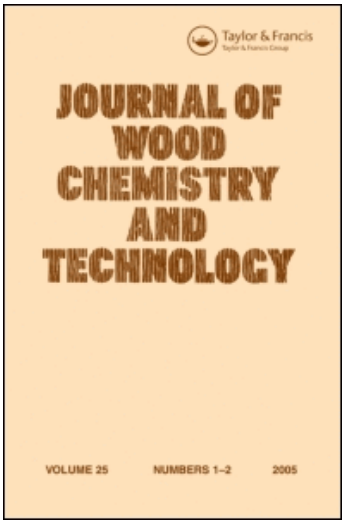

Journal of Wood Chemistry and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

BEHAVIOR OF *EUCALYPTUS GLOBULUS* LIGNIN DURING KRAFT PULPING. II. ANALYSIS BY NMR, ESI/MS, AND GPC

Paula C. Pinto[a]; Dmitry V. Evtuguin[a]; Carlos Pascoal Neto[a]; Armando J. D. Silvestre[a]; Francisco M. L. Amado[a]
[a] Department of Chemistry, University of Aveiro, Aveiro, Portugal

Online publication date: 08 August 2002

To cite this Article Pinto, Paula C. , Evtuguin, Dmitry V. , Neto, Carlos Pascoal , Silvestre, Armando J. D. and Amado, Francisco M. L.(2002) 'BEHAVIOR OF *EUCALYPTUS GLOBULUS* LIGNIN DURING KRAFT PULPING. II. ANALYSIS BY NMR, ESI/MS, AND GPC', Journal of Wood Chemistry and Technology, 22: 2, 109 — 125
To link to this Article: DOI: 10.1081/WCT-120013356
URL: http://dx.doi.org/10.1081/WCT-120013356

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

JOURNAL OF WOOD CHEMISTRY AND TECHNOLOGY
Vol. 22, Nos. 2 & 3, pp. 109–125, 2002

BEHAVIOR OF *EUCALYPTUS GLOBULUS* LIGNIN DURING KRAFT PULPING. II. ANALYSIS BY NMR, ESI/MS, AND GPC

Paula C. Pinto, Dmitry V. Evtuguin, Carlos Pascoal Neto,* Armando J. D. Silvestre, and Francisco M. L. Amado

Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

ABSTRACT

Residual and dissolved lignin from different phases of kraft delignification of *Eucalyptus globulus* wood were isolated and characterized by 1D and 2D ^{1}H NMR, ^{13}C NMR, Electrospray Ionization Mass Spectrometry (ESI/MS), and gel permeation chromatography (GPC). During the temperature rise period, below 70°C, about 20% of the lignin was dissolved without significant structural changes. Above 70°C, the lignin suffered significant degradation/fragmentation in the cell wall prior to dissolution. The lignin ether-linked syringyl units were the most susceptible to alkaline degradation. Through the course of pulping, the residual

*Corresponding author.

DOI: 10.1081/WCT-120013356
0277-3813 (Print); 1532-2319 (Online)

www.dekker.com

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

lignin (RL) revealed a gradual increase of aliphatic moieties of unknown structure, as well as a decrease of native structures such as phenylcoumaran and pino-/syringaresinol lignin units. A significant decrease of the β-*O*-4 structures content in RL was detected only at the final cooking temperature. The lignin dissolved in the black liquor (BL) consisted of highly branched oligomers with rather low molecular weight (average mass 800–1000 u). A part of BL (about 30%) was chemically linked to carbohydrates and possessed a large molecular weight distribution (500–4000 u). BL showed a progressive decrease in β-*O*-4 and pino-/syringaresinol structures and formation of enol ether and stilbene structures. The GPC analyses showed a continuous decrease of the molecular weight of both the residual and dissolved lignins during the pulping process, particularly in the residual stage.

INTRODUCTION

Understanding the lignin behavior during kraft pulping of wood is of crucial importance for process optimization and improvement of pulp quality. Despite the growing interest in *Eucalyptus globulus* wood, due the high quality of the pulp and paper obtained after kraft pulping and bleaching, no significant efforts have been made to improve the knowledge of the chemical behavior of lignin during the industrial pulping process.

In the first paper of this series,[1] residual and dissolved lignin samples were submitted to structural analysis by chemical degradation methods. It was suggested that lignin of vessel cells and in the middle lamella of libriform cells (rich in H and G units, highly condensed and associated with sugars) is dissolved in the earliest stage of pulping and that the delignification of secondary wall fibers occurs in the subsequent stages of the pulping. The extent of lignin condensation and the degradation of β-*O*-4 structures were monitored by permanganate oxidation and thioacidolysis techniques.

In the present study, attempts were made to identify the lignin structural changes during kraft pulping, using ^{1}H and ^{13}C NMR techniques, and to estimate the changes in the lignin molecular weight using Electrospray Ionization Mass Spectrometry (ESI/MS) and gel permeation chromatography (GPC).

EXPERIMENTAL

Materials

The pulping experiments were carried out with standard wood chips of 12 year old *E. globulus* trees. The chemical composition (%, o.d. wood) was as follows: Klason lignin, 20.0%; extractives (ethanol/toluene), 2.2%; and holocellulose (chlorite method), 78.0%. All solvents and chemicals used were pro-analysis grade products supplied by Aldrich and Sigma Chemical Co. (Madrid).

Pulping Experiments

Ten pulping experiments were performed in an M/K Systems batch digester (model 409 MII) using standard conditions as described previously.[1] The pulp and the black liquor preparations for analyses were the same as described in the first part of this paper.[1] Kappa number and Klason and acid soluble lignin were determined by standard TAPPI methods.

Isolation and Purification of Lignins

Wood and residual lignins from kraft pulps were isolated following the published method of mild acidolysis.[2] The ash and sugar contents of the isolated materials were rather low, *ca.* ut 1% and 1.5% (w/w), respectively).

Lignins from black liquors were precipitated with aqueous H_2SO_4 and extracted with ethyl ether.[1] Sugars analyses revealed a high level of carbohydrate contamination. Xylose was the most abundant sugar. These crude black liquor lignins were submitted to purification by dissolution in dioxane, yielding a purified black liquor lignin (soluble in dioxane) and a carbohydrates-rich lignin fraction (non-soluble in dioxane).

The designations used for lignin samples were as follows: EDL, lignin isolated from wood; RL, residual lignins; BL, black liquor lignin without purification; BLp, purified black liquor lignin (soluble in dioxane); and and BLs, black liquor lignin fraction remaining after purification (insoluble in dioxane, rich in sugars).

Quantitative ^{13}C MR Spectroscopy

Quantitative ^{13}C NMR spectra were recorded on a BRUKER AMX 300 spectrometer operating at 75.2 MHz. The lignin samples were dissolved in DMSO-d_6 (300–-500 mg/2.0–2.5 mL of solvent), placed into 10 mm diameter tubes, and the spectra were recorded at 318 K with TMS as an internal reference. An inverse gated decoupling sequence, which allowed quantitative analysis and comparison of signal intensities, was used with the following parameters: 90° pulse angle; 12 s relaxation delay; 16 K data points, and 18 000 scans.

^{1}H NMR Spectroscopy

Prior to ^{1}H NMR analysis, the lignin samples were acetylated following known procedures.[3,4] The acetylated lignins were dissolved in $CDCl_3$ (15–20 mg/0.7 mL solvent), placed into 5 mm diameter tubes, and the spectra were recorded at room temperature using TMS as an internal reference. The acquisition parameters used were as follows: 12.2 μs pulse length (90°); 2 s relaxation delay; and 300 scans.

2D ^{1}H NMR spectra (absolute-mode COSY spectra) of lignin isolated from black liquor were recorded on a BRUKER AMX 300 spectrometer operating at 300.1 MHz by acquiring 2K × 512 increments transformed to a 2K × 1K data matrix after zero-filling, FT and squared sine-bell apodization applied to both dimensions. COSY spectra were acquired over a 9.0 ppm window in both F2 and F1 directions. For each t_1 value, 400 scans were accumulated.

Electrospray Ionization Mass Spectrometry

Negative mode ESI mass spectra were acquired with a VG AutoSpecQ. The instrument was of EBEqQ geometry and equipped with a Micromass ESI source. Lignin solutions (0.05% w/v) in methanol : H_2O (1 : 1) containing 2.5% NH_3 were freshly prepared just before MS analysis. The samples (10 μL) were injected by needle, using 0.25% NH_3 in methanol as the eluent continuously infused at a flow rate 20 μL/min. Calibration of the instrument was done using dodecyl sulfate (SDS) as a standard with a working resolution of 10% peak/valley at m/z 265.

Gel Permeation Chromatography

The GPC analyses were performed with a PL-GPC 110 system equipped with a 10 μm Plgel pre-column, a 10 μm Plgel MIXED D column (300 × 7.5 mm), and a refractive index detector. The pre-column, column, and injection system were maintained at 70°C. The eluent (0.5% w/v LiCl in DMF) was pumped at flow rate of 0.9 mL/min. The lignin solutions (0.5% w/v) were prepared just before analysis using DMF with 0.5% LiCl (w/v) as solvent. The GPC columns were calibrated using lignin preparations previously characterized by ESI/MS.

RESULTS AND DISCUSSION

Four cooks were selected as representative of the different delignification phases: initial (19.0% delignification), initial-to-bulk transition (40.0% delignification), bulk (76.0% delignification) and residual phases (94.4% delignification). The dioxane lignin isolated from *E. globulus* (referred as EDL) represented the lignin in the starting wood material (0% delignification).

^{13}C NMR and 2D ^{1}H NMR Analysis

Qualitative and quantitative differences between *E. globulus* dioxane lignin (EDL), and residual (RL), and black liquor (BLp) lignins isolated after 76% wood delignification (end of temperature rise period, bulk delignification) were estimated by ^{13}C NMR spectroscopy (Fig. 1). The signals in ^{13}C NMR spectra of RL and BLp were assigned using previously published data.[5,6] Additional confirmation for the presence of particular structures in BLp was done using ^{1}H–^{1}H correlation (COSY) NMR spectroscopy (Fig. 2). The assignments for proton chemical shifts were based on known literature data[7,8] and are presented in Table 1.

Taking into account that the abundance of phenylcoumaran structures in EDL is rather low (about 0.03/C_6)[2] and that these structures were not detected in the COSY spectrum of a BLp sample, the integral intensity at 52–54 ppm in ^{13}C NMR spectra is assigned, essentially, to Cβ in pino/syringaresinol structures. The quantitative ^{13}C NMR spectra revealed a significant decrease in the abundance of pino/syringaresinol substructures in both RL and BLp samples (about 50%) as compared to that in EDL (Table 2).

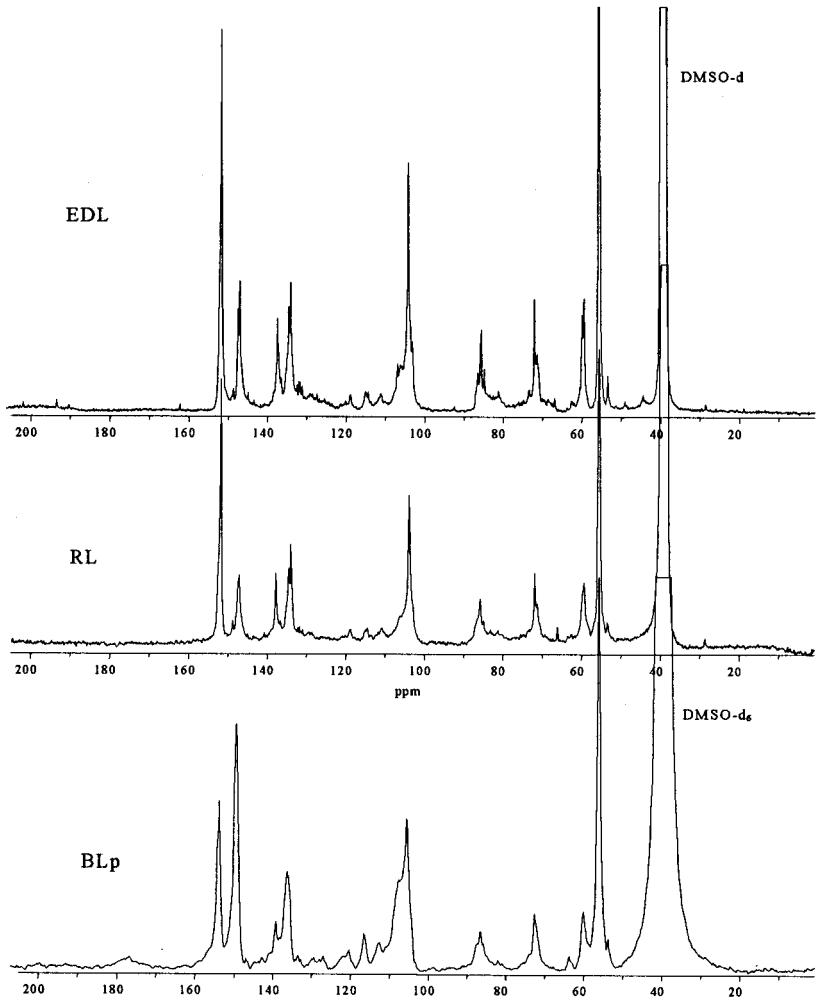

Figure 1. Quantitative ^{13}C NMR spectra of eucalypt dioxane lignin (EDL), residual lignin from pulp (RL) and purified black liquor (BLp) (76.0% delignification).

The rigorous quantification of β-*O*-4 structures per aromatic group (C_6) based on Cγ resonances at 59.0–61.5 ppm is difficult for RL and BLp samples, but trivial for EDL sample.[2] Firstly, the appearance of different unsaturated moieties in the propane chain of lignin structures formed during the pulping makes it difficult to define the region of the

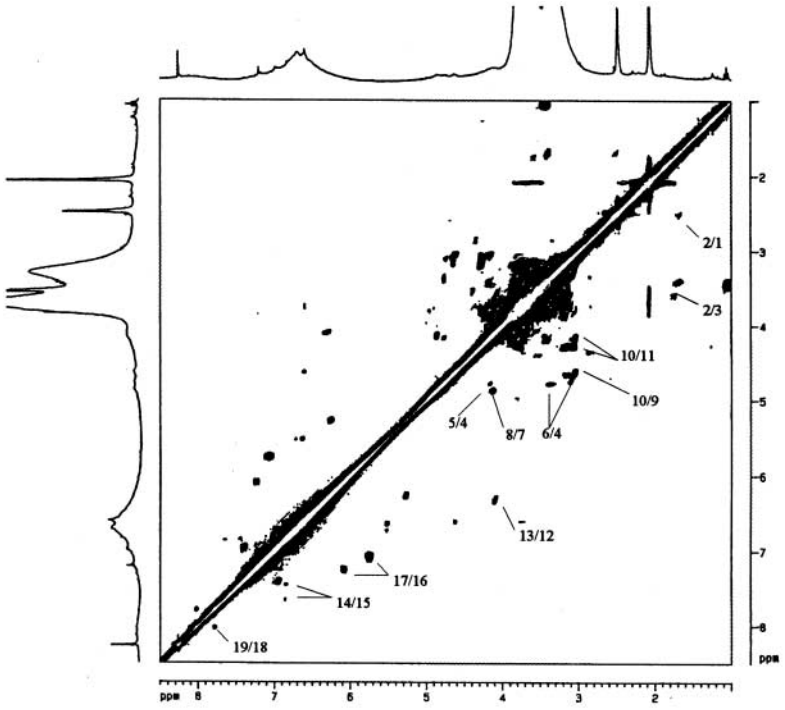

Figure 2. COSY spectrum of purified kraft lignin isolated from black liquor (BLp) (76.0% delignification). Cross-peak designations are presented in Table 1.

spectrum containing only aromatic carbons. For example, the COSY spectrum of BLp clearly shows the presence of cinnamyl alcohol, cinnamaldehyde, vinyl ether and stilbene type structures (Fig. 2), which have olefinic carbons in the side chains contributing to the aromatic region of 100.0–156.0 ppm in the ^{13}C NMR spectra. The cinnamyl alcohol-type moieties are much more abundant in RL and BLp than in EDL, as deduced from the intensity of signals at 127–128 ppm and 129–130 ppm, assigned to Cβ and Cα in these lignin substructures, respectively (Fig. 1). Secondly, although the resonances at 59.0–61.5 ppm in the BLp ^{13}C NMR spectrum were assigned, using HMQC technique,[9,10] essentially to Cγ signals in β-*O*-4 structures (without Cα=O), a small proportion of other lignin subunits may contribute to the same spectrum region. As indicated by the BLp COSY spectrum (Fig. 2), this sample contains dihydroconiferyl/dihydrosinapyl alcohol-type structures with Cγ resonances around 60.2–60.5 ppm. Additionally, Cγ resonances in cinnamyl alcohol structures (61.7 ppm)

Table 1. Assignments of Signals in COSY Spectrum of Eucalypt Kraft Lignin

Signal Number	^{1}H Shift, ppm	Lignin Structures
1	2.55	Hα in dihydroconiferyl alcohol
2	1.75	Hβ in dihydroconiferyl alcohol
3	3.42	Hγ in dihydroconiferyl alcohol
4	4.75–4.90	Hα in β-*O*-4
5	4.10–4.25	Hβ in β-*O*-4
6	3.10–3.35	Hγ in β-*O*-4
7	4.95	Hβ in β-*O*-4 structures with Cα=O
8	4.12	Hγ in β-*O*-4 structures with Cα=O
9	4.62–4.70	Hα in pino-/syringaresinol
10	3.02–3.08	Hβ in pino-/syringaresinol
11	4.12–4.20	Hγ in pino-/syringaresinol
12	6.35	Hβ in cinnamyl alcohol
13	4.12	Hγ in cinnamyl alcohol
14	6.88	Hα in cinnamaldehyde
15	7.48; 7.69	Hβ in cinnamaldehyde
16	7.10; 7.28	Hα in vinyl ether
17	5.75; 6.11	Hβ in vinyl ether
18	8.08	Hα in stilbene type
19	7.82	Hβ in stilbene type

Table 2. Analysis of EDL, RL and BLp (76.0% Delignification) by ^{13}C NMR Spectroscopy (Structural Elements Per C_6)

Structural Elements (Spectrum Region Used for Integration)	EDL	LR	BLp
β-β, β-5 (Cβ 52.0–54.0 ppm)	0,15	0,08	0,06
β-*O*-4 without Cα=O (Cγ, 59.0–61.5 ppm)	0,52	0,44	0,33
β-*O*-4 with Cα=O (Cγ, 63.0–64.5 ppm)	0,04	0,04	0,08*
Ar-H (100.0–125.0 ppm)	1,95	1,98	2,26

*Significant contribution of enol ether substructures.

interfere with Cγ signals in β-*O*-4 structures. Thus, the calculation of number of β-*O*-4 lignin subunits per C_6 based on the integral of signals in the range 59.0–61.5 ppm is less rigorous for RL and BLp than for EDL. However, in spite of these limitations, the relative difference in abundance of β-*O*-4 structures in RL and BLp with respect to EDL, calculated based on Cγ resonance at 59.0–61.5 ppm, was very similar to that

previously obtained by thioacidolysis.[1] In fact, the analysis of the thioacidolysis products showed that the content of β-*O*-4 structures of RL and BLp was 12 and 43% lower than that of EDL[1] while ^{13}C NMR revealed correspondent values of 15 and 37% for RL and BLp, respectively (Table 2). The coherent results obtained by wet chemistry and NMR indicate that in spite of the possible lack of accuracy in the determination of the absolute content of β-*O*-4 structures in RL and BLp, its relative abundance may be estimated by ^{13}C NMR spectroscopy using the integral of Cγ signals in the range 59.0–61.5 ppm.

A fraction of β-*O*-4 structures are degraded under kraft pulping giving vinyl ether-type structures.[1] The problem of the identification of such structures in eucalyptus kraft lignin was discussed recently.[9,10] Enol ether lignin structures, formed via quinone methide intermediates by β-elimination of γ-hydroxymethyl groups, show in the HMQC spectrum cross-signals of the vinylic moiety at δ_C/δ_H 112/6.1 and 146/7.1 assigned to Cβ/Hβ and Cα/Hα correlations respectively.[9,10] Enol ether structures were identified in the BLp COSY spectrum (Fig. 2). These structures are absent in the RL sample because of their high reactivity under the acidic conditions applied in the isolation procedure.

The group of signals at 63.0–64.5 ppm in EDL (Table 2) was assigned, using the HETCOR NMR technique, to Cγ in β-*O*-4 structures containing Cα=O.[2] In spite of the presence of such structures in RL and BLp (Figs. 1 and 2), the strong signals centered at δ 64.2 in BLp cannot be assigned exclusively to Cγ signals in β-*O*-4 structures containing Cα=O. The expected intensity increase of signals from those structures at 194–198 ppm (Cα in Cα=O) and at around 81 ppm (Cβ) in ^{13}C NMR spectra of BLp was not observed (Fig. 1). Previously, using the HMQC technique, it was shown that the Cγ signal of black liquor lignin at around 64 ppm has a correlation with a proton resonance at 3.2–3.3 ppm.[9,10] This is very different from the known data on correlation of Cγ/Hγ (63.8 ppm/4.1 ppm) in β-*O*-4 structures with Cα=O. The Cγ/Hγ correlation at 64.2 ppm/3.2–3.3 ppm suggests that these atoms belong to enolic structures containing γ-hydroxymethyl groups. Thus, Cγ is deshielded by the presence of an electron-withdrawing moiety (Cα=Cβ) and Hγ remains at the same chemical shift as in β-*O*-4 structures. The anisotropy of Hγ in the corresponding vinyl ether structures can be tentatively assigned to the strong interaction (shielding) with π-electrons and/or to hydrogen bonding with oxygen atom in methoxyl group of neighbouring (Cβ linked) aromatic group. This proposition should be confirmed using lignin model compounds.

The content of tertiary aromatic carbons in BLp is higher than that in EDL and RL samples (Table 2). This difference is attributed essentially to the different syringyl (S)/guaiacyl (G) ratios in these samples. As shown

previously by wet chemistry analyses,[1] BLp is richer than EDL and RL in G units, because of the preferential removal of guaiacyl lignin fragments in the initial phase of the delignification.

The ^{13}C NMR spectra of lignins showed important changes in the relative intensity of the carbon signals centered at *ca.* 148 ppm and at *ca.* 153 ppm. The former signal is assigned to C3 in etherified G units and C3,5 in non-etherified S units while the later is assigned to C3,5 in etherified S units (Fig. 1). The ratio of the intensity of signals at 153 and 148 ppm was 3.0 for EDL, 4.0 for RL and 0.7 for BLp. These differences cannot be explained by a simple increase in the relative abundance of G units in BLp (less than 10% when compared to RL[1]). It suggests that EDL and RL are composed essentially by linear chains constituted by ether-linked S units, while BLp possesses a highly branched structure where the relative abundance of ether-linked G and S units is similar. This means that the structural segments constituted by ether-linked S units should be the weak points in the alkali-induced lignin degradation.

In general, the structural features of EDL and RL samples are rather similar and very different from those of BLp.

^{1}H NMR Analysis

Lignin structural changes occurring along the pulping were monitored by ^{1}H NMR. Spectra of RL and BLp samples as acetate derivatives were acquired. Proton signals were assigned based on known literature data.[3,4] Calculations of all structural elements were made per C_9 unit using the resonance of methoxyl protons as an internal standard.

The NMR data of RL series (Table 3) shows that the content in $-CH_2-$ and $-CH_3$ moieties increased as the cook progressed, particularly

Table 3. Analysis of EDL and RL by ^{1}H NM R Spectroscopy (Structural Elements per Phenylpropane Unit)

	% Delignification				
Structural Elements	EDL	19.0	40.0	76.0	94.4
$-C\underline{H}_2-$ and $-C\underline{H}_3$ (0.7–1.5 ppm)	0.27	0.49	0.65	0.83	2.23
H in acetylated aliphatic OH (1.7–2.2 ppm)	1.00	1.43	1.24	1.14	1.06
H in acetylated phenolic OH (2.2–2.5 ppm)	0.31	0.33	0.31	0.32	0.52
Hβ in β-β (2.9–3.2 ppm)	0.15	0.08	0.08	0.05	Traces
Hα in β-*O*-4 without Cα=O (5.9–6.2 ppm)	0.50	0.49	0.52	0.49	0.33

in the residual phase. This is one of the structural changes responsible for the reduction of lignin reactivity in the delignification as reported in the literature.[6,11] A significant decrease of the amount of β-*O*-4 structures is evidenced only at the final temperature of the pulping (degree of delignification higher than 76%). This fact can be explained by the low lignin degradation rate during the temperature rise period, when diffusion of fragments with partially cleaved β-*O*-4 bonds to pulping solution is faster than chemical reactions leading to notable structural changes in bulky lignin in cell wall. In contrast to β-*O*-4 subunits, pino- and syringaresinol structures are readily degraded during all the temperature rise period. The amount of hydroxyl aliphatic groups increases in the initial stage and, then, decreases until the end of the cooking. This last feature could be due to the loss of primary hydroxyl groups in β-*O*-4 structures (with liberation of formaldehyde)[11] and also to the fragmentation and progressive dissolution of structures carrying hydroxyl groups. The high amount of phenolic groups on residual phase of delignification (due to the cleavage of aryl ether linkages) comparatively to that in wood lignin is an important feature since these functional groups contribute to the residual lignin reactivity during bleaching.

BLp samples represent the purified lignin fractions with low carbohydrate contents. Table 4 summarizes the results of ^{1}H NMR analysis of these samples. The content of $-CH_2-$ and $-CH_3$ moieties, aliphatic and phenolic hydroxyl groups in BLp is rather abundant in the initial phase of the delignification decreasing, however, along the pulping (Table 4). This result can be explained, at least partially, by the easy removal of condensed tannins and saponified extractives in the initial phase of the pulping, which may co-precipitate with lignin during its isolation and purification.

The content of β-*O*-4 structures in BLp samples decreases progressively during the pulping due to their intensive degradation both in fibre

Table 4. ^{1}H NMR Functional Group Analysis of BLp (Number of Functional Groups per Phenylpropane Unit)

	% Delignification			
Structural Elements	19.0	40.0	76.0	94.4
$-CH_2-$ and $-CH_3$ (0.7–1.5 ppm)	2.10	0.90	0.77	0.72
H in acetylated aliphatic OH (1.7–2.2 ppm)	1.42	1.25	1.10	0.89
H in acetylated phenolic OH (2.2–2.5 ppm)	1.20	0.83	0.98	1.05
Hβ in β-β (2.9–3.2 ppm)	0.06	0.06	<0.05	Traces
Hα in β-*O*-4 without Cα=O (5.9–6.2 ppm)	0.48	0.38	0.39	0.34

cell wall and in free pulping solution (Table 4). An especially notable decrease in β-*O*-4 structures content occurs in the transition point between initial and bulk phases of the cooking (40% delignification). High content of β-O-4 structures in BLp isolated in the initial phase of the delignification (19% delignification) indicate the insignificant fragmentation of lignin removed during this cooking period. Such a statement is supported by results of RL and BLp analysis by thioacidolysis which showed an high yield of degradation products originated from β-*O*-4 substructures.[1] The intensive cleavage of alkyl–aryl ether linkages during bulk and residual delignification (degree of the delignification from 76% to 94%) generates new phenolic moieties, justifying the observed increase of hydroxyl phenolic groups. The content of aliphatic hydroxyl groups decreases along the pulping due to the continuous degradation of C_9 propane side-chains reflected by the diminishing abundance of lignin subunits linked by β-*O*-4 and α-γ bonds in pino-/syringaresinol substructures (Table 4).

ESI/MS Analysis

Electrospray ionization, combined with mass spectrometry (ESI/MS), provides information on lignin molecular weight and structural features.[12] The ESI-MS spectrum of eucalypt dioxane lignin (EDL) showed a large number of non-resolved peaks with m/z from several hundreds to more than 7000, with a gravity center (Mp) found at m/z 2400.[15] Spectral features similar to EDL were observed for RL sample series (results not shown).

The ESI/MS spectrum of kraft black liquor lignin (BL) from residual phase of the cooking (about 94.0% delignification) (Fig. 3) showed much lower molecular weights and less pronounced fine structure than was observed previously for EDL,[12] reflecting a higher variety of oligomeric ubstructures. The centre of gravity of the mass distribution (Mp) in BL was about m/z 1000. Such a low Mp value reflects lignin degradation in black liquor and removal of low-molecular weight lignin fractions from the cell wall**.** Since BL analysis revealed the presence of carbohydrates,[1] signals in ESI/MS spectra should belong to lignin and lignin–oligosaccharide complexes. Hence, it is not obvious that Mp value, determined for BL, reflect correctly the average molecular weight of lignin in black liquor. In order to estimate the molecular weights of kraft lignin itself and its fragments bounded with oligosaccharides, the BL sample (sugars content 6.0%) was purified by selective precipitation in dioxane (see experimental part) yielding two fractions: BLp (about 70% yield, sugars content 1.5%) and BLs (about 30% yield, sugars content 18.9%). The ESI/MS spectra of BLp and BLs are represented in Fig. 3. The BLp fraction showed lower

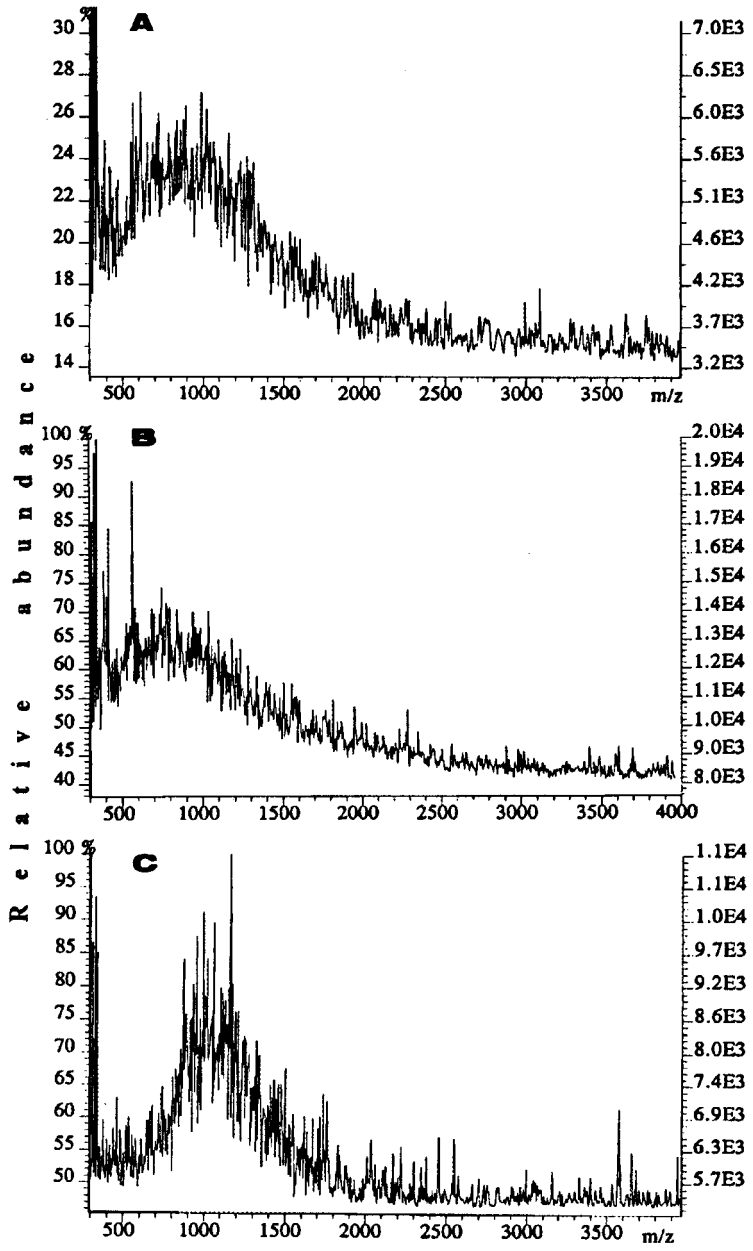

Figure 3. Negative mode ESI-MS spectrum of BL (A), BLp (B) and BLs (C) isolated from black liquor after 94.4% delignification of eucalypt wood.

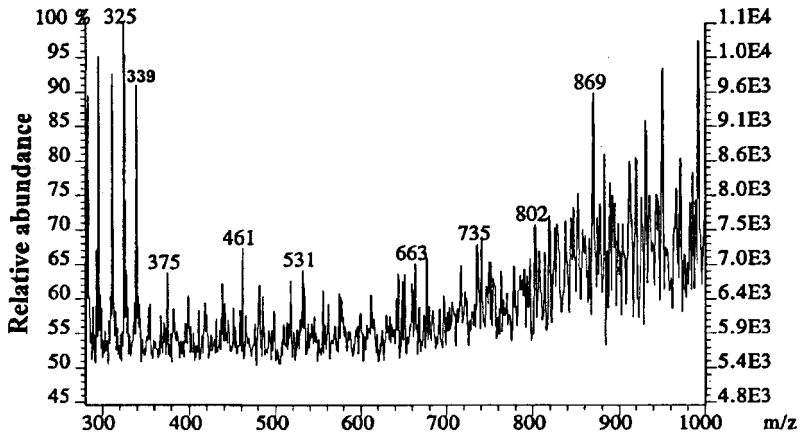

Figure 4. Negative mode ESI-MS spectrum (expanded mass range from m/z 300 to 1000) of BLs isolated from black liquor after 94.4% delignification of eucalypt wood.

molecular masses (Mp about 800) when compared to those of integral sample (BL). On the other hand, the mass distribution of BLs fraction was shifted to higher values (Mp 1100), suggesting that the lignin bounded with oligosaccharides is the high molecular weight fraction of BL sample.

The expansion of the BLs mass spectrum (Fig. 4) showed a series of lignin oligomers bearing m/z differences of 202–206 u (461, 663 and 869; 531, 735 and 938), which is the approximate molecular weight of the phenylpropane unit (C_9). A peak at m/z 339 was assigned to the dissaccharide composed by 4-*O*-methyl-D-glucuronic acid and D-xylose residues (Glc*p*A-Xyl*p*) previously detected in the composition of *E. globulus* xylan.[13]

Gel Permeation Chromatography Analysis

The comparison of weight-average molecular weights (M_w) of RL samples obtained from GPC data (Table 5) indicated that, in general, the molecular weight of lignin decreases as the pulping proceeds, particularly in the residual phase. These observations are in agreement with the predicted continuous depolymerization of lignin along the cooking due to inter-unit bond cleavage. However, when the delignification degree increased from 40.0% to 76.0% (bulk phase), a small increase in molecular

Table 5. Weight-Average Molecular Weight (M_w) of RL and BLp

	M_w	
% Delignification	RL	BLp
19.0	3150	–
40.0	2930	965
76.0	3100	1000
94.4	2170	870

weight of LR was observed. This can be attributed to an intensive dissolution of lignin fragments from fibre bulk to solution at this phase of the delignification.[1]

GPC data for BLp samples (Table 5) showed, in general, a decrease in molecular weights of dissolved lignins as the degree of delignification increased. The comparison of M_w values of RL and BLp shows that lignin in pulp possessed about three times higher molecular weight than that in black liquor along all the delignification process.

CONCLUSIONS

The study of the lignin behaviour during kraft pulping of *E. globulus* wood showed that in the initial stage of delignification, when temperature rises to 70°C and 19% delignification is attained, lignin is dissolved without significant degradation/fragmentation. Notable degradation of β-*O*-4, phenylcoumaran and pino-/syringaresinol type structures in the cell wall was observed at the temperature rise period from 70 to 160°C, corresponding to bulk phase of the delignification. A structural part of lignin macromolecule constituted by ether-linked S units is the most susceptible towards alkaline degradation.

Residual lignin (RL) in pulp showed an important increase in aliphatic moieties ($-CH_2$-, $-CH_3$) content and progressive decreasing of pino-/syringaresinol structures along the pulping. The β-*O*-4 structures content decreased substantially only in the residual phase of the delignification, reaching about half their abundance in the initial wood. Simultaneously, the content of phenolic hydroxyl groups in RL increases while aliphatic hydroxyl groups content decreases along the delignification, reflecting alkali-induced degradation of lignin network. The molecular weight of RL decreases gradually along the pulping being in the M_w range from

about 3200 (initial phase of the delignification) to about 2200 (residual phase).

Lignin dissolved in black liquor (BL) represents highly branched oligomers with rather low molecular weight (average mass about 800–1000 u), i.e., tetramers–pentamers. A part of BL (about 30%) is bonded with carbohydrates and possesses a large molecular weight distribution (from around 500 to several thousands). A significant decrease in abundance of β-*O*-4 structures in BL was observed in the transition point between initial to bulk phases of cooking (about 40% delignification), reflecting the predominant release in pulping solution of fragments with partially cleaved β-*O*-4 bonds. Phenylcoumaran and pino-/syringaresinol structures were not detected in BL isolated in the residual phase of the delignification.

ACKNOWLEDGMENTS

The authors thank FCT-PRAXIS XXI for the financial support of the research project 3/3.2/PAPEL/2327/95 and for the awarding of a research grant (BIC/3700/96) to Paula Pinto. The authors also thank RAIZ—The Forest and Paper Research Institute for their collaboration and pulp preparation facilities.

REFERENCES

1. Pinto, P.C.; Evtuguin, D.V.; Neto, C.P.; Silvestre, A.J.D. Behaviour of *Eucalyptus globulus* Lignin during Kraft Pulping. Part 1. Analysis by Chemical Degradation Methods. J. Wood Chem. Technol. **2002**, Accepted for publication.
2. Evtuguin, D.V.; Pascoal Neto, C.; Silva, A.; Domingues, P.M.; Amado, F.M.L.; Robert, D.; Faix, O. Comprehensive Study on the Chemical Structure of Dioxane Lignin from Plantation *Eucalyptus globulus* Wood. J. Agric. Food Chem. **2001**, *49*, 4252–4261.
3. Chen, C.-L.; Robert, D. Characterization of Lignin by ^{1}H and ^{13}C NMR Spectroscopy. In *Methods in Enzymology*; Wood, W.A., Kellogg, S.T., Eds.; Academic Press, Inc.: San Diego, California, 1988; Vol. 161, 137–174.
4. Lundquist, K. Proton (^{1}H) NMR Spectroscopy. In *Methods in Lignin Chemistry*; Lin, S.Y., Dence, C.W., Eds.; Springer-Verlag: Berlin, Germany, 1992; 242–249.
5. Kringsstad, K.P.; Mörck, R. ^{13}C-NMR Spectra of Kraft Lignin. Holzforschung **1983**, *37*(5), 237–244.

6. Gellerstedt, G.; Robert, D. Quantitative ^{13}C NMR Analysis of Kraft Lignins. Acta Chem. Scand. **1987**, B *41*, 541–546.
7. Fukagawa, N.; Meshitsuka, G.; Ishizu, A.A. Two-Dimentional NMR Study of Birch Milled Wood Lignin. J. Wood Chem. Technol. **1991**, *11*(3), 373–396.
8. Nose, M.; Bernards, M.A.; Furlan, M.; Zajicek, J.; Eberhardt, T.L.; Lewis, N.G. Towards the Specification of Consecutive Steps in Macromolecular Lignin Assembly. Phytochemistry **1995**, *39*(1), 71–79.
9. Balakshin, M.Y.; Capanema, E.A.; Chen, C.-L.; Gratzl, J.S.; Gracz, H. The Use of 2D NMR-Spectroscopy on Structural Analysis of Residual and Technical Lignins. In *Proceedings of 6th European Workshop on Lignocellulosics and Pulp*; Bordeaux, France, 2000; 11–14.
10. Capanema, E.A.; Balakshin, M.Y.; Chen, C.-L.; Gratzl, J.S.; Gracz, H. Structural Analysis of Residual and Technical Lignins by ^{1}H–^{13}C Correlation 2D NMR-spectroscopy. Holzforschung **2001**, *55*(3), 302–308.
11. Gierer, J. Chemistry of Delignification. Part 1: General Concept and Reactions During Pulping. Wood Sci. Technol. **1985**, *4*, 289–312.
12. Evtuguin, D.V.; Domingues, P.M.; Amado, F.L.; Neto, C.P.; Correia, A.J.F. Electrospray Ionization Mass Spectrometry as a Tool for Lignins Molecular Weight and Structural Characterization. Holzforschung **1999**, *53*(5), 525–528.
13. Shatalov, A.A.; Evtuguin, D.V.; Pascoal Neto, C. (2-*O*-α-D-Galacto-pyranosyl-4-*O*-methyl-α-D-glucurono)-D-xylan from *Eucalyptus globulus* Labill. Carbohydr. Res. **1999**, *320*, 93–99.

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016