



Obtaining lignin nanoparticles by sonication



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ABSTRACT

Lignin, the main natural aromatic polymer was always aroused researchers interest. Currently around 90% of this biomaterial is burned for energy. It has a very complex and complicated structure which depends on the separation method and plant species, what determine difficulties to use as a raw material widely. This research presents a physical method to modify lignin by ultrasonic irradiation in order to obtain nanoparticles. The nanoparticles synthesized were dimensionally and morphologically characterized. At the same time the preoccupations were to determine the structural and compositional changes that occurred after sonication. To achieve this, two types of commercial lignins (wheat straw and Sarkanda grass) were used and the modifications were analyzed by FTIR-spectroscopy, GPC-chromatography, ³¹P-NMR-spectroscopy and HSQC0. The results confirm that the compositional and structural changes of nanoparticles obtained are not significantly modified at the intensity applied but depend on the nature of lignin.

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1. Introduction

Lignin was always attracted the interest of researchers being the second natural polymer as spreading after cellulose and categorized as a renewable resource. This made lignin a very interesting topic for researchers and companies. However, its complexity and unevenness has made it difficult until now to know its precise structure and also how it can be transformed into a widely used raw material. Due to the important modifications that occur in the native lignin structure during the extraction procedure, lignin can be mainly classified by separation methods [1]. Lignin is poorly understood in terms of chemistry and structure compared with other wood compounds such as cellulose. The knowledge of this complex biopolymer is still delayed due to its various covalent bonds, resistance to degradation, heterogeneous nature, the absence of selective and convenient analysis methods and the lack of efficient isolation methods [2,3]. The control and tailoring of lignin multifunctional nature is the basis for the exploitation of this fascinating still under exploration material. Depending on lignin modifications a wide array of different applications can be envisaged: fuels, binders, biocides, chemicals, paper additives, concrete additive, dispersants, cosmetics, lubricants, paints. Recently, with

the aim to extend/develop special applications lignin was considered a possible starting material to obtain new nanostructured products. Wood cell wall is biosynthesized basically by self assembly principles of cell wall proteins, polysaccharides and lignin [4]. Fundamentally lignocellulosic materials are made up by a nanometer scale building. This confers important properties (mechanical, optical) for biomass [5]. More specifically lignin displays fractal structure and interesting aggregation behavior [6]. Aiming to conveying the above fundamental findings into new materials we decided to focus and exploit the lignin aggregation processes to synthesize nanoparticles. Nanotechnology allows the use of chemical, physical and biological effects that do not occur outside the nano scale which are environmental friendly in comparison with inorganic materials. Sonochemical synthesis solution is an effective method to prepare various nanomaterials [7]. Zimmiewska et al. [8] prepared nanoparticles based on lignin by ultrasonication for textile fiber treatments. After treatments fiber acquired antistatic, antibacterial and ultraviolet radiation absorbing properties. In 1993 Schilling [9] obtained lignin nano/microparticles (~300 Å) by hydroxymethylation. These were integrated into the production of water-based ink. Another way to obtain lignin micro/nanoparticles is represented by epoxydation reaction. By the epoxydation of some commercial lignin (Protobind type), nanoparticles based on modified lignin were detected in liquid phase [10,11]. Both hydroxymethylated and epoxydated nano

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lignins were used in biocide systems in wood protection [12]. Preliminary results on obtaining nanoparticles based on lignin transformation by ultrasound were presented in a previous paper [13]. In this effort the authors carried out an extensive structural and morphological characterization of such interesting materials.

The development of a variety of chemical reactions due to the action of ultrasound is strongly related to the phenomenon of cavitation. Chemical processes induced by ultrasound field occur only when the ultrasound intensity met conditions to develop of cavities. Regardless, the duration of ultrasonic irradiation intensities that do not reach a certain threshold result in no chemical reactions occurring [14]. The parameters: pressure, temperature, ultrasound settings and type of liquid, influence the formation of the radicals in different ways. The amount of radicals formed due to sonication is a function of the number of cavities created and the number of radicals that are formed per cavitation bubble [15]. Cavitation is the phenomenon of sequential formation (increase and decrease) of millions of vapor bubbles in liquid. Collapse or implosion of these cavities creates local high temperatures and pressures in a very short period of time, forming hot spots in cold liquids. Cavitation is induced by high frequency sonic waves (16 kHz – 100 MHz) through the mass of liquid [16]. The transient state of the local high pressures and temperatures gives rise to the primary chemical reactions occurring during and after micro bubbles collapse [17]. Ultrasound treatments typically induce the formation of radical species. In aqueous solution and in the presence of air both hydroxyl and superoxy radical species are formed. In case of polymers, ultrasounds can lead to homolytic scission of the macromolecular chains resulting pair radicals or polymerization of monomers. Ultrasound energy was used to increase lignin extraction yield and purity or to remove the major inorganic load from the alkali lignin with no significant modifications observed in the properties (composition, structure; at 20 kHz, 100 W) of the isolated lignin samples [18–20] or to polymerization for high molecular weight lignin [21]. Seino et al. [22] claim that the β -O-4 majority [23,24] bonds of lignin can be homolytically cleaved to some extent by sonication.

In this paper the results obtained in the modification of two types of commercial alkali lignins (from wheat straw and Sarkanda grass) using ultrasounds which were analyzed by FTIR-spectroscopy, GPC-chromatography, ^{31}P -NMR-spectroscopy and HSQC0 are presented.

2. Materials and methods

2.1. Materials

Wheat straw lignin (L1) and Sarkanda grass lignin (L2) were kindly supplied by Granit Recherche Development Company Switzerland. Their characteristics have been previously reported [25].

2.2. Lignin nanoparticles synthesis

Aqueous suspensions of lignin (0.7%) were used. The lignin suspensions were sonicated for 60 min. Finally, a homogeneous stable nanodispersion was obtained. The samples resulted in sonication of lignin were dried in mild conditions to be used in further analysis. Ultrasonication was performed by using an ultrasonic horn (Sonics & Materials VC600/CV17) at 20 KHz frequency, 600 W power [13].

2.3. Characterization

2.3.1. Light scattering

Particle size distribution analysis of nanoparticles was done with Shimadzu SALD 7001 instrument. This device allows the determination of the size of particles in a range of 15 nm to 500 μm by laser diffraction method.

2.3.2. TEM

Transmission electron microscopy investigations were performed on a HT7700 type Transmission Electron Microscope operating at 100 KV.

2.3.3. Gel permeation analysis

Gel permeation chromatograms were recorded by a Shimadzu LC 20AT liquid chromatograph equipped with a SPD M20A ultraviolet diode array (UV) detector set at 280 nm. Two columns in series: Agilent PL gel MIXED-D 5 μm , 1–40 K and PL gel 5 μm , MW range 500 Da – 20 kDa were connected for measurements. Calibration was achieved with polystyrene standards produced by Fluka. Tetrahydrofuran (THF) was used as eluent [26,27]. The lignin samples were acetylated with acetyl bromide before the injection, following the procedure described by Asikkala et al. [28].

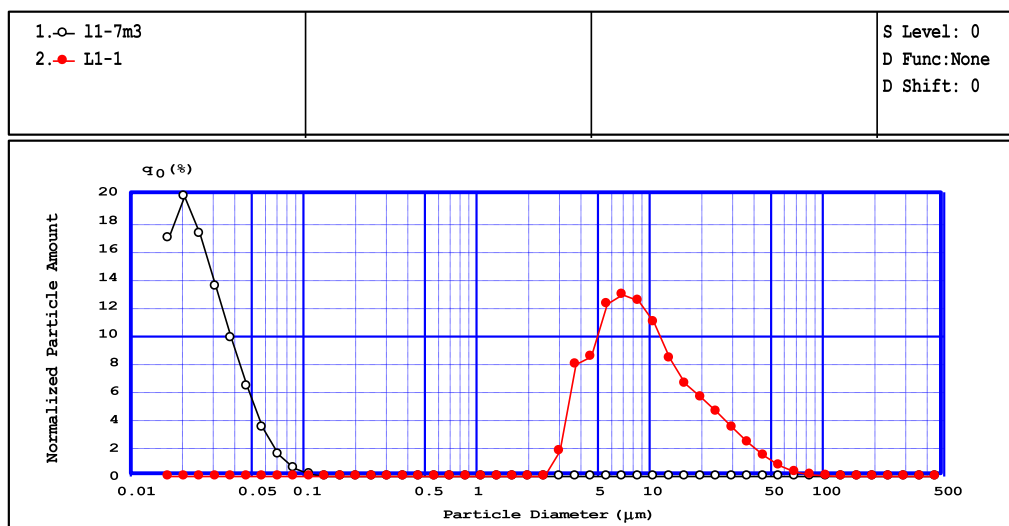


Fig. 1. Average particle size distribution analysis for wheat straw lignin (L1-1 L1) and for sonicated wheat straw lignin (l1-7m3).

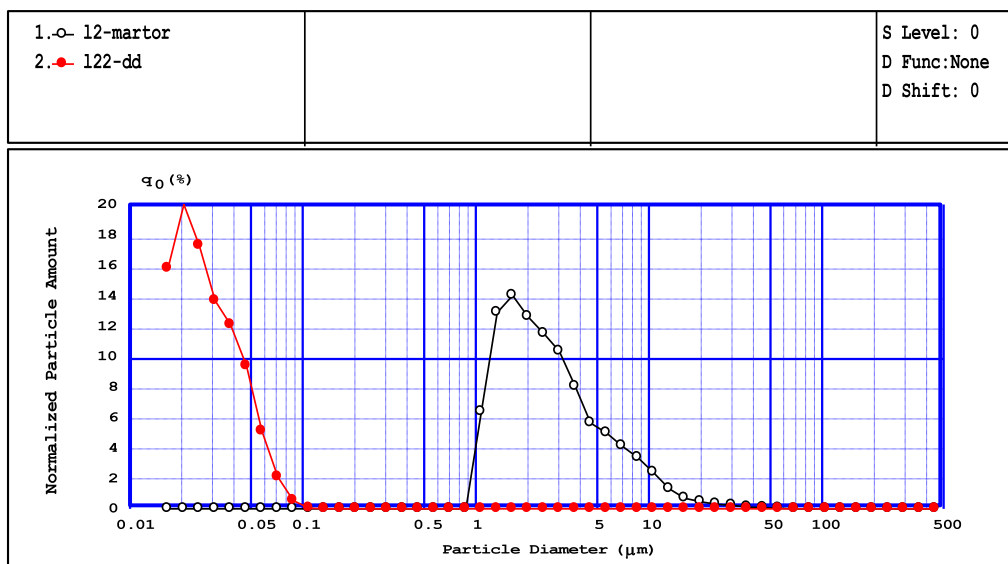


Fig. 2. Average particle size distribution analysis for Sarkanda grass lignin (I2-martor) and for sonicated Sarkanda grass lignin (I22-dd).

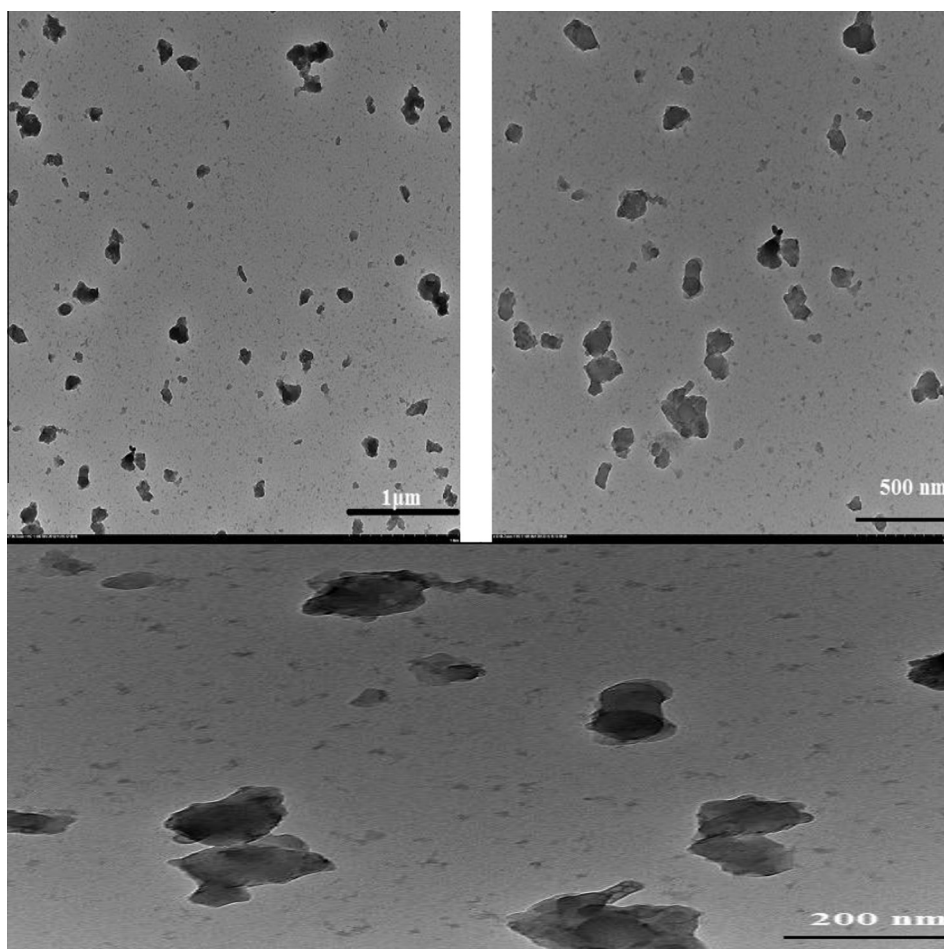


Fig. 3. Sonicated wheat straw (L1 us) lignin morphological characterization by TEM.

2.3.4. ^{31}P NMR analysis

The lignin samples were derivatized with 2-chloro-4,4',5,5'-tetramethyl-1,3,2-dioxaphospholane. Exactly known amounts of the samples were dissolved in a solvent mixture (1.6:1 ratio pyridine/chloroform), and an exactly known amount of a cholesterol

solution in the same solvent system was used as internal standard, and $\text{Cr}(\text{C}_5\text{H}_7\text{O}_2)_3$ was used as relaxation reagent [29,30]. The ^{31}P NMR spectra were recorded on a Bruker 300 NMR spectrometer with 256 scans. The NMR spectra were processed using Bruker-Topspin software.

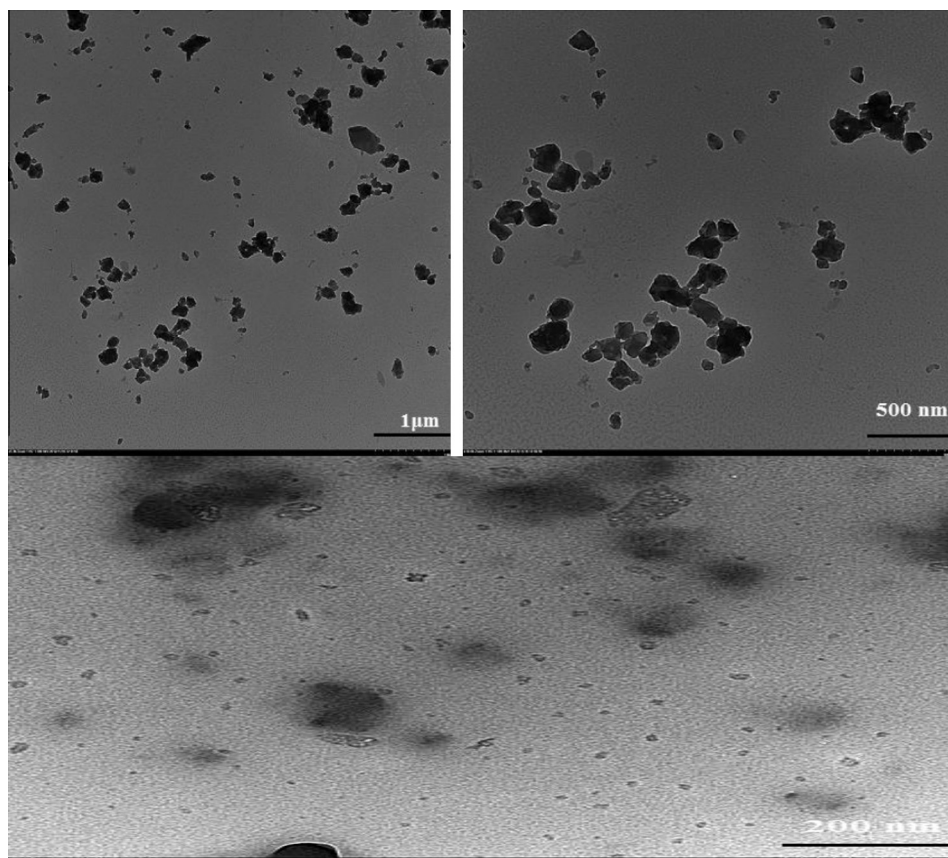


Fig. 4. Sonicated Sarkanda grass (L2 us) lignin morphological characterization by TEM.

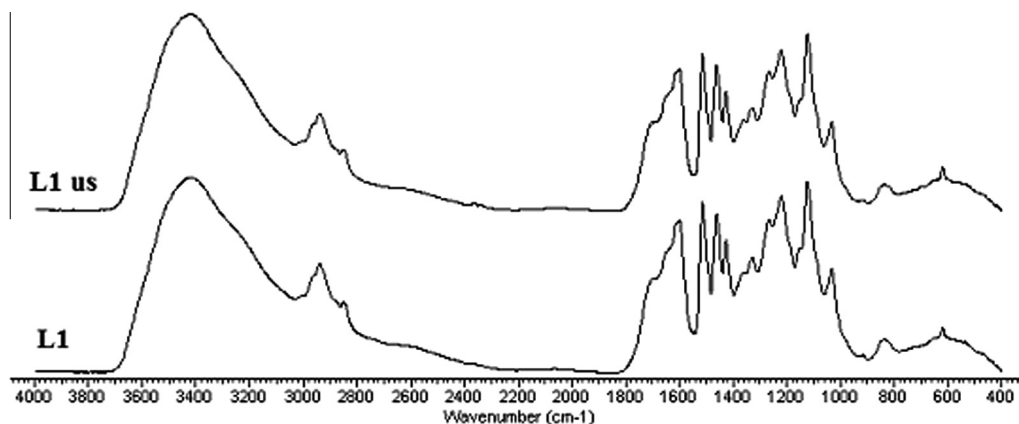


Fig. 5. Wheat straw lignin unmodified (L1) and L1 sonicated lignin FTIR spectra.

2.3.5. 2D NMR analysis

Quantitative 2D NMR was carried out on a Bruker 600 NMR spectrometer using the QQ-HSQC pulse sequence [31].

2.3.6. FTIR analysis

FTIR specters were carried out on Digilab Scimitar FTS2000 at 64 scans at a resolution of 4 cm^{-1} using KBr technique.

3. Results and discussion

The wheat straw and Sarkanda grass lignins were treated by sonication, and different analytical techniques were used to

evaluate modification in morphology, structural and chemical composition after ultrasound irradiation.

3.1. Characterization of nanoparticles. Average particle size distribution and morphological characterization

Figs. 1 and 2 show the light scattering profile of lignin suspensions before and after ultrasound treatment of wheat straw (L1) and Sarkanda grass (L2) lignins, respectively. It is evident that in both cases the average particles diameter is significantly reduced from 1–10 μm to 0.01–0.05 μm . Both in case of L1 and L2 distribution curves move to the left diagram, where the average particles size distribution is in the nano domain. From these results we

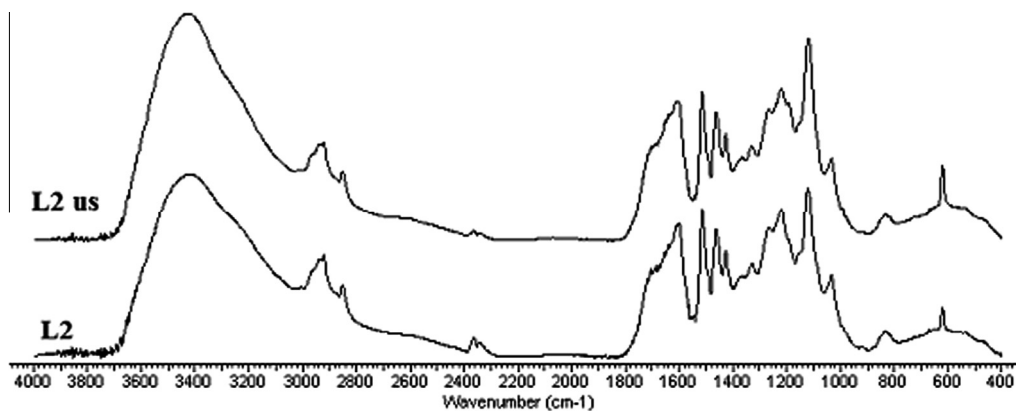


Fig. 6. Sarkanda grass lignin unmodified (L2) and L2 sonicated lignin FTIR spectra.

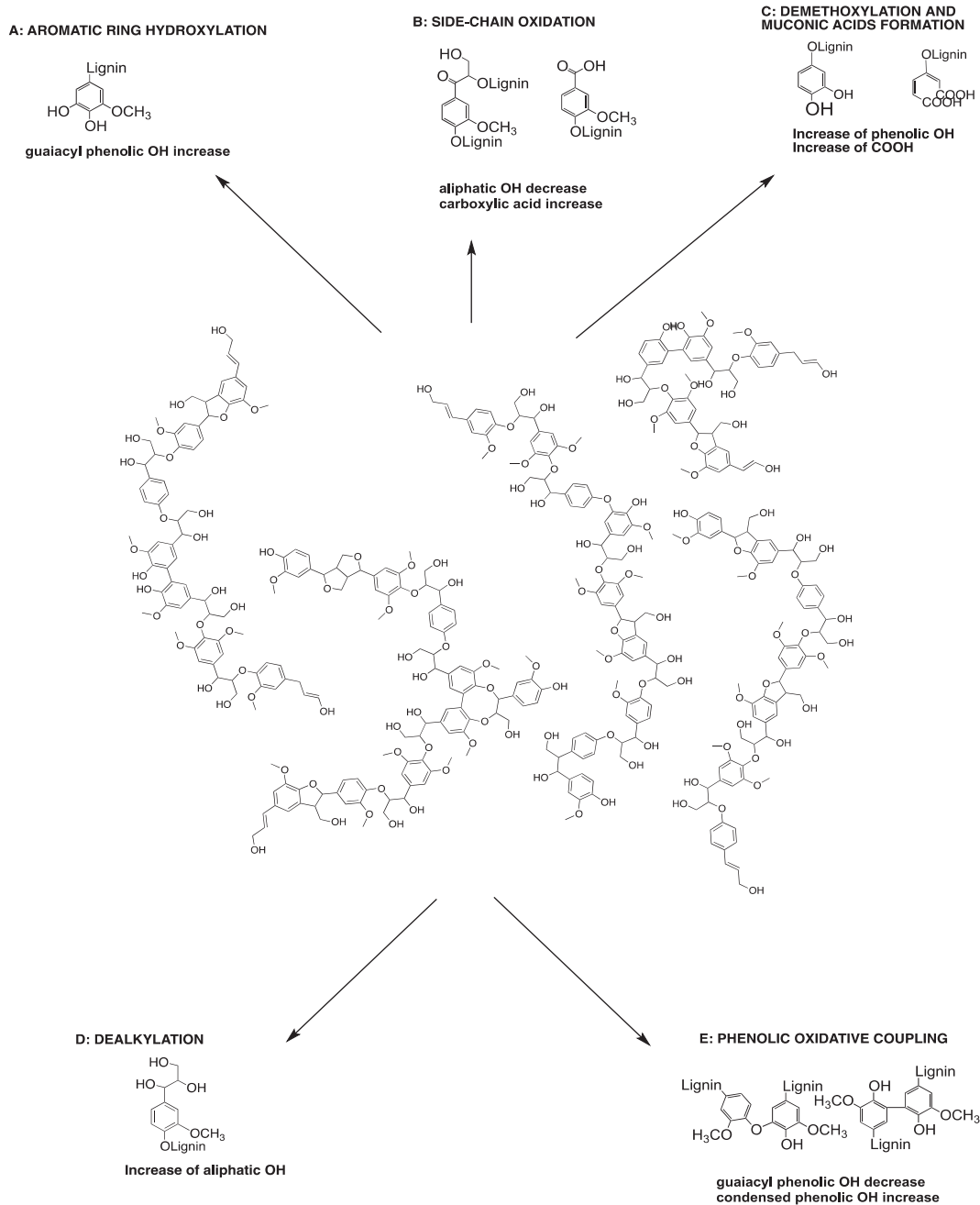


Fig. 7. Proposed mechanism for grass lignin degradation by US generated hydroxy and superoxy radical species.

Table 1

Aliphatic, phenolic, and carboxylic hydroxyl groups content determined in wheat straw (L1) and Sarkanda grass lignin (L2) and ultrasonicated lignin (L1 us, L2 us), derivatized with 2-chloro-4,4,5,5-tetramethyl-1,2,3-dioxaphospholane.

Functional groups	L1 (OH, mmol/g)	L1 us (OH, mmol/g)	L2 (OH, mmol/g)	L2 us (OH, mmol/g)
Aliphatic	2.97	3.27	1.55	2.73
Condensed phenolic	1.80	1.93	1.08	1.59
Guaiacyl phenolic	1.34	1.20	0.64	0.58
<i>p</i> -OH fenol	0.36	0.31	0.36	0.26
Carboxylic acids	1.13	1.01	0.52	1.17

can appreciate that applying ultrasonic irradiation it is possible to obtain nanosized lignin particles. We can mention that the mathematical software used to process the recorded data during the analysis make a number of approximations: the particles are spherical, optical properties of the environment and the samples are known, the particles are homogeneous. By using Transmission Electronic Microscopy (TEM) analysis the above trend is confirmed, as is shown in Figs. 3 and 4. A scale of 100 nm or less is evident in both samples of the nanoparticles.

3.2. FTIR spectroscopy

Lignin infrared absorption spectra show complex bands. This is due to the variety of vibration mode of chemical bonds present in this biopolymer structure. Exceptions are C–H, O–H, C=O stretching present around 1600 cm⁻¹, and aromatic ring stretching around 1510 cm⁻¹ [32]. Lignins analyzed after ultrasound irradiation, show FTIR spectra characterized by a broad O–H band at 3400 cm⁻¹, C–H stretching of methyl or methylene groups around 2900 cm⁻¹, C=O stretching around 1700–1660 cm⁻¹, aromatic skeletal vibration around 1600 cm⁻¹, C–C stretching of aromatic skeletal around 1510 cm⁻¹, C–H stretching of aromatic skeletal around 1460 cm⁻¹, CH vibration of methyl group 1420 cm⁻¹, syringyl units vibration 1330 cm⁻¹, guaiacyl units vibration 1150 cm⁻¹, OH stretching of secondary alcohol 1160–1130 cm⁻¹, OH stretching of primary alcohol 1040 cm⁻¹ [33–35]. The band at 1600 cm⁻¹ was used for normalization and its intensity was always set to 1.00. The IR spectra of ultrasound treated lignins show a significant increase of the intensity of the band at 1120 cm⁻¹ as shown in Figs. 5 and 6. Such band has been previously assigned to condensed aromatic units [23]. Furthermore, ultrasonic irradiation underwent a loss of β-O-4 linkages. This reaction was more pronounced for Sarkanda grass lignin (Fig. 7).

3.3. ³¹P-NMR spectroscopy

NMR methods are currently the most powerful tools used in the structure elucidation of lignin. The ³¹P-NMR technique objective is to obtain qualitative and quantitative information about the lignin functional groups. Thus lignin is marked with reagents containing phosphorus and then subjected to NMR analysis. The modification of lignin with 2-chloro-4,4,5,5-tetramethyl-1,2,3-dioxaphospholane can provide information about the global distribution of hydroxyl groups present in lignin. In addition, this technique may be used to determine the amount of carboxyl, guaiacyl, syringyl and aliphatic hydroxy groups [36,37]. Quantitative data from ³¹P-NMR show an overall increase of the aliphatic OH groups

Table 2

Interunit bondings (% of total aromatic units) in wheat lignin before (L1) and after (L1 us) ultrasound treatment as evaluated by QQ-HSQC.

Type of lignin	β-O-4	β-5	β-β	Cinnamyl alcohol	Ferulates	Other aliphatic protons
L1	12.1	1.2	2	1.1	1.91	23.4
L1 us	10.8	0.1	0.1	0.3	1.86	18.6

Table 3

Molecular mass distribution of straw (L1), Sarkanda grass (L2) and sonicated wheat straw (L1 us) and Sarkanda grass lignins (L2 us) respectively.

Type of lignin	Mw (weight molecular mass, g/mol)	Mn (number average molecular mass, g/mol)	PID (polydispersity index)
L1	210,000	31,000	6.8
L1 us	162,000	26,000	6.2
L2	190,000	18,000	10.7
L2 us	63,000	16,000	4.4

content upon ultrasound treatments. This is a general behavior found during lignin oxidation processes, probably due to side-chain cleavage processes [38].

The modification of the phenolic OH groups content showed a clear rationale. In both cases the guaiacyl and *p*-hydroxy phenol groups decreased upon ultrasound treatment. This is consistent with cross-coupling processes and in agreement with the above reported IR data. Accordingly, the amount of condensed phenolic OH groups was found increased. The Table 1 reports the relative quantitative data.

The radicals species formed during sonication can in fact undergo oxidative coupling to form condensed phenolic units (5-5 type) (Fig. 7). On the basis of these data one could hypothesize that during ultrasound treatments the hydroxyl and superoxy radical species generated might cause the occurrence of different simultaneous oxidative processes that can alternatively produce side chain cleavage with depolymerization and oxidative coupling with corresponding polymerization, as reported in Fig. 7.

Aiming to characterize in detail the modifications induced by ultrasound treatments onto lignins we submitted our wheat straw samples to QQHSQC analysis. This recently developed technique allows to quantitatively evaluate the amount of the different interunit bondings in lignin. Table 2 shows the relative data. The main lignin interunit bondings, β-O-4 were found decreased by about 10%, while both phenylcoumaran and pinoresinol subunits were disappeared. This suggests that ultrasound degrade preferentially the latter interunit bondings. On the other hand, ultrasound treatment did not significantly modify the ferulate content of the wheat straw lignin.

3.4. Gel permeation chromatography

The gel permeation chromatography carried out on acetobrominated samples of the treated and untreated lignins showed an interesting trend (Table 3). The number average molecular weight (Mn) and weight average molecular weight (Mw) values were found decreased after treatment with ultrasounds. This clearly indicates that under the present experimental conditions the oxidative process occurring on lignins results in the prevalence of depolymerization over oxidative coupling. This probably results from the degradation of longer lignin chains. In fact the polydispersity index was also found reduced particularly in case of Sarkanda grass lignin.

4. Conclusions

The paper present a physical method to obtain nanoparticles based on lignin by acoustic irradiation. The nanoparticles were

characterized from morphological and dimensional point of view. The structural and compositional modification resulted after sonication where characterized by FTIR-spectroscopy, GPC-chromatography QQ-HSQC and ^{31}P -NMR-spectroscopy. Two main reaction patterns were identified causing side chain cleavage/depolymerization and oxidative coupling/polymerization, respectively. Under the present reaction conditions the depolymerization process was prevalent; however it could be probably tuned by modulating ultrasound irradiation time and/or power. An increase in condensed units was shown by IR spectra and indirectly from QQ-HSQC analysis. Phenyl coumaran and pinosresinol subunits were found completely degraded and aryl glycerol aryl ether subunits ($\beta\text{-O-4}$) were found reduced.

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