CHARACTERIZATION OF NON-WOOD LIGNIN AND ITS HYDOXYMETHYLATED DERIVATIVES BY SPECTROSCOPY AND SELF-ASSEMBLING INVESTIGATIONS

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Two types of non-wood lignins, namely wheat straw lignin (L1) and Sarkanda grass lignin (L2) (offered by Granit Recherche Dévloppement S.A., Lausanne) were characterized, together with their hydroxymethylated derivatives. To this end, the afore-mentioned lignins have been subjected to hydroxymethylation, under optimal conditions of temperature, time and pH. The unmodified and the hydroxymethylated lignins were analyzed by spectroscopic methods (FTIR, UV-VIS and fluorescence spectroscopy), and atomic force microscopy (AFM). The results point out that the reactivity level of lignin depends on its origin and structure of lignin, while the characteristics of the synthesized products result from the modifications induced by the new functional groups.

Keywords: lignin, hydroxymethylation, FTIR, UV-VIS, fluorescence, AFM

INTRODUCTION

Lignin, one of the main structural polymers present in plant tissue, represents an important participant to the complex formation and transformation of organic material in the biosphere.[1]

It is the second most abundant polymer on the Earth, following cellulose, about 50 million tons of lignin being produced annually as a residue of pulp production. However, due to its numerous functional groups and bio-properties, lignin offers new important possibilities in the preparation of ecological adhesives, in water treatment and agriculture.[2,3] Based on these considerations, chemical modifications are performed to adjust the functional properties for specific applications.

Several attempts have been made to analyze and understand the chemical structure of lignin and its mechanism of self-assembly.[4-6] Although some of its main characteristics, such as functional groups and general topography have been investigated, the researchers could not reach a general agreement on its structure, mainly due to the existence of several parameters determining its properties, such as the origin of lignin, the procedure of sample preparation and the type of substrate used for the analysis.[7]

The investigation on the characteristics of lignin in a monolayer may help understand the way in which these macromolecules are arranged on the cellulose fibers. Obtained directly from bio-resources and annual plants, lignin is available in large quantities, is an easily renewable and cheap product, it can be integrated into ecologic adhesive systems or can be used as coating for smooth surfaces.[8-10]

The present paper discusses the results obtained from the characterization of nonwood plant lignins and of their derivatives, by spectroscopic investigations (FTIR, UV-VIS and fluorescence spectroscopy).[5,6,11] At the same time, based on AFM investigations, the differences between the chemical structures and self-assembling characteristics are compared for four types of lignin polymers: wheat straw lignin (L1), Sarkanda grass lignin (L2) and hydroxymethylated lignins (L1H and L2H).

EXPERIMENTAL

Materials

The lignins used in the present study were obtained from non-wood plants of different origins: one from wheat straw (L1) and the other from Sarkanda grass (L2), provided by Granit Company, with the properties presented in Table 1.

Table 1
The characteristics of unmodified lignins L_1 and L_2

Characteristics	L1	L2
Acid insoluble lignin, %	90	87
Acid soluble lignin, %	1	2
COOH, mmole/g	3.8	3.3
Aromatic OH, mmole/g	1.7-1.8	1.8-1.9
OH/C9 (groups chemical method)	1.02	1.07
OH/C9 (FTIR)	1.06	1.05
pH (10% dispersion)	2.7	3.2
Mw	3510	4310
T softening, °C	170	163
Solubility in furfuryl alcohol, %	88.5	84
Solubility aqueous alkali, pH = 12.0, %	98.5	98.5
Ash, %	2.5	4.1

Methods

Lignin hydroxymethylation

The lignin samples were modified through hydroxymethylation, by a procedure presented elsewhere.[6]

FTIR spectroscopy

The FTIR spectra of the unmodified and hydroxymethylated lignin samples, registered in a KBr pellet, with a DIGILAB–EXCALIBUR FTS 2000 spectrometer, were recorded in the 4000÷400 cm^{-1} range, at a resolution of 4 cm^{-1}.

UV-VIS spectroscopy

UV-VIS absorption spectra were registered using solutions of lignins and of their derivatives, dissolved in a sodium hydroxide solution (0.2 N), on a JASCO 550 spectrophotometer equipped with a quartz cell (1 mL volume for liquids): absorption region 200÷800 nm, scan speed – 200 nm/min and resolution – 1 nm. The processing of the spectra obtained from the tests was carried out with a special Spectra Manager series program.

Fluorescence spectroscopy

The fluorescence emissions of both unmodified and modified lignins were registered on a luminescence spectrometer Perkin Elmer LS 50 B, using fluorescence cells with a liquid volume of 1 mL, 10 mm path length and 350 nm excitation wavelength, in the 400÷600 nm absorption region. The lignins and their derivatives were dissolved in a sodium hydroxide solution. The processing of the spectra was performed with a specialized FLWinLab series program.

Atomic force microscopy

Sample preparation. For each type of lignin, a stock solution was prepared by dissolving lignin powder in NaOH (0.1 N). The solution was stored for no less than 48 h, to allow lignin dissolution. Thin lignin layers have been prepared on freshly-cleaved 1.5 x 1.5 cm mica substrates,[20] without any pre-treatment, to avoid the introduction of inappropriate influencing factors. A drop of 50 µL solution was placed on a stable horizontal mica surface and left for 15 min, to let the lignin polymer begin the self-assembling process and adhere to the surface, after which it was spread by the spin-coating technique assuring uniform distribution.

AFM scans. All microscopic measurements were carried out on a Ntegra Prima AFM device. The scans were accomplished in semi-contact AFM mode, by utilizing Etalon tips purchased

from the NT-MDT Company. All samples were first analyzed on the 5 x 5 μm scanned surface and then the scan was focused to 2 x 2 μm, the statistic data being calculated for 2 x 2 μm. 2D and 3D graph representations (resolution of 256 x 256 points) were performed using a Nova software. No other modifications, except substrate corrections, have been made on the scanned images. The tapping mode consisted in oscillating the cantilever at its resonance frequency (120-200 kHz) and lightly "tapping" the tip on the surface during scanning, in order to avoid the scratching of the scanned surface.

RESULTS AND DISCUSSION

The chemistry of lignin, especially the presence of hydroxylic (aromatic and aliphatic) groups explains its large utilization.[6,8,9,13,14] As any lignin macromolecule, the studied lignins also consist of p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units. Figure 1 presents the structure of wheat straw lignin, as proposed by Digabel F. Le.[12]

The abundance of guaiacyl units in the structure of non-wood lignins provides an important possibility to improve both the interaction capacities and the reactivity through hydroxymethylation. That is why, lignins L1 and L2 have undergone the reaction of hydroxymethylation with formaldehyde. This transformation is based on the introduction of hydroxymethyl groups mainly at three positions of the aromatic nucleus, the possible reactions being represented[5,15] schematically in Figure 2.

These transformations offer new possibilities to extend the potential applications of lignin, which could be anticipated by spectroscopic investigations,[16,17] thus allowing to establish new functionalities for determining new properties.

FTIR spectroscopy

Both unmodified and modified lignin samples were characterized by FTIR spectroscopy, the numerous modifications observed (Table 2) being due to the hydroxymethylation reaction performed with formaldehyde.

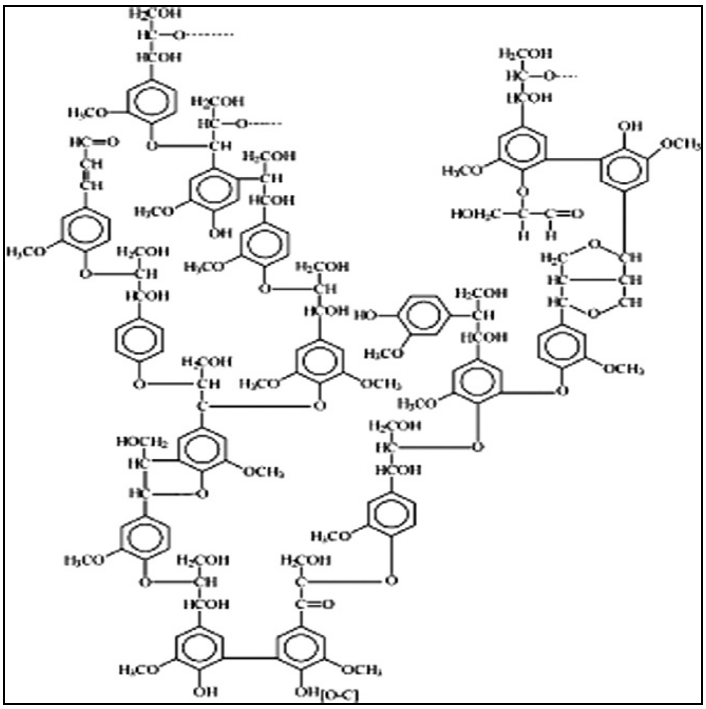


Figure 1: Structure of wheat straw lignin

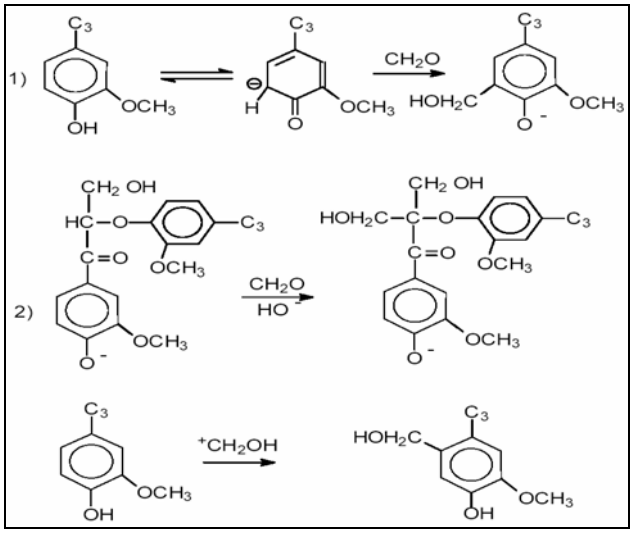


Figure 2: Hydroxymethylation reaction of lignin units

Table 2
FTIR characteristics of analyzed lignins

Wavenumber (cm^{-1})				Type of vibration
L1	L1H	L2	L2H	
3431	3433	3423	3417	Hydroxyl groups in phenolic and aliphatic structures CH stretching in aromatic methoxyl groups and aliphatic methyl and methylene groups of the side chains
2937	2937	2922	2920	CH stretching in aromatic methoxyl groups and methyl and methylene groups of the side chains
2848	–	2850	2848	C=O stretching in conjugated *p*-substituted aryl ketones
1701	1710	1703	1705	C=C stretching of the aromatic ring (S), CH deformation
1602	1635	1602	1600	C=C stretching of the aromatic ring (G), CH deformation
1512	1576	1512	1510	C–H asymmetric deformation in CH_2 and CH_3
1460	1460	1460	1460	C–H asymmetric deformation in $–OCH_3$
1425	1423	1425	1425	Guaiacyl ring breathing, C–O stretch in lignin, C–O linkage in guaiacyl aromatic methoxyl groups
1357	1379	1361	–	Syringyl ring breathing with C–O stretching
1328	–	1328	1327	Aromatic C–H in-plane deformation, typical of G units,
1267	1263	1267	1263	Aromatic C–H in-plane deformation (typical of S units) plus secondary alcohols plus C=O stretching
1219	1219	1226	1219	C–O deformation in secondary alcohols and aliphatic ethers
1086	1080	1085	1079	Deformation vibrations of the C–O bands in primary alcohols

The overall adsorption band range is between 3000 and 2848 cm^{-1}, which is specific to both hydroxyl groups, and C-H aromatic and aliphatic bonds.

Unmodified lignin presents an intensified FTIR adsorption band in the 1710-1600 cm^{-1} region, which is specific to the ether and carbonyl groups bonded to the aromatic nucleus. The 1700-900 cm^{-1} region is characteristic especially of the guaiacyl (G) and syringyl (S) units. The valence vibrations of carbonyl and carboxyl groups may be observed in the region of the 1710 cm^{-1} adsorption band. On the other hand, the

modified lignin samples present lower peak intensity; also, few bands characteristic of the hydroxymethyl groups may be noticed, as a result of the hydroxymethylation reaction.

The higher number of methyl and methylene groups can be correlated with the large number of syringyl units, which also agrees with the intensity of the band at 1460 cm^{-1}, assigned to C–H deformations, in syringyl derivatives. This band was observed to be more intense in modified lignins as compared to the unmodified ones.

The FTIR spectra evidence several significant differences. Thus, in the case of unmodified lignins, the intensity of the band at 1576 cm^{-1} (assigned to the C=C stretching of the aromatic ring – syringyl and C–H deformation) is very weak, compared to that of the modified ones. The higher intensities are explained by the higher ratio of Ak/Ar, S/G groups and methoxy groups (OCH_3) and carbonyl groups (C=O) in its structure.

The intensities of the bands at 1330 cm^{-1} (C–O vibrations in syringyl derivatives), 1230 cm^{-1} (syringyl ring breathing with C–O stretching) and 1115 cm^{-1} (aromatic C–H in-plane deformation of the syringyl units, secondary alcohols and C=O stretching) are higher in the modified lignins; in the case of unmodified lignins, these bands are either overlapped or absent.

UV-VIS spectroscopy

The lignin macromolecules can absorb light in the UV or visible regions of the electromagnetic spectrum, due to the high conjugation degree of the aromatic nucleus, while the spectral patterns show that the free hydroxyl and ether groups contribute[5] significantly to the absorption maximum around 280 nm. Some data obtained from the spectral analysis of lignins dissolved in a 0.2 M NaOH solution are listed in Table 3.

Table 3
UV-VIS characteristics of lignin samples

	Types of lignin			
λ, *nm*	L1	L1H	L2	L2H
u_1	245	250	246	248
u_2	285	280	285	281

u_1 – first shoulder, u_2 – second shoulder

As to the unmodified lignin samples, the biphenyl derivatives present absorption around 240 nm and at 288 nm wavelength, and guaiacyl and syringyl units can be identified. The high S/G units ratio is obvious, owing to the hydroxymethyl groups present in a higher number in the modified lignins. The low intensity probably appears due to the substitution with hydroxymethyl groups on the aromatic nucleus, or to the possible crosslinking reactions through methylene bridges. Meanwhile, the differences appearing in the spectra of the two types of investigated products, caused by the shifting of the absorption maxima from higher to lower wavelength values, could be explained by a higher number of substituted units in the modified lignins. The specific structure of the unmodified and modified lignin macromolecules can be confirmed by UV-VIS spectroscopy analysis, on taking into account the specific absorption wavelength, λ = 280 nm. The UV-VIS method is uniquely suited to monitor any changes in the conjugated phenolics. The hydroxymethylation reaction of the lignin macromolecule induces a slight movement of the π electron in the aromatic structures, that is why, the absorption peaks are shifted to lower wavelength values, so that a hypsochromic effect may be noticed.

Although the UV-VIS spectra do not permit to specify precisely several detailed elements on the reactions observed, these spectra, along with other investigation methods used, offer additional arguments about the introduction of hydroxymethyl groups in the lignin macromolecule, as well as some information on the differences in

lignin reactivity, which causes characteristic changes in its functionality.

Fluorescence spectroscopy

Study of the fluorescence phenomena involved dissolution of the lignin samples in a 0.2 M concentrated NaOH solution. The results are shown in Figures 3-4. The fluorescence method involves excitation of the electrons from the lignin macromolecule of lignin, induced by the chromophore groups (excitation wavelength – 350 nm).

The analysis of the spectra plotted in Figures 3 and 4 shows that the emissions were evaluated in the 400-600 nm range. A slight shift of the peaks can be noticed in the case of modified lignins (L1H and L2H), which indicates increased functionalities.

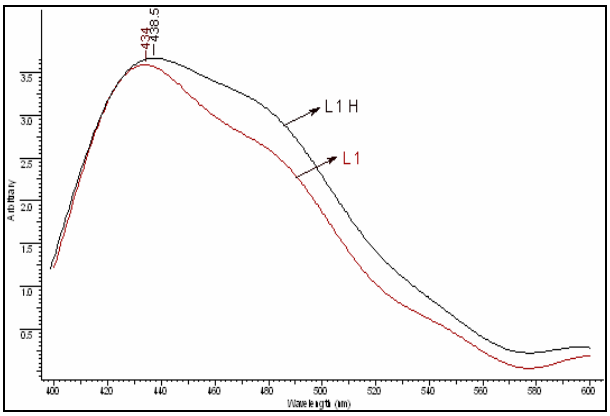


Figure 3: Fluorescence spectra of wheat straw (L1) and hydroxymethylated lignins (L1H)

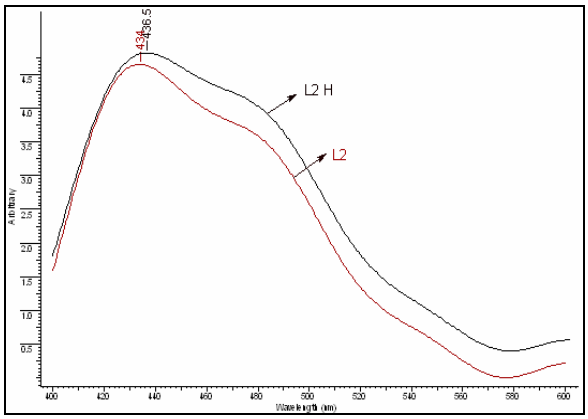


Figure 4: Fluorescence spectra of Sarkanda grass (L2) and hydroxymethylated lignins (L2H)

Atomic force microscopy investigations

The AFM technique provided relatively similar images for the unmodified lignins; although, in the case of modified lignins, at first sight, the surface seems to be almost entirely different.

The AFM images of mica after lignin deposition are shown in Figures 5-8. It has been observed that, in all cases, both lignins are presented in the form of globular particles, occurring in sizes ranging from 100 to 500 nm, with the only exception of the hydroxymethylated lignin (L2H), in which the distribution is approximately uniform. This globular structure of the lignin macromolecule has been also observed by other researchers, who utilized AFM or SEM techniques for either isolated or photochemically polymerized lignin.[18-20,24]

Highly-ordered structures can be observed when larger surfaces are scanned (5 x 5 μm), especially in the case of L1, where large circular cluster structures (over 1 μm) are formed. These self-assembling characteristics can be useful in different lignin applications (*e. g.* as adhesives or adsorbents).

This law is well-respected on small surfaces (1 x 1 μm) for all investigated polymers; in the case of larger surfaces, a slight tendency for aggregation can be observed. In most cases, the granules are evenly distributed on the sample and individual granules can be distinguished. The changes in the nanoparticle size as a function of chemical composition were assessed using height histograms of the same size surfaces, for both lignin types (Fig. 9). The analysis of the histograms reveals that each sample is characterized by a specific average height (the interval limited by the inflection points of the Gaussian curve); comparatively with wheat straw lignin, Sarkanda grass lignin (L2) is characterized by a broad height distribution, thus the 3D image of its surface seems to be covered by a quite thin layer.

To observe the differences induced by hydroxymethylation, a comparative graphical representation was presented in Figure 10 (a and b).

The average height is visibly diminished for both lignins (L1H and L2H), especially in the case of Sarkanda grass lignin (L2H). The 3D organization seems to be different in the case of freshly prepared samples of hydroxymethylated lignins; on the other hand, histogram representations appeared quite similar 10 days after the sample preparation.

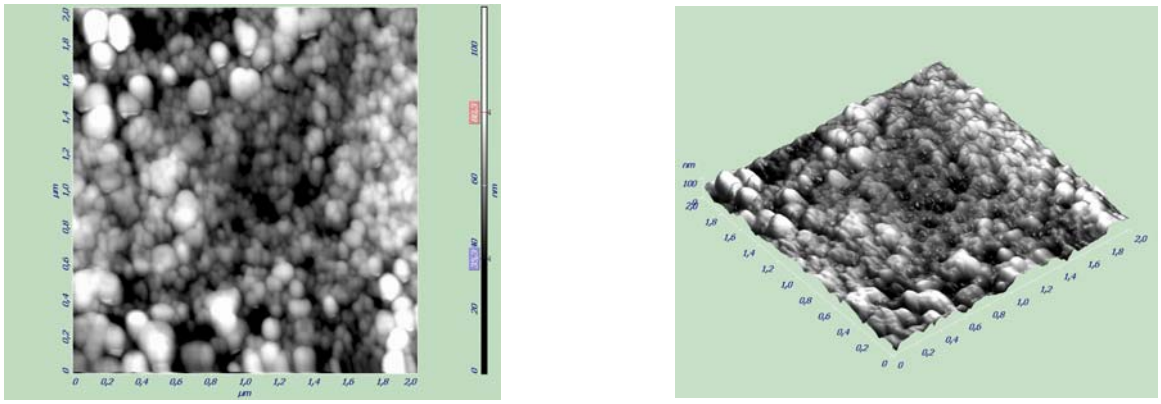


Figure 5: AFM image (2 x 2 µm) of lignin L1 on the surface of mica

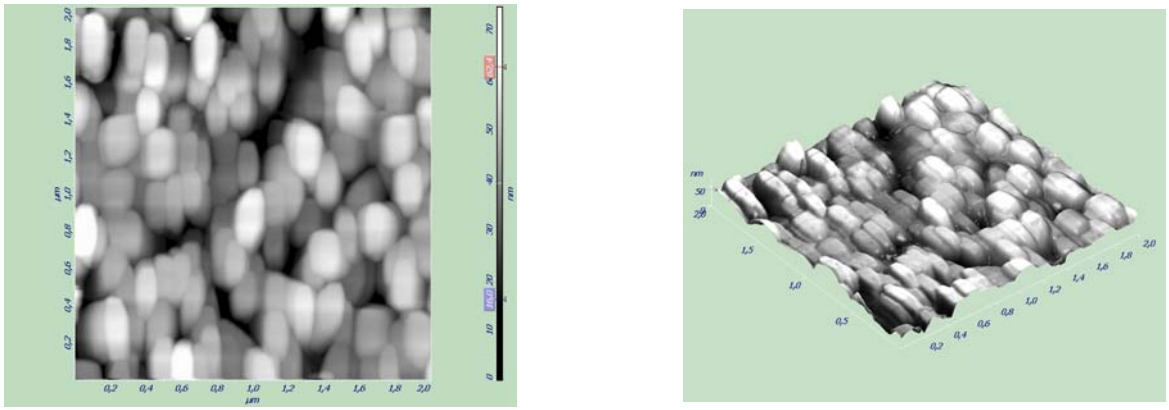


Figure 6: AFM image (2 x 2 µm) of lignin L1H on the surface of mica

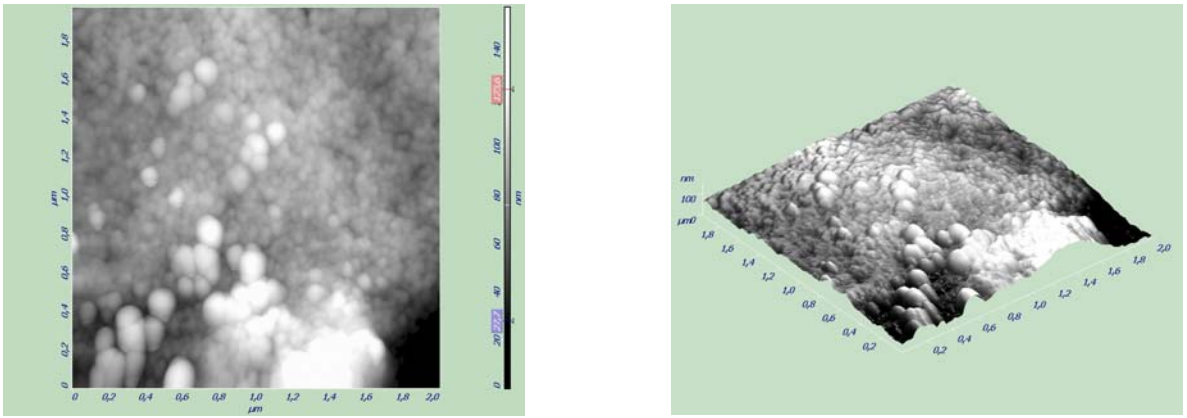


Figure 7: AFM image (2 x 2 µm) of lignin L2 on the surface of mica

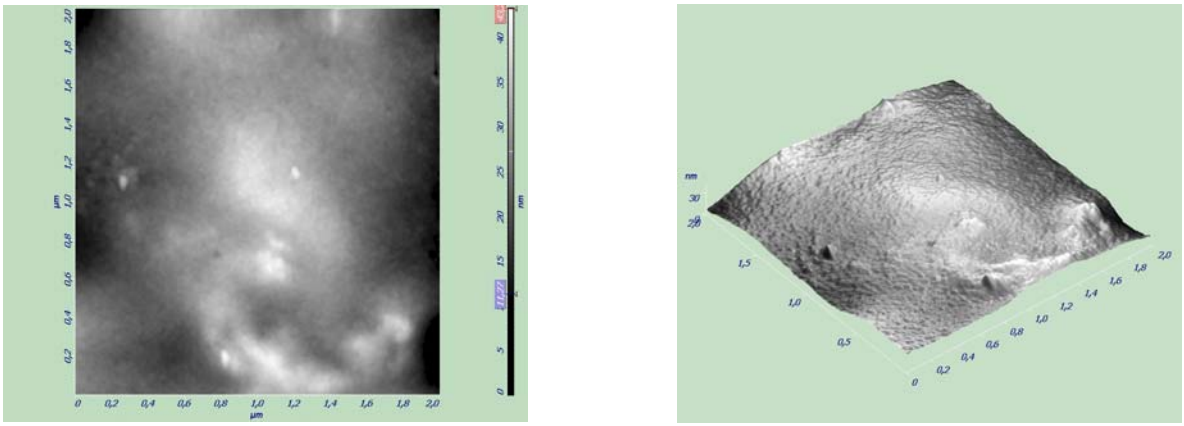


Figure 8: AFM image (2 x 2 μm) of lignin L2H on the surface of mica

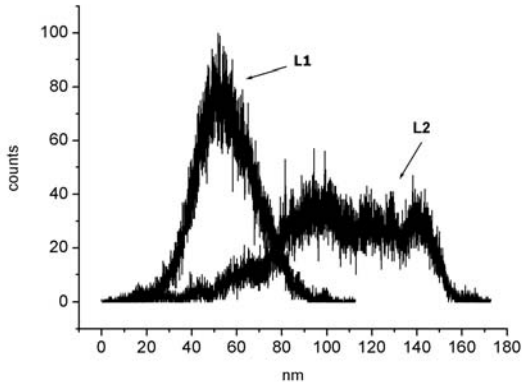
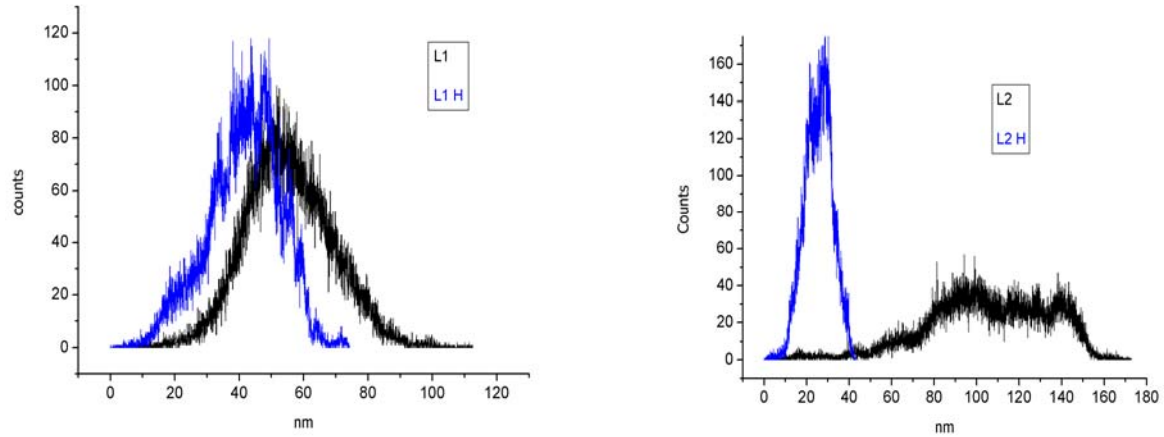


Figure 9: Graphical representation of height variations of wheat straw (L1) and Sarkanda grass (L2) lignins



a b

Figure 10: Comparative representations of histograms for unmodified and hydroxymethylated lignins: L1 and L1H (a) and L2 and L2H (b)

The topographic analysis indicates that the hydroxymethylated forms of both lignins tend to cover the surface in better organized thin layers. This behavior can be determined, on the one hand, by the chemical modifications induced directly by hydroxymethylation while, on the other hand, it can be attributed to the changes appearing in the Ak/Ar ratio and C=O groups. These observations agree with the

information obtained from UV-VIS and FTIR spectra. Also, all chemically induced modifications lead to changes in the S/G ratio (Table 4),[21,22,23] influencing the secondary and tertiary structures of the lignin macromolecules which, in their turn, may influence the self-assembling behavior by increasing their linkage to the selected substrate.

Table 4
Functional groups of non-wood lignin samples and of their derivatives

Samples	T, °C	pH	OH total groups	Ar-OH groups	OCH_3 groups	Ak/Ar ratio	C=O groups	S/G ratio
L1	Initial		1.06	0.93	0.94	0.72	0.80	0.82
L1H	90	10.5	1.16	0.98	1.13	1.20	0.93	0.97
L2	Initial		1.05	0.91	0.96	0.88	0.88	0.82
L2H	90	55	1.14	0.95	1.10	0.96	0.92	0.85

Table 5
Statistical analysis of lignin samples

Samples	Characteristics		
	RMS	AR	S
L1	13.64	9.10	10.47
L1H	11.27	9.01	10.18
L2	28.87	23.09	11.00
L2H	6.54	5.32	9.40

The statistical data and the index of analysis (Table 5) show that the 2 x 2 μm surface is relatively smooth in all samples. The most important difference appears in the roughness of the surfaces. Examining the statistic indexes of topography, one may observe that the Root Mean Square (RMS) and Average Roughness (AR) values changed insignificantly for L1, both before and after modification, while its weight decreased when L2 is chemically modified (Table 5). The values of entropy (S) are surprisingly the same (10 ± 1), which may be due to the same basic structure of the lignin macromolecule.

CONCLUSIONS

1. FTIR analysis evidences the main structural characteristics of lignin, both non-modified and modified through hydroxymethylation.

2. The specific structure of lignin was confirmed by UV/VIS spectroscopy; absorption at λ = 280 nm is characteristic of non-modified lignin. The hydroxylmethylation reaction induces a hypsochromic effect, involving the excitation of electrons in the lignin macromolecule, induced by the chromophoric groups.

3. Fluorescence spectroscopy of lignin is based on the excitation wavelength at λ = 350 nm, being induced by a high lignin activity. Fluorescence emission measurements were performed in the 400-600 nm range.

4. AFM characterization offers a general overview on the self-assembling properties of the lignin polymer.

5. The morphology of lignin in the self-assembled layers depends on its chemical composition and origin. After hydroxymethylation, roughness of the self-assembled layers decreases in the case of Sarkanda grass lignin while, in the case of wheat straw lignin, the RMS parameter is nearly unchanged.

6. Probably, the basic initial association characteristics remain the same, as certified by the entropy of the arrangement on the surface of mica.

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