CHEMICAL AND SPECTRAL CHARACTERISTICS OF ANNUAL PLANT LIGNINS MODIFIED BY HYDROXYMETHYLATION REACTION

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This paper aims at the synthesis and chemical and spectral characterization of lignins from annual plants (L1 – lignin from wheat straw, and L2 – lignin from grass), modified by hydroxymethylation with formaldehyde in alkaline environment. The derivatives synthesized by this reaction were characterized by the introduced functional groups, which has been related to the consumption of formaldehyde under different conditions. Subsequently, additional information on the changes has been obtained by spectral studies (FTIR, ^{1}H NMR) and high performance steric exclusion chromatography (HPSEC). Studies have revealed some functional changes, related to both different reactivity of lignins and reaction conditions.

Keywords: lignin, hydroxymethylation, FT-IR, ^{1}H NMR, HPSEC

INTRODUCTION

In the last decades, research within the field of lignin has sought not only perfection of extraction processes, but also the elucidation of structures of products separated from various vegetal sources, their chemical characterization, reactivity, functional properties and the development of new applications. [1]

At a global level, lignin resulted from cellulose fabrication or technologies of hydrolysis of vegetal mass can be considered as raw material with high capitalization potential, due to its origin from regenerating sources and low price. Lignin is a macromolecular compound, much more active than cellulose or other natural polymers, due to the functional groups contained in its macromolecule, constituting the main aromatic component of vegetal tissues, standing for 20%-30% of the mass in higher plants, where it is present within the cellular membrane and in intercellular spaces.[2]

The reactivity of lignin is determined by its particular structure, in which specific functional groups are identified, as well as by structural modifications induced by methods used for its separation from wood.[3] Thus, it has been concluded that lignin reactivity is determined by its constituting structural units, which, as we know, depend on the species. It is known that lignin has a very complex structure, which varies depending on the plant species, separation method and modification reactions that may induce particular characteristics. Lignin presents at least three basic functional groups in its structure: methoxylic, hydroxylic (alcoholic and phenolic) and the lateral propanic chain. Next to these functional groups, in lower amounts, there can be found carbonylic groups (approx. 1 group of CO at 5 C9 units), usually fixed on the lateral chain.[4,5]

Sometimes, the presence of carboxylic groups as phenol carboxylic acids or small amounts of lactonic groups (in some species of deciduous trees, tropical plants and annual plants) is noticed in lignin.[6,7] One of the pursued research directions consists in improving the properties of lignin

resulted from processes of chemical wood and annual plants processing through reactions that may lead to increased functionality and diversification of lignin applications. Due to its regeneration capacity through photosynthesis, vegetal biomass and its components (including lignin) will become in the future sources of raw material with a high degree of capitalization. From this standpoint, the hydroxymethylation reaction offers important possibilities of increasing the functionality of lignin, which can thus become an important component in various adhesive systems.[8-12] This paper presents the results of a comparative study on the reactivity of two lignins isolated from annual plants (wheat straw and Sarkanda grass) and modified with formaldehyde, as well as the characterization of the derivatives synthesized, from a functionality and polymolecularity standpoint, by various spectral techniques (FTIR, UV-VIS, ^{1}H NMR, fluorescence) and chromatographic techniques (HPSEC).

EXPERIMENTAL

Materials

In this study, we used two types of lignin isolated through alkaline delignification process from annual plants: wheat straw (L1) and Sarkanda grass (L2), offered by Granite Company, Switzerland.

Methods

Lignin hydroxymethylation

37 g of lignin (u.a.) were gradually dissolved in 130 mL of 3% NaOH solution, at room temperature, under mechanical stirring for 1 h. The pH of the solution thus obtained was adjusted to 10.5, and respectively 12, using a solution of 3N NaOH. Then, a 24.1 mL solution of formaldehyde (37%) was added, while continuing the stirring at a temperature of 50 °C, respectively 90 °C, for 3 h. Periodically, each hour, samples were taken out of the reactor to determine the reactioned formaldehyde. After 3 h, the reaction mixture was cooled and treated with a solution of lN HCl, to obtain pH = 2. The hydroxymethyl derivatives were separated through centrifugation at 2500 rpm for 10 min and the precipitate was washed with distilled water and dried.[1,2,11] The chemical and spectral analyses of the products obtained allowed to determine the optimal reaction conditions as follows: temperature – 90 °C, pH = 10.5 and reaction time – 3 h. The lignin derivatives thus obtained were characterized using various spectral techniques and chromatography.

Analysis techniques

FT-IR spectroscopy

FT-IR spectroscopy can be used to track functional changes that occur in the structure of lignin derivatives as compared to the initial samples. FT-IR spectra were recorded in KBr pellet using a spectrometer DIGILAB-Excalibur FTS 2000, equipped with a heating device. The working parameters were the following: spectral range – 4000-400 cm^{-1}, resolution – 4 cm^{-1} and the number of scans – 24. The spectra obtained were processed using a specialized program of the Merlin-Biorad series, baseline correction and their normalization.

Nuclear magnetic resonance (NMR)

Nuclear magnetic resonance (NMR) offers the richest and most complete information regarding the structure of organic compounds. A Bruker Avance DRX 400 MhZ spectroscope was used for this purpose. For investigations, acetylation of lignin and derivates with the purpose of a better dissolution in DSMO-D_6 was necessary. [3] In order to obtain an adequate spectrum, 0.2 mmol/mL concentrations of the solutions are required. Spectrum processing was performed with a specialized program from the SpectraManager series.

High performance steric exclusion chromatography (HPSEC)

A TSK-GEL GMhr-M type column, with 5 μm granulation (produced by Sigma-Aldrich), was used for chromatography. The HPSEC system was equipped with a 9010 model pump, Waters 717 plus autosampler, UV-VIS Waters 486 detector. Tetrahydrofuran was used as mobile phase, with a 0.5 mL/min flow, the elution volume being of 100 μL, detection wavelength λ = 280 nm, and volume for injection of 50 μL. For the calibration of the HPSEC column, a polymer mix (standard) was used, with known molecular masses (polystyrene with molecular mass ranging from 484 to 682000, produced by Fluka), dissolved in tetrahydrofuran (the column separation field must be considered when choosing it). Data interpretation was realized with the aid of the Origin Lab 6 program.

RESULTS AND DISCUSSION

Chemical characterization of unmodified and modified lignin products

The hydroxymethylation reaction of lignin is one of the most well-known and frequently applied reactions and results in the introduction of most hydroxymethyl groups in the aromatic nucleus and even in the lateral chain. The performed experiments allowed highlighting the reactivity differences of the two lignin types during the interaction with formaldehyde in alkaline environment, for two values of temperature (50 °C and 90 °C) and pH (10.5 and 12.0). The data recorded evidence the transformations produced after the applied reaction on the one hand, and the concordance

between the results offered by different analysis techniques on the other hand. Applying the chemical and spectral investigation methods led to determining the functional group content (Table 1).

As can be seen in Table 1, the functional group content is modified for each type of lignin, depending on its nature and applied conditions. This information has been used in order to select the optimum reaction conditions (temperature – 90 °C, pH=10.5 and reaction time – 3 h), which allowed the synthesis of derivatives, with the characteristics presented in Table 2.

The characteristics were explained to highlight lignin reactivity through formaldehyde consumption. Following chemical and spectral analyses (Table 2), it was noticed that grass lignin modified at 90° C, for a three-hour reaction time, was characterized by a higher reactivity compared to the wheat straw product. The derivative synthesized from grass lignin presented a higher total OH group content, which was proved by the results obtained through the chemical method, as well as by the FT-IR spectroscopy data. The lignin derivatives were also characterized from a solubility standpoint in various solvents: furfuryl alcohol, NaOH 0.1 N solutions and acetylating mix (acetic anhydride and pyridine – v/v proportion of 1:1). Solubility determination had the purpose of identifying the solvents that could be used in characterization and usage of lignin. At the same time, determinations of humidity and ash content were performed (Table 3).

Table 1
Chemical characterization of unmodified and modified lignins

Samples	T, °C	pH	OH total groups[1]	Ar-OH groups[2]	OCH_3 groups[3]	Ak/Ar ratio[4]	C=O groups[5]	S/G ratio[6]
L1 (100-W-A)	Initial		1.06	0.93	0.94	0.72	0.80	0.82
	90	12.0	1.17	0.92	1.14	1.08	0.83	0.97
	90	10.5	1.16	0.98	1.13	1.20	0.93	0.97
	50	12.0	1.16	0.99	1.09	1.08	0.89	0.93
	50	10.5	1.24	0.95	1.11	1.05	0.86	0.86
L2 (100-S-A)	Initial		1.05	0.91	0.96	0.88	0.88	0.82
	90	12.0	1.19	0.95	1.16	1.40	1.00	0.89
	90	10.5	1.14	0.95	1.10	1.11	0.92	0.85
	50	12.0	1.20	0.96	1.11	1.19	0.94	0.91
	50	10.5	1.34	0.78	1.18	3.20	0.77	0.57

(L1) – 100-W-A – wheat straw, and (L2) – 100-S-A – Sarkanda grass
[1]OH total groups – content of OH total groups; [2]Ar-OH – content of OH phenol groups; [3]OCH_3 groups – content of metoxyl groups; [4]Ak/Ar ratio – ratio of aliphatic and aromatic groups; [5] C=O groups – content of carbonyl groups; [6] S/G ratio – ratio of syringyl (S) and guaiacyl (G) units

Table 2
Characteristics of initial and hydroxymethylation modified lignins under optimum conditions

Samples	Moles CH_2O reacted/100 g lignin[1]	OH/C_9[2]	Ar-OH, mmole/g lignin[3]	OH/C_9 groups FTIR[4]
L1 (100-W-A)	-	1.02	1.7-1.8	1.06
L1H	0.23/0.20	1.17	1.76	1.16
L2 (100-S-A)	-	1.07	1.8-1.9	1.05
L2H	0.21/0.17	1.18	1.32	1.34

L1H and L2H – hydroxymethyl derivatives
[1]The values corrected without CH_2O consumption in Cannizzaro reactions; [2]The OH groups content was determined by the chemical method with acetic anhydride in pyridine medium;[13] [3]The Ar-OH groups content was determined by the UV-Vis method;[11] [4]The OH groups content was determined from FTIR spectra[11]

Table 3
Characteristics of unmodified and hydroxymethylated lignins

Samples	T, °C	pH	Humidity, %	Ash, %	Solubility in 0.1N NaOH solution, %	Solubility in furfuryl alcohol, %	Acetylation mixture solubility, %
L1	Initial		5.0	2.5	98.5	88.50	97.5
(100-W-A)	90	12.0	4.2	1.43	96.2	56.0	96.3
	90	10.5	3.9	1.2	95.4	34.0	97.2
	50	12.0	2.8	1.8	98.0	89.80	95.3
	50	10.5	4.0	1.1	98.4	90.15	97.4
L2	Initial		5.4	4.1	98.5	92.10	97.0
(100-S-A)	90	12.0	5.0	1.5	95.7	87.9	96.8
	90	10.5	4.0	2.8	89.2	79.8	95.6
	50	12.0	5.5	3.4	90.6	71.6	93.1
	50	10.5	2.5	1.3	80.8	45	94.1

We can notice selective lignin solubility in the 0.1N NaOH solution: hydroxymethylated products present lower solubility due to condensation reactions with methylene links accompanying hydroxymethylation. Thus, from the two types of lignin studied, L1 and L1H, present better solubility compared to L2 and L2H. At the same time, it is noticed that in the case of lignins modified at pH 12 and reaction temperature of 50 °C, derivative solubility is lower compared to that recorded for products synthesized under other reaction conditions – a fact highlighted in the case of furfuryl alcohol usage and acetylation mix as solvents. The lowest solubility is encountered in the case of furfuryl alcohol usage.

Spectral characterization of studied lignin products

FT-IR spectroscopy

FT-IR spectroscopy can be used successfully for estimating the content of various functional groups, such as total alcoholic, phenolic, carbonylic or carboxylic groups.[14] The spectral data are analyzed comparatively with those for unmodified lignins, in order to determine the differences appearing at a functional level. For example, FT-IR spectra for the two unmodified and hydroxymethylated lignin types are presented in Figures 1 and 2.

In the presented specta, in the 3000-2848 cm^{-1} range, vibrations specific to C-H aromatic bonds, aliphatic bonds and hydroxyphenolic groups are noticed. Unmodified lignin presents higher peak intensity in the 1710-1600 cm^{-1} range, which corresponds to etheric bonds and carbonylic groups bound by the aromatic nucleus. The 1700-900 cm^{-1} range constitutes a region, where absorptions corresponding to guaiacyl and syringyl structural units, with different substitutions, are encountered. The obtained data highlight a higher S/G proportion in modified lignin than in witness lignin, an increase that may signify the presence of nuclei substituted with hydroxymethyl groups (Table 1). Absorptions specific to valence vibrations of carbonyl and carboxyl groups are encountered at 1710 cm^{-1}, and hydroxymethylated lignin samples have lower peak intensity because of substitute introduction. The 1400-1460 cm^{-1} range is affected by the excitation of the aromatic nucleus, which reacts with formaldehyde, and possible condensations with the formation of methylene chains that determine stronger vibrations and increased intensity of absorption bands may occur.

As for the spectral analysis of unmodified and hydroxymethylated grass lignin, it has been proven that the product separated from Sarkanda grass is characterized by an IR spectrum where signals can be found in the 3000-2848 cm^{-1} range, specific to aromatic C-H bonds, hydroxylic groups, as well as to hydroxymethyl groups introduced in the lignin structure through the hydroxymethylation reaction. Peak intensity is decreased in the case of modified lignin (L2H) in the 1700-900 cm^{-1} range, belonging mainly to substituted guayacil and syringyl structural units and to interactions among functional groups. The presence of absorption bands in the 1260-835 cm^{-1} range is considered specific especially to grass lignin, which presents a higher content of C-H bonds.[1,6] These data confirm modifications produced in the chemical structure of lignin.

NMR characterization of unmodified and hydroxymethylated lignins

Of all spectral techniques, nuclear magnetic resonance (NMR) offers the richest and most

complex structural information on organic compounds. For ^{1}H NMR spectroscopic characterization, lignin samples were subjected to acetylation in order to favor dissolution in the used solvent (DMSO-d_6).[1]

Figures 3 and 4 present ^{1}H NMR spectra recorded for unmodified and hydroxymethylated lignin; the results have been interpreted using literature data.[15,16]

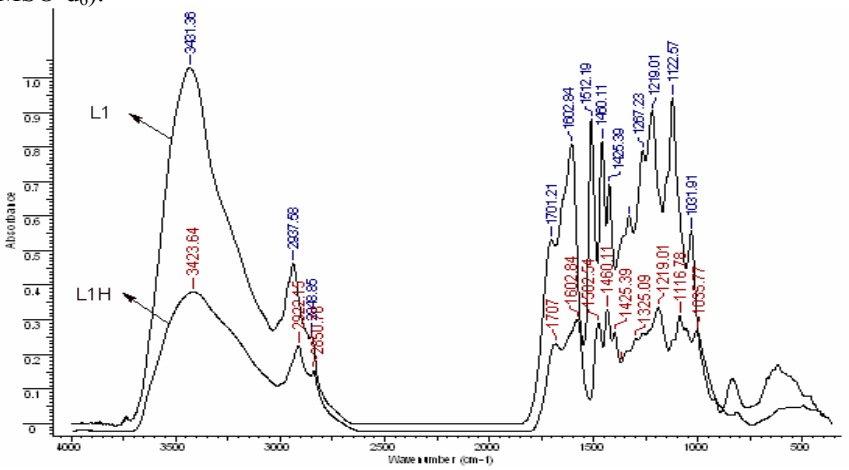

Figure 1: FT-IR spectra for L1 and L1H

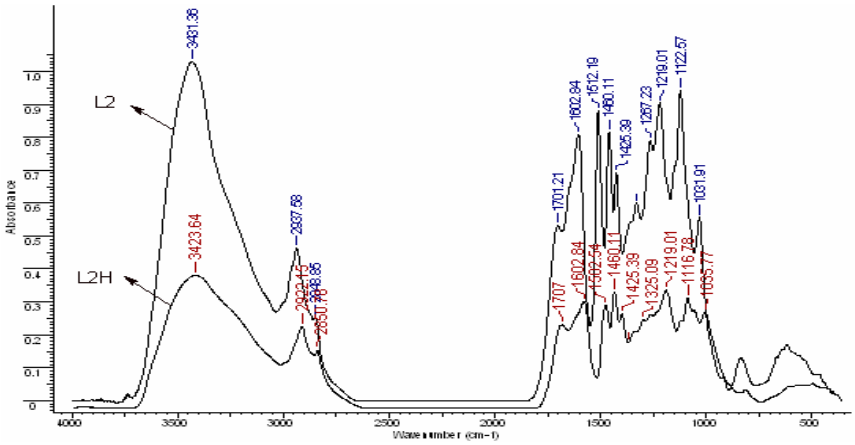

Figure 2: FT-IR spectra for L2 and L2H

From the analysis of spectra presented in Figures 3 and 4, it can be noted that lignin reacted with formaldehyde, introduced in the reaction environment, which is proven by the clear signals offered by ^{1}H NMR spectroscopy and completes the previously presented results obtained by chemical and spectral analyses. In the 9-8 ppm range, modifications of peak intensity take place, along with a clear delimitation of the peaks. This situation is determined by the reaction of lignin with formaldehyde, which is followed by the introduction of hydroxymethyl groups in aromatic nuclei, especially those with unbound phenolic OH. Signals appear in the 5.47-4.98 ppm range, specific to protons from the methoxyl group.

Modifications between 3.82-2.73 ppm also take place, this range being specific to methylene groups. The absorption peak intensity is lower in the case of unmodified lignin (L1) and the recorded signals do not present any clear delimitation. For hydroxymethylated lignin, there is a possibility of signal overlapping characteristic to functional groups introduced through the hydroxymethylation reaction and as a consequence of the acetylation reaction. Proton signals from the 2.2-0.8 ppm can be attributed to methyl and methylene groups from the lateral chain of phenyl propanic groups.

The presence of signals at 6.0 ppm in unmodified lignin proves the presence of a higher amount of syringyl units compared to guayacil,

which was also highlighted through chemical analyses on wheat straw lignin. Figures 5 and 6 present ^{1}H NMR spectra for hydroxymethylated Sarkanda grass lignin. The peaks in the 7-6 ppm range are wide, and the identification of signals specific to functional groups is more difficult, due to structural complexity. The signals at 6.97-6.6 ppm are characteristic to the protons from guaiacyl units, which are present in the lignin structure in greater amounts than the syringyl units. The most important spectral modifications are noted within the 5.99-4.83 ppm range, which can be attributed to the protons from methoxyl and hydroxymethyl groups.

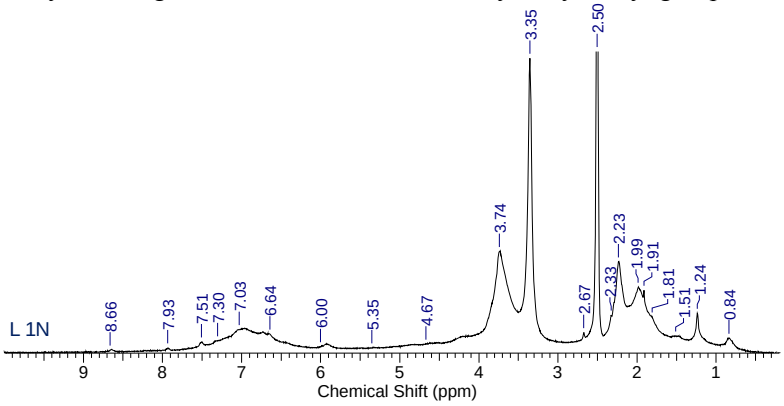

Figure 3: ^{1}H NMR spectrum for unmodified wheat straw lignin (L1N)

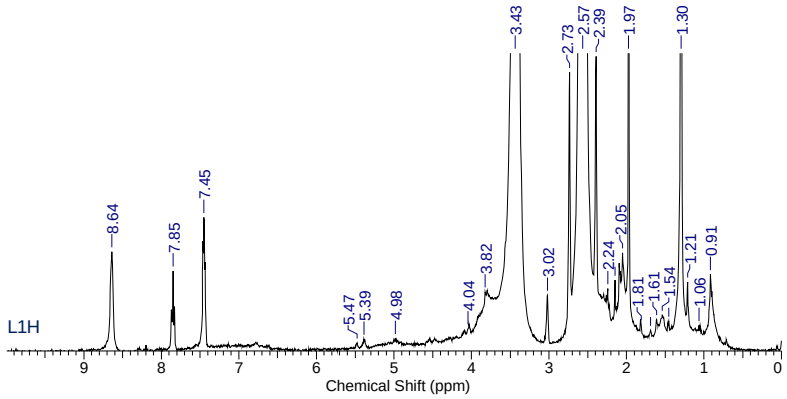

Figure 4: ^{1}H NMR spectrum for hydroxymethylated wheat straw lignin (L1H)

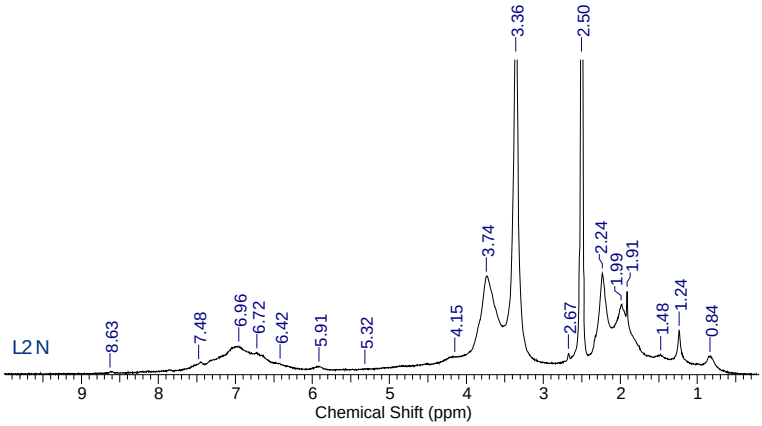

Figure 5: ^{1}H NMR spectrum for unmodified Sarkanda grass lignin (L2N)

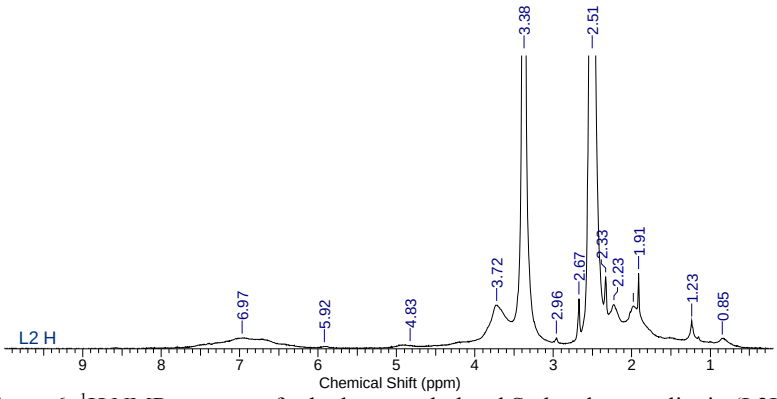

Figure 6: ^{1}H NMR spectrum for hydroxymethylated Sarkanda grass lignin (L2H)

Table 4
Values of Mw, Mn, Mw/Mn, retention time and elution volume for acetylated derivatives of unmodified and hydroxymethylated lignins

Samples	Retention time (min)	Ve initial/Ve final (mL)	Mw (g/mol)	Mn (g/mol)	Mw/Mn (g/mol)
L1	32.99	6.42/16.49	1535	46	33.37
L1H	29.12	4.133/14.563	1651	47.2	35.33
L2	35.94	7.5/17.97	831	89	9.34
L2H	34.78	5.651/17.391	930	90	47.40

Initial Ve- initial elution volume, final Ve – final elution volume, Mw – average molecular mass, Mn – numeric average molecular mass, Mw/Mn – polydispersity

HPSEC characterization of hydroxymethylated lignin

The method consists in passing a polydispersed polymer solution through a stuffing layer with pores of certain dimensions; the pores retain macromolecules of adequate dimensions (smaller than the average pore diameter), while macromolecules of greater dimensions leave the column with the solvent. Detection can be performed with a UV-VIS detector, IR, fluorescence or the conductometric method. Literature sources[17] recommend the use of this technique for evaluating the polydispersity of lignins in acetylated form, dissolved in tetrahydrofuran.[1]

This technique has been used for the characterization of both unmodified and modified lignins. Unfortunately, the adopted procedure did not always allow the complete solubilization of the analyzed samples, so the presented results must be considered strictly for orientation purposes, in terms of directions to be followed in carrying out some processes.

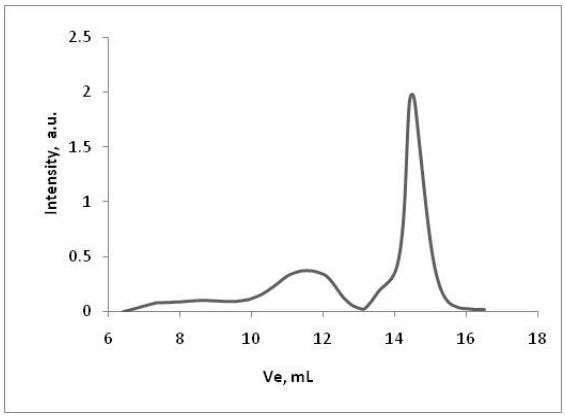

Figure 7: HPSEC chromatogram for unmodified wheat straw lignin (L1)

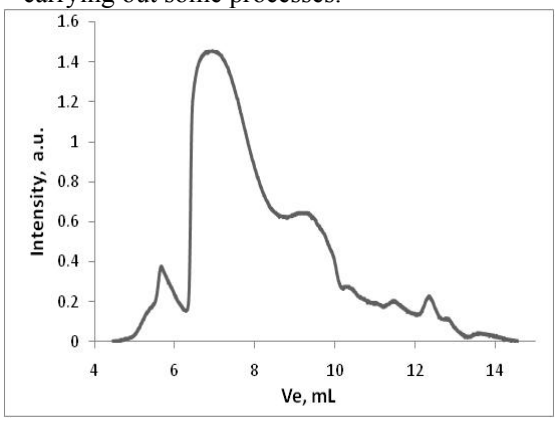

Figure 8: HPSEC chromatogram for hydroxymethylation wheat straw lignin (L1H)

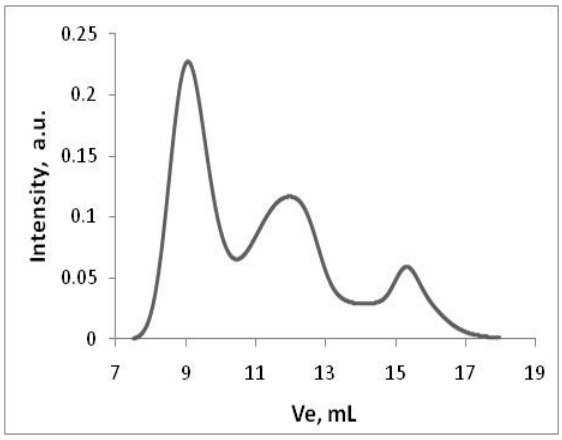

Figure 9: HPSEC chromatogram for unmodified Sarkanda grass lignin (L2)

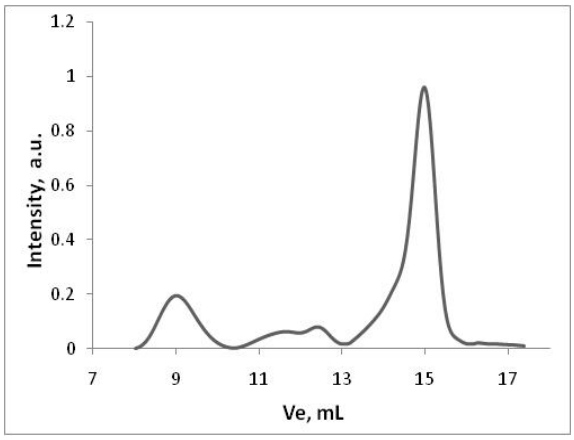

Figure 10: HPSEC chromatograms for hydroxymethylated Sarkanda grass lignin (L2H)

Figures 7-10 present the chromatograms of unmodified and hydroxymethylated lignins, and Table 4 systematizes the values of average molecular masses.

The obtained data can be considered characteristic of the modifications that took place in hydroxymethylated lignin on the molecular level.

A more pronounced tendency of homogenization towards the level of fractions of low molecular mass is noticed in the case of grass lignin, as a consequence of hydroxymethylation in correlation with its higher reactivity and the initial structural uniformity of the under-layer subjected to the reaction. In this case, a visible separation of fractions takes place at lower values of the elution volume – those with lower molecular dimensions, prevailing in this case, follow fractions with high molecular mass, which are the first eluted on the column.

CONCLUSION

The study focusing on the reactivity of the two types of lignin to formaldehyde, in alkaline environment, at two different temperatures (50 °C and 90 °C) and pH values (10.5 and 12.0), revealed that the Sarkanda grass product (L2) exhibited the highest reactivity. The following reaction conditions were determined as the optimum for obtaining the maximum content of functional groups: CH_2-OH/L = 0.285 (w/w), NaOH/L = 0.08 (w/w), T = 90 °C, pH = 10.5).

The use of spectral techniques, such as FTIR, UV-VIS, fluorescence, ^{1}H NMR allowed for highlighting the functionality modifications occurring consequently to hydroxymethylation.

Information on polymolecularity transformations that corresponded to the reactivity of the two lignins and their functionality were obtained through high-performance steric exclusion chromatography applied to initial and hydroxymethylated products.

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