



Association behaviour of lignins and lignin model compounds studied by multidetector size-exclusion chromatography

B. Cathala^{a,*}, B. Saake^b, O. Faix^b, B. Monties^a

^a INRA/URCA, Equipe Parois et Matériaux Fibreux, CRA, 2 Esp R. Garros, BP 224, 51686 Reims Cedex, France

^b Federal Research Centre of Forestry and Forest Products, Institute of Wood Chemistry and Chemical Technology of Wood, Hamburg, Germany

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Abstract

SEC elution curves of spruce milled wood lignin (MWL) and guaiacyl lignin polymer models (G-DHPs) in *N,N*-dimethylformamide (DMF) exhibited a bimodal elution profile. Light scattering measurements indicated that these elution profiles were due to association effects between the molecules. This became apparent from the determination of high molar masses in the range 10^5 – 10^8 g/mol. To study this effect, MWL and DHP were fractionated by precipitation in tetrahydrofuran (THF). The THF-insoluble fractions were found to be the fractions corresponding to the apparent high molar mass part of the DMF elution profiles. The THF-soluble fractions proved to be the less-associated fractions, with lower apparent molecular mass. The individual fractions proved to be rather stable in DMF. Accordingly, the bimodal elution profiles of the starting materials were not the result of an equilibrium between associated and molecular dispersed molecules but of different structures exhibiting a specific and stable association pattern. The different fractions were further characterised by SEC in THF after acetylation to determine molar masses in molecular disperse solutions.

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1. Introduction

Lignins are natural polymers occurring in plant cell walls and represent, after cellulose, the most abundant polymer in nature [1]. Their basic units are linked at least by 10 different linkages (Fig. 1) [2] and form branched chains of interpenetrating network

within the cell wall [3–5]. Due to their very complex structure, lignins are amorphous polymers with rather limited industrial use. Increasing lignin use in valuable applications is still a great challenge and requires a better understanding of their basic properties. Between dissolved lignin molecules there are always association forces which are effective [6,7] both in aqueous solution [8,9] and in organic solvents [10–12]. Accordingly, an understanding of association driving forces is relevant for delignification,

* Corresponding author. Fax: +33-(0)3-26-77-35-99.

E-mail address: cathala@reims.inra.fr (B. Cathala).

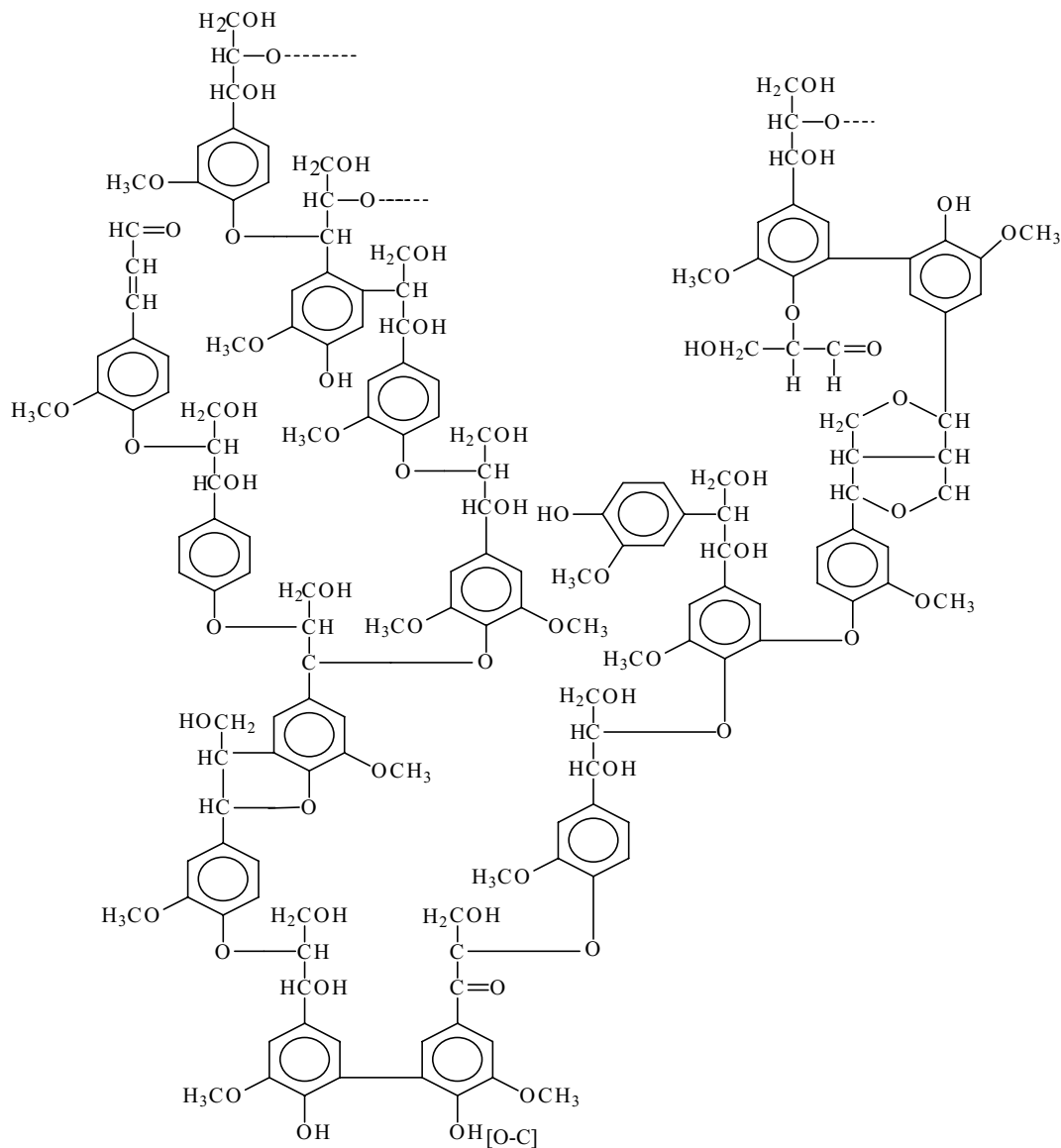


Fig. 1. Prominent structures in Conifer lignin according to Adler [2].

biodegradation processes and in the course of preparation of lignin-based biopolymers [13–16]. Moreover, this phenomenon can interfere with molar mass determination, which is a critical information needed for industrial applications [13,17]. Our paper addresses this topic and focus on spruce milled wood lignin (MWL, Björkman lignin) and different synthetic polymer analogues. In the quest of a better under-

standing of the association phenomena and the limitations of molar mass determination we performed experiments using multi-detection SEC in various solvents.

First, we selected *N,N*-dimethylformamide (DMF) as solvent, because DMF is well known for promoting association effects. In the case of lignin macromolecules, association pattern results in bimodal

elution profiles [11,18,19]. Monomodal elution curves can be obtained by adding lithium chloride to DMF or by acetylation of hydroxyl groups demonstrating the association effect. Our paper addresses a better understanding of this phenomena and the underlying mechanism of lignin association. In contrast to earlier investigations in this study DMF-SEC was performed using multi-angle laser light scattering (MALLS). This method is suitable for determination of molar mass and root-mean-square (RMS) radii of molecular dispersed polymers [20,21] and also of particle masses and RMS of associated and aggregated materials [22,23].

For both lignin and synthetic polymers, two different associated fractions could be identified and were preparatively separated according to their tetrahydrofuran (THF) solubility. In a second experimental set, all samples and their fractions were acetylated to break the association forces, in which OH groups are involved. A new SEC analysis was performed, but this time in THF and THF–lithium bromide (LiBr), since these eluents are believed to be well suited for acetylated lignins. For these studies, viscosimetric detection and universal calibration was used for detection of relevant data of polymer characterisation. The different synthetic polymer models were included in the THF and THF–LiBr investigation as reference materials.

2. Experimental

2.1. Chemicals and materials

DMF and THF were HPLC grade and purchased from SDS and J.T. Baker. All the solvents were filtered on 0.45 μm Teflon filters (Waters) prior to use. Pyridine, acetic anhydride lithium chloride and chemicals required for the DHPs and linear analogues were purchased from Aldrich and used without further purification.

2.2. Instrumentation

2.2.1. Size-exclusion chromatography in dimethylformamide

Experiments were performed on a multidetection system consisting of online vacuum degasser (ERC

3215, ERC Inc.), HPLC pump (Waters 510), autosampler (Waters 717), columns oven (Waters), UV detector (Waters 486), a refractive index detector (Waters 410) and a multi-angle laser light scattering detector (Dawn MALLS; 632.8 nm; Wyatt Corp.). The MALLS detector worked simultaneously at 18 angles. Lignin is known to exhibit fluorescence [24], which interferes strongly with molar mass determination. Therefore, the apparatus was equipped with nine interference filters (on odd-numbered detectors) with a bandwidth of 1 nm. Average molar masses in DMF were measured with the MALLS detector applying a refractive index increment (dn/dc) of 0.147. The dn/dc was experimentally determined on DHP by injecting 1 ml of DHP solutions varying in concentration from 0.2 to 0.5% directly into the ERC refractometer. The ASTRA software (Wyatt) was used for light scattering data collection during SEC runs. Sample solutions (100 μl) were injected at a concentration of 0.2%. Separation was performed on thermostatically controlled Waters columns (50 °C; mixed-bed HR1, HR4, HR5, 4.6 mm \times 300 mm each; Waters) with a flow rate of 0.3 ml/min, resulting in a pressure of 2.06 MPa. The photometer was calibrated with spectrometric-grade toluene (Aldrich). The response of the MALLS detectors were normalised to the 90° detector signal. The normalization of the apparatus detector was performed with two low-molecular-weight monodisperse polystyrene standards (20 000 and 11 000 g/mol). The same polystyrene standards were used to determine the interdetector volumes. Since for high molar mass particles the Zimm plot is not recommended, the determination of molar masses were obtained using the Debye plot procedure ($R(\theta)/K \times C$ versus $\sin^2(\theta/2)$) with first-order fit furnish by the Astra software Random coil and Berry plot were also tested. Difference in absolute values were observed, but the same general trends of M_w and RMS were obtained. For all calculations only the odd-numbered detectors equipped with fluorescence filters were used.

2.2.2. Size-exclusion chromatography of acetylated samples in THF and THF–LiBr

The analysis was performed using a multi-detection system consisting of a degasser (VT 7510, Viscotek), pump (520, Kontron), autosampler (AT 100, TSP), column oven (Croco Cil, ERC) UV-detector (LC

1200, Polymer Laboratories), a refractive index detector (RI-71, Shodex), a two-angle light scattering detector (PD 2000, Precision Detector), and a viscosity detector (H502B, Viscotek). The light scattering detector was not equipped with fluorescence filters and could only be used for qualitative monitoring aggregates. Molar masses of the samples were determined with the viscosity detector and universal calibration [25]. The universal calibration curve was based on polystyrene standards (Polymer Standard Service) using the molar masses determined by the manufacturer and the intrinsic viscosities measured by viscosimetric detection on the system applied in this study. Mark–Houwink coefficients were calculated by plotting $\log[\eta]$ versus $\log M$. The software WINGPC 4.0 from Polymer Standard Service was used for data collection, calibration and evaluation.

Separation in THF was performed by injecting 100 μl of 0.4–0.8% solutions of acetylated samples into thermostatically controlled Microgel columns (40 °C; 50, 100, 1000, 10 000, and 100 000 Å, each 250 mm \times 7.7 mm; Chrompack). The flow rate was 1 ml/min, resulting in a pressure of 5.79 MPa. For analysis in THF with addition of LiBr (0.05 M) a $\mu\text{Styragel}$ 1000 Å column (300 mm \times 7.8 mm; Waters) was used at 40 °C. The flow rate was 1 ml/min, resulting in a pressure of 0.82 MPa.

2.3. Experimental procedure

2.3.1. DHP synthesis

Dehydrogenation polymers are synthetic analogues of lignin. They are polymerised by a peroxidase from lignin monomers. DHP were prepared in a reactor that was previously dried and purged with nitrogen. Three solutions were prepared as follows: 20 mg of peroxidase (HRP type VI, 250–330 units/mg; Sigma) were dissolved in 800 ml of phosphate buffer (pH 6.1, 0.033 M; Aldrich) prepared with degassed ultra-pure water and placed in the reactor. Coniferyl alcohol (1.2 g, 6.64 mmol) was dissolved in 40 ml of acetone and 360 ml of phosphate buffer. Two millilitres of hydrogen peroxide (30% (w/v) in water, 14.6 mmol; Aldrich) were mixed in 400 ml of phosphate buffer. The coniferyl alcohol and hydrogen peroxide solutions were added at the same addition rate (5 ml/h) by pumping the solutions into the reaction vessel

for 72 h. Then the mixture was left to react for another 16 h. The precipitate was centrifuged and, after removal of the aqueous layer, washed three times with 50 ml of ultra-pure water. The yield was 82%.

2.3.2. Spruce MWL preparation

MWL was prepared according to Björkman's procedure [26] after 150 h of dry milling followed by dioxan/water (95:5) extraction for 2 days. Methoxyl groups were determined in a Zeisel apparatus and yielded an OCH₃ value of 16.61% [27]. Carbohydrate determination in MWL was performed by H₂SO₄ hydrolysis and borate ion-exchange chromatography, and yielded 1.93% carbohydrate content [28]. Total OH content, as determined by acetylation, amounted to 10.85%.

2.3.3. Synthesis of linear polymers

A dimeric quinone methide was synthesised via guaiacolglycerol- β -guaiacyl ether [29] according to Ralph and Young [30]. This compound was a quasi-precursor for linear polymers (LP) obtained via Michael addition according to Chum et al. [31]. Two compounds were synthesised (Fig. 2). The reaction was performed with 100 mg quinone methide in 40 ml acetonitrile. In case of LP-A, 140 μl of LiOH (0.064 M) were added while LP-B was synthesised with addition of 140 μl NaOH (0.064 M).

2.3.4. Fractionation in THF

Fractionation was achieved by suspending the non-acetylated spruce MWL and G-DHP in THF under stirring for 1 h (50 mg in 40 ml of anhydrous THF). The suspensions were centrifuged (5 min, 12 000 rpm) and separated into a THF-insoluble (IS) and THF-soluble fraction (S). Yields of the two fractions were: MWL, S: 45%, IS: 55%; DHP, S: 48%, IS: 52%.

2.3.5. Acetylation procedure

Samples were submitted to acetylation in a mixture of acetic anhydride and pyridine (1:1 (v/v)) for 24 h at room temperature. The reaction products were then poured into ice water and extracted with dichloromethane. Organic layers were washed with saturated sodium chloride solutions.

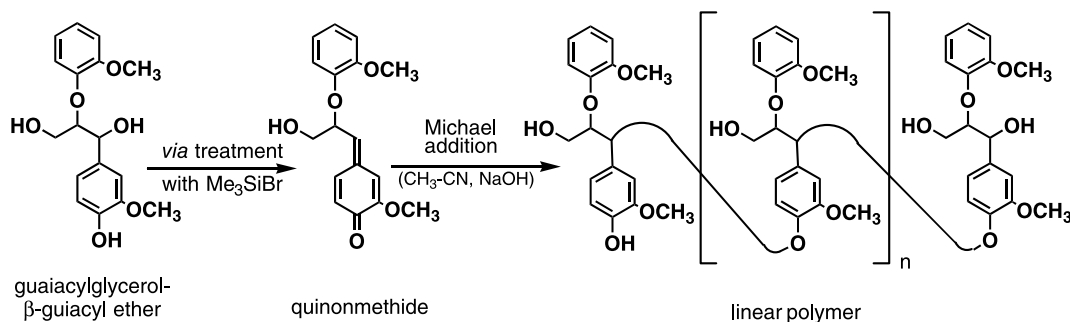


Fig. 2. Synthesis of a lignin-like linear polymer. Guaiacylglycerol-β-guaiacyl ether according to Nakatsubo et al. [29]; Quinonmethide according to Ralph and Young [42]; Michael addition according to Chum et al. [42].

3. Results and discussion

An MWL was prepared according to Björkmann [26], in the course of which wood is subjected to ball milling for several days to break down the supramolecular architecture of the cell wall. Afterwards the lignin was obtained by dioxane extraction followed by precipitation and purification steps. MWL may suffer structural changes compared to in situ lignin but still it shows a complex and statistic intermonomeric linkage pattern (Fig. 1) [2]. It is commonly held that the process of the lignin polymer formation occurs according to a dehydrogenation reaction involving the formation of resonance-stabilised radicals. Coupling of radicals lead—after rearrangement reactions—to at least nine kinds of intermonomeric linkages [32]. The relative proportion of each linkage is related to the ratio of the mesomeric forms of the radicals which are influenced by the physico-chemical environment and kinetics parameters of the reaction [33]. Isolated lignins always contain carbohydrate impurities which can interfere with their solubility and chromatographic behaviour. Polymer models of lignin are prepared by radical coupling of coniferyl alcohol in vitro in the absence of any polysaccharide contamination. The resulting product is referred to as dehydrogenation polymer (DHP) [3]. It has a great importance for fundamental understanding of lignin formation and reactions [24,34,35]. While DHPs have the same type of linkages as MWLs, their binding frequencies are different, since DHPs are not synthesised in the exact conditions of the lignin biosynthesis, especially from the kinetic point of view. Another difference between

the polymers is that DHPs have lower molar masses compared with MWLs. Nevertheless they are useful model compounds for chemical and physico-chemical studies since their chemical structure can be influenced by several parameters during in vitro synthesis and they are free of carbohydrate impurities. DHPs and lignin both present branched structures (Fig. 1). In order to complete our set of model structures, we synthesised a linear polymer model consisting of α,β-bis(*O*-4-guaiacyl) (Fig. 2) units to investigate the effect of linear and branched moieties of lignin on association effects and the molar mass determination of lignins.

3.1. SEC–MALLS analysis of MWL and DHP in DMF

First, the starting materials were analysed by SEC and light scattering in DMF. This study was performed to investigate the association phenomena occurring in this solvent and not to determine true molar masses of the polymers. The bimodal elution profiles typical for SEC of lignins in DMF (refractive index signals, RI), and the molar mass (MW) and RMS radii obtained by MALLS are compiled in Fig. 3. Based on the RI curves, both, MWL and DHP showed bimodal elution profiles with two maxima (21–22 and 27–29 min). For MWL the first maximum was more intense than the second one, and the opposite was true for DHP. To confirm that the high particle masses discussed in the following are true association phenomena and not covalently bound structures, the addition of LiCl to the DMF was also tested (data not shown). In this case the elution curves exhibited a monomodal pattern as

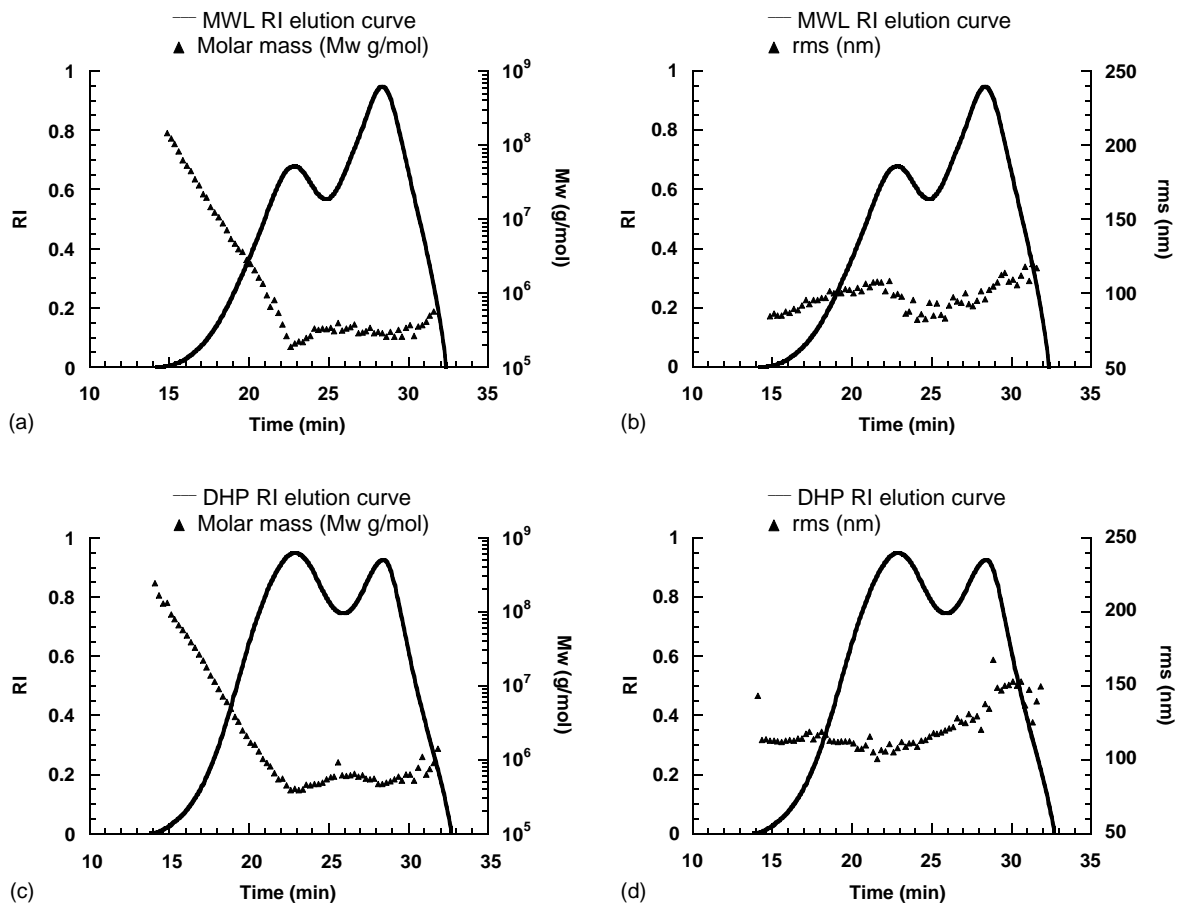


Fig. 3. Elution profile, molar mass (M_w) and RMS radius of MWL (a and b) and DHP (c and d) in DMF.

previously described [11,18,19]. Aggregation in DMF was also evidenced by a slight shift in elution patterns observed as a result of different shear forces applied by variation of flow rates and sample concentrations (data not shown). However, besides the shift in elution volume the change of shear force always resulted in the typical bimodal elution pattern. Therefore, it could be excluded that the aggregates eluting in the second peak are degradation products from the first one as a result of shear-force-induced degradation.

The observed bimodality was not a result of exclusion effects. The nearly Gauss-like shape of RI curves between 15 and 25 min, however, and the quasi-linearly decreasing M_w 's between 10^8 and 10^6 g/mol furnished evidence on regular separation conditions, as far as the pore size of the column was

concerned. Moreover, the tremendously high M_w 's were an unambiguous sign for molecular associations, so much the more as MWL and DHPs seldom possess M_w 's higher than 50 000–60 000 [24,36].

In sharp contrast to the molar masses belonging to the first part of the chromatogram, between 25 and 33 min the corresponding data of the eluates did not decrease further. Here, the M_w 's fluctuated roughly around $(5 \text{ to } 6) \times 10^5$ g/mol for MWL and around 2×10^5 g/mol for DHP. These high molar masses observed showed that the material eluting in the second part of the chromatogram was also strongly associated even when particle masses were lower, as for the first peak. Regarding the accuracy of absolute molar mass determination, the dn/dc value is always a critical parameter. This holds especially true for polymers

without a well-defined repeating unit and bonding pattern, since the dn/dc changes according to chemical structures of the polymer. Since lignins present an heterogeneous and complex structure, theoretically a dn/dc determined for various fractions of the elution would be desirable. But, practically, this is difficult to pursue due to rather large sample quantities required for a reliable dn/dc determination. In the present study the calculations were performed with the dn/dc determined for the total DHP sample. These might have a certain effect on the absolute molar mass but not on the RMS values, and it will not change the general tendencies found in this study.

As far as the M_w 's are concerned, it is worthwhile to mention that Merkle et al. [10] investigated the particle masses of dissolved organosolv lignin using static laser light scattering in a batch mode. These authors found values which were very similar to those reported here. Also the gyration radii deduced from the organosolv lignins (135–285 nm) were similar to the RMS radii of MWL (100 nm for the first peak and 150 nm for the second peak) found in our study (see Fig 3b). The DHP RMS radii fluctuate on a slightly lower level than the MWL RMS radii. This reference also confirms the magnitude of the effects observed in our study.

Surprisingly, however, both samples exhibited increased RMS radii in the range of low molar masses eluting after 25 min. This behaviour is so far unexpected as usually high molar masses coincide with greater RMS radii and not the reverse. The course of RMS radii also documented that the two peaks were not separated according to their hydrodynamic volume, indicating that the two peaks must be retarded to a different extent by the column materials. Johnson et al. [19] reported that strong interaction of lignin model compounds with polystyrene–divinylbenzene columns occurred in DMF. The authors documented that the extent of this interaction is more intense for model compounds with OH groups compared to derivatised samples.

From the ratio of molar mass and RMS, one can deduce that the molecule associates eluted in the high molar mass range have higher densities than those in the low molar mass range. However, the intensity of this densification effect is somehow surprising and will require further investigation. Density variations can be rationalized by various molecular shapes. This could

result, for instance, from different degrees of branching and cross-linking. Another explanation might be that irregular distribution of OH, CO or COOH groups in lignins and lignin polymer models leads to different molecule-to-molecule interactions, in a similar way as those functionalities that influence the interaction of the molecules with the column material.

3.2. Separation of MWL and DHP in THF-soluble and -insoluble parts

Underivatized lignins are only partly soluble in THF, a solvent with lower polarity in comparison with DMF. Therefore, it was interesting to evaluate if the solubility in THF was correlated to the elution pattern in DMF.

As pointed out in the experimental section, 45% of the MWL and 48% of the DHP are soluble in THF. The THF soluble and non-soluble fractions were submitted again to SEC in DMF. The elution profiles as detected by RI in Fig. 4a and b demonstrates the following: The THF-insoluble fraction corresponds to the apparently high molecular mass peaks of MWL and DHP while the THF-soluble fraction contributes mainly to the aggregates in the lower molecular mass region, though the curves of soluble and insoluble material are heavily overlapping for both MWL and DHP. The procedure cannot provide a complete separation but an enrichment of the two fractions, leading to the conclusion that solubility in THF and the association pattern in DMF are related.

It should be pointed out that the elution profiles of the two fractions—both for MWL and DHP—are very stable and differ clearly from that of non-fractionated (original) samples. To test stability, we submitted the solutions of fractionated polymers to prolong stirring for 22 days. Besides very small differences, the SEC elution profiles of fresh and stirred solutions were nearly identical. This proves that the divergent elution patterns of the two fractions must result from structural differences and that the original bimodal distribution pattern in DMF is not caused by an equilibrium process of molecular dispersed and associated molecules. Connors et al. [11] investigated DHP in DMF on soft gels and separated the apparent high and low molar mass fraction by preparative chromatography. After re-chromatography of the fractions the authors obtained results similar to the investigations

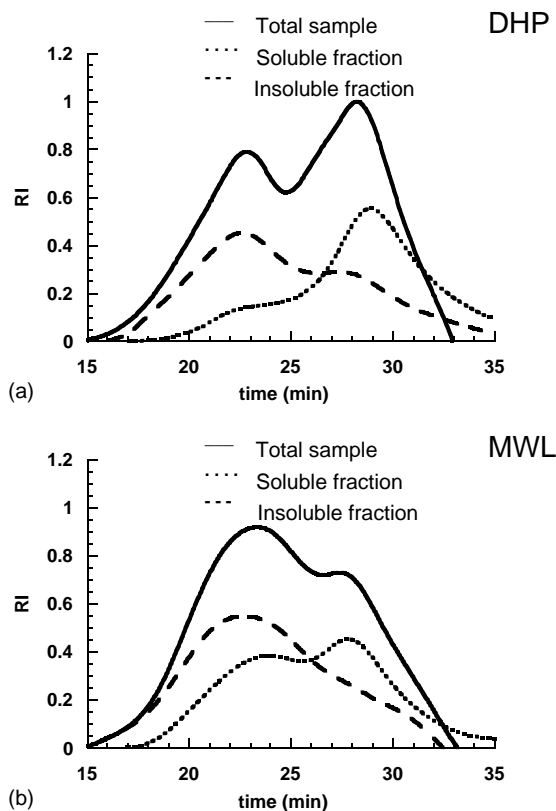


Fig. 4. Elution profiles in DMF for the different fractions isolated from MWL (a) and DHP (b) according to their solubility in THF.

presented here. They also concluded that association effects cannot be driven exclusively by molar mass but did not pursue a further investigation of this phenomenon. Sarkanen et al. [8,9] investigated association phenomena, of kraft lignins in aqueous alkaline systems. In contrast to our finding, these researchers reported that the association phenomena are not stable but influenced heavily by prolonged stirring. This deviation might be due to the fundamental difference in both, the investigated lignin samples and the different solvent systems.

3.3. SEC of acetylated samples in THF and THF–LiBr

The samples described above have been acetylated, dissolved in THF and separated again by SEC (Fig. 5). In contrast to the system “unaltered lignins

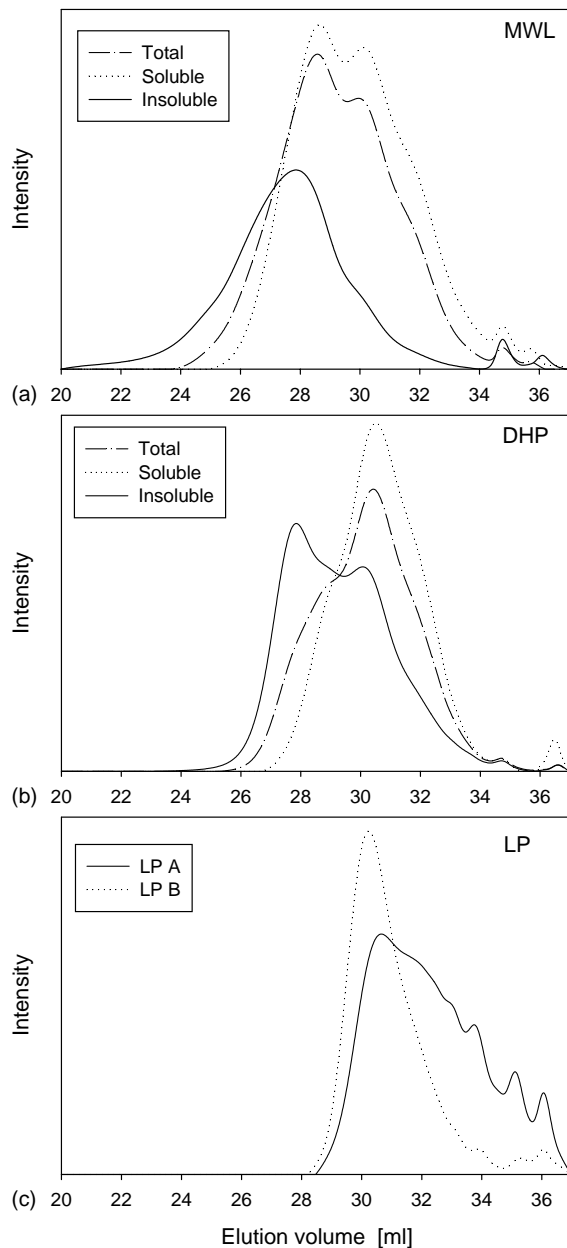


Fig. 5. Elution curves of acetylated MWL (a) and DHP (b) fractions and of linear polymers (LP) (c) as revealed by SEC in THF.

in DMF”, the system “acetylated lignins dissolved in THF” is believed not to be affected too much by association forces. The molar mass determination was performed not by light scattering but by online

Table 1

Intrinsic viscosities, molar mass data and Mark–Houwink coefficients of linear polymers (LP), DHP and MWL fractions as determined by SEC of their acetylated derivatives in THF and THF–LiBr

Solvent	Sample	$[\eta]$ (ml/g)	M_W (g/mol)	M_W/M_N	K (ml/g)	α
THF	LP-A	3.3	3600	1.9	0.78	0.18
	LP-B	3.7	5300	1.5	0.63	0.21
	DHP-T	4.5	10900	2.9	0.77	0.20
	DHP-S	4.0	6700	2.0	0.90	0.17
	DHP-IS	4.5	20700	3.0	0.80	0.23
	MWL-T	4.5	21700	4.3	0.46	0.28
	MWL-S	4.6	14700	3.4	0.68	0.21
	MWL-IS	7.6	54000	4.8	1.02	0.20
	LP-A	3.5	4900	1.6	0.28	0.30
	LP-B	3.9	6200	1.4	0.35	0.28
THF–LiBr	DHP-T	4.9	16300	2.3	0.37	0.28
	DHP-S	4.3	9000	1.9	0.47	0.25
	DHP-IS	5.3	22600	2.4	0.68	0.21
	MWL-T	6.1	24700	3.2	0.40	0.28
	MWL-S	5.7	19000	3.0	0.42	0.27
	MWL-IS	7.5	37300	2.4	0.41	0.28

Parameters K and α were determined for all the samples, but their significance is low under the 10 000 g/mol limit (see text). T: total sample; S: THF-soluble fraction; IS: THF-insoluble fraction.

viscosimetric detection and universal calibration. This approach was selected because light scattering has a low sensitivity for low molar mass polymers and the RMS radii cannot be calculated under the limit of 5–10 nm. Monodisperse dissolved MWL and DHP are below this limit and accordingly no information on the degree of branching of molecules can be obtained. From the viscosimetric detection, Mark–Houwink coefficients were calculated by plotting $\log[\eta]$ versus $\log M$ (Table 1). Variation in the branching of the different polymer fractions should be revealed by lower alpha values calculated for highly branched polymers. The linear lignin-like polymers (LP-A, LP-B) were included as un-branched reference material (see Fig. 2 and experimental section). They were not subjected to acetylation, since according to Johnson et al. [19] a big deviation in the retention behaviour of acetylated and non-acetylated lignin model compounds in THF results mainly from phenolic–OH groups, a functionality absent in the samples.

The elution profiles for the total MWL and DHP samples agree with previous publications on the SEC analysis of lignins and DHPs using divinylbenzol cross-linked polystyrene columns [37–40]. The THF-soluble fractions, both of DHP and MWL, had lower molar masses, and the THF-insoluble fractions

higher molar masses than the total samples (Table 1). The eluting profiles and molar mass data of the two linear polymers are in a range, which is representative at least for the soluble fraction of DHP and MWL.

Differences in molar mass could partly be responsible for the association found in DMF. However, molar mass cannot be the only driving force of association. Both the THF-insoluble and -soluble fractions of MWL always have much higher molar masses than those of DHP, while there is relatively small difference between the THF-insoluble DHP (20 700 g/mol) and THF-soluble MWL (14 700 g/mol) fraction. Therefore, the huge variation in their association pattern must result from variation in the chemical structure, which might be either due to differences in the degree of branching or specific functional groups.

The alpha values calculated from Mark–Houwink plots amounted to 0.18 and 0.28, while K values varied from 0.46 to 1.02 (Table 1). Compared to synthetic polymers, the K values are very high and the alpha values very low. This is in accordance with results obtained by Glasser et al. [41] for 11 commercial lignin samples. The authors obtained alpha values between 0.17 and 0.35 and K values between 0.37 and 1.38. The low alpha coefficients were explained with the branching of the molecules. According to this explanation,

higher coefficients could have been expected at least for the linear polymers, which are a model for an unbranched and well-dissolved phenylpropane polymer. However, the values corresponding to 0.18 and 0.21 for the linear polymers were smaller than expected.

The SEC separation was repeated in the presence of LiBr, which eliminates potential residual association by the shielding of dipole effects. The outcome was a small increment in viscosity and molar masses for all samples except the THF-insoluble MWL (Table 1). The Mark–Houwink plots once more revealed no differences between DHP and MWL fractions. The calculation resulted in alpha coefficients between 0.21 and 0.30, which are significantly higher than those in THF without LiBr. However, the hydrodynamic properties are in a similar order of magnitude as obtained previously. Accordingly, the influence of hydrogen bonds between lignin molecules has to be rejected as an explanation for the low alpha values determined for acetylated samples.

The Mark–Houwink plot of polymers often shows low alpha values for low molar masses, while for the latter higher alpha values are obtained. For many polymers, these high alpha values are only established for molar masses above 10 000 g/mol [37–40]. This leads to the conclusion that for the acetylated lignin and DHP, and even for linear polymers, the chains are collapsed in THF and in THF–LiBr. Therefore the SEC analysis cannot support the hypothesis on the relevance of molecular branching. These results emphasise that THF and THF–LiBr are poor solvents even for acetylated samples and that new systems for molar mass investigations of lignin would be desirable. Therefore future research on versatile solvent systems for the analysis of lignins should be performed.

4. Conclusion

The light scattering investigation on the bimodal elution profile of MWL and DHP in DMF revealed, on the one hand, intense associations, and the insoluble fractions both for DHP and MWL were of high density. The soluble fractions, on the other hand, had higher RMS radii and might be retarded in the elution by interaction with the column material. The separation of THF-soluble and -insoluble fractions yielded an enrichment of these materials. Chromatography of the

individual fractions demonstrated that they are rather stable. Accordingly, the bimodal elution is not the result of an equilibrium between associated and molecular dispersed molecules but of different structures which exhibit a specific and stable association pattern.

SEC analysis of acetylated samples in THF and THF–LiBr revealed that THF-insoluble fractions had higher masses than THF-soluble fractions both for MWL and DHP. However, a comparison of MWL and DHP fractions suggested that molar mass could not be the driving force of association.

The relationship between viscosity and molar masses was in a similar order of magnitude for all acetylated samples. The low alpha coefficients of the Mark–Houwink equation indicate that the polymer coils are collapsed both in THF and THF–LiBr. Based on this observation, the THF-soluble and -insoluble fractions may differ from each other to some extent also in terms of covalent branching. However, since no differences in hydrodynamic behaviour for the acetylated samples could be detected it is unlikely that branching can explain the huge differences of the associated fractions in DMF.

The different RMS radii of the associated fractions are probably due to intermolecular and intramolecular interactions, which are most likely a result of the differences in the chemical structure (functional groups) of the fractions. The relation between chemical structure, association pattern of lignin and solvent properties is a topic of our ongoing research activities. This future work should answer the two main remaining questions: What are the causes for the association pattern showing two distinct fractions with different particle masses, and which physical phenomena can explain the extreme differences in their particle density.

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