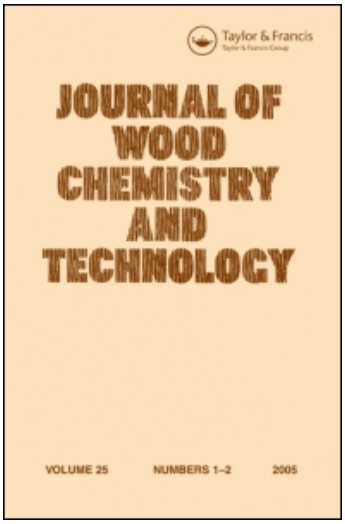

Journal of Wood Chemistry and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

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Online publication date: 18 November 2009

To cite this Article Marques, A. P. , Evtuguin, D. V. , Magina, S. , Amado, F. M. L. and Prates, A.(2009) 'Structure of Lignosulphonates from Acidic Magnesium-Based Sulphite Pulping of *Eucalyptus globulus*', Journal of Wood Chemistry and Technology, 29: 4, 337 — 357
To link to this Article: DOI: 10.1080/02773810903207762
URL: http://dx.doi.org/10.1080/02773810903207762

Journal of Wood Chemistry and Technology, 29: 337–357, 2009

ISSN: 0277-3813 print / 1532-2319 online
DOI: 10.1080/02773810903207762

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Structure of Lignosulphonates from Acidic Magnesium-Based Sulphite Pulping of *Eucalyptus globulus*

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Abstract: The structure of lignosulphonates (LS) from acidic magnesium-based sulphite pulping of *Eucalyptus globulus* wood has been studied. LS from thin and thick spent liquors were purified by dialysis and characterised by 1D/2D Nuclear Magnetic Resonance (NMR) and Electrospray Ionization Mass Spectrometry (ESI-MS). It was suggested that major part of LS is comprised of partially sulphonated low molecular weight oligomers formed via extensive cleavage of β-*O*-4 bonds in eucalypt lignin upon sulphite pulping. More than ten types of LS structures derived from different lignin structural units have been identified. A significant proportion of LS (>20%) is comprised by monomeric compounds, mainly 4-allyl-2,6-dimethoxyphenyl-α-sulphonic and 4-propyl-2,6-dimethoxyphenyl-α,γ-disulphonic acids. LS revealed partial degradation during the thin liquor evaporation leading to cleavage of ether and carbon-carbon linkages in the side chain and to the increasing of the condensation degree.

Keywords: *Eucalyptus globulus*, ESI-MS, NMR, lignosulphonates, structure, sulphite pulping, sulphite spent liquor

INTRODUCTION

Lignosulphonates (LS) are the major component of sulphite spent liquors (SSL) produced during the acidic sulphite or bisulphite pulping of wood.[1] According to industrial practice SSL are concentrated and burned for the base and for the energy recovering (excepting the calcium base). At the same time, LS

This work was financially supported by the Foundation of the Science and the Technology (FCT), grant reference SFRH/BD/31463/2006, and by the innovation PRIME Program (grant SIME I&DT no. 00/22689).

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from Ca-based and some proportion of LS from Mg/Na-based acidic sulphite pulping find out a large number of practical applications and are valuable side products of pulp industry.[1−3] In unmodified state LS are widely used as drilling muds, cement and concrete additives, emulsifies/stabilisers, grinding aids, binders/adhesives, resin ingredients, rubber additives, tanning agents, and so on.[3] The modification of LS allows the production of phenol resins and phenolics, which are valuable raw materials for polymer formulations,[4] fine chemicals,[5,6] and flavors.[7] The commercialization of LS improves the sustainability of sulphite mills and fits well to the biorefinery concept.

Historically, according to industrial availability, LS from sulphite pulping of softwoods (mainly spruce) were considered for structural studies and applied research. It was highlighted, however, that the molecular weight and the structural features of LS from softwoods and hardwoods are rather different.[1] Although the information on the chemical composition of SSL regarding its eventual utilization for different purposes is essential, only scarce knowledge is available on the chemical structure of hardwood LS. This is true, in particular, for LS from the magnesium-based acidic sulphite pulping of *Eucalyptus globulus* wood.

The preliminary studies on the chemical composition of LS from *Eucalyptus globulus* wood showed that these are highly sulphonated lignin oligomers (up to 20% of SO_3H groups) constituted predominantly by syringyl units and possessing rather low molecular weight ($M_w = 1000 - 1300$ Da).[8] It was proposed, based on the results obtained using size exclusion chromatography (SEC) and on the analysis of products from permanganate oxidation, that eucalypt LS contain a notable proportion of highly sulphonated low molecular weight fraction (monomers/dimers) constituted predominantly by syringyl units. It was also suggested that eucalypt wood lignin during acidic sulphite pulping suffered substantial depolymerization and condensation reactions, the origin of which could be disclosed after the elucidation of LS structure.

Aiming to provide basic information on LS structure and structural changes occurring during acidic sulphite pulping LS from acidic magnesium–based sulphite pulping of *Eucalyptus globulus* has been studied using 1D/2D NMR techniques and tandem electrospray ionization mass spectrometry (ESI-MS/MS).

MATERIALS AND METHODS

Materials

Industrial thin (SSL) and thick (THSL) spent liquors from magnesium-based acidic sulphite pulping of *Eucalyptus globulus* were supplied by Caima-Industria de Celulose SA (Constância, Portugal). The industrial pulping was carried out at 130°C and pH 1.5 (liquid-to-wood ratio about 3) to obtain a

pulp with kappa number of 20–22 (total pulping time was about 8 h). The evaporation of SSL at the pulp mill was carried out in a set of multiple-effect evaporators (totally 7 evap*o*rators) under vacuum. The average residence time in the evaporator train was around 50–60 min. The evaporation temperature varied from 142°C (first effect) to 74°C (last effect). SSL and THSL were purified to obtain the lignosulphonate-rich fractions by dialysis with partially benzoylated cellophane membrane of 2,000 NMWCO (Sigma-Aldrich) giving raise to the LS samples LSF0 and LSG, respectively. Alternatively, SSL was dialyzed using membrane of 5,000 NMWCO (Pierron) giving rise to the LS sample LSF. In all experiences the dialysis was carried out against distilled water during 8 h followed by a freeze-drying of dialysate.

Analyses

The SEC analysis and fractionation of LS have been carried out using two PL aquagel-OH MIXED 8 μm 300 × 7.5 mm columns protected by a PL aquagel-OH Guard 8 μm pre-column on a PL-GPC 110 system (Polymer Laboratories, UK). The columns, injector system and the detector (RI) were maintained at 36°C during the analysis. LS were dissolved in 0.1 M aqueous solutions of $NaNO_3$ to a concentration of about 0.5 % (5 mg/mL). The eluent (0.1 M aqueous solution of $NaNO_3$) was pumped at a flow rate of 0.9 mL/min. The analytical columns were calibrated with PSSNa standards (Pressure Chem. Comp.) in the range of 1–100 kDa.

All NMR spectra were recorded on a Bruker Avance 300 spectrometer operating at 300.1 MHz for proton and at 75.2 MHz for carbon. The ^{1}H NMR and ^{13}C NMR spectra of LS were registered in D_2O (at 30 or 50°C) using typical sample concentrations of 2.5% for proton and of 32% for carbon spectra. Sodium 3-(trimethylsilyl) propionate-d_4 was used as an internal standard (δ = 0.00) in proton spectra. The relaxation delay was 2.0 s and about 200 scans were collected (90° pulse). The quantitative carbon NMR spectra were acquired using a pulse width of 4.8 μs (90° pulse), 16 s relaxation delay and 18.000 scans were collected. The internal standard was acetone ($\delta = 30.89$). The DEPT (Distortionless Enhancement by Polarization Transfer) spectra were recorded with a $\theta = 135°$ and a coupling constant ^{1}J (^{1}H−^{13}C) = 150 Hz. Data were processed using a X-Win-NMR software (version 3.1).

2D ^{1}H NMR spectra (absolute-mode COSY spectrum) of lignins isolated from spent liquors were recorded acquiring 2 K × 512 increments transformed to a 2 K × 1 K 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 600 scans were accumulated. The phase sensitive ^{1}H-detected HSQC (Heteronuclear Single Quantum Coherence) spectra were acquired over a F1 spectral weight of 12000 Hz and a F2 width of 2000 Hz with a 2048 × 1024 matrix and 128 transients per

increment. The delay between scans was 2 s and the delay for polarization transfer was optimized for $^{1}J_{C-H} = 150$ Hz. The heteronuclear multiple-bond correlation (HMBC) spectra were recorded using coupling evolution time of 110 ms ($^{3}J_{C-H} = 4.5$ Hz).

Mass spectrometry (MS) and tandem mass spectrometry (MS/MS) analyses using electrospray ionisation (ESI) in the negative mode were obtained with a Q-TOF 2 instrument (Micromass, Manchester, UK) using a MassLynx software system (version 4.0). The mass spectrometer was operated with a capillary voltage of 3000 V, a cone voltage of 35 V, the source block temperature set to 80°C and the desolvation temperature set to 150°C. Mass spectra (MS) were obtained at a resolution of 10,000 (50% valley). MS/MS experiments were carried out by selecting the precursor ion of interest using Q1 and collision-induced fragmentation in a hexapole collision cell using argon as a collision gas, and the collision energy was varied according to the ion of interest (typically 20–45 eV). LSI was dissolved in 0.01 M aqueous solution of ammonia (negative mode analyses). The methanol-water solution (1:1, v/v) was used as an eluent. Samples were introduced at a flow rate of 10 μL/min into the electrospray source.

RESULTS AND DISCUSSION

Purification of Lignosulphonates by Dialysis

Industrial thin (SSL) and thick (THSL) liquors were the same as used in our previous work in which chemical composition is already discussed.[8] LS from SSL and THSL were purified by dialysis prior to the qualitative/quantitative analyses by ^{13}C NMR spectroscopy. The use of dialysis membranes with different cut-off pre-determined some differences in the purified LS composition. Thus, the purification of LS from SSL with membrane of 2000 NMWCO (sample LSF0) did not allow the effective elimination of concomitant carbohydrates, which significant proportion is still present in LSF0 (about 13%, w/w). This was explained by the presence of xylo-oligosaccharides (XOS) possessing similar to LS molecular weight.[8] The application of dialysis membrane of 5000 NMWCO (sample LSF) allowed much better purification of LS (residual sugars about 4%, w/w), but a low molecular LS fraction was removed in this case.[8] In fact, the weight-average molecular weight of LSF (2400 Da) was almost two times higher than for LSF0 (1350 Da). About 20% of SSL dry matter was lost in dialysis with a 2000 NMWCO membrane and about 40% in dialysis with a 5000 NMWCO membrane. Some basic characteristics of obtained LS samples are presented in Table 1.

The purification of LS from THSL (sample LSG) with a membrane of 2000 NMWCO was more successful than the purification of LS from SSL (sample LSF) due to partial degradation of XOS to xylose during SSL evaporation.

Table 1. Chemical composition of *Eucalyptus globulus* lignosulphonates (%, w/w) isolated from thin liquor by dialysis with membrane of 2,000 NMWCO (LSF0) and of 5,000 NMWCO (LSF) and from thick liquor by dialysis with membrane of 2,000 NMWCO (LSG).*

	LSF0	LSF[8]	LSG[8]
Ash	9.0	6.9	8.2
Sugars	12.8	4.1	7.3
Rhamnose	0.2	0.3	0.2
Fucose	<0.1	<0.1	<0.1
Arabinose	0.3	<0.1	0.4
Xylose	9.8	2.7	5.0
Mannose	0.3	0.2	0.3
Galactose	1.2	0.5	0.8
Glucose	1.0	0.4	0.6
C	36.0	48.2	39.1
H	6.2	5.6	5.8
S	6.6	5.5	7.6
SO_3H	19.5	11.2	19.8
Phenolic OH	1.9	1.4	1.9
OCH_3**	—	17.8	15.2
M_{ppu} (g/mol)***	—	259.7	324.9
Approximate yield of retentate	80	60	80

*Analysis of LSF0 was carried out by the same methodology as previously reported.[8]

**Corrected for the ash content.

***Empirical formula for LSF and LSG per phenylpropane unit: $C_9H_{9.77}O_{3..92}S_{0.10}(SO_3H)_{0.36}(OCH_3)_{1.51}$ and $C_9H_{13.20}O_{5.61}S_{0..02}(SO_3H)_{0.79}(OCH_3)_{1.59}$, respectively.[8]

LSG contained about half the amount of residual carbohydrates (7%, w/w) when compared to LSF (Table 1). All three LS samples (LSF0, LSF, and LSG) were submitted to structural analyses by NMR and ESI-MS. LSF, containing the lowest contamination with carbohydrates, was the most suitable sample for structural studies. However, LSF0 and LSG were more representative in relation to the low molecular weight LS fraction when compared to LSF and reflected the structural changes occurred during SSL evaporation.

Assignment of Lignosulphate Structures in NMR Spectra

The assignments of signals in ^{13}C and ^{1}H NMR spectra of LS were made based on known NMR database of eucalyptus lignin,[9] eucalypt heteroxylan/glucan,[10,11] and sulphonated lignin model compounds.[12–16] Additionally, all structural assignments were confirmed by DEPT edited ^{13}C NMR

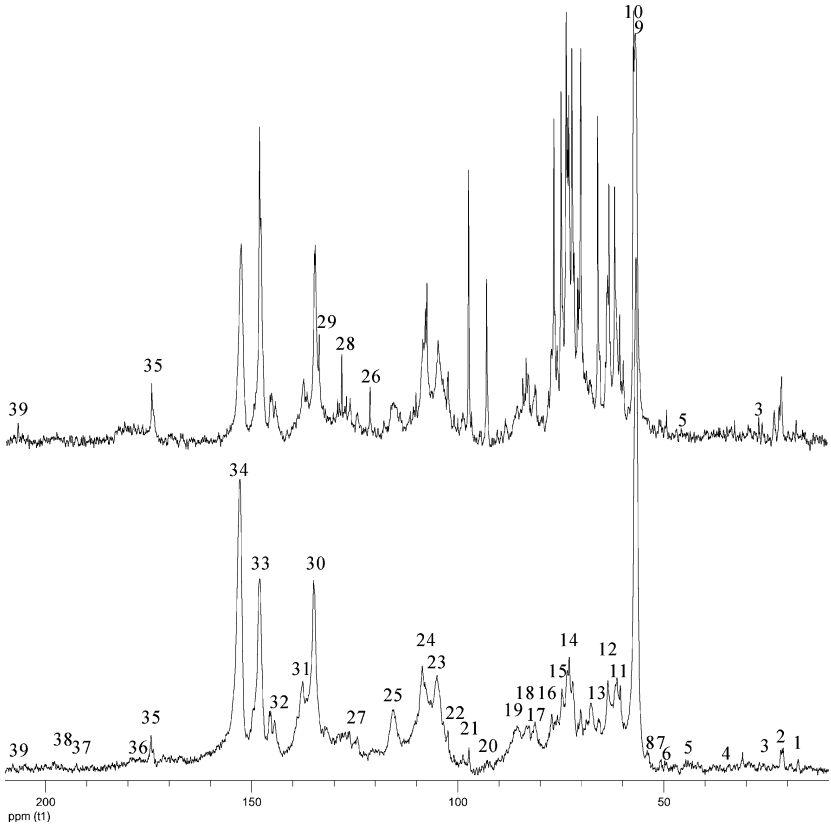

Figure 1. ^{13}C NMR spectra of purified eucalypt lignosulphonates from sulphite spent liquor: LSF0 (top figure) and LFS (bottom figure) (D_2O, 50°C).

subspectra, proton-proton (COSY), and proton-carbon (HSQC) correlation 2D NMR spectra.

^{13}C NMR spectra of LSF0 and LSF in D_2O are presented in Figure 1. ^{13}C NMR total and DEPT (θ 135°) spectra of LSG are shown in Figure 2. The carbon assignments for major LS structures, as depicted in ^{13}C NMR spectra of LSF0 and LSF, are presented in Table 2. The designations for the identified structures are shown in Figure 3.

The sulphonated at benzylic carbon β-*O*-4 structures (**A** type) were easily distinguished in HSQC spectra of LSF (Figure 4), LSG (Figure 5) and in COSY spectra of LS (spectrum of LSG is presented in Figure 5). The chemical shifts for C/H in **A** structures were very close to those reported for the corresponding model compounds taking into consideration the differences in solvents used and the temperature of spectra acquisition.[12]It is important to note that no

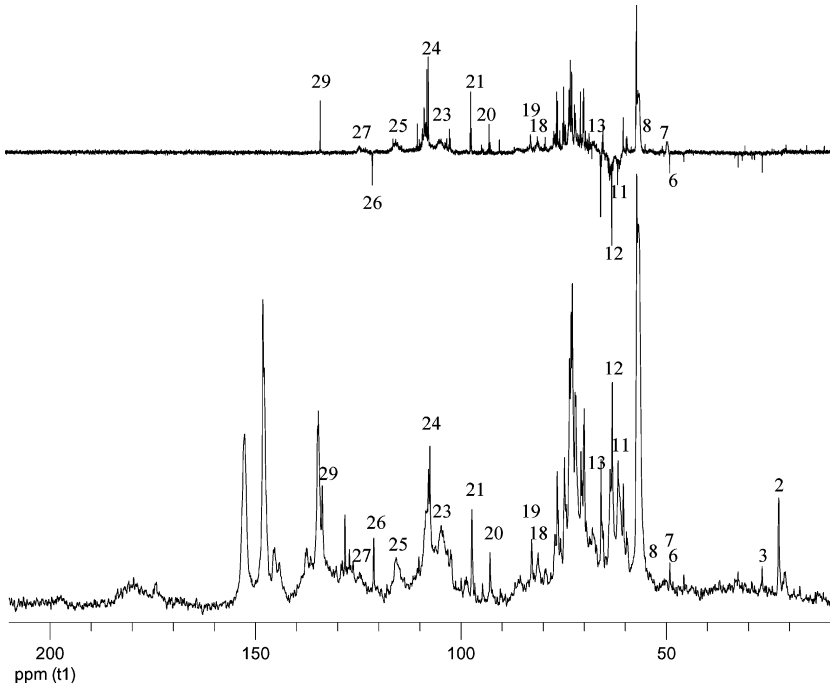

Figure 2. DEPT ($\theta = 135^\circ$) (top figure) and ^{13}C NMR (bottom figure) spectra of purified eucalypt lignosulphonates from thick liquor (LSG) (D_2O, 30°C). Signals designations are the same as in Figure 1.

significant changes in carbon chemical shifts were found in the temperature range of 30–50°C, whereas rather notable differences in proton chemical shifts (δ 0.05–0.10) were registered within the same temperature range (shift to upper field while temperature increases).

The sulphonated at Cα phenyl coumaran (**B** type) and α-methyl benzyl alcohol (**E** type) structures were identified based on carbon and proton assignments reported for corresponding model compounds[12,13] and according to proton-proton correlations in COSY spectra. 4-allylphenol-α-sulphonic acid type structures (**C** type), abundant in LSG and LSF0, were identified based on HSQC and COSY spectra (Figure 5) using database on parent non-sulphonated model 4-allyl-2,6-dimethoxyphenol.[17] DEPT (θ 135°) spectra showed characteristic negative signal for Cγ (CH_2 moiety) at 121.1 ppm and a positive signal for Cβ (CH group) at 133.8 ppm in these structures (Figure 2). The α, γ- disulphonic acid type structures (**D** type) were identified based on reported proton assignment in correspondent lignin model degradation product,[16] lignin models with sulphonated Cγ[12] and proton-proton correlations in COSY spectra of LSF0 and LSG. Thus, Hα at 4.25 ppm in **D** structures showed the correlation to

Table 2. Assignments of carbon signals in ^{13}C NMR spectra of lignosulphonates

Signal	^{13}C shift, ppm	Assignment*
1	17.8	Cβ in **E** structures
2	21.3	CH_3 in acetyl groups
3	26.3	Cβ in **I** structures
4	33.0	Cβ in **D** structures
5	44.5	Cβ in **J** structures
6	49.6	Cγ in **D** structures
7	50.8	Cα in **J** structures
8	53.9	Cβ in **B** structures
9	56.8	Carbon in OCH_3 bonded to aromatic ring
10	57.3	Cα in **F** structures
11	60.6/61.1	Cγ in **A** structures
12	63.6	Cγ in **B** structures and C5 in xylan (int. units)
13	67.6	Cα in **A**, **B** and **J** structures (overlaped)
14	72.0	C2,5 in glucan (int. units)
15	74.6	C2,3 in xylan (int. units)
16	77.1	C4 (int. units) and C3 (red. units) in xylan
17	81.2	Cβ in **A** structures
18	83.1	Cβ in non-sulphonated β-*O*-4 structures
19	85.6	Unknown assignment
20	92.7	C1 in xylose reducing unit (α-isomer)
21	97.3	C1 in xylose reducing unit (β-isomer)
22	102.5	C1 in xylan (int. unit)
23	105.0	C2,6 in S units without sulphonic group at Cα
24	107.7	C2,6 in S units with sulphonic group at Cα
25	115.4	C2,5 in G units with sulphonic group at Cα
26	121.2	Cγ in **C** structures
27	124.2	C6 in G units with sulphonic group at Cα
28	128.3	C1 in **C** structures
29	133.8	Cβ in **C** structures
30	134.9	C1 in **A**, **B**, **D**, **E**, **F**, and **I** structures
31	137.6	C4 in non-phenolic 4-*O*-5 and in E structures
32	145.1	C4 in phenolic G structures
33	148.0	C4 in phenolic S and C3,4 in non-phenolic **G** structures; C3,5 in phenolic S structures
34	152.8	C3,5 in non-phenolic S structures
35	174.2	C=O in acetyl groups
36	178.8	COOH in $COOH\text{-}CH_2\text{-}CH(SO_3H)\text{-}Ar$
37	192.8	CHO in benzaldehyde type structures
38	196.2	C=O in **I** structures
39	206.7	Non-conjugated C=O

*Structure assignments are presented in Figure 3.

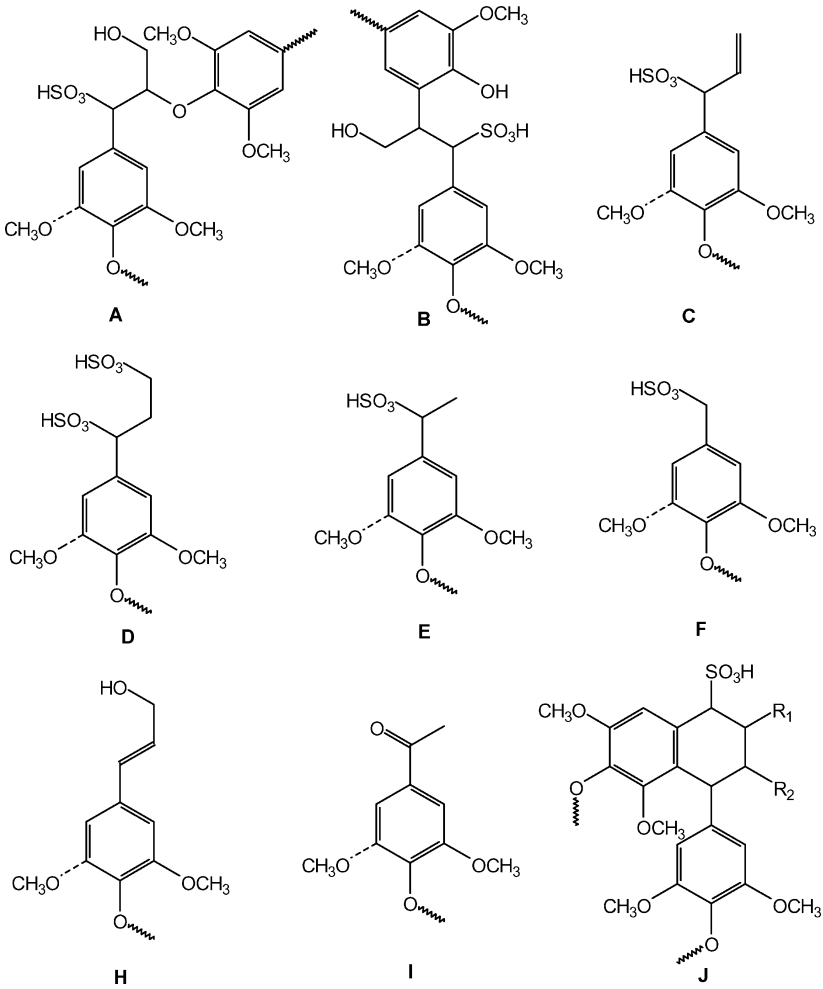

Figure 3. Lignosulphonate structures detected by NMR spectroscopy.

Hβ at 2.83 ppm, which, in turn, correlated to Hγ at 3.45 ppm (chemical shifts for the spectra acquired at 50°C). The sulphonated benzyl alcohol type structures (**F** type) were identified based on Cα/Hα signals (δ 57.3/4.00) reported for sulphonated vanillin alcohol[12] and due to the characteristic coupling of benzylic germinal protons ($^2J = 13.5$ Hz).

Non-sulphonated lignin structures of **H** and **I** types were assigned based on available databases for carbon and proton shifts in the corresponding model lignin compounds and lignin substructures.[17,18] No original $\beta - \beta$ structures of pino-/syringaresinol types were found in all isolated LS samples. These

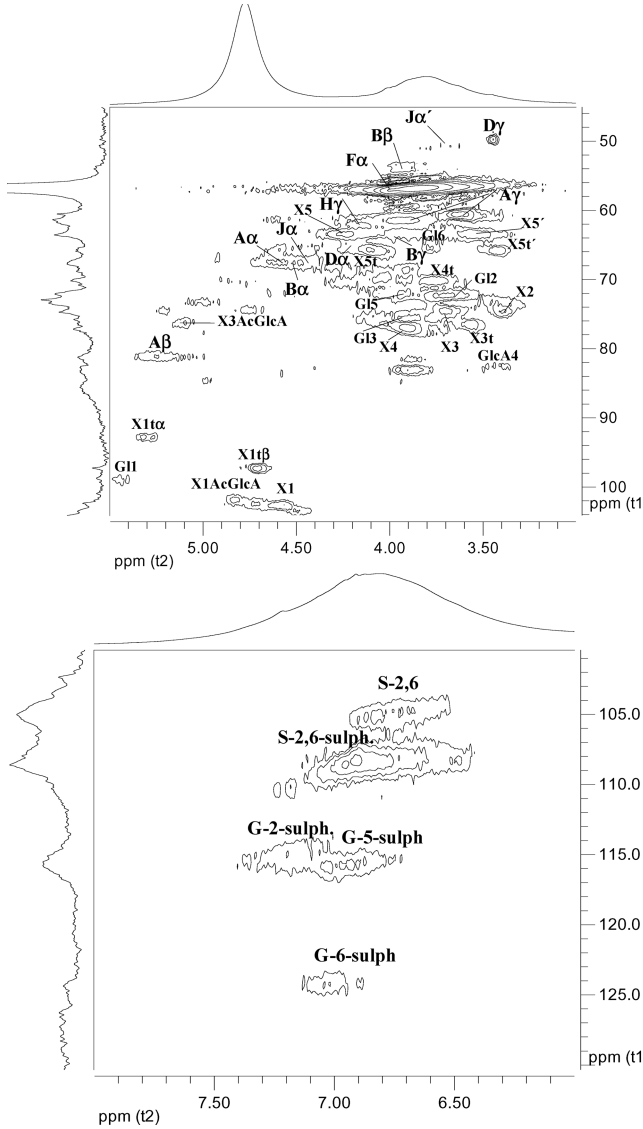

Figure 4. Expanded aliphatic (top figure) and aromatic (bottom figure) regions of the LSF HSQC spectrum (D_2O, 50°C). Designations for the lignin structures are depicted in Figure 3. Guaiacyl (G) and syringyl (S) units possessing sulphonic group at Cα are marked as G-sulph and S-sulph, respectively. Designations for oligosaccharides are as follows: X, internal β-D-Xyl$p$ unit; Xt, terminal non-reducing Xyl$p$ unit; Gl, internal α-D-Glc$p$ unit; Glt, terminal non-reducing Glc$p$ unit; XAcGlcA, Xyl$p$ unit branched at O-2 with MeGlcA residue and O-3 acetylated; α- and β-isomers in the terminal Xyl$p$ reducing units marked as Xtα and Xtβ, respectively; GlcA, terminal MeGlcA residue.

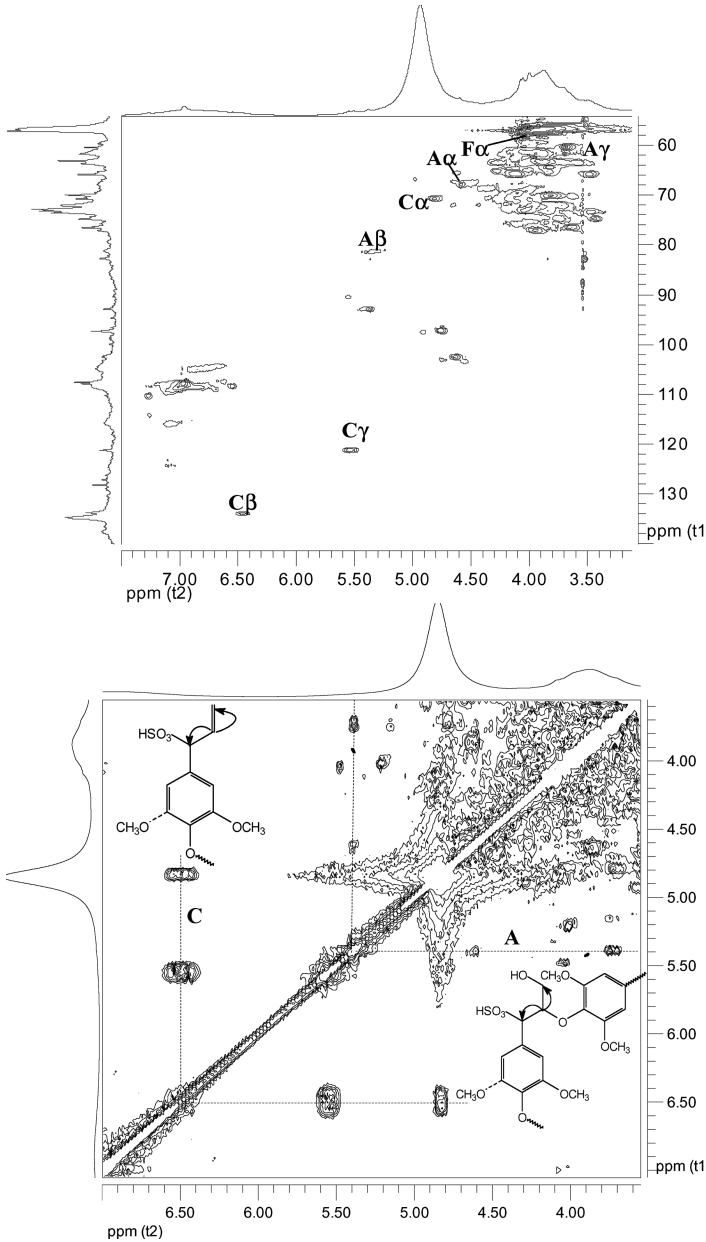

Figure 5. Expanded regions of HSQC (top figure) and COSY (bottom figure) spectra of purified eucalypt lignosulphonate from thick liquor (LSG) (D_2O, 30°C). Designations for the lignin structures are depicted in Figure 3.

structures should degrade under conditions of sulphite pulping producing a large variety of sulphonated structures with cleaved $\alpha - O - \gamma$ linkages.[13,16] Intra-molecular condensation reaction of $\beta - \beta$ structures leads to the formation of cyclic 1-phenyltetraline type structures ($\beta - \beta/\alpha$-6) with or without sulphonic groups.[13] Similar structures (**J** type) were suspected to be present in eucalypt LS based on the characteristic carbon and proton resonances of Cα/Hα at δ50.8/3.73 reported for structurally similar cyclic lignans[17] and among products of acidolytic degradation of $\beta - \beta$ structures in eucalypt lignin.[9] The correlations of H'α at 4.48 ppm (proton linked to sulphonated benzylic carbon) and H'β at 2.36 ppm, as well as correlation of Hα at 3.73 ppm and Hβ at 2.23 ppm allowed proposal of **J** type structures with $R_1 = R_2 = H$ (Figure 3). The structures of benzylaldehyde and sulphonated sinapinic acid type structures (COOH-CH_2-$CH(SO_3H)C_6H_2(OCH_3)_2OH$) were suggested based on characteristic carbon resonances reported for the corresponding model compounds.[12,18] The presence of identified LS structures in spent liquors was additionally confirmed by ESI-MS/MS analysis, the results of which are discussed later.

HSQC spectra of isolated LS samples (Figures 4 and 5) showed well-defined signals from non-acetylated and partially acetylated xylo-oligosaccharides (XOS) and from gluco-oligosaccharides (GOS). According to sugars analysis and ESI-MS/MS analyses, XOS and GOS are still presented in LS samples despite their purification by dialysis.[8] It is rather probable that at least part of XOS and GOS are chemically linked to lignin structures. In particular, a part of non-sulphonated β-O-4 structures had the chemical shift of Hβ to the upper field (Figure 4), which may be due to the presence of benzyl ether linked XOS/GOS.

Analysis of Lignosulphonates by Tandem ESI-MS

LS were analyzed by tandem ESI-MS in order to confirm some structures suggested by NMR spectroscopy and aiming to assess the primary structure of the simplest lignin oligomers. Thus, LSG was submitted to fractionation by SEC and two fractions eluted at 20.4–21.0 min (fraction 1) and at 21.0–21.8 min (fraction 2) were isolated and analyzed by ESI-MS in the negative mode (Figure 6). Negative ESI mode is preferable for lignin analysis, especially in collision-induced dissociation (CID) experiments, thus providing more sensitive and simple mass spectra when compared to those acquired in the positive mode.[19] In this case lignin oligomers are detected in ESI-MS spectra essentially as monocharged adducts $[M\text{-H}]^-$. According to calibration of SEC column with PSSNa standards, the fractions eluted at $>$21.8 min contained liquor components of $<$200 Da and those eluted at $<$20.4 min contained liquor components of $>$1000 Da. These fractions were not considered for the analysis.

The ESI-MS spectra of fractions 1 and 2 are presented in Figure 6. The most intensive signal at m/z 273 in the spectrum of fraction 2 was

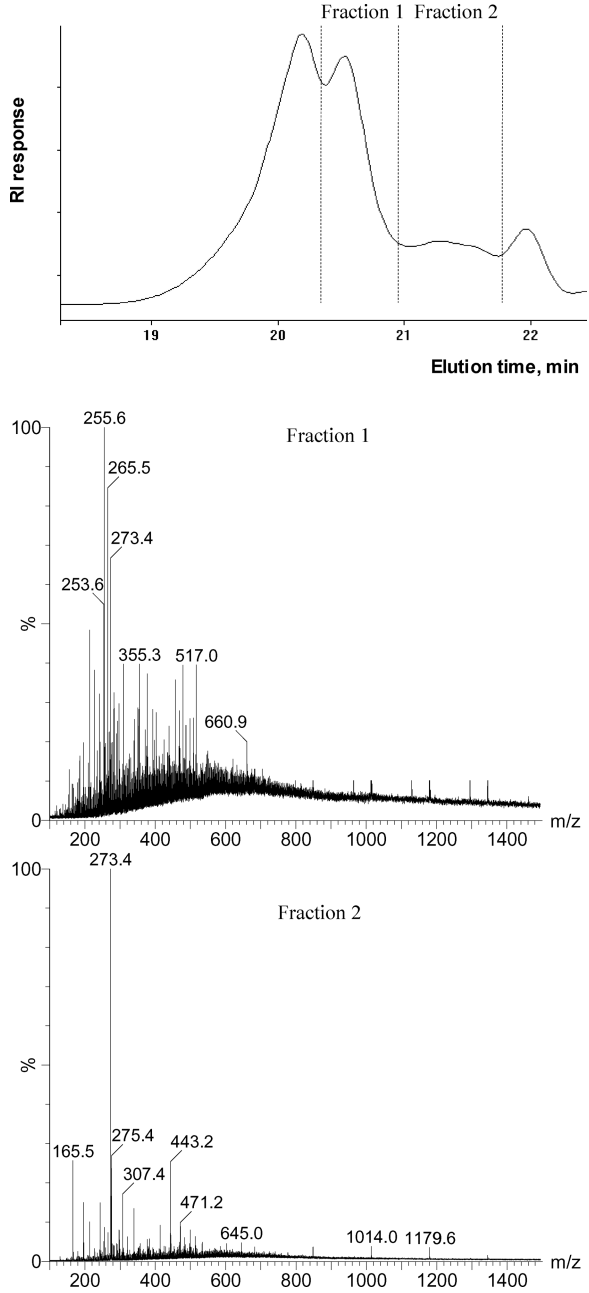

Figure 6. SEC elution curve of purified eucalypt lignosulphonate from thick liquor (LSG) and negative mode ESI-MS spectra of SEC fractions eluted at 20.4-21.0 (fraction 1) and 21.0–21.8 min (fraction 2).

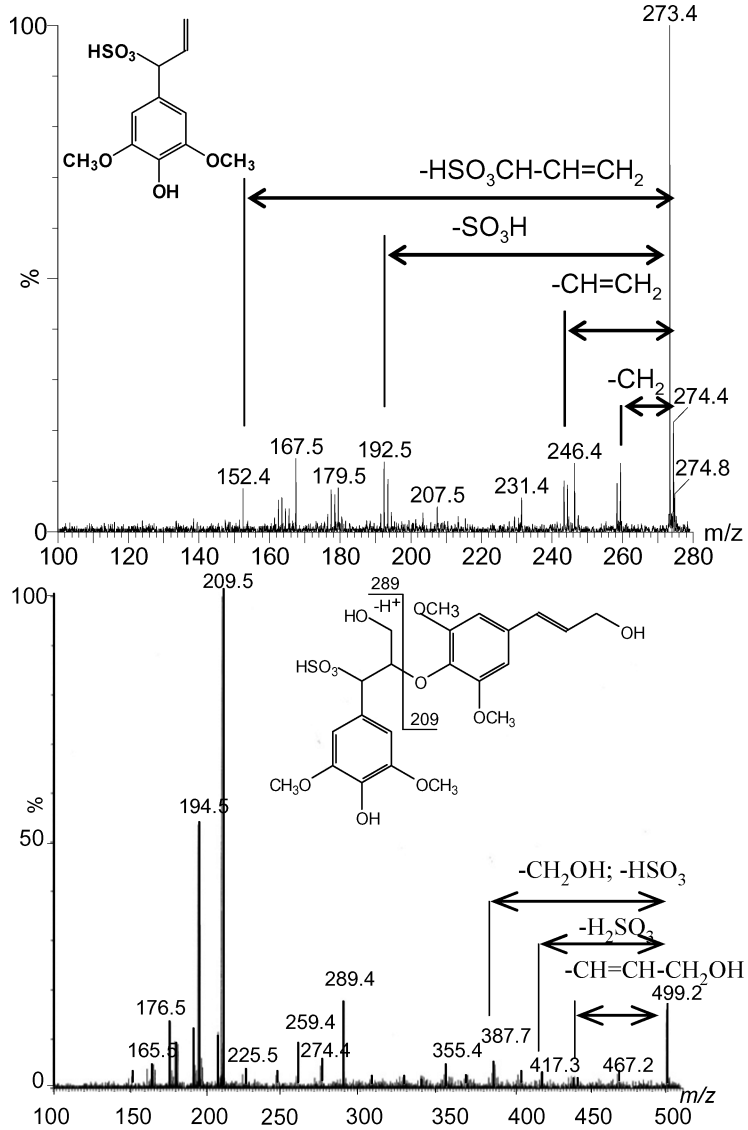

Figure 7. MS/MS spectra (negative mode) of monomeric (*m/z* 273) and dimeric (*m/z* 499) lignosulphonic acids.

assigned, according to fragmentation patterns in the MS/MS spectrum (Figure 7, Table 3), to 4-allyl-2,6-dimethoxyphenyl-α-sulphonic acid (compound **I**, Figure 8). The peak at m/z 273 was also one of the most abundant signals in ESI-MS spectrum of the fraction 1 indicating that monomeric

Table 3. Data on ESI-MS/MS analysis of sulphonated lignin structures in the negative mode (structure assignments are presented in Figure 8)

Structure	MS/MS ions (m/z, (relative abundance, %))
I	273([M-H]$^-$, 100); 259(12); 246(12); 231(6); 207(4); 192(13); 179(10); 167(13); 152(10)
II	355([M-H]$^-$, 100); 325(12); 273(6); 259(8); 245(6); 193(7); 179(15); 165(18); 149(10)
III	499([M-H]$^-$, 18); 467(4); 387(5); 289(20); 259(9); 209(100); 194(56); 176(18); 165(7)
IV*	551([M-H]$^-$, 48); 469(9); 289(25); 259(100); 245(21); 209(39); 195(66); 176(23); 165(12)
V	443([M-H]$^-$, 48); 361(8); 346(25); 331(20); 246(100); 231(14); 195(7); 181(23); 166 (12)
VI	439([M-H]$^-$, 100); 357(14); 273(3); 259(11); 245(7); 179(16); 165(21); 152(5)

*The relative intensity of signals may be inaccurate due to the superposition of peaks from different structures at the same *m/z*.

compound **I** should be the predominant **C** type structure of LS (Figure 3). This is in agreement with the almost absence of these structures in LSF sample (Figure 1) due to their obvious release to dialysate during the SSL purification with membrane of relatively large diameter of pores. Another relatively

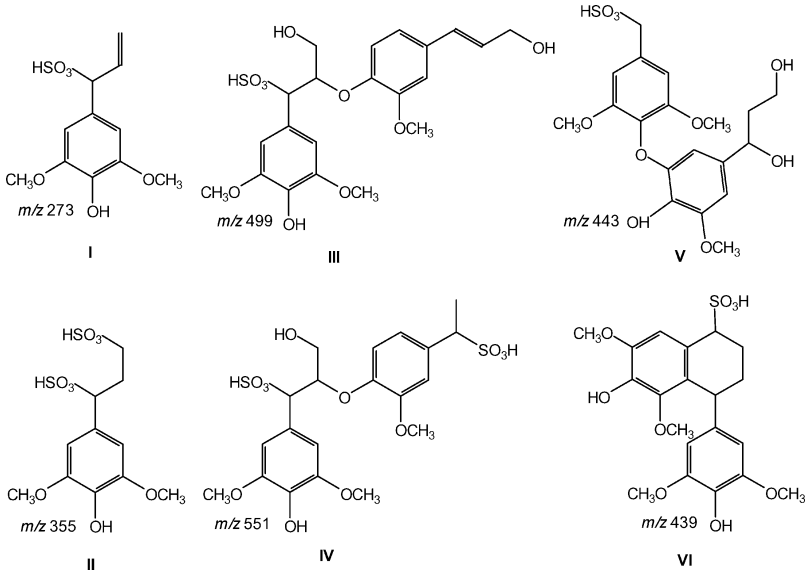

Figure 8. Lignosulphonate structures detected by ESI-MS/MS.

abundant monomeric LS was 4-propyl-2,6-dimethoxyphenyl-α, γ-disulphonic acid (compound **II**, Figure 8), which molecular ion at m/z 355 was detected in the ESI-MS spectrum of fraction 1 and that structure was inferred from the MS/MS spectrum analysis (Table 3). It may be proposed, based on relative abundance of signals at m/z 273 and at m/z 355 that the ratio of compound **I** to compound **II** should be as high as 2–3 (Figure 6).

The dimeric LS were detected in the range of m/z 400–600. These were mainly ether linked sulphonated structures and derivatives of syringaresinol structures (compounds **III–VI,** Figure 8). The molecular ions $[M\text{-H}]^-$ of sulphonated β-O-4 and 4-O-5 dimers always showed in tandem mass spectra the characteristic loss of bisulphite anion (–81 Da) or sulphurous acid (–82 Da). However, the major intensity fragments of β-O-4 and 4-O-5 dimeric structures in MS/MS spectra were those arisen from the cleavage of ether bonds (Figure 7, Table 3). The ions at m/z 289, 259, 209 and 195/194 in the MS/MS spectra were considered as characteristic signals of sulphonated at benzylic position terminal β-O-4 linked syringyl units (Figure 7, Table 3). The detected compounds **IV** and **V** (Figure 8) evidenced the Cα-Cβ and Cβ-Cγ cleavage in LS structures during acidic sulphite pulping, thus confirming the NMR assignments concerning **E** and **F** type structures (Figure 3).

The identified compound **VI** was detected among LS of fraction 1 and, more likely, belongs to the degradation product of syringaresinol type structures. These cyclic 1-phenyltetraline type structures (**J** type) are one of the C-6 condensed structures found in the permanganate oxidation analysis of LS.[8] Due to the high diversity of **J** type structures in LSG, their molecular ion intensities were rather small and difficult to assess by ESI-MS/MS.

The most abundant XOS identified by tandem ESI-MS in fraction 2 were xylobiuronic (MeGlcA-Xyl, m/z 339) and xylobiosebiuronic (MeGlcA-Xyl_2, m/z 471) acids. The fragmentation patterns of these ions were identical to those previously published for these XOS.[20]

Structural Characterization by ^{13}C NMR

The analysis of ^{13}C NMR spectra allowed some quantitative estimation on inter-unit linkages in LS and to compare their abundances with those in eucalypt wood lignin (Table 4). According to previously established methodology,[9,18] all LS structural elements were calculated per one aromatic group (C_6). The integral of aromatic carbons at 103.5–162.0 ppm (I_{ar}) was corrected for the Cγ and Cβ carbon signals from 4-allyl-2,6-dimethoxyphenyl-α-sulphonic acid structures ($I_{ar}^{cor} = I_{ar} - I_{121.2} - I_{133.8}$). The amounts of particular structural groups were calculated ($n_x = 6 \cdot I_x / I_{ar}^{cor}$) using integrals of characteristic carbon signals (I_x), according to suggested assignments (Table 2).

Due to the strong overlapping of lignin aliphatic oxygenated carbons with multiple signals from oligosaccharides, the quantitative estimation of lignin

Table 4. Structural analysis of lignosulfonates by quantitative ^{13}C NMR (per one aromatic group)

Structural elements*	Lignosulphonate samples			EDL[9]
	LSF0	LSF	LSG	
β-O-4 structures	n.d.	0.34	n.d.	0.56
β-5 tructures	0.03	0.06	0.04	0.03
β-βstructures	n.d.	n.d.	n.d.	0.13
4-allylphenolic type structures	0.15	<0.02	0.16	—
α, γ-disulphonic acid structures	0.06	<0.02	0.05	—
OCH_3	1.45	1.36	1.40	1.47
Ar-O	2.70	2.80	2.60	2.50
Ar-C	1.20	1.20	1.30	1.30
Ar-H	2.10	2.00	2.1	2.20
S:G ratio	78:22	71:29	81:19	85:15

*Sulphonated and non-sulphonated structures.

substructures in LSF0 and LSG was particularly difficult. Therefore the amount of β-O-4 structures was calculated only for the relatively pure LSF sample (Table 4). The integrals of signals at 80.0–84.0 ppm and at 60.0–62.0 ppm (Cβ and Cγ in β-O-4 structures, respectively) were similar and revealed the reduced number of β-aryl ether structures in LSF (0.34/C_6). These structures are even less abundant in LSF0 and LSG, which contained significant proportion of monomeric LS. This fact evidence a strong depolymerization of eucalypt wood lignin in acidic sulphite pulping via cleavage of β-O-4 structures. In fact, the abundance of β-O-4 structures in lignosulphonates from SSL was practically the same as in black liquor lignin after kraft pulping of *Eucalyptus globulus*.[21] The relative intensity of signal at 81.2 ppm, assigned to Cβ in sulphonated β-O-4 structures (Table 2), was about 15% lower for LSG than for LSF0, thus indicating the partial cleavage of those structures upon liquor evaporation. This conclusion is supported by the lower ratio of signals at 152.8 ppm (C3,5 in non-phenolic S structures) to signals at 148.0 ppm (contribution of C3,5 in phenolic S structures) in LSG when compared to LSF0.

The abundance of β-5 structures in LS was calculated based on integral of resonance centred at 53.4 ppm (Cβ in sulphonated phenyl coumaran structures). Some higher amounts of β-5 structures in LS than in eucalypt dioxane lignin (EDL) may be explained by preferential occurrence of these structures in oligomers of relatively high molecular weight and removal of monomeric syringyl units upon purification by dialysis.

The amounts of 4-allyl-phenyl-α-sulphonic acid type structures (**C** type, Figure 3) and α, γ-disulphonic acid type structures (**D** type, Figure 3) was

estimated based on resonances at 121.2 and 49.2 ppm, respectively, assigned to Cγ in corresponding structures (Table 2). According to ESI-MS analysis these structures are predominantly monomeric and this was a reason for their minor abundance in LSF, which was dialyzed with membrane of a relatively large diameter of pores. Both structures contributed to more than 20% of LS in LSF0 and LSG. The real amounts of these two structures in SSL may be even higher, because their undetermined amounts were released during the purification by dialysis. More likely, monomeric mono- and di-sulphonated structures (**I** and **II** in Figure 8) were derived from sinapyl alcohol in reaction with sulphurous acid.[16,22,23] Sinapyl alcohol type structures and its derivatives were also detected by ESI-MS in LS dimers (Figure 8). These features indicate that sinapyl alcohol structures are basic intermediates of syringyl lignin depolymerization upon acidic sulphite pulping of eucalypt wood.

The massive release of **I** and **II** structures from SSL during dialysis is the main reason for lower proportion of S units in LS, when compared to EDL (Table 4). The ratio of S and G units was calculated as described previously based on the relative intensities of signals at 103–110 ppm (tertiary aromatic carbons in S units) and at 110–125 ppm (tertiary aromatic carbons in G units).[9] Practically no *p*-hydroxyphenyl propane units were detected in LS (the absence of resonances at 159-163 ppm belonging to C-4 in corresponding structures). These structures count to about 2% units in eucalypt lignin[9] and may be removed during lignin purification or were preferentially retained in pulp.

The sulphonated vanillin/syringyl alcohol structures (**F** type, Figure 3) were relatively abundant in all analyzed LS samples (Figures 1 and 2). However, their accurate quantification was difficult because of overlap of signals from benzylic carbon in **F** structures and carbon in methoxyl groups. A rough estimation allowed account to about 0.1–0.2/C_6 of **F** type structures in LS. The significant proportion of **F** structures indicates the important contribution of Cα-Cβ scission in side chain to the lignin degradation. The amount of **F** structures in LS of thick liquor is higher than in LS of thin liquor (comparing the carbon spectra of LSF0 and LSG, Figures 1 and 2). Hence, during the evaporation of thin liquor LS suffered additional degradation leading to the Cα-Cβ scission of propane chain.

A set of characteristic resonances at 181–185 ppm indicated the eventual presence of quinone type structures in LS (Figures 1 and 2). These signals were especially pronounced in LS purified by dialysis with membrane of relatively low diameter of pores from thin (LSF0) and thick (LSG) liquors and much less abundant in LSF dialyzed with membrane of larger diameter of pores. Therefore, quinone structures should be localized mainly in the low molecular weight LS oligomers. The quinone structures were more abundant in LFG (about 0.14/C_6) than in LSF0 (about 0.09/C_6) thus indicating their possible formation during the liquor evaporation. To our knowledge, quinone structures in LS were never reported before and may be some kind of resonance-stabilised *para*-quinones formed during LS desulphonation. The presence of

quinone structures can explain the pronounced shoulder at 1680–1740 cm^{-1} in FTIR spectra of LS.[8] At the first glance, comparing the ^{13}C NMR spectra of LSF/LSG and lignin isolated from kraft black liquor,[21] the amounts of quinone structures in those technical lignins were of the same order.

The proportion of aromatic quaternary oxygenated (Ar-O), non-oxygenated (Ar-C) and tertiary (Ar-H) carbons were formally determined based on integration of corresponding characteristic spectra intervals at 136–162, 125–136, and 103–125 ppm (Table 4). Although no global conclusions could be made comparing the data for EDL and for the three isolated LS samples, it may be proposed that among LS samples studied LSG possessed highest amounts of quaternary non-oxygenated carbons (Ar-C). This finding corroborates with results of permanganate oxidation analysis, which showed the higher proportion of condensed structures in LS from thick than from thin liquor.[8]

The results of this work showed that the eucalypt lignin degradation pathways in acidic sulphite pulping (especially the extent of cleavage for the main interunit bonds) were rather different from those reviewed for the softwoods.[1,13,23] Apparently, the mechanisms of delignification upon acidic sulphite pulping of hardwoods differ from those suggested for softwoods and need additional explanation.

Small differences in the amounts of methoxyl group in wood lignin (EDL) and in LS from spent liquor (LSF0) may be indicative of the low significance of the demethylation reactions during acidic sulphite pulping. The same conclusion was reached previously based on wet chemistry analyses of LS.[8]

The ^{13}C NMR spectra of LSG and LSF0 revealed also some changes in XOS that occurred during SSL concentration. Besides eventual hydrolysis, XOS suffered notable deacetylation as followed from the significant decrease (about four times) of resonance at 174.2 ppm in ^{13}C NMR spectrum of LSG assigned to the carbonyl carbon in acetyl groups (Figures 1 and 2).

CONCLUSIONS

The structural studies on lignosulphonates (LS) have revealed substantial depolymerization and structural modification of *Eucalyptus globulus* lignin during acidic magnesium–based sulphite pulping. Thus, the β-O-4 and α-O-4 bonds were readily cleaved upon acidic sulphite pulping giving raise to partially sulphonated, mainly at the benzylic carbon, oligolignols of relatively low molecular weight (<1500 Da). A significant proportion of depolymerization products arisen from β-O-4 structures were monomeric derivatives of sinapyl alcohol: 4-allyl-2,6-dimethoxyphenyl-α-sulphonic and 4-propyl-2,6-dimethoxyphenyl-α,γ-disulphonic acids. These monomeric structures contributed to more than 20% of LS. Pino- and syringaresinol lignin structures were readily degraded during sulphite pulping giving raise to large variety of residues

including condensed cyclic 1-phenyltetraline type structures. Depolymerized lignin oligomers suffered notable cleavage of propane chain at Cα-Cβ and Cβ-Cγ. In particular, the proportion of sulphonated vanillyl/syringyl alcohol type structures in LS was especially remarkable. LS also contained a relatively high proportion of quinone type structures (as least about $0.10/C_6$). At the same time the demethylation reactions of aromatic methoxyl groups in lignin were less significant. In fact, the extent of lignin degradation (decrease in the molecular weight, scission of the main inter-unit ether linkages, etc.) during the acidic sulphite pulping and the kraft cooking of eucalypt wood were very similar.

LS analysis revealed also structural changes occurred during the concentration of SSL by evaporation under industrial conditions. Namely, the additional cleavage of ether and carbon-carbon linkages in the side chain of LS and the increase of their condensation degree has been detected.

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