

Exploring chemical reactions to enhance thermal and dispersion stability of kraft and organosolv lignin

Nagore Izaguirre^a, Xabier Erdocia^b, Jalel Labidi^{a,*}

^a Biorefinery Processes Research Group, Chemical and Environmental Engineering Department, Engineering Faculty of Gipuzkoa, University of the Basque Country UPV/EHU, Plaza Europa 1, 20018 Donostia, Spain

^b Biorefinery Processes Research Group, Department of Applied Mathematics, University of the Basque Country UPV/EHU, Rafael Moreno "Pichichi" 3, 48013 Bilbao, Spain

ARTICLE INFO

Keywords:

Kraft lignin
Organosolv lignin
Carboxymethylation sulfomethylation
Methylation

ABSTRACT

Lignin has been overlooked and used as a waste for long due to its complex and partially hydrophobic structure. Many efforts have been carried out to overcome these deficiencies and apply it as a high-value product, which are insufficient to reach the full potential of lignin in various advanced applications, since they require with procedures for the obtaining of more specific and fine-tuned chemical structures. This work focuses on the obtaining of differently structured hydrophilic lignins derived both from Kraft and organosolv isolation processes. The chemical structures of the different lignin types were studied, and the effect of the structural differences in the modification processes and their subsequent properties analyzed, valorizing their potential application for diverse purposes. The carboxymethylation and sulfomethylation reactions were carried out with the aim of enhancing the polarity of the lignin samples, while the methylation reaction aimed to obtain lignins with higher stability. The physicochemical analyses of the samples, carried out by FTIR, GPC, ³¹P NMR, ¹³C NMR, and HSQC NMR, verified the effectiveness of the chemical reactions and conditions selected, obtaining lignins with lower hydroxyl content, due to their substitution and insertion of carboxymethyl, sulfomethyl and methyl groups, therefore obtaining more condensed, aromatic and oxygenated aromatic carbon structures. While the methylation reaction was the most efficient in substituting the OH groups, due to its non-selectivity, OL showed higher modification yields than KL. In terms of the thermal and morphological properties, analyzed by DLS and TGA respectively, it was observed that the modified samples showed lower Z potential values, along with higher conductivity, being the sulfomethylated organosolv lignin the one showing the best results, which was also the one with the smallest particle size and polydispersity index. Finally, all the modified samples showed higher T_{50%} values, suggesting a better stability towards degradation.

1. Introduction

Lignin is one of the main components of lignocellulosic materials, along with cellulose and hemicellulose, and its complex chemistry is designed to give structural rigidity and protection against pathogens of the plant species [1]. Its high heterogeneity and non-organized formation, where three different phenolic monomers primarily cross-link through carbon-carbon and ether bonds, entail the uncertainty of the chemical structure of the lignin obtained, highly dependent on the raw material, the environmental conditions in which they have been grown, and lignin extraction method employed, for instance [2]. This is the main reason why it has been considered as waste and its implementation

as a value-added product for numerous applications has been neglected, albeit its interesting chemistry and great potential to substitute the currently used petroleum-based chemicals and materials [3].

There are numerous lignin extraction methods, some of the most employed being the Kraft pulping process, the sulfite process, and the organosolv process. The Kraft method is the most widely employed in the pulp and paper industry, with over 90 % of the chemical pulp produced by this process due to its highly optimized conditions [4]. The lignin can be isolated through acid precipitation. Nevertheless, it is predominantly incinerated to fulfill the energy requirements of the pulping process, while the chemicals employed are recovered [5]. The second most important pulping process is the sulfite process, from which

* Corresponding author.

E-mail address: jalel.labidi@ehu.es (J. Labidi).

<https://doi.org/10.1016/j.ijbiomac.2024.130518>

Received 6 November 2023; Received in revised form 22 February 2024; Accepted 27 February 2024

Available online 28 February 2024

0141-8130/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

the lignosulfonate type of lignin is obtained. This chemical characteristic makes this type of lignin interesting for its further implementation, due to its water solubility [6]. They have been widely employed as commodity products [7] and more recently, as advanced specialty products [8], paving the way for the incorporation of even energy storage materials [9]. Nevertheless, its outdated pulping process is leading to a decrease in production [10], favoring other extraction methods like the organosolv pulping process, considered key in future multiproduct and integral biorefineries, due to its sustainable production process and high yield in numerous lignocellulosic components [11].

Processing Kraft and organosolv lignins is more complex compared to the lignosulfonates, primarily due to their partial hydrophobic nature towards water [12]. Even though hydrophilic functional groups are present in lignin particles, these are often hindered due to the complex folded structure. Therefore, the introduction of various functional groups into the molecules is an interesting path for its further dissolution in polar solvents like water [13].

Several modifications have been suggested for the synthesis of lignin with tuned properties, for the obtaining of materials with enhanced hydrophilicity, which eases the processability and optimizes the performance of the material for several applications [14–18].

The carboxymethylation reaction is a widely employed modification path to obtain water-soluble particles. It has extensively been used with cellulose, whose high crystallization degree makes its solubilization in water difficult. The obtained carboxymethyl cellulose (CMC) can be applied in industries like food packaging [19], biomedical [20], pharmaceutical [21], and textile [22], along with other products like cleaning supplies and personal care products [21]. Its water solubility and viscosity make it interesting to apply as a thickener, stabilizer, and viscosity modifier of foods and personal care products like toothpaste and lotions, and as a binder in pharmaceutical tablet preparation and in paperboard and boxes for paper and packaging materials [22].

More recently, a similar strategy has been applied to other biomaterials like chitosan, where carboxymethyl chitosan (CMCS) has been considered a promising material in cosmetics [23], drug delivery systems [24], wastewater treatments, food preservation, and membrane technology. Carboxymethylated lignin (CML), on the other hand, is still under research but shows great potential to be applied as a dispersant for clay suspension [25,26], flocculant [27], and afterglow material with excellent phosphorescence [28], a hybrid modifier to improve physical and mechanical properties of fast-growing wood [29], and adsorption for heavy metal ions [30]. Moreover, it is expected to also be potentially implemented in similar advanced application as the CMC, such as the pharmaceutical or energy storage material.

Another common lignin modification for its further water solubility relies on mimicking the functionalization obtained from the sulfate pulping process into other types of lignin like Kraft and organosolv. Sulfomethylation (SM) reaction is considered a sustainable procedure since no hazardous reactions are needed, and properties like the previously mentioned water solubility, surface activity (enhancing the water dispersibility), ionic properties (being able to ionize in water), and compatibility with polymers are brought to the lignin. These properties make this modification attractive for applications like dispersants and emulsifiers for several formulations, being the main target in many recently published works. For instance, different molecular weight lignins were studied to find the optimal dispersibility of TiO₂ [31], and different sulfonic group contents were obtained for the study of the formulation for dye dispersion [16]. They have also been applied as dispersants for kaolin [32] and emulsifiers for stabilizing oil-in-water nanoemulsions [18]. Enzymatically hydrolyzed lignin (EHL) [17] was also employed for these types of emulsions, as for the employment as dispersants for graphite [33]. Lastly, sulfomethylated lignin (SML) was grafted with other compounds like pyrrolidone or polyacrylic acid as a dispersant for cobalt blue [34] or as hydrogels in the removal of heavy metal ions in wastewater treatments [35], respectively.

Another modification quite commonly employed is the alkylation of

the lignin hydroxyl groups, in this case, to obtain more stable molecules. The reactivity and functionality can be quite easily modulated by different alkylation reactions, also bringing thermal stability that prevents self-polymerization reactions [36]. It is a way of blocking the hydroxyl groups present in lignin particles, enabling the study of the effect of these OH groups in processes like the formation of lignin nanoparticles, for instance. Blocking these specific functional groups, along with determining the methylation degree, the interaction created between the hydroxyl groups of lignin and the medium can be regulated, consequently controlling the nanoparticle size obtained [37]. This modification, apart from being interesting for the obtaining of nanolignins, the hydrophobic skeleton of methylated lignin (ML) has also been proven as an effective dispersant for carbon nanotubes [38].

Therefore, this work is focused on obtaining different modified lignins by carboxymethylation, sulfomethylation, and methylation reactions, both from technical Kraft lignin (KL) and lab-scale organosolv lignin (OL), and determining their properties by different physicochemical, chemical, thermal, and morphological analyses. Fourier Transformed Infrared (FTIR) spectroscopy, Gel Permeation Chromatography (GPC), ¹³C Nuclear Magnetic Resonance (NMR) spectroscopy, Heteronuclear Single Quantum Coherence Nuclear Magnetic Resonance (HSQC NMR) spectroscopy, Dynamic Light Scattering (DLS), and Thermogravimetric Analysis (TGA) were carried out to determine the effectiveness of the reactions and changes in the properties compared to the initial KL and OL and between the different modification reactions. It could be observed that the obtained modified lignins had more condensed structures, especially in the case of carboxymethylation and sulfomethylation, with less OH content. This resulted in more thermally stable products and higher water-solubility, with better overall processability for a wide variety of applications ranging from dispersants to water-based battery electrode materials.

2. Materials and methods

2.1. Materials

Eucalyptus chips and Kraft liquor were supplied by the local paper and pulp industry, Papelera Zikuñaga (Hernani, Gipuzkoa). Kraft lignin (KL) was obtained by the acid precipitation using H₂SO₄ (7664–93–9, 96 % technical grade, PanReac), and organosolv lignin (OL) was extracted following the method described in a previous work, along with the small (S) and big (B) lignin fractions obtained by the sequential organic solvent extraction method, expressed as SKL (Small Kraft Lignin) and BKL (Big Kraft Lignin) for Kraft lignin fractions, and SOL (Small Organosolv Lignin) and BOL (Big Organosolv Lignin) for organosolv lignin fractions. The pulping process carried out by the Kraft method use high pH conditions to separate lignin from cellulose, generated by the addition of sodium hydroxide and sulfides are used. Moreover, temperatures ranging from 150 to 180 °C for 2 h are usually applied [39]. On the other hand, organosolv lignin was performed at 180 °C for 90 min, with an ethanol/H₂O 50/50 (v/v) solvent with oxalic acid as catalyst. The black liquor obtained from the industrial Kraft pulping, the organosolv liquor obtained from the organosolv pulping, and the eucalyptus chips from which both liquors were derived were characterized in a previous work. Briefly, the eucalyptus chips had a 20 % of lignin content, 36.9 % of cellulose content, and 37.3 % of hemicellulose content. The Kraft liquor had a density of 1.09 ± 0.01 g/cm³, pH of 13.02 ± 0.03, total solid content of 15.49 ± 2.94 %, organic content of 8.55 ± 0.82 %, inorganic content of 8.74 ± 0.00 %, and dry lignin content of 41.67 ± 5.65 %. Alternatively, the organosolv liquor had a density of 0.95 ± 0.01 g/cm³, pH of 4.02 ± 0.08, total solid content of 5.57 ± 0.21 %, organic content of 5.54 ± 1.17 %, inorganic content of 0.04 ± 0.15 %, and dry lignin content of 29.11 ± 2.92 %. The Kraft lignin was precipitated from the liquor by slowly adding concentrated H₂SO₄ (96 %) dropwise under the fumehood to avoid any inhalation of the toxic H₂S gas liberated in the process. In the case of organosolv lignin

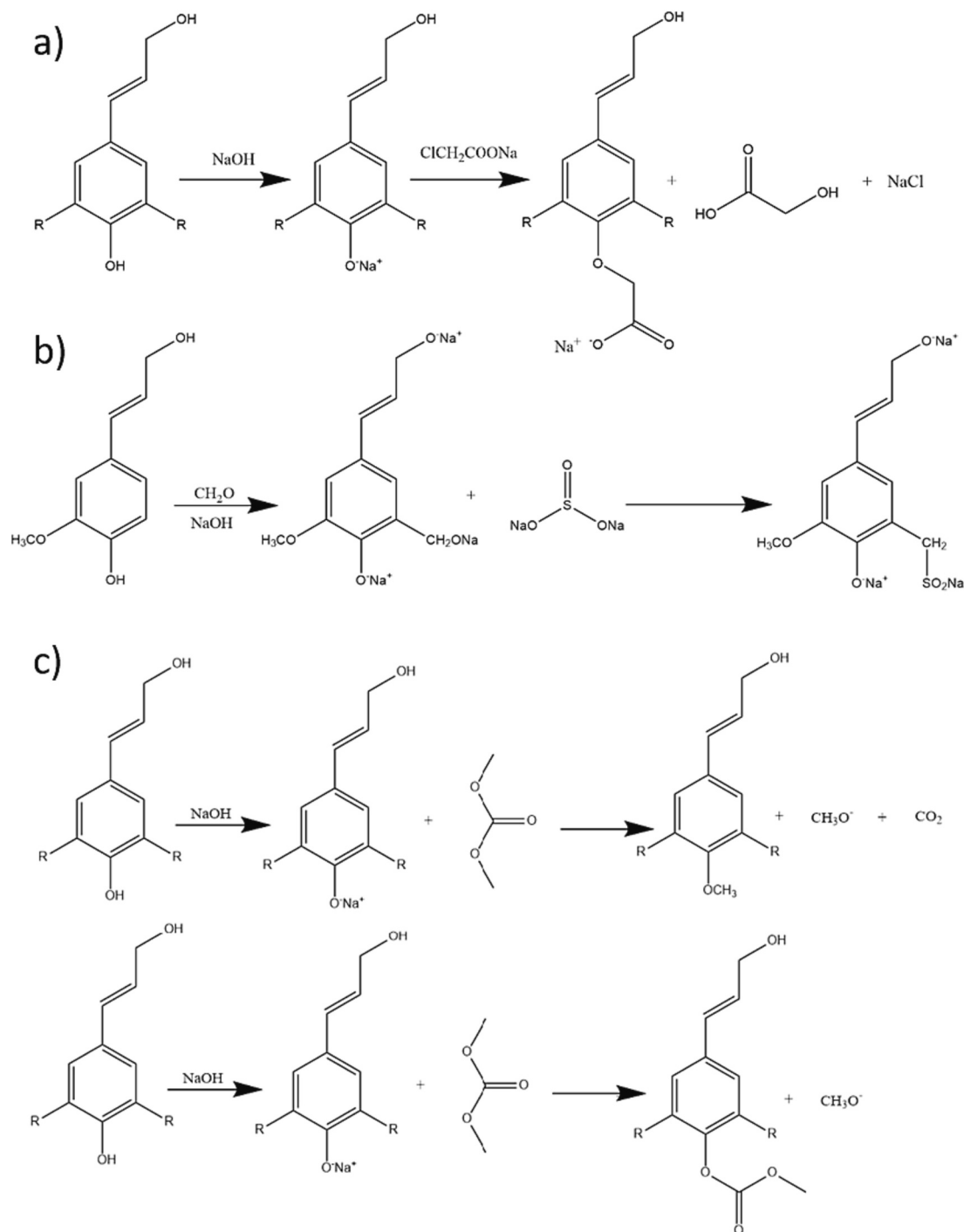


Fig. 1. Reaction mechanisms of the modifications: a) carboxymethylation; b) sulfomethylation; and c) methylation.

precipitation, an acidified water solution prepared by adding H₂SO₄ until pH 2 was added to the liquor [40]. NaOH (1310-73-2, for Analysis, CS, ISO, PanReac), sodium chloroacetate (SCA) (3926-62-3, for synthesis, Sigma), formaldehyde (37 %, 50-00-0, Panreac), sodium sulfate (Na₂SO₄) (7757-82-6, Sigma), dimethyl sulfoxide (DMSO) (ACS, 67-68-5, PanReac), dimethyl carbonate (DMC) (99 %, 616-38-6, Alfa Aesar), and hydrochloric acid (37 %, 7647-01-0, PanReac) were used as reactive in the reactions. *N,N*-Dimethylformamide (DMF, 68-12-2, 99.5 %, HPLC grade, Fisher), and lithium bromide (LiBr) (7550-35-8, PanReac) were used for the GPC. Pyridine (Py, 613-002-00-7, anhydrous, 99.8 %, Sigma-Aldrich), deuterated chloroform (CDCl₃, 865-49-6, "100 %", 99.96 atom % D, Aldrich), Chromium(III) acetylacetonate (Cr(acac)₃,

21679-31-2, 97 %, Aldrich), *endo*-*N*-hydroxy-5-norbornene-2,3-dicarboximide (NHND, 21715-90-2, 97 %, Aldrich) and 2-Chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP, 14812-59-0, 95 %, Sigma-Aldrich) were used for the ³¹P NMR. DMSO-*d*₆ (99.5 %, 2206-27-1, Aldrich) was used as a solvent for ¹³C NMR and HSQC NMR analysis.

2.2. Carboxymethylation reaction

The carboxymethylation reaction conditions followed were the optimum addressed from the work by Konduri et al. [25]. 1 g of KL and OL were dissolved in 60 mL of 0.15 M NaOH. 3 mol SCA per lignin mol was added, and the reaction was carried out in a 100 mL round bottom flask,

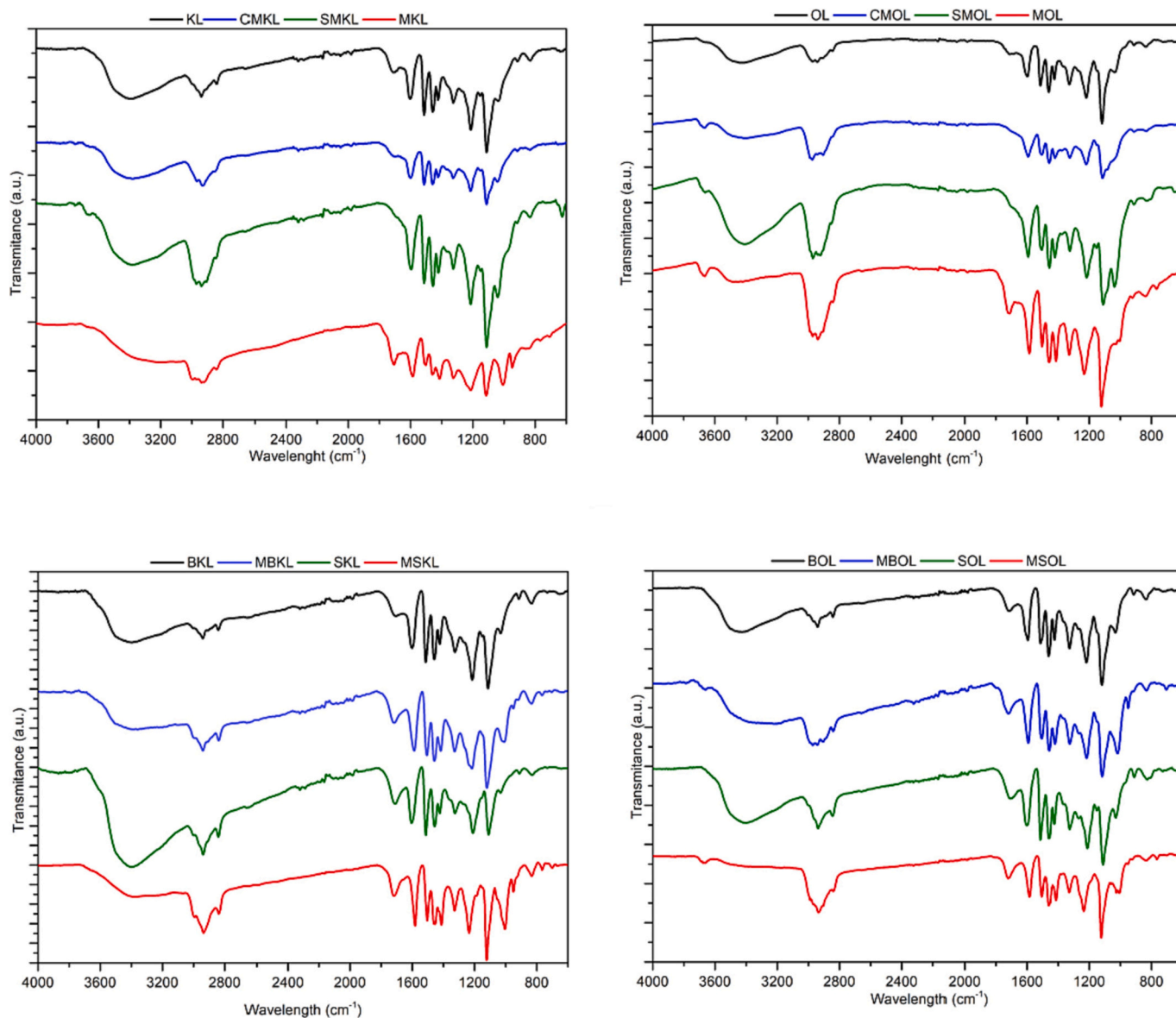


Fig. 2. FTIR spectra of the original lignin samples (KL and OL), their fractions (SKL, SOL, BKL and BOL), and their modified analogues (CMKL, CMOL, SMKL, SMOL, MKL, MOL, MSKL, MSOL, MBKL and MBOL).

with 150 rpm agitation and 40 °C for 4 h. Once the reaction time finished, the solution was cooled down and pH was adjusted to 7 adding 1 M H₂SO₄ dropwise. The solution was introduced in a dialysis bag (6–8 kDa pore size) and osmosis was carried out against distilled H₂O for the removal of unreacted reagents. Water was changed every 12 h for 2 days, and the solution was dried in an oven at 105 °C.

2.3. Sulfomethylation reaction

The sulfomethylation reaction conditions followed are the ones addressed by Yang et al. [41]. 1 g of lignin (KL and OL) was dissolved in 60 mL of 0.15 M NaOH solution. The solution was transferred to a 100 mL round bottom flask and heated to 70 °C. 0.041 g of 37 % formaldehyde was added and stirred for 1 h. 0.6 g of Na₂SO₄ was added, and the temperature increased to 95 °C for 3 h. Once the reaction finished, the solution was brought to room temperature (RT), pH was adjusted to 6, and the solution was dialyzed for 2 days using a 6–8 kDa dialysis bag, changing water every 12 h, to remove any unreacted reactive. The modified lignin was dried in an oven at 105 °C overnight.

2.4. Methylation reaction

The methylation reaction followed was described by Sen et al. [36]. 1 g of lignin (KL and OL) was dissolved in 15 mL of DMSO. 2 equivalents of NaOH to the total phenolic hydroxyl groups of the lignin were added, along with 10 equivalents of DMC, which acted as methylation agent, also to the total phenolic OH groups. The solution was heated to 150 °C for 15 h. Once the reaction finished, it was cooled down to RT and acidified with the addition of 50 mL of 2 M HCl. The precipitate was filtered using a 0.45 μm pore-sized nylon filter and washed 4 times with 50 mL of deionized water each time. Once the modified lignin was washed, it was dried at 60 °C overnight.

2.5. Characterization methods

FTIR spectra were recorded in a Perkin-Elmer Spectrum Two FT-IR Spectrometer. The wavenumber range was defined from 4000 to 600 cm⁻¹, with a 4 cm⁻¹ resolution and 64 scans per measurement [42].

GPC measurements were carried out to determine the molecular weight (M_w), number molecular weight (M_n), and polydispersity index

Table 1
M_w, M_n, and PI values for the original lignins and their modified analogs.

	Yield (%)	M _w (g/mol)	M _n (g/mol)	PI		Yield (%)	M _w (g/mol)	M _n (g/mol)	PI
KL		5048	1149	4.39	OL		6374	1537	4.15
SKL		3312	1128	2.94	SOL		1856	953	1.95
BKL		9131	2407	3.79	BOL		8753	2439	3.59
Carboxymethylated									
CMKL	90.8	19,154	1456	4.54	CMOL	91.2	10,710	2503	4.28
Sulfomethylated									
SMKL	89.7	23,234	3124	7.44	SMOL	88.9	11,618	2554	4.55
Methylated									
MKL	85.9	9080	1669	4.39	MOL	86.1	14,203	2222	6.39
MSKL	87.3	6474	1371	4.72	MSOL	90.1	6594	1414	4.66
MBKL	89.4	15,374	3948	3.89	MBOL	87.6	19,979	4014	4.98

(PDI). A Jasco LC-Net II/ADC device equipped with a RI-2031 Plus Intelligent refractive index detector, two PolarGel-M columns (300 mm × 7.5 mm) placed in series, and a PolarGel-M guard (50 mm × 7.5 mm) was used. 20 µL of 50 ppm lignin solution was injected for each measurement. The solution used to dissolve the samples was DMF with 0.1 % LiBr, also used as the mobile phase of the column. The column eluted the mobile phase at 40 °C and a flow rate of 0.7 mL/min. The standard used for the calibration was polystyrene with molecular weights ranging from 266 to 70,000 g/mol.

³¹P NMR spectroscopy was used to quantitatively determine the hydroxyl groups of the samples, and Py and CDCl₃ were used as a solvent in a ratio of 1.6:1 (v/v). 50 mg of each lignin sample was solubilized in 0.5 mL of the solvent mixture, and 0.1 mL of internal standard (IS) was added to each sample. The IS solution prepared had 5 mg/mL of Cr (acac)₃ and 18 mg/mL of NHND concentration. Once the samples were dissolved, 0.1 mL of TMDP was added to carry on the phosphitylation. ³¹P NMR spectra were recorded in a Bruker AVANCE 500 MHz, using the parameters recommended by Meng et al. [43], and MestReNova 11.0 was used for the data processing. The peak shown in the spectral range of the NHND, at 151 ppm, was used as reference for the integration of the other peak in the spectra that belong to the lignin sample.

¹³C NMR spectroscopy was used to further elucidate the chemical structures of the samples. DMSO-*d*₆ was used as the solvent, and sample solutions of 100 mg/mL were prepared. The spectra were recorded in a Bruker AVANCE 500 MHz. These spectra provide information on the nature of all the carbon atoms of the molecules.

HSQC NMR spectra were recorded on a Bruker AVANCE NEO 500 MHz equipped with a z-gradient BBO probe. The ¹H NMR spectra were recorded using a zg sequence from Bruker's library. A time domain of 32 k, a spectral width of 10 KHz, and 16 scans were used for the measurements, with a 1 s interpulse delay and 3 s of acquisition time. The HSQC spectra were recorded using the hsqcetgp sequence at 500.132 Mhz for ¹H and 125,7672 MHz for ¹³C. A time domain of 2048 F2 × 256 F1, and a spectral width of 5 Khz in ¹H and 20 Khz in ¹³C were also used. The number of scans was 16 per increment, with a 1 s interpulse delay and a 0.1 s acquisition time. A 50 g/L concentration solution was prepared with each sample, dissolved in DMSO-*d*₆.

DLS was used for the determination of the Z potential (ZP), Z average, polydispersity, and conductivity values of the lignin and their modified analogs. A ZetaSizer Ultra (Malvern Panalytical) equipped with a He–Ne laser source (=633 nm) and a scattering angle of 173° was used. Water dispersions of 0.1 wt% were prepared, left stirring overnight, and treated in an ultrasound bath for 15 min before the measurements for the obtaining of homogeneous dispersions. An average of 20 measurements was recorded for each measurement.

Thermogravimetric analyses were carried out using a Mettler Toledo TGA 851 with a heating circulator type F32-HL from Julabo GmbH.

Around 5 mg of sample were introduced in 70 µL alumina crucibles and heated from 25 to 800 °C at a 10 °C/min heating rate under inert conditions (Ar), in a flow rate of 40 mL/min.

3. Results and discussion

3.1. Lignin modification reaction schemes

The different reaction mechanisms of the modifications are shown. Fig. 1a shows the carboxymethylation reaction mechanism, where the hydroxyl groups of the lignin become a nucleophile by the NaOH of the medium, making it adequate to react with the SCA and obtain the carboxymethylated lignin. Fig. 1b shows the reaction of sulfomethylation, which similarly to the previous one, the nucleophilic lignin reacts with the Na₂SO₃. Finally, Fig. 1c depicts the reaction mechanism of methylation, displaying the possibility of two distinct reaction pathways.

3.2. Modified lignin characterization

All the FTIR spectra obtained for the lignins employed for the different modification reactions, and their modified analogs are shown in Fig. 2, and a list of the main functional groups identified, and their band associations can be found in Table S1.

The broad band at around 3400 cm⁻¹ for the O–H stretching vibrations, 2940–2840 cm⁻¹ for methyl and methylene C–H stretching, 1708 cm⁻¹ for C=O stretching, and other band around 1610 and 1150 cm⁻¹ for different skeletal aromatic vibrations were the main bands found in lignin FTIR spectra. KL showed a considerable intensity on the OH band, more intense compared to OL, albeit being derived from the same raw material (*Eucalyptus globulus*). This was a consequence of the delignification process, since the Kraft process employed harder conditions than the organosolv, breaking up more lignin bonds and therefore creating a smaller and more functionalized lignin. Both KL and OL were fractionated in a previous work [40] by a solvent extraction method, where the biggest and smallest fractions obtained were modified in the current work. It can be observed that SKL had a very high hydroxyl and carbonyl content compared to the BKL and the initial KL, like the case with OL, where SOL also showed more intense bands at 3400 cm⁻¹ and 1708 cm⁻¹.

In terms of the effect seen on the carboxymethylation reactions, both CMKL (Carboxymethylated Kraft Lignin) and CMOL (Carboxymethylated Organosolv Lignin) show a significant decrease in the O–H stretching band compared to their KL and OL analogs. Alkaline conditions of the reaction make the aromatic hydroxyl groups strong nucleophiles, reacting with the sodium chloroacetate and yielding carboxymethylated aromatic rings [25]. Therefore, a decrease in the O–H vibration band was observed for all the reactions, and an increase

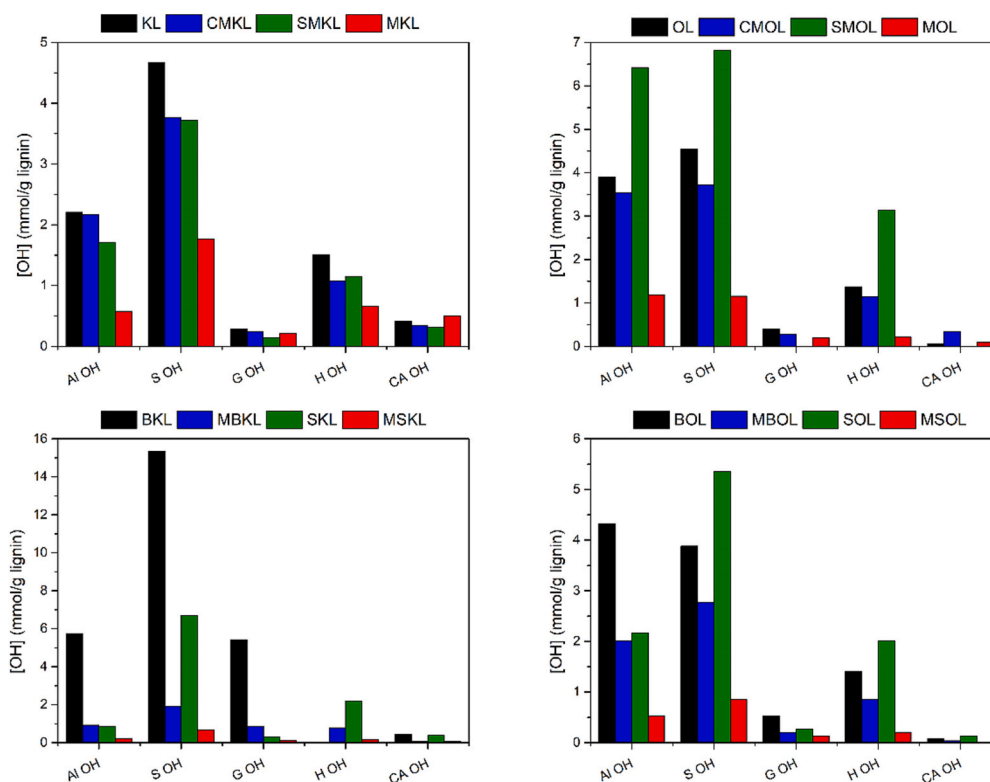


Fig. 3. ^{31}P NMR results obtained for all the samples.

of the methyl and methylene C—H stretching vibrations, due to the new $-\text{CH}_2\text{COOH}$, $-\text{CH}_2\text{SOOH}$, $-\text{OCH}_3$, and $-\text{COOCH}_3$ groups present in the modified lignins, seen in the 2940 and 2840 cm^{-1} spectra region.

The rest of the bands associated with the aromatic skeletal vibrations and aliphatic hydroxyl groups did not show major differences after the modification [30,44], as a consequence of the selectivity of the reactions of CM (carboxymethylation) and SM (sulfomethylation), in which only the aromatic OH groups were modified while maintaining the aliphatic OH groups. Therefore, the band at 3400 cm^{-1} for samples CMKL, CMOL, SMK (sulfomethylated Kraft Lignin), and SMOL (sulfomethylated Organosolv Lignin) can be attributed to aliphatic OH and unreacted phenolic OH groups. Moreover, the insertion of sulfur in the case of SM reactions, lead to an increase in the bands at 644 and 916 cm^{-1} . Contrarily, the M (methylation) reaction does not show the same selectivity, reacting with all types of OH groups, therefore showing a more significant change in the 3400 cm^{-1} band intensity. Another difference observed in all the methylated samples, MKL (methylated Kraft Lignin), MOL (methylated Organosolv Lignin), MBKL (methylated Big Kraft Lignin), MBOL (methylated Big Organosolv Lignin), MSKL (methylated Small Organosolv Lignin), and MSOL (methylated Small Organosolv Lignin), was the increase of the band at 1700 cm^{-1} , attributed to ketones, due to the insertion of the acetate groups of the sodium chloroacetate compound.

The yields of the reactions were calculated gravimetrically, and the molecular weights and their polydispersity index were calculated by GPC. The values obtained are listed in Table 1 and the chromatogram curves obtained for all the samples are shown in Fig. S1. Overall, it can be observed that the chemical reactions increased the M_w of the lignins. In the case of the carboxymethylation reactions, both KL and OL considerably increased their M_w . In the case of KL, the M_w almost quadrupled in CMKL, while for OL, CMOL less than doubled in size. This might be because larger lignin molecules tend to have less functionalized groups like OH, and the ones present are more hindered and less reactive, obtaining a lower carboxymethylation degree with larger-sized particles [25]. The carboxymethylation reaction overall increases the

M_w of the lignin not only due to the replacement of hydroxyl groups for carboxymethyl groups but also due to the condensation reactions happening during the process [27].

An almost identical behavior could be observed with the sulfomethylation reactions, where the M_w of both SMK and SMOL increased in the same proportions as for CMKL and CMOL [31]. Nevertheless, with the methylation reaction, considerably smaller molecules were obtained, probably due to the more stable lignins obtained, which have been reported to prevent self-polymerization reactions [36].

Since these three chemical reactions are based on the generation of alkoxide ions of the lignins to act as a strong nucleophile and subsequently react with the different reactives to introduce new functionalities, the quantification of the OH groups present in both the unmodified and the modified lignins is essential for the determination of the efficacy of the chemical reactions. The most efficient method for the quantitative analysis is the ^{31}P NMR, where different OH groups are shown in different parts of the spectra, enabling the individual identification, integration, and subsequent quantification of the concentrations of each type of OH group present in the sample. The results obtained are shown in Fig. 3, values are listed in Table S2, and the spectra obtained for each sample are shown in Figs. S2 and S3.

The carboxymethylation reaction was shown to be effective in the substitution of the phenolic hydroxyl groups on both KL and OL. Almost 20 % of the syringyl (S) OH groups (equivalent to the C5 substituted OH groups) of KL and OL react, while 16 % and 31 % of the guaiacyl (G) OH groups of KL and OL reacted, respectively. In the case of *p*-Hydroxyphenyl (H) OH, almost 30 % of the groups quantified for KL reacted, compared to 17 % of the OL. This tendency can be justified by the different pK_a values of the hydroxyl group of the monolignols, which vary depending on the substitution degree of the aromatic ring. The hydroxyl groups of the H units have a pK_a of 10.2, which tends to decrease with the presence of a methoxy group in the aromatic ring (in the case of the G unit), even further decreasing with an additional methoxy group, as in the case of the S units. Therefore, more OH groups in the G units react than S units, yielding lower G OH mmol concentrations after the

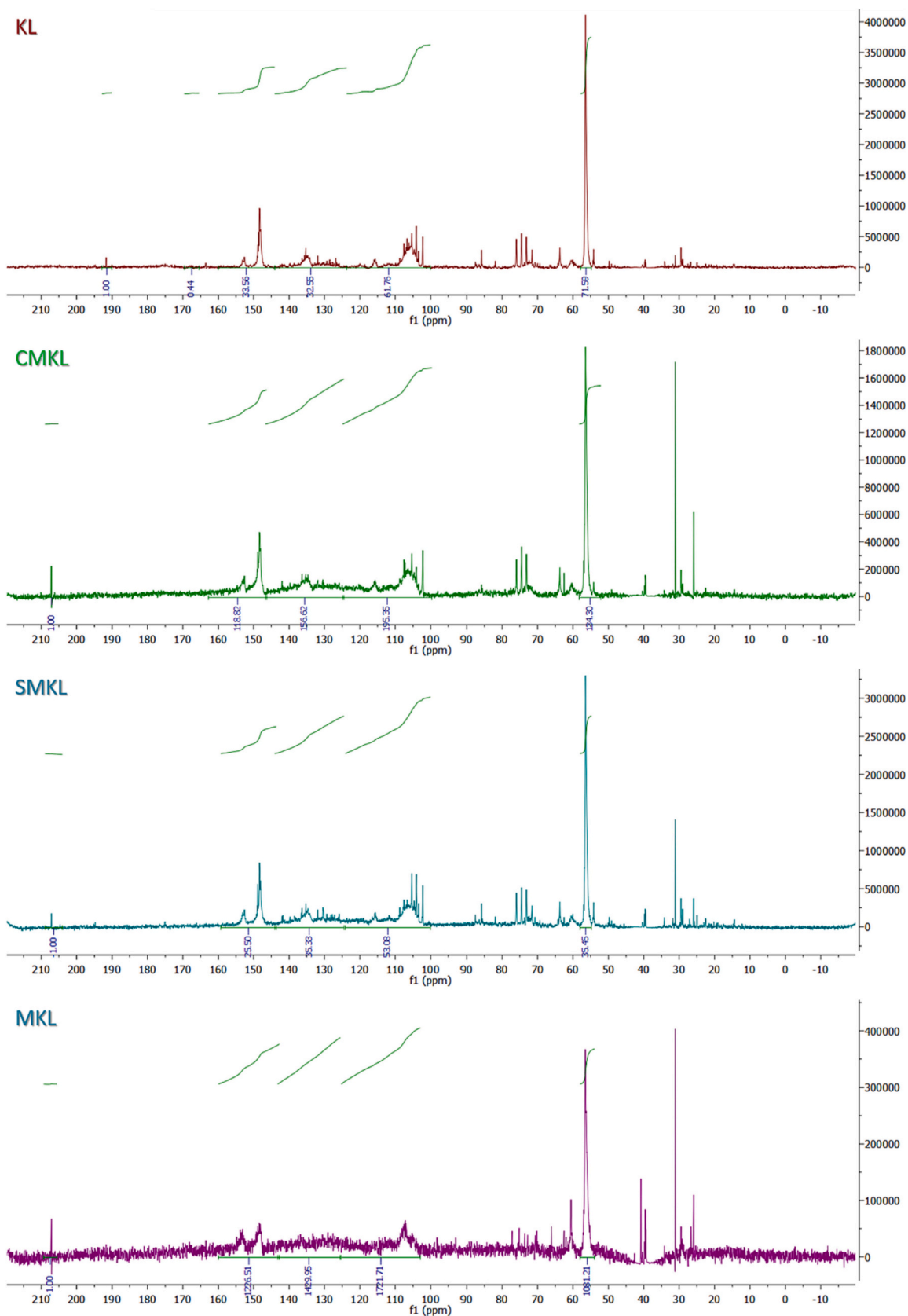


Fig. 4. ¹³C NMR spectra of KL and the modified analogs.

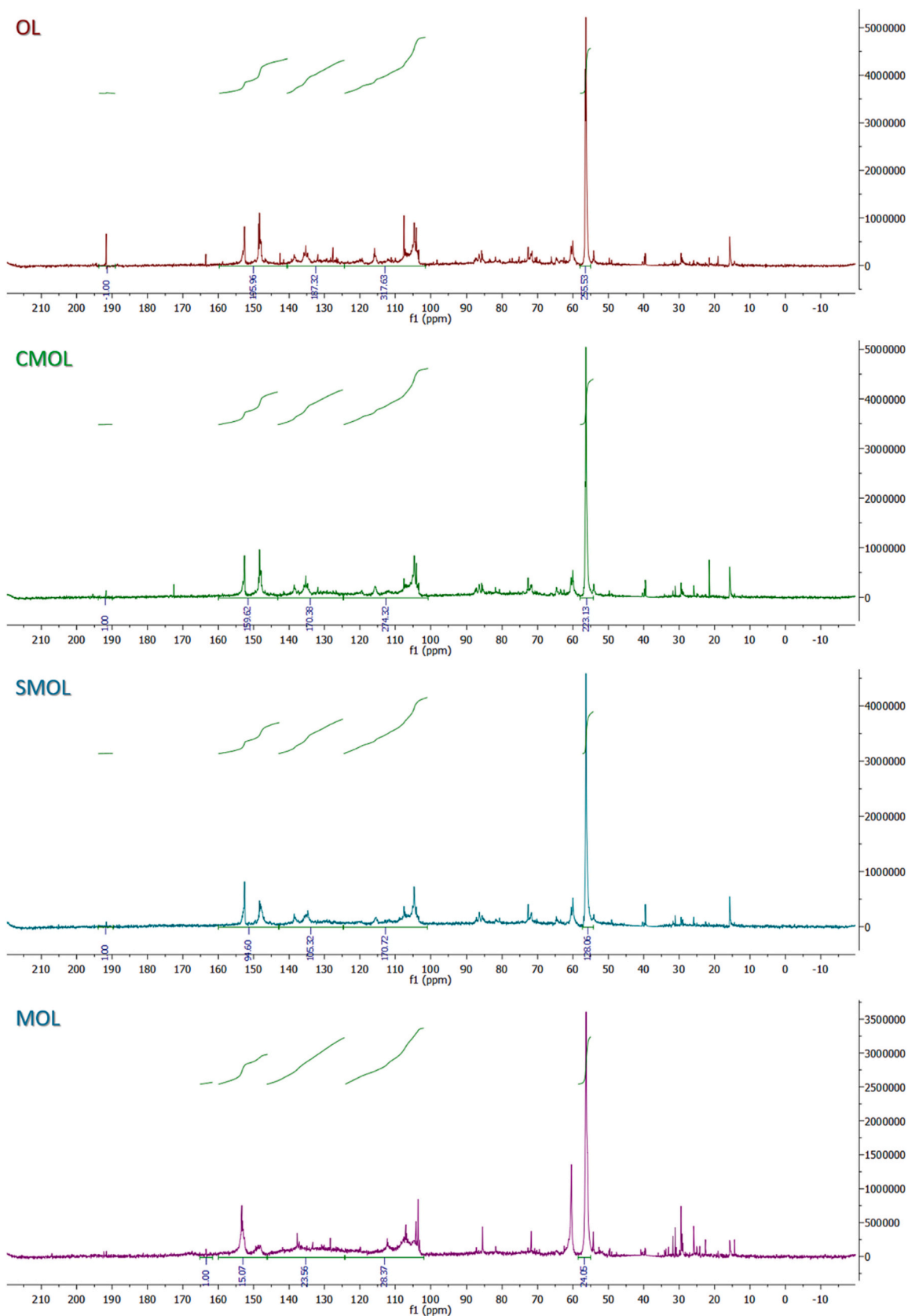


Fig. 5. ¹³C NMR spectra of OL and the modified analogs.

Table 2

Integration values of the different spectral regions identified in the modified and unmodified KL and OL samples.

Spectral region	Chemical shift range (ppm)	Number of moieties per aromatic ring							
		KL	CMKL	SMKL	MKL	OL	CMOL	SMOL	MOL
Methoxyl content	57–54	6.12	6.12	6.12	6.12	6.12	6.12	6.12	6.12
Aromatic methine carbons	125–103	5.28	8.90	9.16	9.75	7.61	7.52	8.16	7.22
Aromatic carbon-carbon structures	141–125	2.78	7.14	6.10	–	4.49	4.67	5.03	6.00
Oxygenated aromatic carbons	160–141	2.87	5.41	4.40	6.94	4.69	4.38	16.54	3.83
Carbon from carbonyl-type structures	195–190	0.09	–	–	–	0.02	0.03	0.05	0.25
Degree of condensation	125–103	5.28	8.90	9.16	9.75	7.61	7.52	8.16	7.22

carboxymethylation and sulfomethylation reactions. In terms of the aliphatic (Al) OH content, no significant changes could be observed [29] due to the selectivity of the reaction [25]. In terms of the sulfomethylation reaction, the hydroxyl group leaves the aromatic ring due to the attack of the electrophilic sulfur into the carbon atom of the methoxy group [45]. Although it also showed selectivity in the reactivity of the Al OH groups, a decrease of around 20 % could be observed, probably due to the condensation and self-polymerization side reactions happening rather than the actual sulfomethylation reaction.

In terms of the methylation reaction, it was observed to be the most efficient for the reduction of all types of OH groups, being the most effective when the lignin molecules were smaller, as the OH groups were more accessible.

The ^{13}C NMR spectra obtained are shown in Figs. 4 and 5, and the values obtained after the integration of different spectral regions are listed in Table 2. The peak attributed to the carbonyl content at 191 ppm showed that the modification reactions affected the presence of the functional group on the sample, disappearing completely the signal in all the modified Kraft samples, and almost completely disappearing the signal for the organosolv modifications. The signal at ~ 175 ppm, attributed to the $-\text{COOH}$ content [30,46], was only visible in the case of CMKL and CMOL, verifying the effectiveness of the carboxymethylation reaction. Another visible change observed from all the reactions (the carboxymethylation, the sulfomethylation, and the methylation) was the significant increase in the signals in the range of 0–50 ppm, confirming the insertion of additional alkyl branches, due to the increase of C–C bond signals.

In the case of ^{13}C NMR, the methoxyl content of all the integration values obtained were normalized to obtain 6.12 at this region, and compared with the integration values of the rest of the peak at different regions, as suggested in previous works [47,48]. Overall, it can be observed that the modified lignins contained more condensed structures, as can also be deduced from the M_w values obtained, and the FTIR peak in the 2940 and 2840 cm^{-1} spectra region, due to the new $-\text{CH}_2\text{COOH}$, $-\text{CH}_2\text{SOOH}$, $-\text{OCH}_3$, and $-\text{COOCH}_3$ functional groups present in the modified lignins, composed by methyl and methylene groups. The content of aromatic methynes did not significantly increase in the case of the modified OL samples, while a more considerable increase was observed in the case of the modified KL samples, possibly due to undesirable reactions occurred because of the lower purity of the Kraft lignin. The aromatic carbon-carbon structures did not significantly alter either, meaning that the reactions did not affect the aromatic structure of the lignin and that the conditions employed were adequate. Nevertheless, an increase of oxygenated aromatic content was observed, due to some reactions that happened from the new functional groups inserted in the lignin molecules, such as the acetate, sulfate and carbonate. It can therefore be deduced, that these modifications, apart from being appropriate to apply in water-based formulation, can also be interesting due to their aromaticity, and electron conjugation, increasing the electron movement along the molecules and enhancing their performance as electroactive material or components.

HSQC NMR experiment determines the proton-carbon single bond correlations, showing the protons in f2 (X axis) and the carbons in f1 (Y axis), resulting in a two-dimensional spectrum. All lignin samples,

original and modified, were submitted to this analysis and compared to determine structural differences. The side chain regions observed were the methoxyl (MeO) and β -O-4 aryl ether (A) groups (A_{β} , A_{α} , $A_{\beta(G)}$, and $A_{\beta(S)}$), while the aromatic regions identified were the $S_{2,6}$ and $S'_{2,6}$, corresponding to the $\text{C}_{2,6}\text{-H}_{2,6}$ in etherified and oxidized S units respectively, and the G_2 and G_5 samples the cross-correlations [49]. The results obtained for KL and its modified analogues are shown in Fig. 6, while Fig. 7 shows the OL and its modified analogues. The identified cross-signals and their assignments, based on the works previously published by Yuan et al. 2011 [50] and Ying et al. 2022 [49], are listed in Table S3. From the spectra obtained in Fig. 6 it can be concluded that the modification reactions were effective in obtaining structurally different lignin molecules. In the case of the carboxymethylation reaction, the CMKL sample showed weaker signal in B_{β} , $A_{\beta(G)}$, $S_{2,6}$, and $S'_{2,6}$, and disappeared signals for A_{α} , $A_{\beta(G)}$, and G_2 , compared to the original KL, proving the breakage of β -O-4 linkages in the case of sulfomethylation reactions, however, the cross-correlation signals (G_2 and G_5) maintained quite similar to the original. Finally, the methylation reaction also showed significant structural differences compared to KL, with the disappearance of several signals (B_{β} , I_{β} , A_{α} , $A_{\beta(G)}$, $A_{\beta(S)}$, $S'_{2,6}$, G_2 and G_5). In the case of OL and its analogues, shown in Fig. 7, the main differences observed occurred in the cross-correlation signals of the sulfomethylated and methylated samples (SMOL and MOL). Therefore, from the results obtained from the HSQC NMR, along with the previously discussed FTIR, ^{31}P NMR and ^{13}C NMR, that the chemical reactions were effective for obtaining lignin with diverse structure.

Dynamic Light Scattering (DLS) was used for the Z potential (mV), conductivity (mS/cm), size average (nm), and dispersity index determination. ZP determines the stability of the particles, measuring the electric charge present on the surface of the particles, and concluding the tendency of the particles to aggregate. High ZP values, positive or negative, mean that there are many changes in the particle surfaces, creating more repulsion between them and difficulting the aggregation or precipitation. Low ZP values ranging from -30 to $+30$ mV do not have the necessary repulsive force to avoid interactions, leading to the formation of Van der Waals forces that create aggregates.

Fig. 8 shows the ZP values and conductivity values obtained for the modified and unmodified lignin particles. Unmodified lignins, KL, and OL showed a ZP value around -30 mV, in the limit of incipient and good stability. The carboxymethylation reaction had different effects on the particle stability depending on the lignin type, with increased ZP values for CMOL, and slightly deteriorated ZP values for CMKL. The sulfomethylation reaction maintained a similar ZP value with a slight improvement for SMKL, while considerable changes occurred for SMOL, with a ZP value of almost -45 mV. Methylation reaction, on the other hand, did not affect the ZP values of either MKL or MOL significantly, obtaining very similar values to the KL and OL. When methylation reaction was carried out with the lignin fractions (SKL, SOL, BKL, and BOL) a difference in the tendency could be observed. On one side, the small fractions (the ones with smaller M_w) showed a lower ZP value, resulting in worse stability, than the big fractions, with a higher difference shown in the Kraft lignin fractions. Higher ZP values were obtained for MSKL and MSOL compared to the unmodified fractions, reaching highly stable particles with MSKL. Although better results were obtained

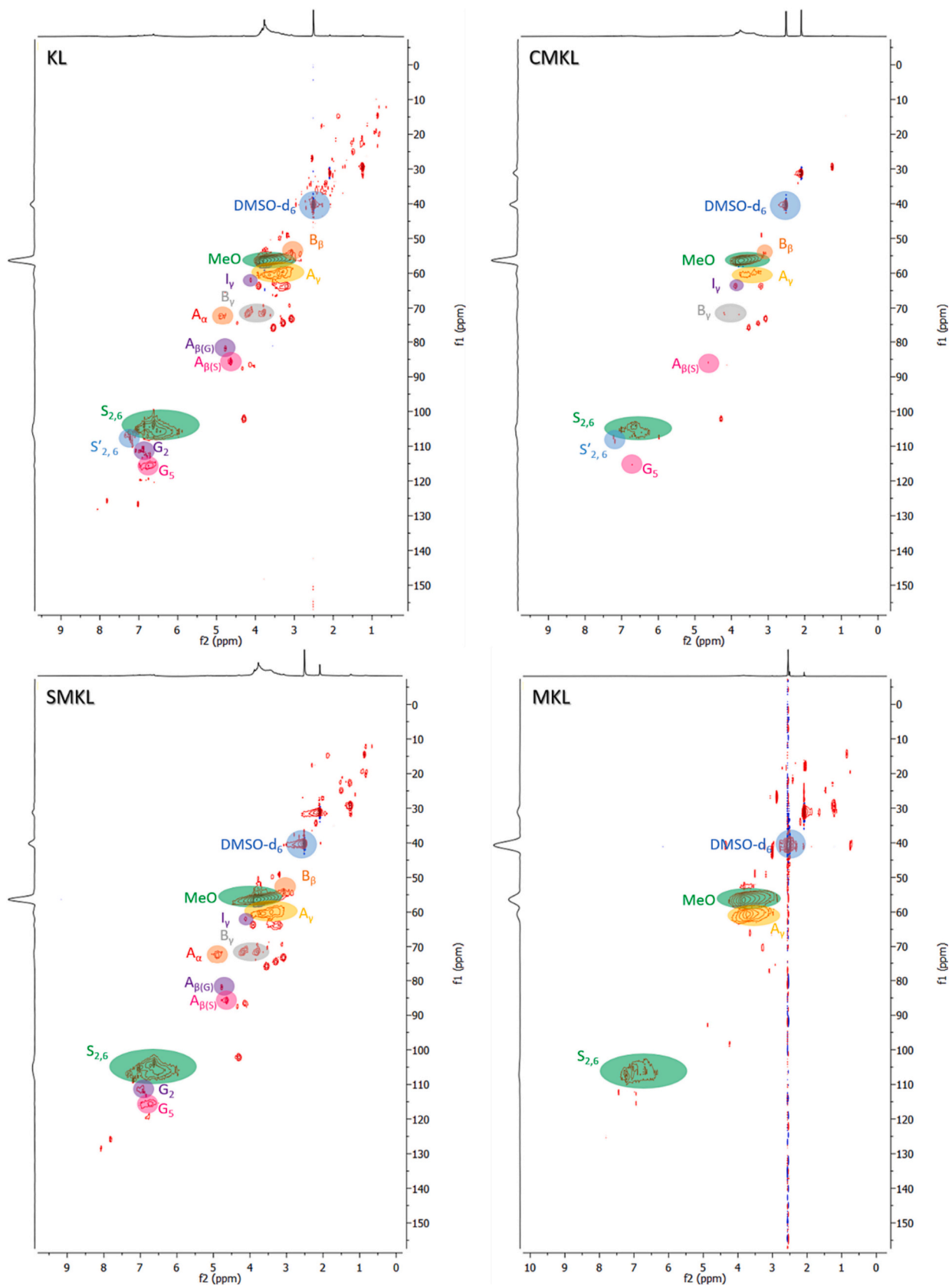


Fig. 6. HSQC NMR spectra of KL and the modified analogs.

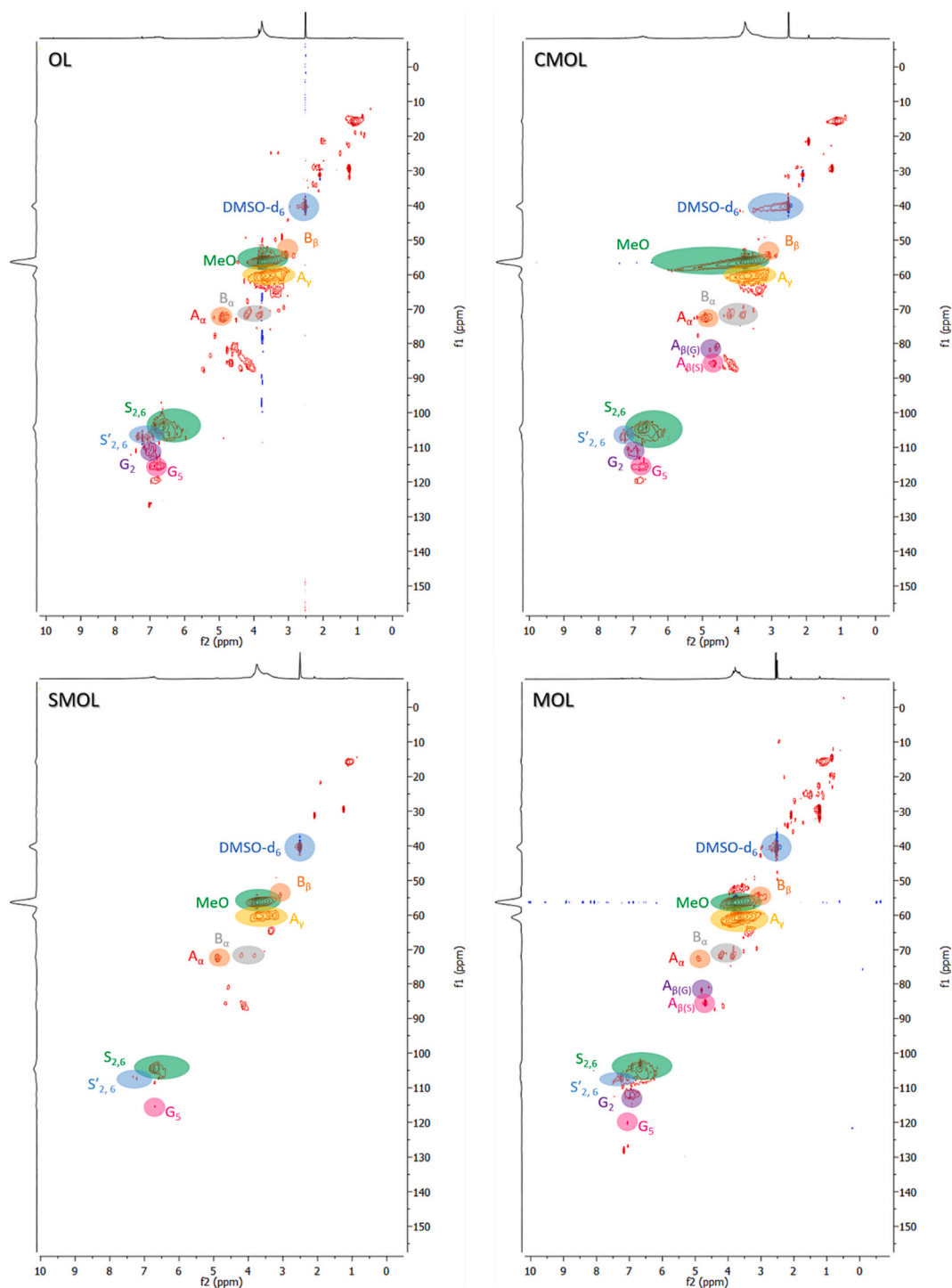


Fig. 7. HSQC NMR spectra of OL and the modified analogs.

with MSKL and MSOL, these values also showed higher error bars, which might be related to the high PI index and the fact that the methylation reaction had two possible pathways, with consequently two end products. Nevertheless, this tendency was not maintained with BKL and BOL, where lower values of ZP were obtained after the modification, but considerably lower error bars.

It can be deduced that different lignin types obtained their most stable version with different modifications. While KL obtained the molecules with the lowest ZP and highest conductivity with the methylation reaction, obtaining an even more significant improvement when the precursor of this modification was small KL molecules, in the

case of OL it was the sulfomethylation reaction the one that obtained the most stable particles.

In terms of the Z average, different tendencies could be observed depending on the lignin type, as shown in Fig. 9. CMKL showed the highest particle size of all the samples, with more than tripled the size of the original KL particle, which slightly decreased for SMKL and minorly increased with MKL. When comparing the fluctuation of the values obtained for the ZP, it could be observed that these two values were inversely related, obtaining the biggest particles when the ZP show was the smallest. When the particles have a small ZP value, it means that the ions present on the surface are not abundant enough to obtain a stable

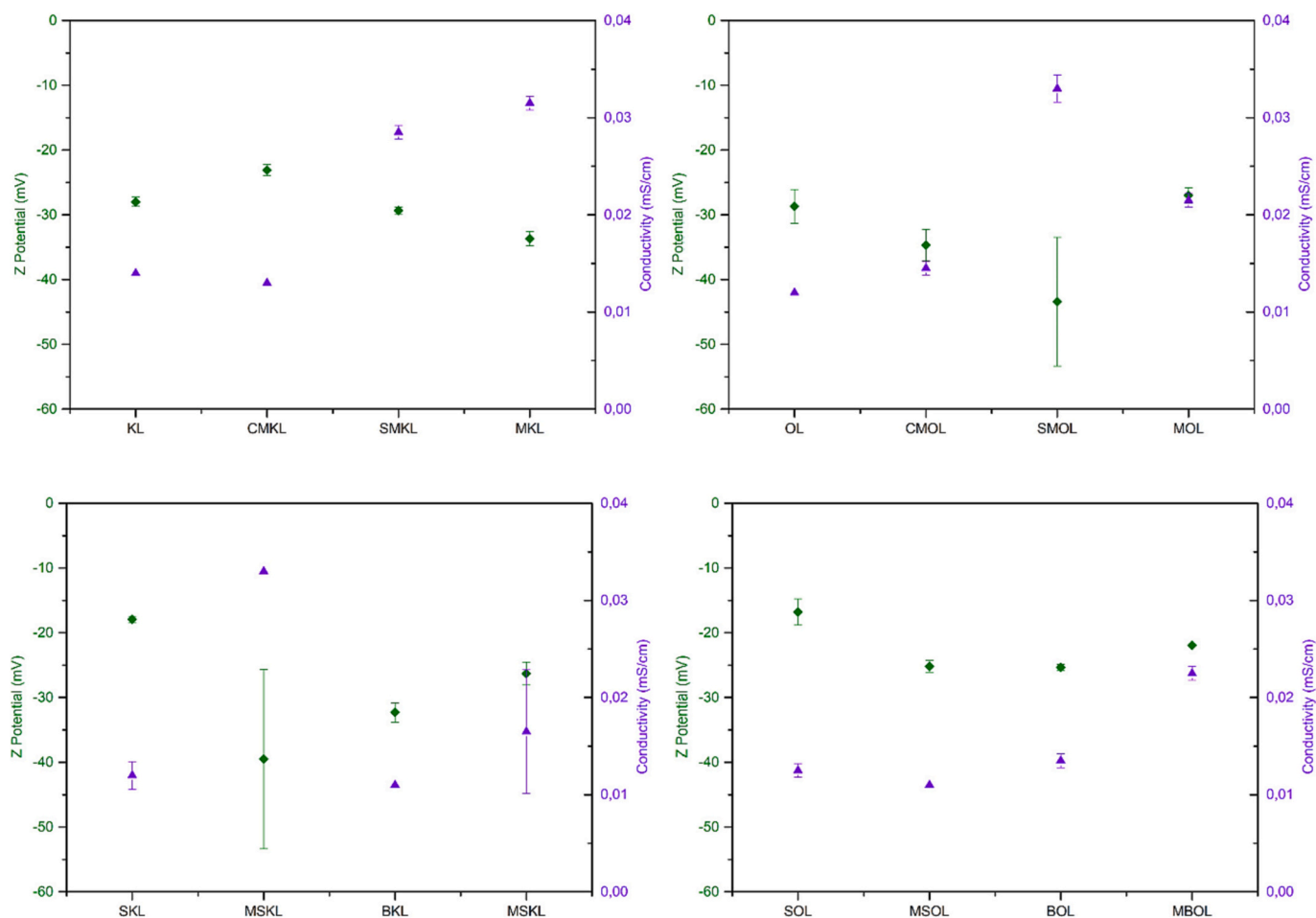


Fig. 8. Z Potential and conductivity values for the modified and unmodified lignin particles.

dispersion in the media, which promotes their aggregation. This phenomenon was observed to not necessarily correlate with the actual M_w values, as can be observed in the case of OL and CMOL. OL had a M_w of 600 g/mol, while the CMOL analog obtained resulted in a M_w of 10,700 g/mol due to the self-polymerization side reactions happening during the process. Nevertheless, the CM functional groups added brought a slight increase in the ZP value, which consequently resulted in slightly more stable water dispersions and a decrease in the tendency to agglomerate, obtaining 4 times smaller lignin particle sizes (1200 nm for OL vs. 300 nm for CMOL). This trend can be extrapolated to other samples, where similar Z average values were obtained for samples with similar ZP values, and bigger Z average values were obtained for samples with lower ZP values.

Thermogravimetric analysis of the lignins and their modified analogs was carried out. The $T_{5\%}$, $T_{50\%}$, different degradation stages identified, and the char (%) residue amount values are listed in Table 3, and the TGA and DTG curves are depicted in Figs. S4 and S5.

The modifications were effective in obtaining more thermally stable molecules, with considerably more mass content (char residue) when high temperatures of 800 °C were reached under an inert atmosphere. In the case of CM and SM reactions, the modified lignins obtained showed lower degradation stages, the main one being at 307 °C in both cases and compared to the 385 °C of KL. Moreover, the $T_{50\%}$ was reached at considerably high T-s, increasing it to more than 200 °C. In the case of the methylation reactions, an improvement in the thermal stability of the lignin could also be observed, although the difference was not as considerable, due to the obtaining of smaller M_w particles. An interesting difference compared to the other modification also relies on the degradation stages, where two main degradation stages could be

observed in the methylated samples. Since the methylation reaction can take two different paths, obtaining two different molecules, the scheme shown in Fig. 3, and therefore, there are two different T to degrade each modified lignin.

4. Conclusions

The three selected modification reactions, the carboxymethylation, sulfomethylation and methylation, proved to be effective for obtaining lignins with different properties. Diverse resulting products with differentiated characteristics were obtained depending on the lignin type employed, showing distinct behaviors for each modification and final properties. The different physicochemical techniques employed showed a decrease in the quantity of the main functional group (hydroxyl groups), which was reacted to obtain new functionalities. Other signals corresponding to new ones such as methyl and methylene groups and ketones increased the intensity. Moreover, with the modified samples obtained, higher molecular weight, and greater thermal stability were achieved. More condensed structures and higher aromaticity were obtained, and side chain regions and cross-correlation signals generally reduced, due to the breakage of β -O-4 linkages mainly. All of these physicochemical properties consequently showed variations in other properties like thermal stability, morphology and water stability. All the modified samples showed higher $T_{50\%}$ temperature values, suggesting more resistance to degradation. Moreover, the Z potential and conductivity values obtained suggested better behavior towards water and hydrophilicity in general. It was interesting to observe that whereas carboxymethylation reaction was effective for obtaining stable water dispersions for organosolv lignin, it was the least efficient in the case of

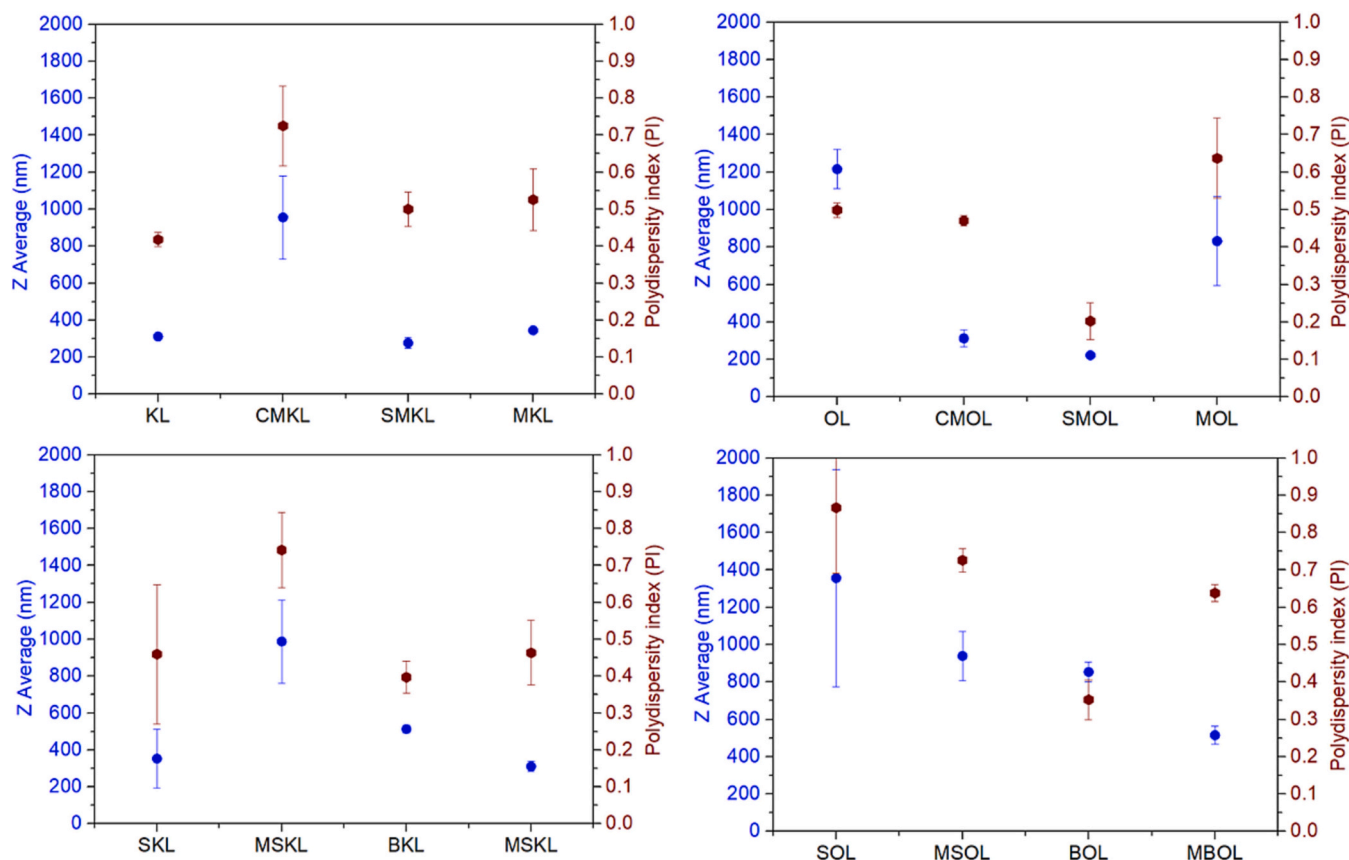


Fig. 9. Z average and polydispersity (PI) values for the modified and unmodified lignin particles.

Table 3

$T_{5\%}$, $T_{50\%}$, different degradation stages identified, and the char (%) residue of the modified and unmodified lignin particles (values in bold refer to the temperature of the main degradation stage).

	$T_{5\%}$ (°C)	$T_{50\%}$ (°C)	Degradation stages (°C)	Char (%)
KL	166	424	56/150/252/ 385	33.89
CMKL	113	643	74/109/ 307	47.69
SMKL	116	698	85/108/ 307 /380/726	45.32
MKL	120	553	62/ 162 / 407 /530	44.32
OL	103	397	385	23.92
CMOL	101	629	82/ 307 /357/461/530/	47.54
SMOL	96	599	84/ 282 /356/530/661	46.01
MOL	215	673	61/189/ 380 / 411 /531/580	48.45
SKL	212	448	370	36.49
MSKL	141	482	76/153/ 390 /536	41.64
SOL	225	420	362	30.68
MSOL	199	518	59/187/ 385 /535	43.52
BKL	210	502	384	39.30
MBKL	169	751	43/169/ 373 / 411 /533	49.69
BOL	190	480	393	36.96
MBOL	143	470	66/170/ 344 /400/533	41.57

Kraft lignin. Conversely, a reversed pattern was evident with methylated samples, and similar behaviors were observed when sulfomethylation reactions were carried out.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to acknowledge the financial support of the Basque Government for (grant PIF19-183) and Diputación de Gipuzkoa (EZAGUTZ-11/2022). The authors thank for the technical and human support provided by SGiker (UPV/EHU/ ERDF, EU).

Statement

The authors have not used any tool/service for the preparation of the content of the manuscript. The authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijbiomac.2024.130518>.

References

- [1] H. Chen, J. Liu, X. Chang, D. Chen, Y. Xue, P. Liu, H. Lin, S. Han, A review on the pretreatment of lignocellulose for high-value chemicals, *Fuel Process. Technol.* 160 (2017) 196–206, <https://doi.org/10.1016/j.fuproc.2016.12.007>.
- [2] T. Pang, G. Wang, H. Sun, W. Sui, C. Si, Lignin fractionation: effective strategy to reduce molecule weight dependent heterogeneity for upgraded lignin valorization, *Ind. Crop. Prod.* 165 (2021) 113442, <https://doi.org/10.1016/j.indcrop.2021.113442>.
- [3] J.A. Poveda-Giraldo, J.C. Solarte-Toro, C.A. Cardona Alzate, The potential use of lignin as a platform product in biorefineries: a review, *Renew. Sust. Energ. Rev.* 138 (2021) 110688, <https://doi.org/10.1016/j.rser.2020.110688>.
- [4] W. Schutyser, T. Renders, S. Van Den Bosch, S.F. Koelewijn, G.T. Beckham, B. F. Sels, Chemicals from lignin: an interplay of lignocellulose fractionation, depolymerisation, and upgrading, *Chem. Soc. Rev.* 47 (2018) 852–908, <https://doi.org/10.1039/c7cs00566k>.
- [5] Z.H. Xie, J.H. Zhou, H.M. Li, G.W. Sun, Comparison between green liquor pretreatment-Kraft pulping and conventional Kraft pulping, *Adv. Mater. Res.*

- 781–784 (2013) 2650–2653, <https://doi.org/10.4028/www.scientific.net/AMR.781-784.2650>.
- [6] T. Li, S. Takkellapati, The current and emerging sources of technical lignins and their applications, *Biofuels Bioprod. Biorefin.* 12 (2018) 756–787, <https://doi.org/10.1002/BBB.1913>.
- [7] J.D. Gargulak, S.E. Lebo, Commercial use of lignin-based materials, *ACS Symp. Ser.* 742 (1999) 304–320, <https://doi.org/10.1021/bk-2000-0742.ch015>.
- [8] M. Österberg, M.H. Sipponen, B.D. Mattos, O.J. Rojas, Spherical lignin particles: a review on their sustainability and applications, *Green Chem.* 22 (2020) 2712–2733, <https://doi.org/10.1039/d0gc00096e>.
- [9] X. Yang, Y. Zhang, M. Ye, Y. Tang, Z. Wen, X. Liu, C.C. Li, Renewable lignin and its macromolecule derivatives: an emerging platform toward sustainable electrochemical energy storage, *Green Chem.* 4 (2023) 4154–4179, <https://doi.org/10.1039/d3gc00565h>.
- [10] J. Liu, X. Li, M. Li, Y. Zheng, Lignin biorefinery: lignin source, isolation, characterization, and bioconversion, *Adv. Bioenergy* (2022), <https://doi.org/10.1016/bs.aibe.2022.05.004>.
- [11] D. Sidiras, D. Politi, G. Giakoumakis, I. Salapa, Simulation and optimization of organosolv based lignocellulosic biomass refinery: a review, *Bioresour. Technol.* 343 (2022) 126158, <https://doi.org/10.1016/j.biortech.2021.126158>.
- [12] Q. Yang, X. Pan, Correlation between lignin physicochemical properties and inhibition to enzymatic hydrolysis of cellulose, *Biotechnol. Bioeng.* 113 (2016) 1213–1224, <https://doi.org/10.1002/bit.25903>.
- [13] X. Sun, J. Lin, Y. Cheng, L. Duan, X. Sun, X. Li, C. Lu, Insight into the structure and mechanical performance of high content lignin reinforced poly (vinyl alcohol) gel-spun fibers via the regulation of esterified hydrophilic lignin composition for better sustainability, *J. Appl. Polym. Sci.* 140 (2023) 1–12, <https://doi.org/10.1002/app.53577>.
- [14] H. Zhao, Y. Zhu, H. Zhang, H. Ren, H. Zhai, UV-blocking composite films containing hydrophilized spruce kraft lignin and nanocellulose: fabrication and performance evaluation, *Int. J. Biol. Macromol.* 242 (2023), <https://doi.org/10.1016/j.ijbiomac.2023.124946>.
- [15] N. Alwadani, N. Ghavidel, P. Fatehi, Surface and interface characteristics of hydrophobic lignin derivatives in solvents and films, *Colloids Surfaces A Physicochem. Eng. Asp.* 609 (2021) 125656, <https://doi.org/10.1016/j.colsurfa.2020.125656>.
- [16] Q. Tang, Q. Chen, M. Zhou, D. Yang, Preparation of nano disperse dyes using sulfomethylated lignin: effects of sulfonic group contents, *Int. J. Biol. Macromol.* 234 (2023) 123605, <https://doi.org/10.1016/j.ijbiomac.2023.123605>.
- [17] K. Chen, Y. Qian, S. Wu, X. Qiu, D. Yang, L. Lei, Neutral fabrication of UV-blocking and antioxidant lignin-stabilized high internal phase emulsion encapsulates for high efficient antibacterium of natural curcumin, *Food Funct.* 10 (2019) 3543–3555, <https://doi.org/10.1039/c9fo00032g>.
- [18] L.B. Brenelli, L.R.B. Mariutti, R. Villares Portugal, M.A. de Farias, N. Braganolo, A. Z. Mercadante, T.T. Franco, S.C. Rabelo, F.M. Squina, Modified lignin from sugarcane bagasse as an emulsifier in oil-in-water nanoemulsions, *Ind. Crop. Prod.* 167 (2021), <https://doi.org/10.1016/j.indcrop.2021.113532>.
- [19] M. Yildirim-Yalcin, F. Tornuk, O.S. Toker, Recent advances in the improvement of carboxymethyl cellulose-based edible films, *Trends Food Sci. Technol.* 129 (2022) 179–193, <https://doi.org/10.1016/j.tifs.2022.09.022>.
- [20] M. Pourmadadi, E. Rahmani, A. Shamsabadipour, A. Samadi, J. Esmaili, R. Arshad, A. Rahdar, F. Tavangarian, S. Pandey, Novel carboxymethyl cellulose based nanocomposite: a promising biomaterial for biomedical applications, *Process Biochem.* 130 (2023) 211–226, <https://doi.org/10.1016/j.procbio.2023.03.033>.
- [21] M.A. Raeisi Estabragh, M. Sajadi Bami, G. Dehghannoudeh, Y.D. Noudeh, E. Moghimipour, Cellulose derivatives and natural gums as gelling agents for preparation of emulgel-based dosage forms: a brief review, *Int. J. Biol. Macromol.* 241 (2023) 124538, <https://doi.org/10.1016/j.ijbiomac.2023.124538>.
- [22] M.S. Rahman, M.S. Hasan, A.S. Naitai, S. Nam, A.K. Karmakar, M.S. Ahsan, M.J. A. Shiddiky, M.B. Ahmed, Recent developments of carboxymethyl cellulose, *Polymers (Basel)* 13 (2021), <https://doi.org/10.3390/polym13081345>.
- [23] A. Jimtaisong, N. Saewan, Utilization of carboxymethyl chitosan in cosmetics, *Int. J. Cosmet. Sci.* 36 (2014) 12–21, <https://doi.org/10.1111/ics.12102>.
- [24] V.K. Mourya, N.N. Inamdar, A. Tiwari, Carboxymethyl chitosan and its applications, *Adv. Mater. Lett.* 1 (2010) 11–33, <https://doi.org/10.5185/amlett.2010.3108>.
- [25] M.K. Konduri, F. Kong, P. Fatehi, Production of carboxymethylated lignin and its application as a dispersant, *Eur. Polym. J.* 70 (2015) 371–383, <https://doi.org/10.1016/j.eurpolymj.2015.07.028>.
- [26] D. Schieppati, A. Dreux, W. Gao, P. Fatehi, D.C. Boffito, Ultrasound-assisted carboxymethylation of LignoForce Kraft lignin to produce biodispersants, *J. Clean. Prod.* 366 (2022) 132776, <https://doi.org/10.1016/j.jclepro.2022.132776>.
- [27] M. Aldajani, N. Alipoormazandarani, P. Fatehi, Two-step modification pathway for inducing lignin-derived dispersants and flocculants, *Waste Biomass Valoriz.* 13 (2022) 1077–1088, <https://doi.org/10.1007/s12649-021-01579-8>.
- [28] T. Zhang, J. Zhou, H. Li, J. Ma, X. Wang, H. Shi, M. Niu, Y. Liu, F. Zhang, Y. Guo, Stable lignin-based afterglow materials with ultralong phosphorescence lifetimes in solid-state and aqueous solution, *Green Chem.* 25 (2023) 1406–1416, <https://doi.org/10.1039/d2gc04370j>.
- [29] F. Yu, Z. You, Y. Ma, H. Liu, Y. Wang, Z. Xiao, Y. Xie, Modification with carboxymethylation-activated alkali lignin/glutaraldehyde hybrid modifier to improve physical and mechanical properties of fast-growing wood, *Wood Sci. Technol.* 57 (2023) 583–603, <https://doi.org/10.1007/s00226-023-01465-7>.
- [30] B. Du, L. Chai, Q. Zheng, Y. Liu, X. Wang, X. Chen, S. Zhai, J. Zhou, R.C. Sun, Designed synthesis of multifunctional lignin-based adsorbent for efficient heavy metal ions removal and electromagnetic wave absorption, *Int. J. Biol. Macromol.* 234 (2023) 123668, <https://doi.org/10.1016/j.ijbiomac.2023.123668>.
- [31] Z. Ding, X. Qiu, Z. Fang, D. Yang, Effect of molecular weight on the reactivity and dispersibility of sulfomethylated alkali lignin modified by horseradish peroxidase, *ACS Sustain. Chem. Eng.* 6 (2018) 14197–14202, <https://doi.org/10.1021/acscuschemeng.8b02826>.
- [32] D.Y. Hopa, P. Fatehi, Using sulfobutylated and sulfomethylated lignin as dispersant for kaolin suspension, *Polymers (Basel)* 12 (2020) 11–14, <https://doi.org/10.3390/POLYM12092046>.
- [33] B. Zhang, D. Yang, H. Wang, Y. Qian, J. Huang, L. Yu, X. Qiu, Activation of enzymatic hydrolysis lignin by NaOH/urea aqueous solution for enhancing its sulfomethylation reactivity, *ACS Sustain. Chem. Eng.* 7 (2019) 1120–1128, <https://doi.org/10.1021/acscuschemeng.8b04781>.
- [34] R. Yang, Q. Tang, Y. Qian, Y. Pang, D. Yang, D. Zheng, M. Zhou, Preparation of sulfomethylated lignin grafted by pyrrolidone for utilization as a dispersant in nano pigment paste, *Ceram. Int.* 49 (2023) 16578–16586, <https://doi.org/10.1016/j.ceramint.2023.02.015>.
- [35] G.J. Jiao, J. Ma, Y. Li, D. Jin, J. Zhou, R. Sun, Removed heavy metal ions from wastewater reuse for chemiluminescence: successive application of lignin-based composite hydrogels, *J. Hazard. Mater.* 421 (2022) 126722, <https://doi.org/10.1016/j.jhazmat.2021.126722>.
- [36] S. Sen, S. Patil, D.S. Argyropoulos, Methylation of softwood kraft lignin with dimethyl carbonate, *Green Chem.* 17 (2015) 1077–1087, <https://doi.org/10.1039/C4GC01759E>.
- [37] J.H. Lee, T.M. Kim, I.G. Choi, J.W. Choi, Phenolic hydroxyl groups in the lignin polymer affect the formation of lignin nanoparticles, *Nanomaterials* 11 (2021) 1–13, <https://doi.org/10.3390/nano11071790>.
- [38] Y. Xi, D. Yang, W. Liu, Y. Qin, X. Qiu, Preparation of porous lignin-derived carbon/carbon nanotube composites by hydrophobic self-assembly and carbonization to enhance lithium storage capacity, *Electrochim. Acta* 303 (2019) 1–8, <https://doi.org/10.1016/j.electacta.2019.01.094>.
- [39] J.E. Holladay, J.F. White, J.J. Bozell, D. Johnson, Top value-added chemicals from biomass volume II — results of screening for potential candidates from biorefinery lignin, https://www.pnnl.gov/main/publications/external/technical_reports/PNNL-16983.pdf, 2007.
- [40] N. Izaguirre, E. Robles, R. Llano-Ponte, J. Labidi, X. Erdocia, Fine-tune of lignin properties by its fractionation with a sequential organic solvent extraction, *Ind. Crop. Prod.* 175 (2022) 114251, <https://doi.org/10.1016/j.indcrop.2021.114251>.
- [41] D. Yang, X. Wu, X. Qiu, Y. Chang, H. Lou, Polymerization reactivity of sulfomethylated alkali lignin modified with horseradish peroxidase, *Bioresour. Technol.* 155 (2014) 418–421, <https://doi.org/10.1016/j.BIORTECH.2013.12.017>.
- [42] A.A. Myint, H.W. Lee, B. Seo, W.S. Son, J. Yoon, T.J. Yoon, H.J. Park, J. Yu, J. Yoon, Y.W. Lee, One pot synthesis of environmentally friendly lignin nanoparticles with compressed liquid carbon dioxide as an antisolvent, *Green Chem.* 18 (2016) 2129–2146, <https://doi.org/10.1039/c5gc02398j>.
- [43] X. Meng, C. Crestini, H. Ben, N. Hao, Y. Pu, A.J. Ragauskas, D.S. Argyropoulos, Determination of hydroxyl groups in biorefinery resources via quantitative ³¹P NMR spectroscopy, *Nat. Protoc.* 14 (2019) 2627–2647, <https://doi.org/10.1038/s41596-019-0191-1>.
- [44] J. Zeng, D. Zhang, W. Liu, J. Huang, D. Yang, X. Qiu, S. Li, Preparation of carboxymethylated lignin-based multifunctional flocculant and its application for copper-containing wastewater, *Eur. Polym. J.* 164 (2022) 110967, <https://doi.org/10.1016/j.eurpolymj.2021.110967>.
- [45] A. Eraghi Kazzaz, Z. Hosseinpour Feizi, P. Fatehi, Grafting strategies for hydroxy groups of lignin for producing materials, *Green Chem.* 21 (2019) 5714–5752, <https://doi.org/10.1039/c9gc02598g>.
- [46] K. Chen, L. Lei, H. Lou, J. Niu, D. Yang, X. Qiu, Y. Qian, High internal phase emulsions stabilized with carboxymethylated lignin for encapsulation and protection of environmental sensitive natural extract, *Int. J. Biol. Macromol.* 158 (2020) 430–442, <https://doi.org/10.1016/j.ijbiomac.2020.04.106>.
- [47] K.M. Holtman, H. Chang, H. Jameel, Quantitative ¹³C NMR Characterization of Milled Wood Lignins Isolated by Different Milling Techniques Quantitative ¹³C NMR Characterization of Milled Wood Lignins Isolated by Different Milling Techniques, 2006, <https://doi.org/10.1080/02773810600582152>.
- [48] C.L. Chen, D. Robert, Characterization of lignin by ¹H and ¹³C NMR spectroscopy, *Methods Enzymol.* 161 (1988) 137–174, [https://doi.org/10.1016/0076-6879\(88\)61017-2](https://doi.org/10.1016/0076-6879(88)61017-2).
- [49] W. Ying, J. Yang, J. Zhang, In-situ modification of lignin in alkaline-pretreated sugarcane bagasse by sulfomethylation and carboxymethylation to improve the enzymatic hydrolysis efficiency, *Ind. Crop. Prod.* 182 (2022) 114863, <https://doi.org/10.1016/j.indcrop.2022.114863>.
- [50] T.Q. Yuan, S.N. Sun, F. Xu, R.C. Sun, Characterization of lignin structures and lignin-carbohydrate complex (LCC) linkages by quantitative ¹³C and 2D HSQC NMR spectroscopy, *J. Agric. Food Chem.* 59 (2011) 10604–10614, <https://doi.org/10.1021/jf2031549>.