agents into the lignin backbone were examined by quantitative ^{31}P and ^{13}C NMR spectroscopy. It should be noted that ^{13}C NMR did not show any signals relative to the cellulose and hemicellulose, thus there was no formation of lignin–carbohydrate complexes that could be determined in the precipitated liquid wood sample [28,29]. The typical lignin spectra obtained by ^{31}P and ^{13}C NMR analysis indicate the complete lignin isolation from liquefied cellulose products (Figs. 3 and 4). Therefore, the precipitated liquid wood sample was referred to the term of 'lignin-based polymer' and will be used in the further discussion.

3.3. Quantitative ^{31}P NMR spectroscopy

The change of primary and secondary aliphatic hydroxyl groups content during the wood liquefaction was evaluated by ^{31}P NMR from the samples phosphitylated with 2-chloro-1,3,2-dioxaphospholane.

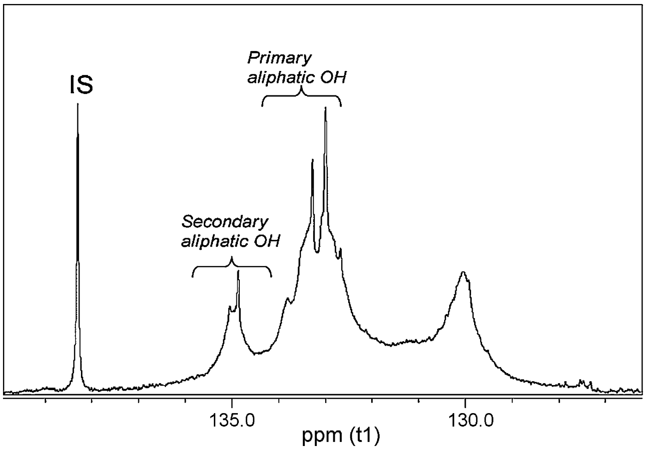


Fig. 4. Quantitative ^{31}P NMR spectrum of LBP sample taken after 30 min of wood liquefaction phosphitylated with 2-chloro-1,3,2-dioxaphospholane.

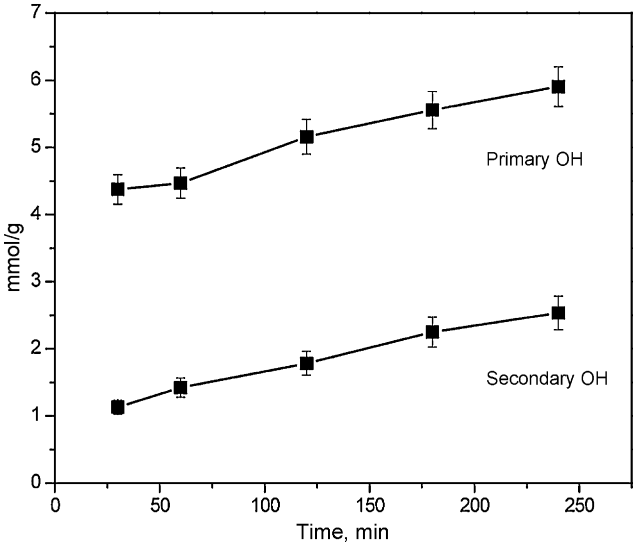
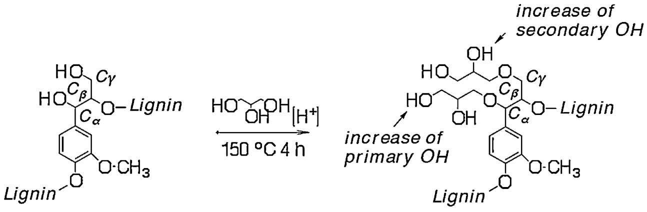


Fig. 5. Primary and secondary aliphatic OH group content in LBP samples with time as evaluated from ^{31}P NMR.

The primary and secondary aliphatic OH content in the precipitated samples was evaluated by integrating the signals in the range of 134.0–132.0 and 136.2–134.0 ppm, respectively (Fig. 4) [29]. The content of both, primary and secondary aliphatic OH increased with time from 4.38 and 1.13 mmol/g to 5.91 and 2.53 mmol/g, respectively, indicating the formation of lignin-based polymer, followed by the introduction of the glycol chains into the lignin structure (Fig. 5).

The incorporation of glycerol and/or diethylene glycol into the lignin network provided new primary and secondary OH groups. From the results obtained the assumption could be made that the introduction of glycerol and diethylene glycol proceeds at Cα and Cγ positions of phenyl propane units as presented in Scheme 1. The introduction of glycerol molecule at Cα position provides one primary and one secondary OH group, resulting in the increased amount of primary OH groups and leaving the amount of secondary OH groups unchanged. The increase of secondary OH group content could be achieved in case of glycerol reaction at Cγ position.

The introduction of additional hydroxyl groups from glycols consequently increased the final product solubility. Moreover, the formation of highly polymerized not soluble residue induced by the interactions between depolymerized cellulose products and the lignin during the liquefaction catalyzed by H_2SO_4 [6] was suppressed by the use of the optimal PTSA concentration. In our recent study, PTSA was found to be strong enough to degrade cellulose micro-fibrils during the liquefaction [15]. Additionally, due to the lower hydronium ion amount obtained after PTSA dissociation compared to the one provided by H_2SO_4, the typical



Scheme 1. A tentative lignin reaction with glycerol during wood liquefaction with PTSA as a catalyst based on carbon assignments.

Table 2
The content (mmol/g) of phenolic and carboxylic hydroxyl groups in LBP samples with time determined by quantitative ^{31}P NMR measurements.

Time, min	Condensed phenolic OH	Guaiacyl phenolic OH	COOH
30	1.79	1.55	0.02
60	1.48	1.20	0.06
120	1.16	0.85	0.05
180	1.36	0.60	0.10
240	1.11	0.48	0.20

Table 3
Content (mmol/g) of aromatic and methoxy units, aliphatic carbons and carbonyl groups with time as determined from quantitative ^{13}C NMR measurements.

Time, min	Aliphatic carbons	Aromatic units	Methoxy units	Carbonyl groups
MWL	1.6 ± 0.02	4.2 ± 0.04	3.2 ± 0.04	0.0 ± 0.00
30	5.0 ± 0.05	3.9 ± 0.04	3.1 ± 0.03	0.0 ± 0.00
120	6.3 ± 0.06	3.2 ± 0.03	1.7 ± 0.02	0.9 ± 0.01
180	7.4 ± 0.07	2.8 ± 0.03	1.5 ± 0.02	1.2 ± 0.01
240	7.6 ± 0.08	2.6 ± 0.03	1.0 ± 0.01	1.2 ± 0.01

intensive lignin self-polymerization in acidic medium [30] was not induced.

The change of condensed phenolic OH, guaiacyl phenolic OH and carboxylic groups content during the wood liquefaction was evaluated by ^{31}P NMR from LBP samples after phosphitylation with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane [31]. The amounts of condensed phenolic OH, guaiacyl phenolic OH and carboxylic acids were evaluated by integrating the signals in the ranges of 144.3–137.5, 140.0–138.6 and 135.5–134.0 ppm, respectively.

The overall decrease of the condensed phenolic OH and guaiacyl phenolic OH group content with time could be attributed to the incorporation of the aliphatic liquefying agents that leads to the formation of the lignin-based polymer as it was later on confirmed by SEC (Table 2, Fig. 7). In addition, the phenolic OH group decrease may be interpreted in terms of lignin self-polymerization via phenolic OH condensation, while the more pronounced increase of aliphatic OH than the phenolic OH decrease could be a result of simultaneous substitution by glycerol or DEG.

The increase of the carboxylic OH group content in 0.18 mmol/g during the wood liquefaction might be addressed to the hydrolysis of the ester linkages present in the LBP sample. The hydrolysis presumably was initiated by water present in initial wood or additionally provided by *p*-toluene sulfonic acid monohydrate used as a catalyst and by water molecules obtained as a side product after the lignin OH groups have been etherified.

3.4. Quantitative ^{13}C NMR spectroscopy

Quantitative ^{13}C NMR analysis was used to study the incorporation of aliphatic chains into the lignin backbone. This reaction is considered to be the cause for the enhanced solubility of liquefied wood.

The aliphatic carbon region of LBP spectra (59.5–90 ppm) can include signals for Cα, Cβ and Cγ in β-O-4 structures. In all spectra obtained in this work, only Cγ signal appears indicating preferential cleavage of α-aryl and β-aryl ether bonds. However, Cγ signal at 63.4 ppm is highly overlapped with the signals (in the range from 59.5 to 69.6 ppm) corresponding to the etherified glycerol and DEG (Fig. 6).

Thus, the overall amount of aliphatic carbons in LBP samples was evaluated by integrating signals in the range of 69.6–59.5 ppm. The increase of aliphatic carbon content with time from 1.6 to 7.6 mmol/g indicates the intensive glycerol and DEG aliphatic chain introduction into the lignin structure (Table 3).

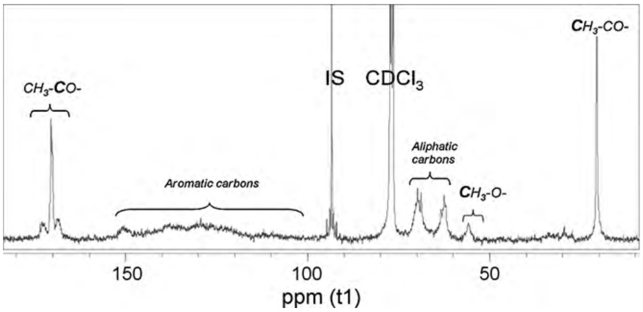


Fig. 6. Quantitative ^{13}C NMR spectrum of LBP sample taken after 240 min of wood liquefaction.

The appearance and the increase of the additional carbonyl group signals with time at 174.5–171.2 ppm were evaluated by subtracting the integral that corresponds to methyl groups of the acetylated aromatic and aliphatic OH at 23.2–16.5 ppm from 174.5 to 171.5 ppm integral that corresponds to the all carbonyl groups. The appearance of the additional carbonyl group signals during the wood liquefaction could be interpreted in terms of the aliphatic glycerol chain oxidation in the formed LBP samples. The induced oxidation of incorporated aliphatic side chains with time could be confirmed by the increase of additional carbonyl group amount that appears to follow the same trend as the aliphatic carbons.

The amount of aromatic units was determined by integrating the signals in the range from 99.8 to 154.8 ppm and was found decreased from 4.1 to 2.6 mmol/g. This is caused by lignin functionalization by glycerol and DEG. The progressive introduction of glycerol and DEG moieties inside the lignin structure severely affects its overall composition, lowering the relative amount of aromatic units.

The methoxy group content evaluated by the integration of the signal at 55.6 ppm was determined to decrease from 3.5 to 1.0 mmol/g over the 4 h of treatment. The decrease in intensity of the signal corresponding to the methoxy groups in LBP samples is more pronounced than the aromatic units decrease. This implies, besides an overall severe modification of the lignin structure, the possible occurrence of demethylation reactions of guaiacyl units within the lignin macromolecule during wood liquefaction.

3.5. SEC analysis

The effects of the liquefaction on lignin structure were elucidated by the evaluation of changes in molecular weight distribution (MWD) of the isolated LBP and GS samples using size-exclusion chromatography (SEC). Samples for SEC analysis were prepared by separating the low-molecular weight lignin derivatives according to the sample preparation scheme shown in Fig. 1. Due to the polarity and the differences of prepared liquid GS and freeze-dried LBP samples the SEC analysis were carried out using two different chromatographic systems as reported in Section 2. It should be noted that with the use of UV detector set at 280 nm, the detection of low-molecular weight aliphatic compounds as the liquefying agents in GS was prevented and the obtained chromatograms shown in Fig. 7a and b correspond only to low-molecular weight aromatic derivatives.

The acetylated MWL sample with average molecular weight ($\bar{M}_w$) of 8800 g/mol was used as a reference material for obtained LBP samples. Additionally, it should be noted that $\bar{M}_w$ of the LBP sample taken from the wood liquefaction mixture after 30 min was determined to be about 49 000 g/mol that is very close to the $\bar{M}_w$ of the enzymatic mild acidolysis lignin (EMAL) as reported in the literature [19]. Accordingly, this observation implies about

Table 4
Weight-average molecular weight ($\bar{M}_w$), number-average molecular weight ($\bar{M}_n$) and polydispersity index (PDI) of MWL (used as a reference), LBP and GS samples with time of reaction.

Time, min	Sample	$\bar{M}_w$, g/mol	$\bar{M}_n$, g/mol	PDI	Sample	$\bar{M}_w$, g/mol	$\bar{M}_n$, g/mol	PDI
0	MWL	8800	6520	1.4	–	–	–	–
30	LBP-30	49 150	5470	9.0	GS-30	590	200	3.0
60	LBP-60	75 720	5900	12.8	GS-60	625	82	7.7
90	LBP-90	115 920	7970	14.5	GS-90	625	102	6.2
120	LBP-120	97 340	8000	12.2	GS-120	670	195	3.4
180	LBP-180	87 770	7460	11.8	GS-180	735	78	9.5
210	LBP-210	79 770	7450	10.7	GS-210	1090	92	11.8
240	LBP-240	136 500	8380	16.3	GS-240	980	235	4.2

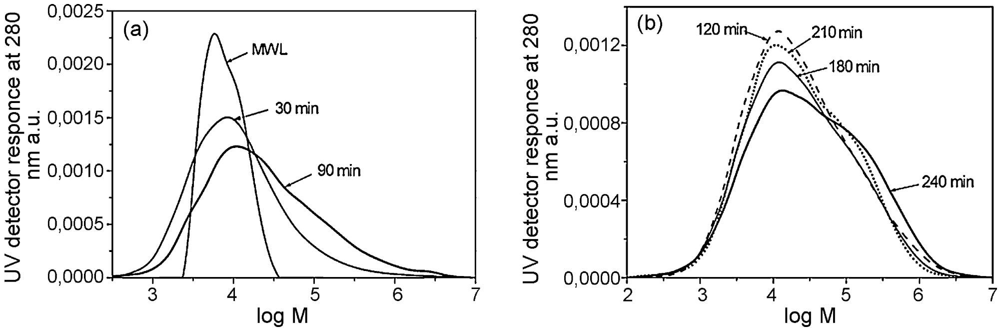


Fig. 7. SEC chromatograms of MWL and LBP with time of reaction.

synchronous acidic degradation of cellulose micro-fibrils and dissolution of accessible lignin during the initial 30 min of treatment. Despite the unimodal molecular weight distribution curve, high polydispersity index of 9.0 was obtained (Table 4). The high polydispersity indices are especially significant for lignin and lignin derivatives as it was reported by Argyropoulos and Menachem [32]. Thus, the data obtained from SEC analysis are in good agreement with the NMR characterization about the primary lignin isolation during the wood liquefaction process.

The extremely intensive growth of $\bar{M}_w$ was observed from 30 to 90 min of the liquefaction. Besides the predominant functionalization of the dissolved lignin by glycerol and DEG at this time interval, the additional lignin self-polymerization reactions under acidic conditions [30], form a highly polydispersed product. The reduced aromatic region absorbance at 280 nm of the LBP chromatograms shown in Fig. 7a confirms the formation of the condensed structures induced by the incorporation of the aliphatic glycerol and DEG moieties inside the lignin structure. Furthermore, the appearance of a high-molecular weight shoulder during the initial 90 min of treatment consequently increases $\bar{M}_w$ to 115 920 g/mol and polydispersity index to 14.5. The polymerization of the complex tridimensional biopolymer with bi-functional and tri-functional monomers as glycerol and DEG formed highly branched LBP macromolecules with broad MWD as it is evident from the PDI values listed in Table 4. The gradual PDI increase during initial 90 min of the reaction is obtained due to the presence of reactants with different functional group reactivity. The intensive decrease in functional group content, and the induced incorporation of aliphatic chains into the lignin structure determined by quantitative ^{31}P and ^{13}C NMR analysis implies that the formation of the highly polydispersed LBP during the initial 90 min of the liquefaction is a result of condensation reactions between highly reactive phenolic OH, secondary aliphatic OH at Cα position in lignin phenyl propane units and excess of less reactive aliphatic OH provided by glycerol and DEG.

Here, low-molecular weight lignin-derived compounds that appeared in GS after 30 min of the liquefaction undergo initial degradation as it is proposed by $\bar{M}_n$ decrease from 200 to 82 g/mol and simultaneous PDI increase from 3.0 to 7.7 (Table 4). The constant growth of $\bar{M}_w$ in GS samples with time could be interpreted in terms of non-etherified syringyl and guaiacyl unit condensation with glycerol and DEG. In addition, the reduced aromatic region absorbance at 280 nm of GS chromatograms with time (Fig. 8a) might be a result of both—simultaneous incorporation of low-molecular weight fragments into intensive LBP formation at the beginning of the liquefaction and the formation of

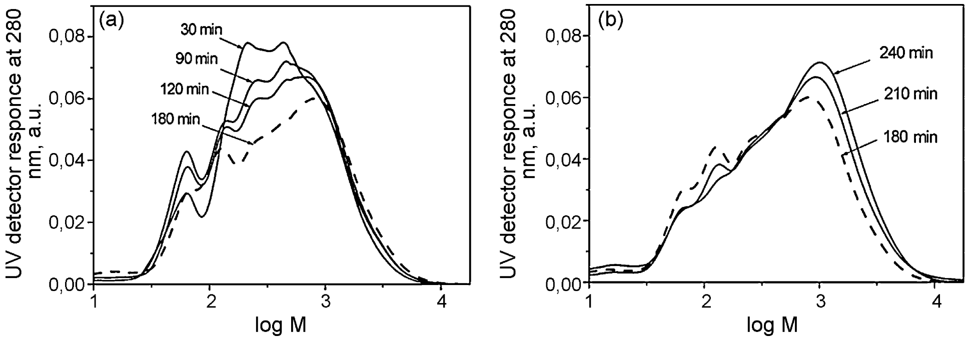


Fig. 8. SEC chromatograms of GS with time of reaction.

the larger lignin-derived structures induced by glycerol and DEG chain incorporation.

After 90 min of the liquefaction, the continuous gradual change in functional group content as well as less pronounced incorporation of the aliphatic carbons determined by quantitative ^{31}P and ^{13}C NMR analysis, implies the presence of condensation reactions involving functional groups with lower reactivity. Therefore, according to quantitative NMR analysis, LBP formation after 90 min of the treatment is based on further glycerol and DEG incorporation into LBP by condensation with less reactive primary aliphatic OH groups at Cγ position in lignin phenyl propane units and/or obtained after glycerol and DEG incorporation (Scheme 1). However, the manner of PDI alternation with reaction time indicated the occurrence of concurrent condensation and polymerization processes. The formation of LBP approached the transitional state at 120 min of the reaction where the equilibrium between condensation and degradation reactions was emphasized by decrease in $\bar{M}_w$ and followed by synchronous increase in $\bar{M}_n$.

After 120 min of the liquefaction, the newly formed large LBP macromolecules undergo degradation, followed by the formation of soluble, low-molecular weight structures. Additionally, the partial degradation of large LBP macromolecules during prolonged times, resulted in loss of high-molecular weight shoulder reducing PDI from 14.5 to 12.2 and $\bar{M}_w$ from 115 900 to 97 300 g/mol. However, the slight increase in $\bar{M}_n$ from 7970 to 8000 g/mol during the same time interval (from 90 to 120 min), verifies the continuation of condensation reactions.

The observed slight PDI decrease from LBP-120 to LBP-210 could indicate both, further LBP degradation and/or condensation reactions. According to the polymerization principles, PDI narrows when functional group reactivity decreases with molecular size [33]. Thus, in this case, narrower MWD were obtained due to the condensation of less reactive primary aliphatic OH groups in large LBP-120 molecule with glycerol and DEG. Moreover, due to the synchronous LBP degradation obtained low-molecular weight LBP fragments in GS-180 and GS-210 consequently enlarged $\bar{M}_w$ from 735 to 1090 g/mol and PDI from 9.5 to 11.8 (Table 4). Additionally, low-molecular weight LBP fragments in GS due to condensation reactions caused gradual $\bar{M}_n$ increase from 78 to 235 g/mol. The presence of the condensation reactions is also evident from GS chromatograms obtained after 180 min of the reaction time (Fig. 8b). The shift of the main peak to the higher molecular weight followed by induced aromatic region absorbance at 280 nm, revealed the formation of larger molecules that, in contrast to the beginning of the liquefaction, involved the incorporation of aromatic moieties, presumably obtained after LBP degradation.

However, the appearance of additional shoulder at higher molecular weights in the chromatogram profile of the LBP-210 (Fig. 7b) shows a tendency towards larger macromolecular structures formation, while the induced intensity of the main peak implies the presence of increased amount of LBP fragments that were eventually susceptible to degradation.

The formation of high-molecular weight products at the end of the wood liquefaction is evident from the increased $\bar{M}_w$ and $\bar{M}_n$ of LBP taken after 240 min of the reaction. The reduced aromatic region absorbance at 280 nm of LBP-240 chromatogram in Fig. 7b and the appearance of the enlarged shoulder in the high-molecular weight region confirmed the repetitive occurrence of the polymerization reactions followed by the progressive introduction of the glycerol and DEG moieties. In contrast to the LBP-240 min, condensed low-molecular weight compounds in GS-240 confirmed by increased $\bar{M}_n$ (from 92 to 235 g/mol) exhibited induced aromatic region absorbance at 280 nm (Fig. 8b) due to more aromatic sample character. Furthermore, from the simultaneously increased PDI of LBP-240 to 16.3 followed by significantly reduced PDI of GS-240 to 4.2, it is evident that the final LBP structure is enriched by condensed low-molecular weight lignin derivatives composed in GS during the prolonged wood liquefaction.

4. Conclusion

Wood sawdust was liquefied at 150° C in the presence of glycerol and diethylene glycol and under catalysis of *p*-toluene sulfonic acid monohydrate. The use of the *p*-toluene sulfonic acid monohydrate as a catalyst enabled to increase the content of wood in liquefaction mixture and to reduce self-polymerization reactions of lignin, consequently preventing the formation of the undesirable insoluble residue over 4 h of wood liquefaction.

Liquefied wood samples were prepared by novel sample separation procedure. They were characterized by quantitative ^{31}P, ^{13}C NMR and by monitoring the changes of molar masses using size-exclusion chromatography. Precipitated initial liquid wood sample (LBP-30), showed typical lignin spectrum as was confirmed by NMR spectroscopy. The introduction of glycerol and diethylene glycol into the lignin at Cα and Cγ positions resulted in raise of primary and secondary hydroxyl group content, respectively, as was confirmed by quantitative ^{31}P NMR. The increasing content of aliphatic chains and consequently decreasing aromatic carbon content in samples with time as was determined by quantitative ^{13}C NMR supported the conclusion that the glycerol and diethylene glycol incorporation in to precipitated lignin samples is followed by the formation of lignin-based polymer.

The formation of highly branched lignin-based polymer with broad molecular weight distribution during the wood liquefaction was determined by size-exclusion chromatography. Final lignin-based polymer was produced by predominant condensation reactions between the aromatic lignin sub-units as indicated by the decrease in total phenolic hydroxyl group content followed by the incorporation of glycerol and diethylene glycol moieties.

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