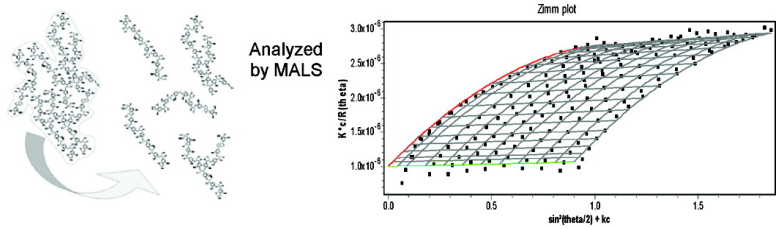
Article

Propensity of Lignin to Associate: Light Scattering Photometry Study with Native Lignins

Sofi#a Contreras, Armindo R. Gaspar, Anderson Guerra, Lucian A. Lucia, and Dimitris S. Argyropoulos

Biomacromolecules, **2008**, 9 (12), 3362-3369 • DOI: 10.1021/bm800673a • Publication Date (Web): 08 November 2008



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Biomacromolecules is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Propensity of Lignin to Associate: Light Scattering Photometry Study with Native Lignins

Sofía Contreras, Armindo R. Gaspar, Anderson Guerra, Lucian A. Lucia, and Dimitris S. Argyropoulos*

Organic Chemistry of Wood Components Laboratory, College of Natural Resources, North Carolina State University, Raleigh, North Carolina 27695-8005

Received June 23, 2008; Revised Manuscript Received August 27, 2008

Many studies of lignins in solution invoke association and aggregation phenomena to explain their solution behavior (e.g., reprecipitation onto pulp fibers, condensation, etc.). Following their colloidal (apparent) molecular weights in solution as a function of time allows us to explore observable dissociation phenomena. These measurements were carried out using multiple angle laser light scattering (MALLS) photometry in the static mode. The challenges and opportunities of measuring the specific refractive index increment (d*n*/d*C*) of lignin solutions and determining the kinetics of the dissociation process were thus investigated. Hardwood and softwood representative lignins were isolated, and method for their full dissolution in THF was further developed, which then lead to accurate d*n*/d*C* values being obtained as a function of time. When coupled to additional work using light scattering static measurements and Zimm plots for the same solutions, this effort offers insight into the aggregation and ensuing dissociative events that operate within the lignin macromolecules.

Introduction

Early work on lignin structural characterization described its structure as a complex 3D polymer network; its structure in wood was visualized as being branched with linear chains cross-linked by a variety of interchain covalent bonds.[1] However, very little is known about the natural variation of lignin content and its compositional traits in the wood of trees[2] despite the fact that 15−35% of it consists of lignin. Nevertheless, its function is essential: lignin is required for adequate retainment of water in the vascular system, for mechanical strength as the binding agent for the cellulose microfibril sheaths, and for resistance to insects and pathogens.[3]

The biopolymer known as lignin is a complex natural polymer that results from oxidative coupling of primarily 4-hydroxyphenylpropanoids (coniferyl alcohol, sinapyl alcohol, and *p*-coumaryl alcohol).[4] The actual structure of the lignin macromolecule has not been absolutely defined as of yet. A currently accepted theory for its structure is that the lignin polymer is formed by combinatorial-like phenolic coupling reactions via radicals generated by peroxidase−H_2O_2 under simple chemical control in which monolignols react endwise with the growing polymer.[5] The randomness of linkage generation and the number of possible isomers leads to a wide distribution of polydispersities. The interunit linkages that characterize the macromolecular assembly are β-O-4, β-5, β-1, β-β, 4-O-5 and 5-5 couplings.[1] The β-O-4 linkage is the most frequent bonding motif and is typically more abundant in hardwoods than in softwoods. Furthermore, there are two forms, the erythro and threo stereoisomers of the β-O-4 linkage.[6−8]

The macromolecule, once isolated from the wood matrix, is insoluble in most neutral solvents. The solubilization of this polymer requires the systematic breaking of bonds, which yields molecular fragments, leading to an observed polydispersity in the apparent molecular weights of soluble lignin derivatives.[9] Previous work showed an increase in the apparent molecular weight of kraft lignin solutions over time.[10−15] These authors suggested the presence of noncovalent interactions among individual lignin molecular components that lead to aggregates. Furthermore, Lindström reported the presence of stable lignin sols in aqueous solution whose extent of aggregation was followed by gel permeation chromatography (GPC) and viscometric measurements; however, no light scattering measurements were made. Lindström[10] explained the aggregation (sol or gel formation) by hydrogen bonding between neighboring lignin group carboxylic groups, ether oxygens, and hydroxylic groups. It was found that the extent of aggregation of kraft lignin sols in the hydrogen form increases with increasing storage time and temperature. Sarkanen[16] made the same study with organosolv lignin and kraft lignin and concluded that the intermolecular associative effects are apparently governed by nonbonded orbital interactions presumably among the aromatic moieties in the components. Despite these various studies that have the prevailing consensus that lignin associates in both aqueous and organic media, the magnitude and the underlying driving forces behind these processes are still a matter of debate.[17]

In additional work, Lindberg[18] and coworkers considered the lignin molecule in solution to consist of a strongly immobilized, tight network core and a looser surface region where random coil-like local motions of the polymer chains are possible. It was later shown that kraft lignin sols are easily destabilized by simple or complex electrolytes in a manner that is typical for lyophobic colloids.[19] Lindström also concluded that van der Waals forces strongly contribute to the interactions among the particles and that the irreversible nature of the association process is essentially a coagulation process in which the particle size is increased and the shape of the aggregates is changed, although the sol is still stable. Sarkanen[16] explained the presence of kraft lignin association complexes in alkaline solutions by means of HOMO−LUMO interactions (interactions of the highest occupied molecular orbital with the lowest unoccupied molecular orbital).

* Corresponding author. Tel: (919) 515-7708. Fax: (919) 515-6302. E-mail: dsargyro@NCSU.edu.

10.1021/bm800673a CCC: $40.75
Published on Web 11/08/2008

Additional work based on lignin association followed by GPC measurements of lignin of several sources and species demonstrated that the molecular weight distributions in lignin solutions changed depending on the method used to isolate the lignin from wood, the type of lignin (softwood or hardwood), the solvent used to prepare the solutions, and the age of the solution.[11,12,16,17,20]

Studies have also been carried out with light scattering[21−26] and have concluded that MALLS (multiangle laser light scattering) can be used for the determination of the molecular weight and the molecular weight distribution of lignins. MALLS is an absolute molecular weight technique that is based on the intensity of the light that is scattered when a laser passes through a sample. The intensity of the light scattered is directly proportional to the molar mass and thus provides a powerful technique for monitoring the presence and formation of aggregates in solution because of its inherent sensitivity to molecular weight. Therefore, light scattering is a good technique for indirectly studying interaction forces characterized by apparent molecular weight changes that are induced by lignin association phenomena. Gidh et al.[26] have therefore concluded that light scattering techniques offer great insight into lignin aggregate characterization.

Among dissolved lignin molecules, there are always association forces that are effective in both aqueous and organic media.[20] The manner used in this research to examine the efficacy of MALLS on resolving apparent molecular weights of lignin was to study two kinds of lignins in solution (Norway spruce and *Eucalyptus globulus*) in both organic (THF) and aqueous (alkaline) media. The change in the apparent molecular weight distributions of the lignin solutions was determined by the use of static mode MALLS at variable incubation times and temperatures.

Another important measurement that is required in determining the weight-average apparent molecular weight distributions of lignins is the refractive index increments of lignin solutions. Differential refractometry (DR) is a widely used technique for the determination of specific refractive index increments, d*n*/d*C*, of macromolecular solutions.[27] Although much work has been published about the d*n*/d*C* values for homopolymers, there has been little in the way of published work that determines the d*n*/d*C* values for heteropolymers.[28]

The d*n*/d*C* needs to be measured accurately to get a precise molecular weight value. There is no previous study of the d*n*/d*C* for lignins in THF. In our study, the measurement of d*n*/d*C* for the two lignin samples that followed the age of the solution was obtained. Surprisingly, the d*n*/d*C* value of THF solutions for EMAL Norway spruce increases with aging, but the d*n*/d*C* of EMAL *Eucalyptus globulus* (*E. globulus*) does not. The d*n*/d*C* values for these two lignins in NaOH solutions were found to be constant with aging. An effort was thus made to understand the origin and manifestation of these changes.

Experimental Section

Sample Preparation. Lignin samples from *E. globulus* and Norway spruce were isolated using the enzymatic mild acidolysis isolation protocol (EMAL).[29] Then, part of the samples was acetobrominated,[17] and the resulting lignin was incubated in THF. The other part (without acetobromination) was incubated in 0.1 N NaOH. The measurements for fresh samples were done immediately proceeding the preparation of the lignin solutions. The solutions were kept at room temperature so that the measurements that follow the aging behavior of lignin could be done. The details of the lignin isolation and solution preparation have been described in previous reports.[17,29]

Chemicals and Solvents. THF was HPLC grade and was previously equilibrated in air by standing under mild agitation in a loosely covered container overnight.

Specific Refractive Index Increment Measurements. Specific refractive index increments (d*n*/d*C*) for the different samples were determined using an Optilab DSP interferometric refractometer (Wyatt Technology). The wavelength of the polarized light was 633 nm. The temperature of the flow cell (P10, 1 mm length) was 25 °C. The solvent and the samples were pumped with an HPLC pump (Waters Millipore, model 510). In an effort to ensure that there were no variations in the compressibility of the various solvents used, THF and 0.01 N NaOH (with THF being the most compressible among all solvents examined), the pumped solvent initially passed through dampers (one Ultrastyragel 10^3 Å and two Ultrastyragel 10^4 Å columns (Waters) connected in series, followed by one Li-Chroma-Damp III (Handy & Harman Tube)). This ensured that the liquid pressure was stabilized, and the solvent was then passed through an injector (U6K Waters) equipped with a 4 mL loop aimed to load the sample solutions prior to flowing through the refractometer.

The samples were loaded into the injector in the load mode by the use of a syringe with a 0.45 μm filter (i.e., solvent was allowed to bypass the loop directly into the refractometer). Once the load of the loop was completed, the injector was switched to the inject mode, and the sample was allowed to flow to the refractometer. All of the data were collected and processed by the software supplied by Wyatt Technology (ASTRA).

Light Scattering Measurements. The light scattering measurements were carried out on a Dawn DSP laser photometer from Wyatt Technology. The laser polarized light was set at 633 nm. Interferometric filters with 1 and 10 nm bandwidths were used at the photodiodes to filter the scattered light so as to avoid an overestimation of the molecular weight of lignin due to fluorescence. In our efforts to optimize the instrument for these measurements, we chose interferometric filters with 1 nm bandwidth to be used for the rest of the experiments after studying the results that were obtained with both bandwidth filters. The filters used were standard green float glass, and all of the spectral characteristics were supplied by the thin-film deposition process. Under these conditions, the fluorescence values were negligible, totaling less than 0.001%.

The cell was set at room temperature (25 °C). The solvent and samples were loaded using a syringe pump (Cole-Parmer Instruments, series 74900, Agilent Model GA80AA). The solvent was passed through a 0.02 μm diameter porous filter (Whatman), and the samples were passed through 0.45 μm diameter porous filters (Alltech). All of the data were collected and processed by software supplied (ASTRA).

Results and Discussion

Light Scattering Photometric Measurements of Lignin. Lignins can be characterized by light scattering (LS) photometry.[30] This is a very useful method, although not as widely used as it could be, especially under the static mode. This work provides details of the use of this method exclusively under the static mode as applied to lignin biopolymers in which the weight-average molecular weights ($\bar{M}_w$) of lignins in solution are accurately determined. In some cases, the average root-mean-square (rms) radius of gyration was also measured.

Multiple angle laser light scattering (MALLS) photometry makes use of multiple (in this case, 18) angles to detect the light that is scattered from a sample. The intensities of the scattered light can be correlated with concentration to allow for the determination of a weight-average apparent molecular weight of a lignin in solution. When the light scattering signal is measured as a function of angle and concentration, a global fit can be obtained. The quality of the fit can be assessed via a Zimm plot. This type of plot is a 2D slice of a 3D data set. The global fit results are presented as a grid, and the data are

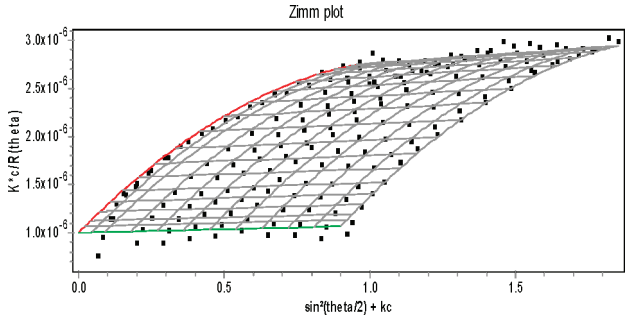


Figure 1. Typical Zimm plot by light scattering photometry from a Norway spruce lignin solution in THF.

presented as points. Figure 1 presents a typical Zimm plot of a Norway spruce acetobrominated EMAL lignin in THF solution with the data points fitted in the final global fit grid.

To obtain reproducible and accurate light scattering measurements of lignins, several optical phenomena must be taken into account, and the associated limitations must be overcome.[30,23] The specific optical properties of lignins that need to be addressed are their anisotropy, absorbance, and fluorescence.[23,30,31]

More specifically, the anisotropy caused by the depolarization of the incident beam gives rise to an enhancement of the scattered light, which causes an overestimation of the measured final apparent molecular weight. A possible adjustment makes use of an analyzing polarizer inserted between the sample and the detector, which provides corrections from the vertical and horizontal excess Rayleigh factors.[32] Another method involves the use of the Cabannes factor in the case of the incident light being vertically polarized.[32] More recently, it has been shown that the anisotropy effect exhibited is mainly due to the solvent itself rather than the lignin.[23] The software used during this effort (ASTRA) provided the compensating method of choice for the accurate correction of all anisotropy effects enumerated.

In addition, the absorption of near-ultraviolet light tailing to the visible light may attenuate the incident and scattered light, leading to an underestimated apparent molecular weight. When the measuring cell is cylindrical and the optical density of the solution is sufficiently weak, the correction becomes partially independent of the scattering angle. In this case, the absorption effect can be corrected by simply dividing the apparent Rayleigh factor by the transmittance of the solution.[31,33,34] In our case, the absorption by the lignin solution is very low because at 633 nm, the absorption is minimal. Alternatively, the concentration of the analyzed lignin solutions were also low; therefore, even for the most concentrated solutions, the transmittance was negligible, keeping the Rayleigh factor constant for all of the concentrations of the lignin solutions.

Finally, optical fluorescence is typically of lower energy (i.e., greater wavelength) than excitation (incident) light, which is primarily due to the Stokes shift. Nevertheless, this fluorescence is more pronounced at lower wavelengths.[34] Without correction, the photomultiplier in the LS photometer will detect both the scattered and fluorescent light. Even at 633 nm, under the acquisition conditions of the LS, the lignin fluorescence effect again leads to an overestimation of the apparent molecular weight. These findings are clearly demonstrated by the measurements carried out with and without interferometric filters (Figure 2). One can clearly observe that the lignin samples that were analyzed without the filters show much higher apparent molecular weights than those analyzed with the 1 nm filters (samples A and B in Figure 2, top graph). The overestimation was between 62 and 111%,

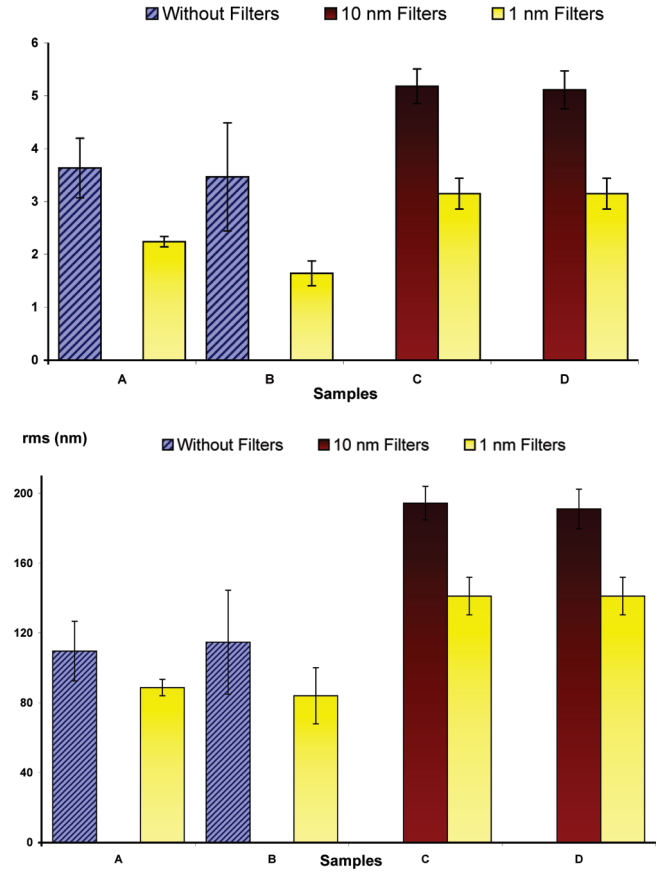


Figure 2. Effect of light scattering filters on the weight-average apparent molecular weight (top graph) and on the root-mean-square radius (bottom graph) of EMAL Norway spruce solutions in THF.

using as a reference the value obtained with 1 nm filters. The error associated with the measurements without the filters is much more pronounced than that when the 1 nm filters are used. The use of interferometric filters with a bandwidth of 10 nm was also investigated. Even with the 10 nm filters, the final measured apparent molecular weights were overestimated by 62−65% compared with the data obtained with the 1 nm filters (samples A and B in Figure 2, top graph). For each sample, its specific d*n*/d*C* was used for the apparent molecular weight calculations with and without the use of filters. Similar findings were thus observed with respect to the radius of gyration measurements (samples A and B in Figure 2, bottom graph). Surprisingly, the use of 10 nm bandwidth filters leads to an overestimation of the radius of gyration. Therefore, for all ensuing measurements, 1 nm filters were used. In a previous report,[33] the nullification of fluorescence from kraft lignins was achieved when a low angle laser light scattering (LALLS) signal was used with a 632.8 nm filter that was placed between the sample solution and the photomultiplier.

When the above optical effects and their detailed adjustments for the light scattering measurements were taken into account, the reproducibility observed for the final results was excellent when the apparent weight-average molecular weight was measured for several samples of the same lignin (i.e., for each acetobrominated lignin that was dried, dissolved in THF, and diluted to the proper concentrations for analysis). In all cases, all of the final values were within experimental error (Figure 3). The apparent molecular weight calculations took into account the specific d*n*/d*C* values for the fresh EMAL Norway spruce solutions in THF that were analyzed by light scattering

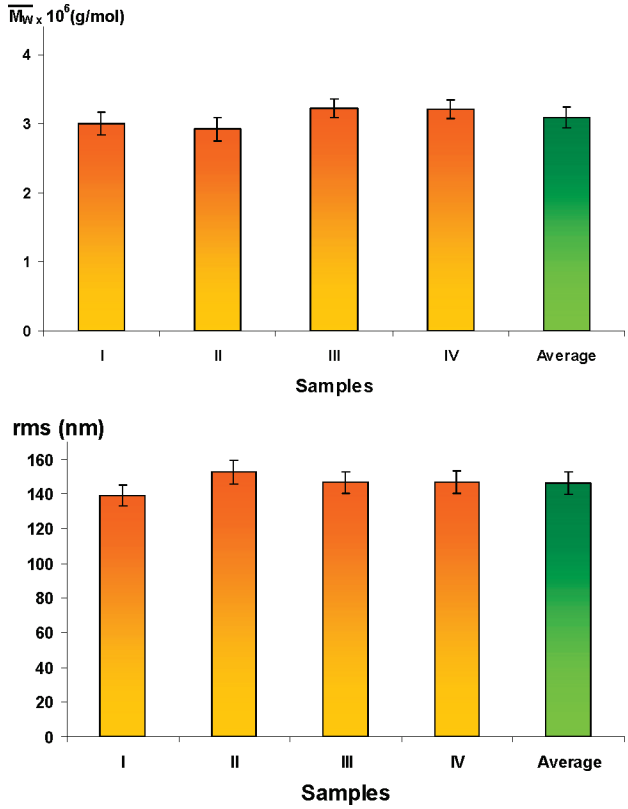


Figure 3. Reproducibility of the weight-average apparent molecular weight (top graph) and of the root-mean-square radius (bottom graph) by light scattering photometry of fresh EMAL Norway spruce lignin solutions in THF.

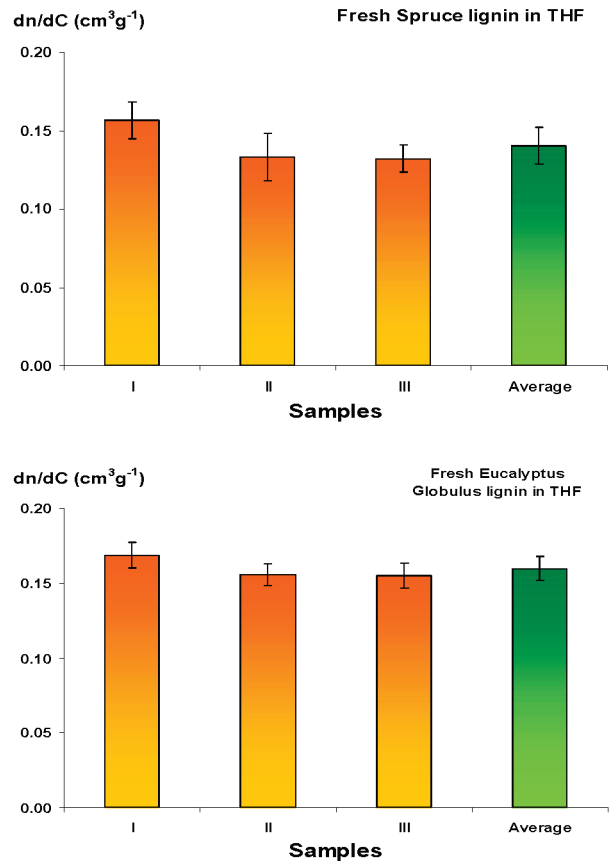


Figure 4. Reproducibility of the specific refractive index increment of fresh (just after dilution) EMAL Norway spruce solutions (top graph) and EMAL *Eucalyptus globulus* solutions (bottom graph), both in THF and at 25 °C.

photometry. Similar results of good reproducibility were observed for the rms radius of the lignin (Figure 3). These measurements allowed us to pursue more detailed investigations confidently, as described below.

Specific Refractive Index Increment. The determination of the apparent weight-average molecular weight by light scattering photometry requires that the specific refractive index increment (d*n*/d*C*) of the material in solution is known. In this respect, our aim was to determine d*n*/d*C* values for each lignin reproducibly. For both fresh EMAL Norway spruce lignin and fresh EMAL *E. globulus* solutions in THF, it was observed that the reproducibility of the d*n*/d*C* values was within experimental error (Figure 4); therefore, the average determined values were considered to be reliable. The d*n*/d*C* values were 0.140 ± 0.011 and 0.165 ± 0.008 cm^3 g^{-1} for the EMAL Norway spruce and the EMAL *E. globulus*, respectively, for freshly dissolved acetobrominated samples in THF.

The d*n*/d*C* value as a function of solution aging time was also investigated. These data are presented in Figure 5. In the case of Norway spruce EMAL in THF, it was observed that significant changes in the d*n*/d*C* values were apparent between a freshly prepared solution and an aged solution,. There was an increase in the d*n*/d*C* from 0.140 ± 0.011 (fresh solution) to 0.28 ± 0.011 cm^3 g^{-1} after 2 days of aging in THF. At prolonged aging times, the d*n*/d*C* values remained constant. The d*n*/d*C* values used for the calculations of the apparent weight-average molecular weights were 0.140, 0.149, 0.250, and 0.284 for fresh, 0.5, 1, and 2 days and beyond aged solutions, respectively. In the case of EMAL *E. globulus* in THF, the d*n*/d*C* values displayed insignificant changes with the aging of the solution. The d*n*/d*C* value was 0.165 ± 0.008 cm^3 g^{-1} for any incubation time. The d*n*/d*C* values of the EMAL Norway spruce

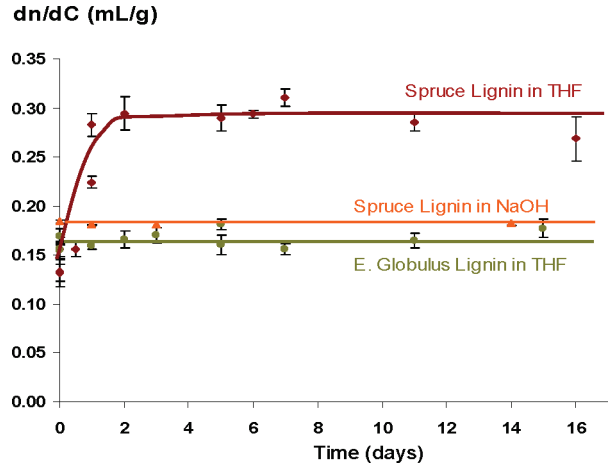


Figure 5. Incubation effect on the specific refractive index increment (d*n*/d*C*) of EMAL Norway spruce and *Eucalyptus globulus* in THF and EMAL Norway spruce lignin in 0.01 N NaOH.

dissolved in 0.01 N NaOH were also investigated as a function of time (Figure 5). It was observed that the d*n*/d*C* values were relatively constant with solution incubation time. The d*n*/d*C* of the lignin in 0.01 N NaOH was 0.182 ± 0.002 cm^3 g^{-1}.

A remarkable observation regarding the associated errors of the d*n*/d*C* measurements is that the accuracy of the measurements in NaOH solution was much greater than that in the case of the lignins in THF. The error associated with the d*n*/d*C* of the lignin in NaOH solution was ~1%, whereas the error for the d*n*/d*C* of the lignins in THF was in the range of 4−8%. These data corroborate the difficulties associated with THF as

a solvent for refractive index measurements. In our view, the compressibility of this solvent is a factor that causes the enumerated determination difficulties.

The d*n*/d*C* of polymers has been shown to change with the charge density of the polymer, degree of substitution, degree of dissociation, chain scission, conformational changes, chemical changes, and molar mass.[35−38] However, we did not find any systematic study that relates the changes of d*n*/d*C* to incubation or association phenomena of polymers in solution. During this work, an unexpected finding was obtained regarding the systematic changes in the d*n*/d*C* values with the aging of the solution with no supporting literature citations because a unique d*n*/d*C* value of lignins in solution was used in all previous work. That value is found to change with the type of lignin and solvent used, but never before was it reported that a change in the d*n*/d*C* value of lignin occurs with incubation time. It is generally accepted that the d*n*/d*C* value for a homopolymer is almost entirely dependent on the monomer and is weakly dependent on (or independent of) molecular weight.[39] Copolymers can be very different, however, because the molecular weight and composition can be closely linked.[28] In almost all cases, the d*n*/d*C* that is once determined for a polymer is assumed to be constant and is used for all ensuing measurements. However, d*n*/d*C* changes due to chemical modifications have been reported once.[35] The values of *dn/dC for* chitosans in 0.1 mol L^{-1} $KClO_4$ were found to be dependent on the degree of acetylation and the degree of dissociation. These changes were rationalized on the basis of the induced adjustments in the ionic/hydrophobic interplay of these biopolymers in solution. For the case of Norway spruce lignin in THF, the lignin chemical environment may interact in a similar manner to what has been previously reported for chitosan solutions. The incubation time of the Norway spruce lignin in THF, showing dissociation phenomena as described by Guerra et al.,[17] may induce changes in the degree of its hydrophobicity. A better knowledge of the factors affecting the dependency of the d*n*/d*C* of polymers, however, is still required.

Dissociation of the Lignins in Solution. To explore the propensity of lignin to associate, the apparent weight-average molecular weights of the lignins in solution as a function of incubation time were investigated by light scattering photometry. The lignins used were Norway spruce and *E. globulus* EMALs, which were acetobrominated and dissolved in THF, and the Norway spruce EMAL, which was dissolved in an aqueous 0.1 N NaOH solution. The incubation of the lignin samples was carried out at 25 ± 3 (room temperature) or 4 ± 1 °C without stirring and at a concentration of 10.0 g L^{-1}. This stock solution was appropriately diluted for the determination of d*n*/d*C*. The apparent weight-average molecular weights were calculated taking into account the previously determined d*n*/*dC* values at specific incubation times.

The incubated acetobrominated Norway spruce EMAL in THF showed a temporal decrease in $\bar{M}_w$ that has a different temperature profile (room temperature or at 4 °C) (Figure 6). It is observed that the apparent molecular weight of the freshly prepared solutions rapidly decreases over the initial 24 h of incubation for measurements at both room temperature and 4 °C. It is also observed that the initial rates of the $\bar{M}_w$ decrease are very similar at both temperatures. From the 24 h point until the 48 h point, only a slight reduction is seen. After 48 h of incubation, steady final values were obtained. In the case of the acetobrominated Norway spruce EMAL in THF incubated at 4 °C, the $\bar{M}_w$ dropped from 3.15 × 10^6 to an averaged steady value of 5.0 × 10^5 g mol^{-1}, which is equivalent to an 84%

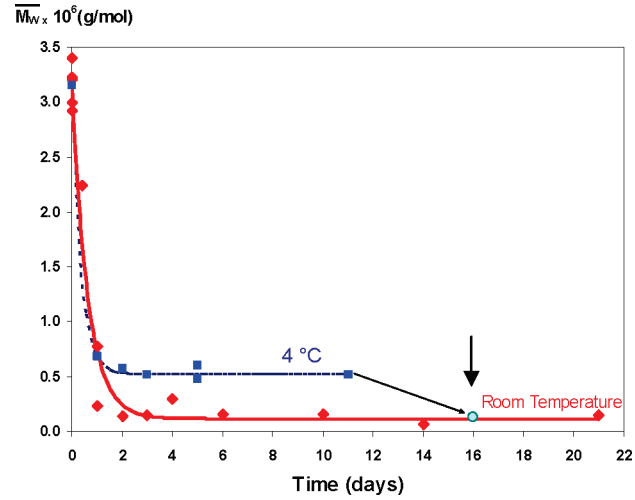


Figure 6. Incubation effect on the weight-average apparent molecular weight of the EMAL Norway spruce solution in THF kept at room temperature and at 4 °C.

reduction. When the acetobrominated Norway spruce EMAL in THF solution was incubated at room temperature, the $\bar{M}_w$ dropped from 3.15 × 10^6 to the averaged final value of 1.5 × 10^5 g mol^{-1}, which corresponds to a 95% decrease. The steady-state apparent molecular weights obtained by the lignin in THF kept at 4 °C were more than 3 times those of the lignin solution kept at room temperature. This singular behavior of the apparent molecular weight reduction is an indication of the dissociation phenomena taking place in lignins. Moreover, this lignin dissociation phenomenon is temperature dependent.

The prevention of any further dissociation at 4 °C versus room temperature may be understandable because molecular motion is related to the average internal energy of the system. (At temperatures of >4 °C, further lignin dissociation may be promoted by only the higher average internal energy of the lignin molecules.) This proves the drop in the apparent weight-average molecular weight of the lignin once its solution was kept at 4 °C and was then allowed to warm to room temperature. After 14 days, the solution at 4 °C was allowed to equilibrate at room temperature, and at 16 days of incubation (14 days at 4 °C plus 2 days at room temperature), the $\bar{M}_w$ was measured again. The new value was 1.4 × 10^5 g mol^{-1} (circle point shown with an arrow in Figure 6); this value was the same as that reached by the lignin solution that was continuously incubated at room temperature.

The data were fitted by the use of a kinetics program. The function that fit the experimental points was a one-phase exponential decay, which is applicable when the decrease in a variable is at a rate that is proportional to its value. The general equation is as shown in eq 1

$$\bar{M}_w = A(\exp(-Kt)) + B \qquad (1)$$

where $\bar{M}_w$ is the weight-average apparent molecular weight of lignin, A is related to the extent of dissociation and its units are g mol^{-1}, K (day^{-1}) is the rate of the dissociation process, and B is the final weight-average apparent molecular weight of the sample in g mol^{-1} or when it is completely dissociated.

After regression of the points for Norway spruce lignin at 4 °C and at room temperature, eqs 2 and 3 were obtained, respectively.

$$\bar{M}_w = 2.625 \times 10^6 (\exp(-2.784t)) + 522\,903 \qquad (2)$$

The corresponding half life, which refers to half of the time needed for complete dissociation, for Norway spruce lignin at

4 °C was 0.2490, and $R^2 = 0.9980$, indicating a good fit between the experimental data and the equation.

$$\bar{M}_w = 3.033 \times 10^6(\exp(1.623t)) + 147\,897 \qquad (3)$$

The half life for Norway spruce lignin incubated at room temperature was 0.4271, almost twice that for incubation at 25 °C. This means that the extent of the dissociation phenomenon is lower for lower temperature, since it takes less time to complete half dissociation; this can also be seen when comparing the coefficient A (extent of dissociation) values of eqs **2** and **3**, because they are (2.625 and 3.033) $\times$ 10^6 g mol^{-1}, respectively. For the fitted points at 25 °C, the R^2 value of 0.9750, also demonstrated a good correlation of the points with the exponential decay. It is also important to note that the rate of dissociation is higher at 4 than at 25 °C because the K values were found to be 2.784 and 1.623, respectively. This was not expected, but it may be that the temperature influences the extent of dissociation but not the rate of dissociation.

Dissociation Behavior of Lignins from Different Species. Besides softwood Norway spruce lignin, a hardwood *E. globulus* lignin was also investigated, aimed at further exploring the described dissociation phenomena on a lignin of a completely different botanical origin (a hardwood lignin). The development of the apparent weight-average molecular weight of acetobrominated EMAL *E. globulus* in THF was followed as a function of incubation time at room temperature by light scattering photometry, and it was compared with what was observed with its counterpart softwood (EMAL Norway spruce lignin, Figure 7). Not surprisingly, the $\bar{M}_w$ incubation profile of the *E. globulus* lignin is very similar to that observed by Guerra et al.[17] that was measured by GPC. For the case of the Norway spruce lignin, compared with the data measured by GPC,[17] the values obtained by LS are much higher. The $\bar{M}_w$ values of the fresh Norway spruce lignin solutions measured by LS were found to be approximately 30 times higher than those measured by GPC. An explanation of this difference may be attributed to the fact that light scattering photometry has a much higher sensitivity to the high apparent molecular weight fractions than does GPC. Furthermore, the values obtained from light scattering are absolute as opposed to GPC measurements.

The apparent weight-average molecular weight of the fresh acetobrominated *E. globulus* lignin in THF was 3.2 $\times$ 10^4 g mol^{-1}. After 4 days of incubation, a further decrease in the apparent molecular weight was observed, and a second plateau was achieved at 1.0 $\times$ 10^4 g mol^{-1} with a reduction of 68% compared with the fresh solution. Previously, it was indicated that the $\bar{M}_w$ reduction for the Norway spruce lignin was 95%. The dissociation phenomena of the hardwood lignin took place at a different degree than that of the softwood Norway spruce lignin.

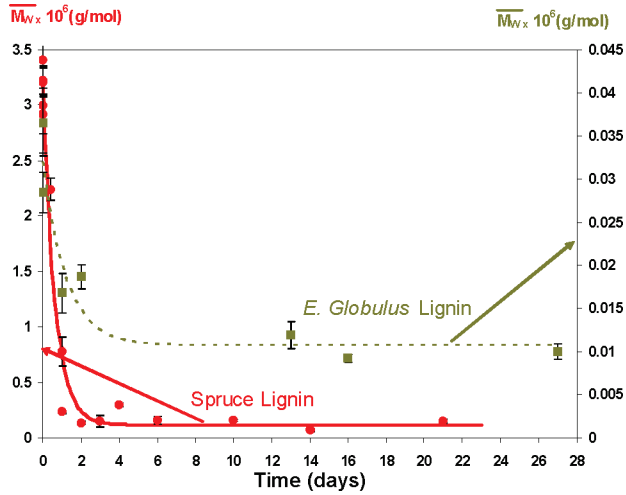


Figure 7. Incubation effect on the weight-average apparent molecular weight of the Norway spruce and *Eucalyptus globulis* lignin in THF solution maintained at room temperature.

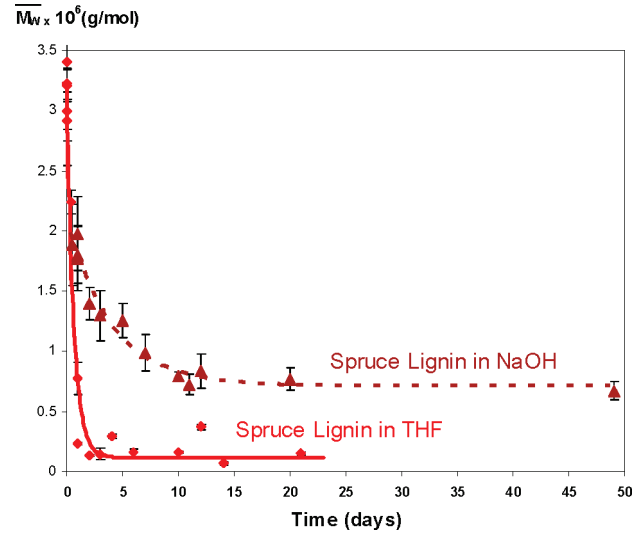


Figure 8. Incubation effect on the weight-average apparent molecular weight of the Norway spruce lignin in THF and in 0.01 N NaOH solutions maintained at room temperature.

These profiles can be seen in eqs 3 and 4, which are the kinetic equations for Norway spruce and *E. globulus* lignin, respectively, at room temperature.

$$\bar{M}_w = 21\,314(\exp(-0.8247t)) + 10\,735 \qquad (4)$$

The half life for the dissociation of hardwood *E. globulus* was 0.8405, and $R^2 = 0.9003$. It can be deduced that the dissociation of *E. globulus* takes more time than the dissociation of Norway spruce in THF when comparing the respective half lives, 0.8405 and 0.4271. The dissociation of *E. globulus* is slower than that for Norway spruce lignin, as concluded when examining the K value for eqs 3 and 4; the K value for Norway spruce is higher than that for *E. globulus* and are 1.623 and 0.8247, respectively. Therefore, one may conclude that softwood lignin dissociates faster than hardwood lignin.

Hardwood lignins consist of guaiacyl and syringyl units in variable proportions, whereas Norway spruce and other softwood lignins consist almost entirely of guaiacyl units.[6] The difference between the behaviors in the dissociation phenomena of hardwood and softwood lignins may be ascribed to their different polymeric structures and the different linkages. The relative proportion of each linkage is related to the ratio of the mesomeric forms of the radicals (present during biosynthesis) that are influenced by the physicochemical environment and kinetics parameters of the biosynthesis reaction.[40]

Such differences between the behaviors of Norway spruce and *E. globulus* lignins as a function of incubation time were observed in a previous study in our laboratory[17] that was carried out using GPC in which a bimodal elution curve was observed for Norway spruce lignin; meanwhile, *E. globulus* lignin displayed an almost unimodal elution profile for freshly prepared samples. This indicated that *E. globulus* did not have a fraction of higher apparent molecular weight, but it did show dissociation behavior with aging. It was thus concluded that the extent and pattern of the associative process appears to depend on the wood

Table 1. Kinetic Factors for EMALs Incubated in Different Solvents at Different Temperatures[a]

EMAL	temperature (°C)	solvent	rate ($days^{-1}$)	A (g mol^{-1})	B (g mol^{-1})	R^2
Norway spruce	25	THF	1.623	3.033×10^6	1.48×10^5	0.9750
Norway spruce	4	THF	2.784	2.625×10^6	5.23×10^5	0.9980
E. Globulus	25	THF	0.8247	2.13×10^4	1.07×10^4	0.9003
Norway spruce	25	NaOH (0.01 N)	0.1677	1.466×10^6	5.38×10^5	0.9319

[a] As shown in eq 4.

species; furthermore, there are different propensities to associate, even among lignins from different species of softwood.

Guerra et al.[17] attempted to correlate the presence of different functional groups in lignin with the change in the apparent molecular weight; the data indicated that there was no clear correlation between the extent of dissociation and the total number of hydroxyl groups, carboxylic acids, or condensed phenolic groups within the lignin. However, there was a correlation found: *E. globulus* contains much more uncondensed β-aryl-ether structures than any of the other softwoods evaluated. This corroborates that hardwood lignin is more linear than softwood lignin. As a result, there is a correlation between the number of uncondensed β-aryl-ethers present in lignin molecules and their extent of association. This finding indicates that the observed effects may have their origin, at least partially, in chain entanglements operating within different macromolecules. Guerra et al.[17] concluded that chain entanglements may play a significant role in the underlying mechanisms of lignin association.

Deassociation Behavior of Lignins in Different Solvents. The apparent molecular weight of EMAL Norway spruce was followed in alkaline solution of 0.1 N NaOH. It is important that EMALs in NaOH were not acetobrominated; therefore, in this case, completely native lignins were examined in an alkaline solution (pH 13), and the changes in the apparent molecular weight were monitored with static light scattering. As previously observed, a decrease in the apparent molecular weight value, from 3.77×10^6 to an average final value of 6.22×10^5 (representing an 84% reduction), was apparent as a function of solution aging. The trend of lignin dissociation phenomenon in NaOH is similar to that observed for THF solutions (Figure 8).

The initial apparent molecular weights for the Norway spruce lignin in the two different solvents are different, as are the values when the plateaus are reached. Furthermore, there is a difference between the lignins that are dissolved in the two solvents. The lignin in THF was acetobrominated, and the lignin in NaOH was not. This means that lignins in the THF solutions do not have any possibilities for hydrogen bonding interactions because all of the hydroxyl groups are acetylated. Meanwhile, in the NaOH solutions, lignin was not pretreated; therefore, it contains a variety of hydroxyl groups. The fact that the two lignins (the one that was acetobrominated and the one that was not) show the same behavior in solution demonstrated that the acetobromination procedure is a good method to use in the study of lignin in organic solutions because it provides excellent dissolution characteristics to the lignin.

For the measurements of fresh lignin solutions in 0.1 N NaOH (with a corresponding pH of 13), the apparent molecular weight was immediately measured following preparation. Then, part of the solution was kept at room temperature to follow the change in the apparent molecular weight with the age of the solution (the same procedure used as for the THF solutions). Once again, there was a decrease in the apparent molecular weight values of lignin with time, which demonstrates a dissociation behavior of lignin in aqueous media. It is important that there is was no oxidation of lignin when it was incubated in alkaline solutions because of its contact with air. Whereas precautions to avoid this effect were taken by carefully excluding ambient air from these solutions (by sealing). Comparative literature studies have shown that exposure to air did not have a detectable effect on the kraft lignin apparent molecular weight distributions.[12]

The curves of Figure 8 can be fitted with an exponential decay equation in a manner similar to that carried out for the dissociation data at different temperatures and different lignins (hardwood and softwood) on the basis of the data of Figure 8. The plateau of eq 5 for the final apparent molecular weight was $\sim 5.4 \times 10^5$ g mol^{-1}, and the time required to achieve the plateau value was twice its half life, 4.132. The R^2 value for this fit was 0.9319, and thus the correlation of the experimental data with the equation is quite acceptable.

$$\bar{M}_w = 1.466 \times 10^6 (\exp(-0.167t)) + 538\,082 \qquad (5)$$

When the half lives for Norway spruce in THF and NaOH, which were 0.4271 and 4.132, are compared, it can be concluded that dissociation in THF is much faster than that in NaOH (about 10 times faster), and this is evidenced in the K values for eqs 3 and 5, which correspond to dissociation kinetics of Norway spruce in THF and NaOH, respectively. The K value for Norway spruce in THF is 1.623, which is higher than that for Norway spruce in NaOH, 0.1677. The extent of dissociation is higher when lignin is dissolved in THF. The pre-exponential A value (eq 1) was found to be 3.033×10^6 g mol^{-1} for Norway spruce lignin in THF, whereas that for the same lignin in alkaline solution was found to be 1.466×10^6 g mol^{-1}

Using a diffusion technique, Benko[41] measured the relative apparent molecular weights of kraft lignins and lignosulfonates. This work showed very large differences in the apparent molecular weights measured in aqueous and organic media. Polymerized hardwood lignosulfonates gave an apparent molecular weight of 50 000 g mol^{-1} in 0.1 N aqueous KC1, which decreased to $\sim$2000 g mol^{-1} when measured in DMSO. Similar trends were apparent during this work. The final apparent molecular weight (when the plateau was reached) for Norway spruce lignin was higher in NaOH than in THF, indicating that the apparent molecular weight value may depend on the solvent that was used to prepare the solutions most likely due to solvent−solute interactions.

In Table 1, one may observe the rate of dissociation of Norway spruce EMAL in the two different solvents examined. These values are different, which means lignin deassociates faster in THF than in 0.1 N NaOH. It is likely that the interactions present between lignin and THF promote deassociation processes at a more rapid rate. Perhaps the fact that the lignin in THF was acetobrominated (before preparing the solutions) has eliminated one of the interactions that occurs among the associated lignin molecules (hydrogen bonding), thus allowing dissociation phenomena to take less time. It is experimentally evident that kraft lignin has a pronounced tendency to associate and form more complex structures to a degree that is dependent on the extent of interaction with the

solvent.[42] The actual disparity in the dissociation of lignin between the different solvents may also indicate that the association forces that operate in aqueous and organic media are not essentially the same.[17] The dissociation phenomenon takes 10 days until the apparent molecular weight reaches a constant value (Table 1).

Conclusions

Lignin obtained via enzymatic mild acidolysis (EMAL) provided valuable insight into the way its molecular weight changes over time. This understanding was made possible by the application of static light scattering measurements that followed hardwood and softwood lignin behavior in solution at two different solvents, THF and aqueous alkaline media. It was found that the propensity of different types of lignin to associate in different solvents is diverse, as was observed by the use of kinetic equations. Furthermore, the dn/dC value of lignin is shown to be constant for all examined cases with the notable exception of Norway spruce EMAL in THF, where it was found to vary with the time of incubation. This was a new finding because in literature, dn/dC is assumed to be of constant value, even if association of polymers in solution is known to take place.

Acknowledgment. This article is dedicated to the memory of Professor Anderson Guerra, a beloved researcher and scholar whose influence both professionally and personally has been wide and far-reaching. The technical assistance of Wyatt Technology and the expert advise of Professor S. Sarkanen (University of Minnesota) are also gratefully acknowledged.

References and Notes

(1) Adler, E. *Wood Sci. Technol.* **1977**, *11*, 169.
(2) Wadenbäck, J.; Clapham, D.; Gellerstedt, G.; von Arnold, S. *Holzforschung* **2004**, *58*, 107.
(3) Chen, C.; Baucher, M.; Christensen, J. H.; Boerjan, W. *Euphytica* **2001**, *118*, 185.
(4) Holmgren, A.; Brunow, G.; Henriksson, G.; Zhang, L.; Ralph, J. *Org. Biomol. Chem.* **2006**, *4*, 3456.
(5) Ralph, J.; Lundquist, K.; Brunow, G.; Lu, F.; Kim, H.; Schatz, P. F.; Marita, J. M.; Hatfield, R. D.; Ralph, S. A.; Christensen, J. H.; Boerjan, W. *Phytochem. Rev.* **2004**, *3*, 29.
(6) Brunow, G.; Karlsson, O.; Lundquist, K.; Sipilä, J. *Wood Sci. Technol.* **1993**, *27*, 281.
(7) Helm, R. F.; Li, K. C. *Holzforschung* **2000**, *54*, 597.
(8) Bardet, M.; Robert, D.; Lundquist, K.; von Unge, S. *Magn. Reson. Chem.* **1998**, *36*, 597.
(9) Gilardi, G.; Cass, A. E. G. *Langmuir* **1993**, *9*, 1721.
(10) Lindström, T. *Colloid Polym. Sci.* **1979**, *257*, 277.
(11) Sarkanen, S.; Teller, D. C.; Abramowski, E.; McCarthy, J. L. *Macromolecules* **1982**, *15*, 1098.
(12) Sarkanen, S.; Teller, D. C.; Stevens, C. R.; McCarthy, J. L. *Macromolecules* **1984**, *17*, 2588.
(13) Norgren, M.; Edlund, H.; Wagberg, L. *Langmuir* **2002**, *18*, 2859.
(14) Bikova, T.; Treimanis, A.; Rossinska, G.; Telysheva, G. *Holzforschung* **2004**, *58*, 489.
(15) Gidh, A. V.; Decker, S. R.; Vinzant, T. B.; Himmel, M. E.; Williford, C. *J. Chromotogr., A* **2006**, *1114*, 102.
(16) Sarkanen, S.; Teller, D. C.; Hall, J.; McCarthy, J. L. *Macromolecules* **1981**, *14*, 426.
(17) Guerra, A.; Gaspar, A.; Contreras, I. S.; Lucia, L.; Crestini, C.; Argyropoulos, D. S. *Phytochemistry (Elsevier)* **2007**, *68*, 2570.
(18) Lindberg, J. J.; Bulla, I.; Tormala, P. *J. Polym. Sci., Polym. Symp.* **1975**, *53*, 167.
(19) Lindström, T.; Westman, L. *Colloid Polym. Sci.* **1980**, *258*, 390.
(20) Cathala, B.; Saake, B.; Faix, O.; Monties, B. *J. Chromotogr., A* **2003**, *1020*, 229.
(21) Merkle, G.; Auerbach, S.; Burchard, W.; Lidner, A.; Wegener, G. *J. Appl. Polym. Sci.* **1992**, *45*, 407.
(22) Moacanin, J.; Felicetta, V. F.; Haller, W.; McCarthy, J. L. *J. Am. Chem. Soc.* **1955**, *77*, 3470.
(23) Dong, D.; Fricke, A. L. *J. Appl. Polym. Sci.* **1993**, *50*, 1131.
(24) Beignon, M.; Bamba, S. E. H.; Evaina, K.; Le Goff, D.; Proutiere, A. *J. Mol. Struct.* **1999**, *477*, 255.
(25) Fredheim, G. E.; Braaten, S. M.; Christensen, B. E. *J. Chromotogr., A* **2002**, *942*, 191.
(26) Gidh, A. V.; Decker, S. R.; See, C. H.; Himmel, M. E.; Williford, C. W. *Anal. Chim. Acta* **2006**, *555*, 250.
(27) Soria, V.; Llopis, A.; Celda, B.; Campos, A.; Figueruelo, J. E. *Polym. Bull.* **1985**, *13*, 83.
(28) Leamen, M. J.; McManus, N. T.; Penlidis, A. *J. Appl. Polym. Sci.* **2004**, *94*, 2545.
(29) Guerra, A.; Filpponen, I.; Lucia, L. A.; Saquing, C.; Baumberger, S.; Argyropoulos, D. S. *J. Agric. Food Chem.* **2006**, *54*, 5939.
(30) Pla, F.; Lin, S. Y.; Dence, C. In Handbook of Size Exclusion Chromatography and Related Techniques; Wu, C., Ed.; Marcel Dekker: New York, 2004; p 689.
(31) Pla, F.; Froment, P.; Capitini, R.; Tistchenko, M. *Cellulose Chem. Technol.* **1977**, 711.
(32) Stacey, K. A. *Light-Scattering in Physical Chemistry*; Academic Press: New York, 1956.
(33) Kolpak, F. J.; Cietek, D. J.; Fookes, W.; Cael, J. *J. Appl. Polym. Sci.: Appl. Polym. Symp.* **1983**, *37*, 431.
(34) Forss, K.; Stenlund, B. *Pap. Puu, Papper Och Trä* **1967**, *8*, 525.
(35) Sorlier, P.; Rochas, C.; Morfin, I.; Viton, C.; Domard, A. *Biomacromolecules* **2003**, *4*, 1034.
(36) Kulicke, W. M.; Horl, H. H. *Angew. Makromol. Chem.* **1983**, 149.
(37) Stejskal, J.; Horská, J. *Makromol. Chem.* **1982**, *10*, 2527.
(38) Dupont, A.-L.; Harrison, G. *Carbohydr. Polym.* **2004**, *58*, 233.
(39) *Polymer Handbook*; Brandrup, J., Immergut, E. H., Eds.; Wiley-Interscience: New York, 1999.
(40) *Lignins: Occurrence, Formation, Structure, and Reactions*; Sarkanen, K. V., Ludwig, C. H., Eds.; Wiley-Interscience: New York, 1971.
(41) Benko, J. *Acta Chim.* **1964**, *41*, 401.
(42) Brown, W. *J. Appl. Polym. Sci.* **1967**, *11*, 2381.

BM800673A