NEW INSIGHTS INTO EUCALYPTUS LIGNIN & PULPING CHEMISTRY

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ABSTRACT

Despite the growing importance of *Eucalyptus* wood as pulp and paper raw material, there is a lack of knowledge on the specific chemistry of the macromolecular components of *Eucalyptus* species. The present paper addresses this issue by combining the recently developed protocol for isolating enzymatic mild acidolysis lignins (EMAL) with the novel combination of derivatization followed by reductive cleavage (DFRC) and quantitative ^{31}P NMR. More specifically, EMALs were isolated from *E. globulus*, *E. grandis*, redwood and White fir, milled under different ball milling conditions. Overall, the yields and purities of EMALs isolated from *Eucalyptus* were found to be higher than those from the examined softwoods. Comparison of lignins isolated from *E. globulus* and *E. grandis* by ^{31}P NMR showed significant differences in their chemical structure. Lignin from *E. globulus* was found to contain higher contents of arylglycerol-β-aryl ether structures, free phenolic hydroxyl groups and syringyl-type units than lignin from *E. grandis*. New insights provided by the combination of DFRC and ^{31}P NMR revealed that up to 62.2% of arylglycerol-β-aryl ether structures in *E. globulus* are uncondensed, while in *E. grandis* the amount of this uncondensed structures were found to be lower than 48%. Syringyl to guaiacyl ratio determined by ^{31}P NMR before and after DFRC indicated that syringyl-type units in both *Eucalyptus* woods are predominantly non-phenolic. Size-exclusion chromatography (SEC) analyses performed on these EMALs showed that lignins from *E. grandis* and softwoods associate in greater extension than lignin isolated from *E. globulus*. Furthermore, green liquor (GL)-modified pulping was confirmed to be a feasible technology for producing *Eucalyptus* and softwood pulps. GL pretreatment of *Eucalyptus* wood chips offered significant savings in the total amount of active alkali, besides reducing the amount of rejects and the residual lignin retained on the fibers. Kinetic studies performed on isolated lignin from *E. grandis, E. globulus* and a mixture of softwoods revealed that the higher the amount of free phenolic hydroxyl contents within the lignin, the faster the degradation of arylglycerol-β-aryl ether structures under GL impregnation conditions.

INTRODUCTION

Hardwoods are important raw materials used in the production of pulp and paper. For example, *Betula Pendula* (birch) is the dominant hardwood species for such applications in Northern Europe, whereas *Eucalyptus* species represent the main fiber sources for the pulp and paper industry in the Iberian Peninsula and South America (*1*).

Despite the growing importance of *Eucalyptus* wood as pulp and paper raw material, there is a lack of knowledge on the specific chemistry of the macromolecular components of *Eucalyptus* wood (*1*,*2*). As far as lignin is concerned, few efforts have been made to better understand its isolation process from this hardwood (2,*3*). Milled wood lignin (MWL) from *Eucalyptus* is always obtained in poor yields (*2*,*4*) and with high proportion of attached hemicelluloses and tannins, which hinder the quantitative analysis of the lignin structural elements (*2*). As such, a study aimed at understanding how to isolate lignin from *Eucalyptus* wood in high yield and purity is warranted.

Recent reports that deal with the isolation of the lignin from wood have shown that a novel procedure, using the combination of enzymatic and mild acidolysis (EMAL), isolates lignin that may be more representative of the total lignin present in milled-wood (*5*). Because a mild acid hydrolysis can liberate lignin from lignin-carbohydrate complexes (known to preclude lignin isolation in high yields), it can be combined with low severity of milling, facilitating the isolation of less modified lignin in high yields and purities from milled-wood (*5*). We have recently shown that the yields of EMAL are from 2 to 5 times greater than the corresponding MWL and cellulolytic enzyme lignin (CEL) isolated from the same batch of milled-wood, depending upon the wood species from which they were isolated (6). Comparison of the chemical structure of EMAL, MWL and CEL has revealed only subtle differences, evidencing that EMAL is released by cleaving lignin-carbohydrate bonds rather than other linkages within lignin macromolecule (*5*,*6*). Consequently, the aforementioned protocol presents a real opportunity to improve yield and purity of lignin from *Eucalyptus*, since such lignin is always obtained in low yields and purities due to the high proportion of attached hemicelluloses (*2*).

A novel approach for the quantification of different lignin structures using the combination of Derivatization Followed by Reductive Cleavage (DFRC) and quantitative ^{31}P NMR was recently described (*5*,*6*,*7*). Since quantitative ^{31}P NMR determines the amounts of the various hydroxyl groups, such spectra "before DFRC" provide quantitative information about the aliphatic hydroxyls, carboxylic groups and condensed and uncondensed units bearing phenolic hydroxyl groups within lignin. Such hydroxyl groups are revealed and quantified by ^{31}P NMR after phosphitylating lignin with 2-chloro-

4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (*8*). Unfortunately, quantitative ^{31}P NMR cannot offer any information about the etherified or carbon-carbon linked bonding pattern of lignin. However, when the aryl ether linkages are selectively cleaved by DFRC, the corresponding phenolic hydroxyls released can be quantified by ^{31}P NMR. In this way, the ^{31}P NMR spectra "after DFRC" offer detailed information about condensed and uncondensed units connected through β-aryl ether linkages (*5*,*7*), as well as dibenzodioxocins.

Overall, therefore, this study applies the recently described procedure to isolate EMAL and then uses the combination of DFRC with quantitative ^{31}P NMR spectroscopy (DFRC/^{31}P NMR) to provide new insights into the structure of lignins from *Eucalyptus grandis* and *Eucalyptus globulus* wood. For comparative purposes, two species of softwood, redwood and White fir, were also included in this study. The yields of EMAL obtained were also compared to MWL and CEL isolated and purified from identical batches of milled wood.

In another front, exploring the details of isolating lignin from wood in high yields and purities may provide new insights into *Eucalyptus* pulping chemistry. For instance, a number of studies have shown that when softwood chips are pretreated with green liquor (GL), a rich and recyclable hydrosulfide source, prior to kraft pulping, it is possible to obtain faster delignification, a decrease in the overall consumption of pulping chemicals, and better pulp strength properties (*9*-*11*). It has also been observed that the [HS^-]/[OH^-] ratio of the treatment liquor affects the later uptake of hydrosulphide in softwoods (*10*). Despite the growing interest in *Eucalyptus* wood, no significant efforts have been made to understand how green liquor interacts with lignin from this hardwood. Understanding lignin behavior during green liquor impregnation of *Eucalyptus* is of crucial importance for process optimization and improvement of pulping quality. The second part of the current work was therefore conducted to compare and contrast how GL interacts with lignin isolated from *E. grandis* and *E. globulus* and selected softwood species.

MATERIALS AND METHODS

Isolation of EMALs, MWL and CEL. Enzymatic Mild Acidolysis Lignins (EMALs) were isolated from white fir (*Abies concolor*), redwood (*Sequoia sempervirens*) and eucalyptus (*Eucalyptus globulus and grandis*). Wood powders from each different wood species were ground to pass a 20-mesh screen in a Wiley mill and Soxhlet extracted with acetone for 48 h. The resulting Wiley-milled wood powder was air-dried and stored in a desiccator under vacuum. The *Eucalyptus* wood-powder was submitted to an alkaline extraction with 0.075 mol/L NaOH for 1h under reflux to remove tannins before use (*2*). Rotary ball milling was performed in a 5.5 L porcelain jar in the presence of 474 porcelain balls (9.4mm diameter), which occupied 18% of the active jar volume. One hundred grams of extractive-free wood powder was loaded into the jar, creating a porcelain ball/wood weight ratio of 16.6. The milling process was conducted at room temperature for up to 28 days with a rotation speed of 60 rpm (*5*). EMALs were isolated from ball milled-wood as previously reported (*5*,*6*), wherein the ground wood meal was treated with cellulase (Iogen, Canada; filter paper activity 130 FPU ml^{-1}) in a previously optimized (*12*) ratio of 40 FPU per gram of wood. The enzymatic hydrolyses were carried out at 40 ^{0}C for 48 h using 50 mM citrate buffer (pH 4.5) at 5% consistency in an orbital water bath shaker. The insoluble material remained after the enzymatic hydrolysis was collected by centrifugation (2000 x *g*), washed twice with acidified deionized water (pH 2) and freeze-dried. The crude lignin obtained was further submitted to a mild acid hydrolysis using an azeotrope (b.p 86 ^{0}C) of aqueous dioxane (dioxane/water 85:15, v/v, containing 0.01 mol/L HCl) under an argon atmosphere. The resulting suspension was centrifuged (2000 x *g*) and the supernatant were carefully withdrawn, neutralized with sodium bicarbonate and finally added drop wise to 1L of acidified deionized water (pH 2). The precipitated lignin was allowed to equilibrate with the aqueous phase overnight and it was then recovered by centrifugation, washed (2x) with deionized water and freeze-dried. It is important to emphasize that the acidolysis residue after centrifugation should be carefully decanted and discarded. Efforts to wash it, so as to increase the lignin yields may cause serious carbohydrate contamination in the final product.

MWL was isolated from the extractive-free wood according to the method described by Björkman (*13*,*14*). CEL was isolated from the insoluble material obtained after isolating MWL according to the method of Chang et al. (*15*) modified by Ikeda et al. (*16*). Both preparations were purified as described elsewhere (*13*).

Acetobromination Derivatization Procedure. Approximately 2.5 mL of a mixture composed of 8 parts of acetyl bromide and 92 parts (v/v) of glacial acetic acid were added to about 10 mg of a lignin sample in a 15 mL round-bottom flask. The flask was sealed and placed in a water bath set at 50 ^{0}C for 2 h with continuous magnetic stirring. The solvent was rapidly evaporated at 25-28 ^{0}C in a rotary evaporator connected to a high vacuum pump and a cold trap. The residue was immediately dissolved in THF (5 mL) and subjected to size exclusion analyses (SEC).

Incubation of EMALs in THF. After acetobromination one aliquot (4.0 mL) of each lignin sample was withdrawn from the starting solution in THF kept at room temperature (25 ± 3 ^{0}C) under vigorous stirring (5000 rpm) for periods of up to 30

days. These samples were used to evaluate the effects of incubation at room temperature on the molecular weight distribution of the evaluated lignins.

Size Exclusion Chromatography. SEC of EMAL samples were performed on size exclusion chromatographic system (Waters system) equipped with a UV detector set at 280nm. The analyses were carried out at 40 ^{0}C using THF as eluent at a flow rate of 0.44 mL/min. One hundred and twenty µL of the sample dissolved in THF (2 mg/mL) were injected into HR5E and HR 1 columns (Waters) connected in series. The HR5E column specifications allow for molecular weights up to 4x10^6 g/mol to be reliably detected. The SEC system was calibrated with polystyrene standards in the molecular weight range of 890 – 1.86 x 10^6 g/mol and Millenium 32 GPC software (Waters) was used for data processing.

Quantitative ^{31}P Nuclear Magnetic Resonance. Quantitative ^{31}P NMR spectra of all lignin preparations were obtained using published procedures (*8*). To improve resolution, a delay time of 5s was used and a total of 256 scans were acquired.

DFRC/^{31}PNMR. The DFRC was performed as described by Lu and Ralph (*17*). The precise amounts of the lignin and precautions due to the ensuing ^{31}P NMR steps were nearly identical to those reported elsewhere (*5*,*6*,*7*).

Green Liquor (GL) Impregnation on Wood Chips. The GL used in these experiments was provided by an industrial sponsor. It was found to contain 15.91g/L NaOH, 25.15 g/L Na_2S, and 85.36 g/L Na_2CO_3 (all concentrations expressed relative to Na_2O) analyzed by ABC titration test methods. Pretreatments were carried out as described before (*9-11*), wherein 1.5 L of green liquor were loaded into a 10-L digester containing 1 kg (o.d.) of wood chips, performing a ratio of GL to wood of 4:1. Pretreatment time was 60 minutes at 120 ^{0}C.

Kraft Pulping. After pretreatment, the GL was drained, and fresh white liquor was added for the post cook. Conventional kraft cooks for comparison were also done in the same digesters with the same conditions. The cooking conditions were as follows: initial temperature of 65^{0}C; the ratio of liquor to wood chips was 4:1 (liquor adsorbed by wood chips was included); sulfidity = 32%. Cooking temperature and alkali charge were adjusted to reach target for kappa number.

Reactions with Isolated Lignins (EMAL). The synthetic green liquor used was prepared by dissolving NaOH, Na_2S and $Na_2CO_3^{2-}$ in distilled water in amounts calculated to obtain final concentrations of 11, 37.2 and 76 g L^{-1}, respectively. The reactions were conducted in glass tubes at 130^{0}C for different periods of time. After reaction, the lignin (EMAL) was recovered from the green liquor by reducing the pH to 2 followed by centrifugation. Before analysis, the lignin was exhaustively washed with water (pH 2), freeze-dried and stored under vacuum. The various functional groups that define the lignin structure were determined before and after green liquor treatment by using quantitative ^{31}P NMR as described before.

RESULTS AND DISCUSSION

LIGNIN ISOLATION AND CHARACTERIZATION

Our continuing efforts to better understand the lignin isolation process from wood have prompted us to examine various salient features of lignin isolation variables. In a recent series of papers (*5*,*6*), we have shown that the combination of enzymatic and mild acidolysis (EMAL) affords for isolating lignin in high yields and purities from different wood species (*6*). The cleavage of lignin-carbohydrate bonds afforded during the mild acid hydrolysis step of the EMAL protocol allows the isolation of lignin fraction that are not accessed by any other isolation procedures (*5*,*6*). Consequently, the aforementioned protocol presents a real opportunity to improve yields and purities of lignin from *Eucalyptus* species, since such lignin is always obtained in low yields due to, at least in part, the high proportion of attached hemicelluloses, which is known to preclude lignin isolation in high yields. In the first part of the present work, therefore, we compare and contrast the yield, molecular weight and structure of lignin (EMAL) samples isolated from *Eucalyptus globulus*, *Eucalyptus grandis* and two different species of softwoods.

Effect of Milling on Lignin Yields from Different Wood Species. During our work, the effect of different isolation conditions on the yield of the resulting lignins was extensively examined and the emerging data are shown in **Figure 1**. Progressive mechanical treatments are seen to facilitate the disruption of the wood cell wall structure allowing for more lignin to be extracted. As a result, the longer the milling time the higher the EMAL yield, regardless of the wood species evaluated (**Figure 1A**). The yields of EMAL from *Eucalyptus* species, however, were tremendously different from those obtained for the examined softwoods. A closer examination of these yields reveals that different species of hardwood and softwoods display different behavior when submitted to the same milling conditions. More specifically, the yields of EMAL from *E. globulus* reached 54% (w/w, based on the amount of klason lignin of the starting wood and the isolated lignin) after 7 days of ball milling, whilst *E. grandis*, white fir and redwood were lower than 45, 25 and 17.5%, respectively. Extending

the milling time to 14 days did not affect the EMAL yield from white fir, but increased the yields of redwood, *E. grandis* and *E. globulus* lignin to 27, 58 and 62%, respectively. While prolonged ball milling beyond 14 days was found to increase the EMAL yield from white fir, redwood and *E. grandis*, it had negligible effect on the yield of EMAL from *E. globulus.* Nevertheless, the yields of lignin from *E. globulus* and *E. grandis* obtained after 28 days of ball milling were found to be quite similar, while a significant difference was observed between the different species of softwoods.

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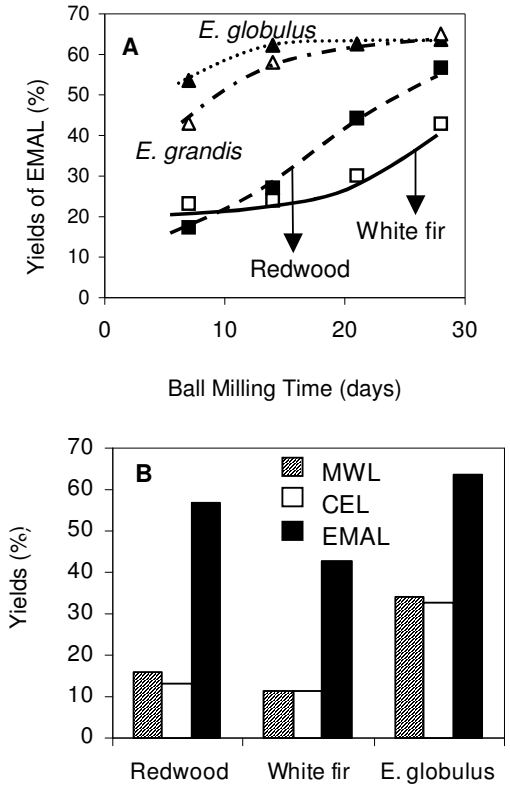

Figure 1. Yields of EMALs isolated from *Eucalyptus globulus* (black filled triangles), *Eucalyptus grandis* (open triangles), white fir (open squares) and redwood (black filled squares) as a function of the ball milling time (**A**); and yields of EMAL, CEL and MWL isolated from the same batch of milled-wood (ball milled for 28 days) (**B**).

Milled wood lignin (MWL), cellulolytic enzyme lignin (CEL) and EMAL were isolated from the same batch of redwood, white fir and *E. globulus* ball-milled for 28 days (**Figure 1B**). The yield of EMAL was always greater than the corresponding MWL and CEL, regardless the wood species from which they were isolated. These data corroborate previous findings that state that the concerted effect of cellulolytic action and mild acid hydrolysis offer significant yield improvements over the traditional procedures for isolating lignin from wood (*5*). Different wood species, however, offered different yields when isolated with the same isolation procedure. For example, the yields of EMAL, MWL and CEL from *E. globulus* were greater than those obtained from softwoods. As previously reported (*6*), different species of softwood also offered different yields of such lignin preparations. The differences in the yields observed in our work may be rationalized by the existence of different microstructure and the presence of a variety and variable abundances of lignin carbohydrate bonds amongst the different wood species.

Determination of Units Bearing Free Phenolic Hydroxyl Groups. ^{31}P NMR spectroscopy is a reliable method to determine the amounts of various hydroxyl groups within lignin macromolecule (*6*,*8*). Such hydroxyl groups are revealed and quantified after phosphitylating lignin with 2-chloro-1,3,2-dioxaphospholane or 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (*18*). The uncondensed phenolic hydroxyls (**Figure 2**) were determined by phosphitylating the lignins with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane. The β-aryl ether content (**Figure 3**) was determined after phosphitylating the Cα hydroxyl groups in these moieties with 2-chloro-1,3,2-dioxaphospholane (*8*). Quantification was then carried out via peak integration using *N*-hydroxynaphthalimide as internal standard. Details of signal acquisition, assignment and integration can be found elsewhere (*18*).

In an effort to better understand how the wood species affects lignin structure when isolated with the same method, we compared the ^{31}P NMR data obtained for the different EMALs (**Figure 2-3**). The data of **Figure 2** shows that *E. grandis* and *E. globulus* have lower total phenolic hydroxyl contents than the examined softwood species. Specifically, while both *Eucalyptus* species were found to contain less than 1000 μmol of phenolic hydroxyl/g of lignin, such content in lignin from softwood ranged from 1340 to 1850 μmol/g. This finding is not surprising, since the total contents of phenolic hydroxyl groups in softwood have been reported to be somewhat higher than in hardwoods (*19*). Moreover, earlier observations on MWL have indicated that the syringyl units present in hardwood lignins are primarily of the non-phenolic type (*19*,*20*). Such low content of free phenolic units in hardwood lignins has long been used to explain their relatively poor responses to sulfite treatments used in the preparation of chemimechanical pulps (*21*). However, the amount of uncondensed phenolic hydroxyls within the EMAL from *E. globulus* (990 μmol/g) was found to be higher than in *E. grandis* (710 μmol/g). Furthermore, the S/G ratio calculated for *E. globulus* (1.78) was higher than for *E. grandis*

(1.46), indicating that *E. globulus* is richer in syringyl units. No such syringyl units were detected in any softwood species. These results are in accordance with the work of Akiyama et al. (*22*) and demonstrate the heterogeneity of lignins within woods of different species.

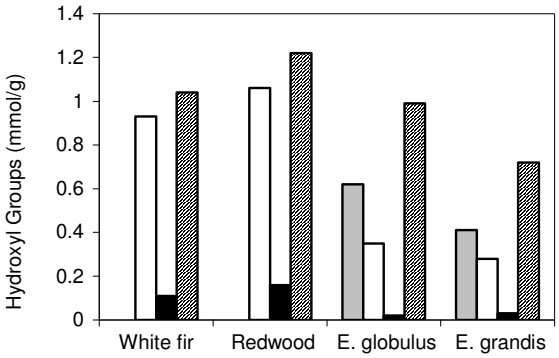

Figure 2. Uncondensed syringyl units (grey bars), uncondensed guaiacyl (open bars), *p*-hydroxyphenyl units (black filled bars) and total uncondensed phenolic hydroxyl units (vertical striped) of EMALs isolated from white fir, redwood, *Eucalyptus globulus* and *Eucalyptus grandis.*

As shown in **Figure 3**, *E. globulus and E. grandis* were found to contain more arylglycerol-*β*-aryl ether structures than all the softwoods evaluated so far**.** Nevertheless, the value of such ether linkage obtained for *E. globulus* by ^{31}P NMR was higher than for lignin from *E. grandis*. Specifically, while the amount of arylglycerol-*β*-aryl within lignin from *E. globulus* was 2780 μmol/g of lignin, *E. grandis* was found to contain only 2100 μmol of arylglycerol-*β*-aryl/g of lignin. Such values correspond to 58.7 and 44.3% *β*-aryl-ether structures within lignins from *E. globulus* and *E. grandis*, respectively, considering that the average molecular weight of one phenylpropane unit (C9) in such lignins is 211 g/mol, derived from elemental composition of *E. globulus* dioxane lignin (*2*). These values correlate very well with the 56 and 47% of arylglycerol-*β*-aryl structures reported for *E. globulus* and *E. grandis*, respectively, by Evtuguin et al. (*2*) and Adler et al. (*23*). The differences observed in the contents of arylglycerol-*β*-aryl structures between *E. grandis* and *E. globulus* is not surprising when viewed in the lights of the recent work of Akiyama et al. (*22*), where analyses of different species of hardwood by ozonation have shown that lignins with a higher syringyl/guaiacyl ratio are richer in arylglycerol-β-aryl structures. Amongst softwoods, white fir was found to contain slightly higher contents of such linkages (1500 μmol/g), while redwood was observed to contain the lowest values (1340 μmol/g).

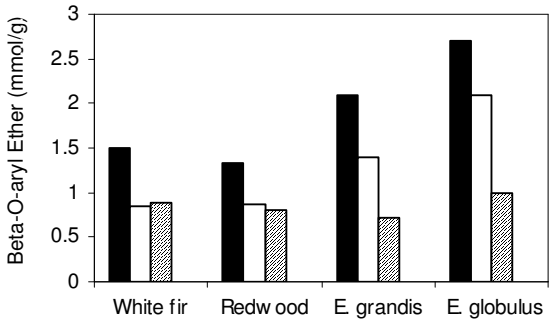

Figure 3. *Erythro* (open bars), *threo* (striped bars) and total (black filled bars) arylglycerol-β-aryl ether functional groups of EMALs isolated from white fir, redwood, *E. globulus* and *E. grandis*.

Both *erythro*- and *threo*-stereoisomeric forms of arylglycerol-*β*-aryl structures can also be determined using ^{31}P NMR after derivatization of lignin with 2-chloro-1,3,2-dioxaphospholane, by integrating the regions from 136 to 134.5 and from 134.5 to 133.4 ppm, which have been attributed to Cα-OH in *erythro* and *threo* forms of β-*O*-4 structures, respectively (*18*). As expected, **Figure 3** shows that the *erythro/threo* ratios were similar in the examined species of gymnosperms, while the predominance of the *erythro*-form was obvious in *E. globulus* and *E. grandis*. Furthermore, *E. globulus* was found to contain higher contents of the *erythro*-form of arylglycerol-*β*-aryl structures than the lignin from *E. grandis*. These data corroborate previous findings reported by Akiyama et al. (*22*), where the proportion and amount of *erythro*- and *threo*-forms were described as very similar in softwoods, while, in contrast, for hardwood species the *erythro*-form of β-*O*-4 structures was found to predominate, the extent being dependent upon wood species. Such selective behavior has been rationalized on the basis of the widely accepted theory for the formation of arylglycerol-*β*-aryl structures in lignin (*20*). According to this theory the first step in the formation of β-*O*-4 structures is the 4-*O*-coupling of an oligolignol phenoxy radical to a monolignol radical at its side-chain *β*-position to form a quinone methide intermediate. The next step is water addition to one of the two faces of the quinone methide, leading to the formation of *erythro* or *threo*-forms. Such water addition, however, depends on the aromatic ring type, solvent and pH (*24*). Model experiments simulations have shown that the water addition leading to the *erythro*-form is preferred when syringyl-type aromatic rings are forming the quinone methide (*24*).

Determination of Units Bearing Etherified Phenolic Hydroxyl Groups in arylglycerol-β-aryl Ether Structures. Although quantitative ^{31}P NMR has contributed significantly to our understanding of the hydroxyl-bearing functional groups, it cannot offer any information about the etherified or carbon-carbon linked bonding pattern of lignin (*7*). To overcome this limitation, Tohmura and Argyropoulos (*7*) have recently proposed the combination of derivatization followed by reductive cleavage (DFRC) with ^{31}P NMR. In this way, when the aryl ether linkages are selectively cleaved by DFRC (*17*), the corresponding phenolic hydroxyls released can be quantified by ^{31}P NMR. Because ^{31}P NMR can distinguish condensed from uncondensed phenolic hydroxyls, the ^{31}P NMR spectra "after DFRC" offer detailed information about condensed and uncondensed units connected through *β*-aryl ether linkages as well as dibenzodioxocins (*6,7*). The total amount of uncondensed *β-O*-aryl structures determined by DFRC/^{31}P NMR and thioacidolysis has been shown to be quite similar when both techniques are applied on the same sample of isolated lignin (*5*).

The quantification of the hydroxyl groups released from arylglycerol-*β*-aryl ether structures by DFRC are given in **Figure 4**. The total amount of uncondensed *β*-aryl ether structures within *Eucalyptus* was significantly higher than that within lignin from softwoods. As well known, lignins from hardwoods have much more uncondensed *β*-aryl ether structures than lignin from softwood (*25*). The value of 1730 μmol of uncondensed *β*-aryl ether structures/g of lignin obtained for *E. globulus* corresponds to 36.5% uncondensed *β*-aryl ether structures within lignin, considering that the average molecular weight of one phenyl propane unit (C9 unit) in such lignin is 211 g/mol (*2*). The data of **Figure 4**, coupled with the data of **Figure 5**, shows that 62.2% of the total amount of *β-O*-aryl ether structures in *E. globulus* is uncondensed (1730 μmol/2780 μmol), which is in good agreement with the value obtained by thioacidolysis for the same wood species (*2*). The S:G ratio obtained "after DFRC" (83:15) **(Figure 4)** is somewhat different from that one observed 'before DFRC" (63:35) (**Figure 3**), indicating that the syringyl units present in *E. globulus* are primarily of the non-phenolic type (*2,20*).

The total amount of uncondensed *β*-aryl ether structures within *Eucalyptus grandis*, however, was somewhat lower than in *E. globulus* (**Figure 4**). For this species of *Eucalyptus* only 47.6% of the total amount of *β-O*-aryl ether structures were found to be uncondensed (1000 μmol/2100 μmol). This finding corroborates previous data obtained by permanganate oxidation, which indicated that *E. grandis* is more condensed that *E. globulus*. Furthermore, S:G ratio before (58:42) and after DFRC (83:17) reveals that syringyl-type units in *E. grandis* are also preponderantly non-phenolic.

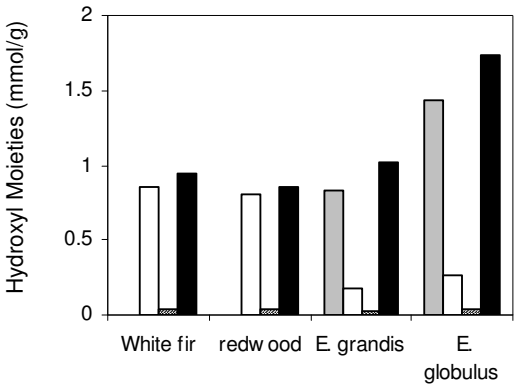

Figure 4. Values of hydroxyl moieties determined by DFRC/^{31}P NMR. The different colours in the bars refer as follow: syringyl units (grey bars), guaiacyl units (open bars) and *p*-hydroxyphenyl units (vertical striped) involved only in uncondensed *β-O*-aryl structures; and total uncondensed *β-O*-aryl structures (filled black bars) of EMALs isolated from white fir, redwood, *Eucalyptus globulus* and *Eucalyptus grandis.*

The amount of condensed phenolic hydroxyls within EMAL from *Eucalyptus* species was not calculated since the condensed syringyl- and guaiacyl-type phenolic hydroxyl signals overlap in the ^{31}P NMR. Nevertheless, due to the absence of syringyl units, DFRC/^{31}P NMR can be used to calculate the amount of dibenzodioxocins and condensed *β-O*-aryl ether units within lignin from softwoods. While the total contents of dibenzodioxocins (220-240 μmol/g of lignin) and condensed *β-O*-aryl ether units (470-490 μmol/g of lignin) were similar among white fir and redwood, the total content of uncondensed *β-O*-aryl ether was found to be slightly different from specie to specie. Condensed β-aryl ether bonds refer to structures that are characterized by the covalent attachment of two macromolecules or oligomers that themselves are interlinked via structures other than *β*-aryl ethers. The total amount of uncondensed *β*-aryl ether structures (**Figure 4**) was found to be slightly higher within the lignin from white fir (900 μmol/g) than redwood (850 μmol/g). The total amount of uncondensed *β*-aryl ether linkages in such softwoods was nearly double that of condensed moieties, indicating that about two-thirds of the etherified phenolic moieties in *β*-aryl ether structures present in EMAL from these species are uncondensed units connected to another phenylpropane unit bearing *β-O*-4, *β*-5, and *β-β* linkages. One-third of the etherified phenolic moieties in the *β*-aryl ether structures contained a subsistent group *ortho* to the phenolic hydroxyl, with the majority been dibenzodioxocins. These findings are similar to those reported by Tohmura and Argyropoulos for MWL lignin from black spruce (*7*).

On the Propensity of Lignins from Different Wood Species to Associate; Effects on Molecular Weight & its Distribution. It has recently been reported that acetobromination represents a facile and rapid alternative to the complete solubilization of sparingly soluble lignin samples, while still allowing for an accurate size exclusion analysis (*5*). By dissolving a lignin sample in neat acetyl bromide diluted with glacial acetic acid (8:92, v/v), the primary alcoholic and the phenolic hydroxyl groups are acetylated, while the benzylic α-hydroxyls are displaced by bromide (*17*). Similarly, benzyl aryl ethers are quantitatively cleaved to yield aryl acetates and acetylated α-bromo products (*17*). The concerted effect of acetylation when coupled with the polarity induced by the selective *α*-bromination caused every lignin sample examined so far to become highly soluble in THF, allowing rapid SEC analyses. Comparison between acetobromination and acetylation with acetic anhydride/pyridine has shown minor differences in the UV responses and elution profiles, which is supportive of the viability of using acetobromination as derivatization technique to sparingly soluble lignin (*5*).

The molecular weight distribution of freshly acetobrominated EMALs was found to be strongly dependent upon the wood species from which they were isolated. A highly polydisperse behaviour is apparent in the SEC chromatograms of the EMAL samples as far as their molecular weight distributions are concerned (**Figure 5).** The elution profiles, however, were found to be different amongst the EMAL isolated from different wood species. While the chromatograms of EMAL isolated from softwoods displayed a bimodal behaviour, the chromatogram of EMAL isolated from *E. globulus* (**Figure 5**) and *E. grandis* showed only a low-Mw peak and a small shoulder extending over 100 x 10^3 g mol^{-1}. The SEC chromatogram of *E. grandis*, which was omitted to prevent data over-crowding in Figure 5, would have overlapped with that of the *E. globulus* curve. Moreover, a high molecular weigh fraction, extending into about 500 x 10^3 g mol^{-1}, was apparent in the chromatograms of EMALs from softwoods. Such a fraction, however, was absent in the lignins from *Eucalyptus* species.

A question that emerges at present is whether such material causing the formation of the aforementioned high molecular weight fractions observed in the chromatograms of softwoods but absent in the SEC of *Eucalyptus* species consists of covalently bound lignin or lignin-lignin association. If such high molecular weight fractions were due to association the incubation of the lignin solution would disrupt such association leading to the release of lignin oligomers (*26*). **Figure 6** shows the effect of incubation on the molecular weight distribution of acetobrominated EMALs. Incubation in the present work refers to the aging of the acetobrominated lignins conducted at 2.0 g L^{-1} in THF at 25 ± 3 ^{0}C under vigorous magnetic stirring (5000 rpm).

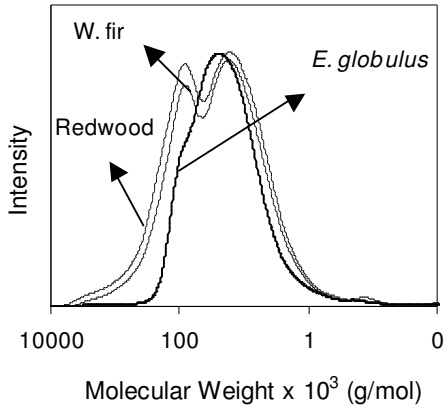

Figure 5. Size exclusion chromatograms (SEC) of lignin samples (EMALs) isolated from redwood, white fir, *Eucalyptus globulus* and *Eucalyptus grandis* (curve is overlapped by *E. globulus*).

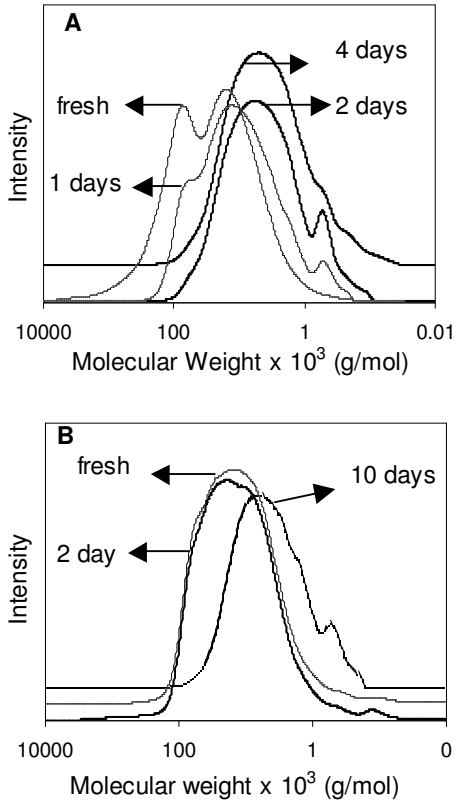

Figure 6. Effects of incubation at room temperature on the molecular weight distribution of redwood (A) and *Eucalyptus globulus* (B).

As illustrated in **Figure 6A** for one EMAL isolated from softwoods (redwood), the bimodality was clearly observed only in the chromatograms of EMALs carried out on freshly prepared lignin solutions (analyzed immediately after derivatization). After 2 days of incubation under stirring the fragments with

high hydrodynamic volumes extending over 500 x 10^3 g mol^{-1} disappeared, while the high-Mw peak became a shoulder, which was no longer observed after 3 days of incubation. Furthermore, the low-Mw peak was found to shift toward longer retention times after 3 days of incubation, demonstrating the accumulation of fragments with lower hydrodynamic volumes. In addition, one well-resolved peak appearing at longer retention times (i.e. lower molecular mass) was also discernible after 3 days of incubation (**Figure 6A**). This peak was found to be due to a significant increase in the proportion of dimeric species, a finding deduced directly from the calibration curve and confirmed by co-elution of 1- (3,5-dimethoxy-4-hydroxyphenyl)-2-(4-methoxy-phenyl)-propanodiol- 1,3 with an EMAL sample (SEC not shown). After longer incubation times, however, the bimodal elution pattern observed for EMALs from softwoods was replaced by a single broad elution peak and the signal due to the dimers was no longer resolved. This could be rationalized on the basis of the accumulation of lignin oligomers released during the incubation, which replaces the bimodal elution pattern by a broad peak eluting at the low molecular weight zone. This finding is supported by the work of Evtuguin et al. (*27*), who evaluated lignin molecular weight by electrospray ionization mass spectrometry (ESI-MS) and distinguished lignin oligomers ranging from trimers to octamers as prominent fragments in the structure of lignins from spruce and *E. globulus.*

The observed dependency of molecular weight distribution upon incubation time is not totally surprising when viewed in the light of the earlier conclusions of Sarkanen et al. (*18*), where a pronounced reduction in the apparent weight-average molecular weight has been reported for organosolv, synthetic, kraft, and Braun's native lignins during incubation in alkaline conditions. It is of significance, however, that such notable incubation effects were not observed for the size exclusion chromatograms of EMAL from both *Eucalyptus globulus*. As illustrated in **Figure 6B** an almost unimodal elution profile was found for freshly derivatized lignin. After 10 days of incubation the low-Mw peak shifted toward longer retention times (lower molecular weights) and the peak due to the accumulation of oligomeric species also appeared. Longer incubation times, however, had negligible effects on the molecular weight distribution of EMAL from *E. globulus*.

The accompanying change in the apparent weight-average molecular weight of the lignins isolated from the different wood species as a function of the incubation time is shown in **Figure 7**. Noteworthy in **Figure 7** is the significant difference in the dissociation behaviour among *E. globulus* and the other wood species, including softwoods (illustrated by redwood and white fir) and *E. grandis*. In an attempt to compare such dissociation behaviour in numerical terms, the Mw reduction factor, F, was calculated from the relation between the Mw of the EMAL freshly acetobrominated and after complete dissociation in THF. For the purposes of calculating F we ensured that when the molecular weight became stable the dissociation process had been completed. This was done by examining the SEC chromatograms of samples incubated for 20-30 days in the presence of LiCl. The latter eliminates potential residual association by the shielding of dipole effects (*28*). No alteration in the molecular weigh distribution in the presence of LiCl was apparent, supporting our contention of complete dissociation. The data of **Figure 7** show that the apparent weight-average molecular weight for lignins isolated from softwoods decreased as a whole by factors F ranging between 5-6, while the F factor was found to be less than 3.0 for *E. globulus*. As anticipated, however, the Mw reduction factor for *E. grandis* (6.2) was found to be closer to those observed for softwoods than for *E. globulus.* This finding indicates that lignins from different species of hardwoods and softwoods have different propensity to associate in THF.

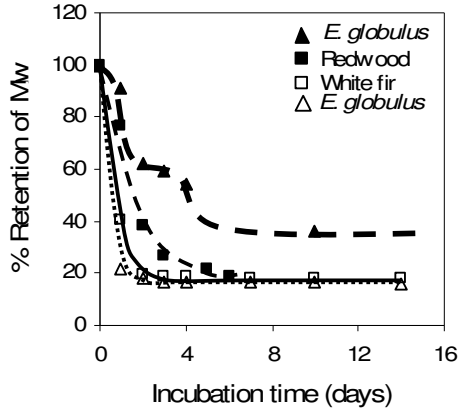

Figure 7. Effects of incubation at room temperature on the apparent weight-average molecular weight (Mw) of lignins isolated from different wood species.

In a further effort to better understand such unexpected dissociation behaviors observed during the incubation of these lignins in THF we examine the presence of any correlation between the observed molecular weight dissociation effects and specific functional groups within the lignin macromolecules. Such approach indicates that there is no clear correlation between the extent of dissociation and total amount of hydroxyl groups, carboxylic acids and condensed phenolic groups within lignin (correlations not shown). Extensive, multiple linear regression efforts did not provide statistically significant models ($P \leq 0.1$) capable of predicting the F factor as a function of these functional groups. The lack of

correlation between the extension of dissociation and the total amount of hydroxyl and carboxylic acids groups indicates that ionization and conjugation of various functional groups cannot explain the large differences observed in the dissociation behavior of lignins from different wood species in THF (**Figures 6** and **7**).

The total amount of uncondensed β-aryl ether bonds present within the examined lignins was also correlated with the extent of dissociation factor F and the data is shown in **Figure 8**. A closer inspection of data reported in this figure reveals that the total amount of uncondensed β-aryl ether linkages correlates with the extent of dissociation when one compares softwoods and *Eucalyptus*, i.e. the higher the contents of uncondensed β-aryl ether linkages are, the lower the extent of dissociation observed during the incubation of the lignin. This finding is indicative that the observed effects may have their origin, at least in part, in chain entanglements operating within different macromolecules. Such effects are anticipated to manifest themselves differently in lignins with different degrees of branching.

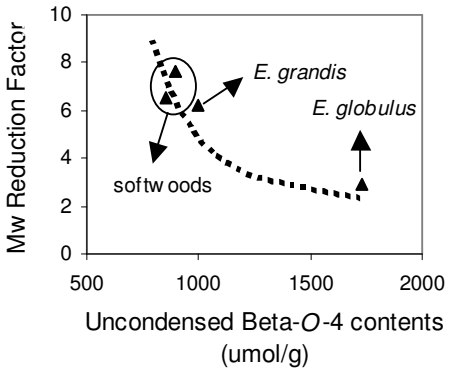

Figure 8. Molecular weight reduction factor ($Mw_{initial}/Mw_{final}$) as a function of the total amount of uncondensed β-aryl ether contents determined by DFRC/^{31}P NMR.

It is also significant to note that the weight-average molecular weights (Mw) calculated for lignins from softwoods and *Eucalyptus* after complete dissociation were found to be quite low as compared to each initial values. More specifically, the Mw after dissociation for the lignins from White fir, redwood, *E. grandis* and *E. globulus* were found to be 7.4, 10.3, 4.9 and 8.2 x 10^3 g/mol, respectively. This finding suggests that the huge variety of Mw reported for lignins from softwoods and hardwoods in the literature may be due to lignin association rather than macromolecules with different size.

FUNDAMENTAL STUDIES OF THE CHEMICAL EFFECTS OF GREEN LIQUOR IMREGNATION (GL) ON EUCALYPTUS

As mentioned before, such advances on the isolation and characterization of lignin may provide new insights into *Eucalyptus* pulping chemistry. In the second part of this paper, therefore, we explored the virtues of EMAL to compare and contrast how GL interacts with lignin isolated from *E. grandis*, *E. globulus* and selected softwood species.

Amongst the various research efforts we have undertaken to further promote our understanding of this cost effective process, is a series of kinetic and mechanistic investigations aimed at fully exploiting this pre-treatment (*10*). GL performed on softwoods was found to increase the rate of pulping by improving the kinetics at the onset of the bulk delignification stage (*10*). A correlation between chemical performance and lignin removal has also been established for GL-modified pulping (*11*). Overall, the efficiency of reactivity for the hydrosulfide and hydroxide anions in delignification was 2 and 1.6 times higher, respectively, than conventional kraft pulping (*11*).

While our previous studies have been focused on the GL features on softwood species (*10*,*11*), in this paper we report our preliminary studies on the effects of GL pretreatment on hardwood pulping. Comparison of GL-modified and conventional kraft pulping performed at similar H-factors illustrates the efficiency of green liquor impregnation performed on *E. grandis* (**Figure 9**). For example, it was found that the total amount of active alkali (AA) required to prepare low-yield *E. grandis* pulps can be significantly reduced by using the GL-modified pulping (saving of up to 14.7%). Furthermore, the GL-treated pulp was found to have a kappa number 13.3% lower than the conventional kraft pulp, while the amount of rejects were 52.9% lower for GL pretreatment. Nevertheless, a slightly decrease in the classified yield was observed when GL pretreatment was applied on *E. grandis* wood chips. Further efforts have been conducted in our laboratory in order to evaluate the mechanical properties of these pulps.

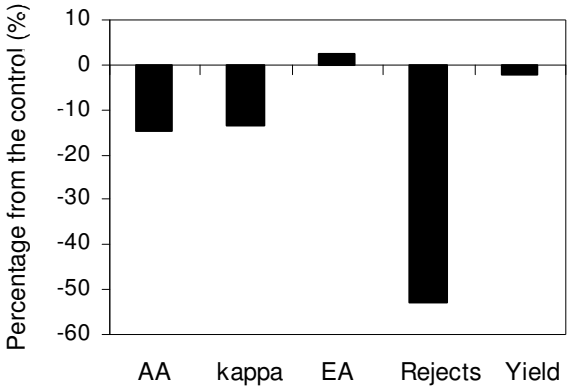

Figure 9. Comparison between GL-modified and conventional kraft cook of *E. grandis* wood chips. Control, AA and EA in this figure refers to conventional kraft cooking, active alkali and effective alkali, respectively.

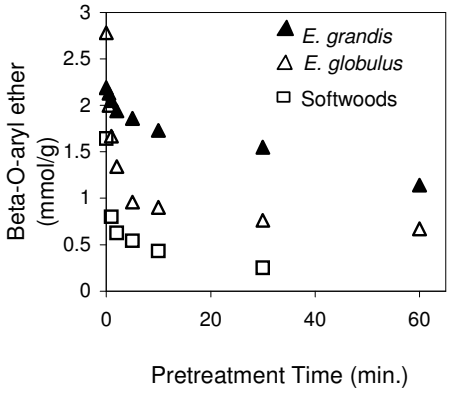

Figure 10. Plot of contents *vs* pretreatment time describing the degradation of the arylglycerol-β-ether structures under GL impregnation at 130 ^{0}C. *Eucalyptus grandis* (black filled triangles), *Eucalyptus globulus* (open triangles) and mixture of softwoods (open squares).

Despite the considerable amount of research endeavors initiated in this promising cost-effective technology, the chemistry of the GL pretreatment stage is not fully understood (*10,11*). As part of a rational fundamental understanding of this reactivity difference, we investigated how the green liquor interacted with EMAL isolated from *E.* grandis, *E. globulus* and selected softwoods. Specifically, the effects of the GL on the lignin structure have been evaluated by impregnating EMAL with synthetic GL. The total amount of arylglycerol-β-aryl ether structures within such "GL-treated lignins" were determined by using quantitative ^{31}P NMR. In addition, the degradation of lignin was also followed by size exclusion chromatography (SEC).

The green liquor treatment was found to significantly affect the amount of β-aryl ether structure in the lignin from both softwoods and hardwoods (**Figure 10**) as well as their molecular weights, as indicated by the results from size exclusion chromatography (SEC not shown), which revealed extensive lignin degradation taking place during the GL treatment. A closer examination of the data reported in **Figure 10** reveals that for the actual process of scission of these ether bonds two kinetics regimes operate. Regardless of the wood specie from which the lignin was isolated, a significant part of the total β-aryl ether structures were degraded in an initial fast phase that was followed by another slower phase.

These data were then fitted to different kinetics expressions in order to determine the sought rate constants (**Table 1**). As such, the rate constant (k) for the reaction describing the cleavage of arylglycerol-β-aryl ether linkages during both stages were found to follow a pseudo-first-order rate low, in agreement with previous publications (*29*).

Table1. Kinetic data describing the degradation of the arylglycerol-β-aryl ether units in enzymatic mild acidolysis lignin (EMAL) from different wood species under GL treatment conditions.

EMAL from	Rate Constant (k x 10^{-2}) (min^{-1})	
	Fast Phase	Slow Phase
E. globulus	34.47	0.9
E. grandis	13.70	0.6
Softwoods	48.34	3.2

The data of **table 1** reveals that the cleavage of arylglycerol-β-aryl ether linkages during GL impregnation of softwoods is faster than in hardwoods. More specifically, the arylglycerol-β-aryl ether degradation rate constants (k x 10^{-2}) for the fast and slow phases of lignin from softwoods were found to be 1.4 and 4.9 times higher, respectively, compared to *E. globulus*. Comparison of rate constants between the two species of *Eucalyptus* indicated that arylglycerol-β-aryl degradation is faster in *E. globulus*. These findings are not surprising when viewed in the light of the recent work of Pinto et al. (*1*) and Gellerstedt (*30*), where lignin units bearing free phenolic groups are reported to be more reactive than etherified units in alkaline conditions. As mentioned before, the total amount of phenolic hydroxyl compounds in such

lignins decreases from the mixture of softwoods (1,46 mmol/g) to *E. globulus* (1.06 mmol/g) and *E. grandis* (0.71 mmol/g). When such amounts of phenolic groups were plotted against the arylglycerol-β-aryl ether degradation rate constants (**Figure 11**) an interesting tendency was observed, confirming the effect of free phenolic hydroxyl groups in the GL impregnation efficacy to degrade these ether bonds.

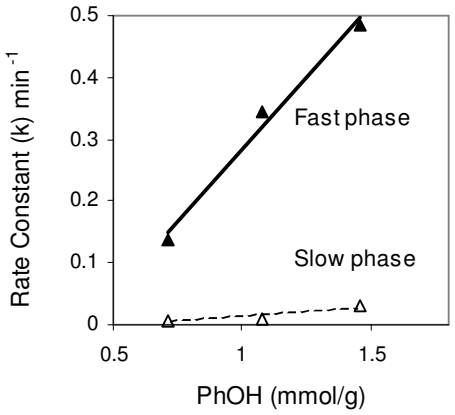

Figure 11. Relationships between arylglycerol-β-aryl ether degradation rate constants and total contents of free phenolic hydroxyl groups within the lignins. Fast phase (black filled triangles) and slow phase (open triangles).

Syringyl-type units, because of the presence of two methoxyl groups in the position 3 and 5 of the aromatic nuclei, are known to be more reactive than their guaiacyl counterparts under conventional kraft conditions (*29*,*30*). Under the milder conditions used during GL pretreatment, however, the presence of free phenolic hydroxyl groups seems to prevail over the S/G ratio. As a result, one may conclude that the higher the phenolic hydroxyl contents, the faster the cleavage of arylglycerol-β-aryl ether linkages during the GL-modified pulping. This finding may explain, at least in part, the differences between the benefits of GL-modified kraft pulping performed on *E. grandis* and the mixture of softwood chips reported in **Figure 10**.

In addition to the aforementioned effect on the amount of arylglycerol-β-aryl ether linkages, ongoing reactive studies in our laboratory has indicated that GL-pretreated EMALs contain a significant amount of sulfur. The exact quantification of the associated sulfur in such GL-pretreated EMALs has been investigated by using quantitative ^{31}P NMR spectroscopy and they will be the subjects of further publications.

CONCLUSIONS

It can be surmised that MWL, CEL, and EMAL when isolated from two different species of softwoods and hardwoods were found to offer different yields. More significantly, the combination of enzymatic and mild acid hydrolysis offered the possibility to isolate lignin in high yield from *Eucalyptus* wood species. A more detailed comparison of the lignin yields revealed that the structures of different species of *Eucalyptus* are significantly different, which can affect their pulping performance.

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