



Title	Determination of the absolute molar mass of acetylated eucalyptus kraft lignin by two types of size-exclusion chromatography combined with multi-angle laser light-scattering detectors
Author(s)	Wang, Linping; Uraki, Yasumitsu; Koda, Keiichi; Gele, Aori; Zhou, Xuesong; Chen, Fangeng
Citation	Holzforchung, 73(4), 363-369 https://doi.org/10.1515/hf-2018-0119
Issue Date	2019-04-24
Doc URL	http://hdl.handle.net/2115/77655
Type	article
Additional Information	There are other files related to this item in HUSCAP. Check the above URL.
File Information	Holzforchung73(4)363-369.pdf



[Instructions for use](#)

Linping Wang, Yasumitsu Uraki*, Keiichi Koda, Aori Gele, Xuesong Zhou and Fangeng Chen

Determination of the absolute molar mass of acetylated eucalyptus kraft lignin by two types of size-exclusion chromatography combined with multi-angle laser light-scattering detectors

<https://doi.org/10.1515/hf-2018-0119>

Received May 23, 2018; accepted September 17, 2018; previously published online October 17, 2018

Abstract: The molar mass (MM) and polydispersity indices (PDI) of acetylated hardwood kraft lignin (Ac-HWKL) and Ac-HWKL fractions were measured by size-exclusion chromatography with multi-angle laser light scattering (SEC-MALLS). The detectors worked at $LL_{658\text{ nm}}$ and $LL_{785\text{ nm}}$. The MM of Ac-HWKL measured at 785 nm was much smaller than that measured at 658 nm. The number-average (M_n) and weight-average (M_w) molar masses of Ac-HWKL determined at 785 nm were approximately 6.2 and 6.5 times larger, respectively, than the values estimated using a conventional calibration curve created with authentic polystyrene standards in tetrahydrofuran (THF). Based on the M_w of Ac-HWKL fractions, the Mark-Houwink-Sakurada equation for Ac-HWKL was established to be $[\eta]/\text{ml g}^{-1} = 0.320 M^{0.24}$ in THF and $[\eta]/\text{ml g}^{-1} = 0.142 M^{0.26}$ in dimethyl sulfoxide (DMSO). These results demonstrate that Ac-HWKL has a more compact structure in an organic solvent than polystyrene.

Keywords: absolute molar mass, acetylated hardwood kraft lignin, fractionation, multi-angle laser light scattering (MALLS)

Introduction

Lignin, the second or third most abundant biomass component after cellulose in terrestrial plants, is an aromatic

polymer that arises from phenylpropane precursors (monolignols). Lignin polymerization is initiated mainly by peroxidase as a one-electron transferase (Higuchi 1990), and the polymerization proceeds via a free-radical mechanism of the monolignol radicals (Yamamoto et al. 1989; Gang et al. 1999). The molar mass (MM) is an important chemical property needed for the characterization and utilization of lignin isolated from woody biomass. Before the 1980s, the MM of isolated lignin was frequently determined by light scattering (Moacanin et al. 1955; Woerner and McCarthy 1988), ultrafiltration (Olivares et al. 1988) and colligative properties, such as vapor pressure osmometry (VPO) (Brown 1967; Dolk et al. 1986; Siochi et al. 1990) and cryoscopy (Powell and Whittaker 1924; Gross et al. 1958; Chuksanova et al. 1961). In the last three decades, size-exclusion chromatography (SEC) became preponderant for MM determination. SEC is based on the hydrodynamic radii of polymers and leads to number-average and weight-average molar masses (M_n and M_w), from which the polydispersity index (PDI, i.e. M_w/M_n) can be calculated. Because the intrinsic viscosity ($[\eta]$) is a measure of the hydrodynamic radius, a universal calibration curve using $[\eta]$ and M (M_w) is applicable to obtain the absolute M_n and M_w values of an unknown sample via SEC (Grubisic et al. 1967; Weiss and Cohn-Ginsberg 1969; Guo et al. 2003). For this approach, $\log [\eta] M$ is plotted against the elution volume, where M is the MM of an authentic standard material. Such calibration plot curves can be created for any polymer. However, the $[\eta]$ determination is a tedious process, and thus the conventional calibration via plotting $\log M$ against the elution volume is still widely used (Mourey et al. 1990). In this approach, a relative MM value is obtained instead of an absolute MM because the hydrodynamic volume is ignored. When the hydrodynamic radii of an unknown sample and authentic standard material are identical at the same retention volume in SEC profiles, the relative MM data are close to those of the absolute MM.

In general, authentic polystyrene standards serve as reference material for lignin SEC calibration, and tetrahydrofuran (THF) is the eluent for native or isolated lignin samples (Siochi et al. 1990; Glasser et al. 1993; Baumberger et al. 2007; Alekhina et al. 2015; Lourencon

*Corresponding author: Yasumitsu Uraki, Research Faculty of Agriculture, Hokkaido University, Sapporo 060-8589, Japan, e-mail: uraki@for.agr.hokudai.ac.jp

Linping Wang: Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

Keiichi Koda: Research Faculty of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

Aori Gele, Xuesong Zhou and Fangeng Chen: State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou, Guangdong, China

et al. 2015). The $[\eta]$ value of kraft lignin in 1,4-dioxane is 6 ml g^{-1} (Lindberg et al. 1964) and that of polystyrene in dioxane is 113 ml g^{-1} (Chee 1987). Polystyrene $[\eta]$ values in THF being 10.2 ml g^{-1} for $10\ 300 \text{ g mol}^{-1}$ and 229 ml g^{-1} for $862\ 000 \text{ g mol}^{-1}$ are also reported in the literature (Alliet and Pacco 1968; Sparatorico and Coulter 1973). The low $[\eta]$ value of kraft lignin is due to the small a value in the Mark-Houwink-Sakurada equation ($[\eta] = KM^a$), which is in the range of 0.12–0.32 for several isolated lignins in alkaline aqueous solutions and organic solvents (Goring 1971). The a for polystyrenes is around 0.725. Accordingly, isolated lignins have a compact Einstein's sphere and therefore, the MM data of lignins estimated by a conventional SEC calibration are smaller than the absolute MM value.

There are publications about matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI-TOF-MS) (Jacobs and Dahlman 2000; Rönnols et al. 2017) and laser desorption ionization time-of-flight mass spectroscopy (LDI-TOF-MS) (Andrianova et al. 2018). MALDI-TOF-MS is the method of choice for MM determination of proteins. On the other hand, MM analysis of lignins via MALDI is problematic because of the PDI distortion due to the suppressed ionization in the range of high MM compared to the low MM range. Therefore, this application of this approach is limited to lignin fractions with a narrow PDI (Jacobs and Dahlman 2000), which is not given for the most lignin samples.

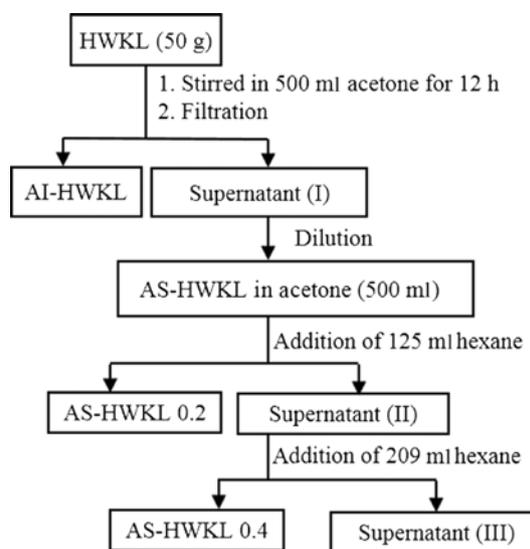
Currently, SEC combined with multi-angle laser light scattering (SEC-MALLS) is used for the determination of absolute MM data (Hokputsa et al. 2003; Han and Lim 2004; Saito et al. 2005; Chemin et al. 2015; Kleen et al. 2016). Only a few articles are dedicated to this topic in the context of lignin analysis (Fredheim et al. 2002; Cathala et al. 2003; Mikame and Funaoka 2006). Cathala et al. (2003) reported that the Mw of spruce-milled wood lignin (MWL) was approximately $5\text{--}6 \times 10^5 \text{ g mol}^{-1}$ and the Mw of a lignin polymer model synthesized from coniferyl alcohol was approximately $2 \times 10^5 \text{ g mol}^{-1}$. Fredheim et al. (2002) found that the Mw of Na-lignosulfonate from spruce was $64\ 000 \text{ g mol}^{-1}$. The Mw values of lignocresol, its fractions and other isolated lignins were approximately $1.7\text{--}12.9 \times 10^4 \text{ g mol}^{-1}$ (being 2–20-fold larger than the corresponding Mw values calculated from a conventional calibration curve), (Mikame and Funaoka 2006). In these studies, MALLS LL_{633 nm} (Fredheim et al. 2002; Cathala et al. 2003) and LL_{690 nm} (Mikame and Funaoka 2006) and band filters were used. The latter approach eliminates the self-fluorescence of lignin, which enhances the intensity of scattered light, leading to MM overestimation (Dong and Fricke 1993; Gidh et al. 2006;

Contreras et al. 2008). However, filter bandwidths with 1–10 nm do not completely remove the lignin self-fluorescence. A new type of MALLS system with LL_{785 nm} was developed (Yamamoto et al. 2017), which removes more self-fluorescence.

Kraft lignin is a promising industrial feedstock as it is available in large amounts. In the last decade, the Ligno-Boost process was developed for kraft lignin isolation from black liquor (BL) of kraft pulping (Tomani 2010). In the present article, two MALLS instruments equipped with LL detectors working at 658 nm and 785 nm were applied for the absolute MM determination of acetylated hardwood kraft lignin (Ac-HWKL). The Mark-Houwink-Sakurada equation was used for calculations and to obtain conformation data. To this purpose, the HWKL was fractionated before MM measurements via SEC-MALLS.

Materials and methods

Preparation and fractionation of HWKL: The lignin was precipitated from black liquors, obtained from kraft pulping of a hardwood species (*Eucalyptus exserta* F.Muell), supplied by the Hainan Jinhai Pulp Paper Co., Ltd. (Danzhou, China), via acidification by the dropwise addition of 20% H₂SO₄ under stirring. The precipitate was collected by filtration and washed with distilled water until the solution reached pH 3. The resultant precipitate was dried *in vacuo* at 50°C for 48 h. The HWKL powder was fractionated according to Cui et al. (2014), see Scheme 1. Fifty grams of HWKL was suspended in 500 ml of acetone under stirring for 12 h at room temperature. An acetone-insoluble fraction (AI-HWKL) was separated by filtration and washed with acetone. Acetone was further added to the supernatant in a volumetric vessel (500 ml). Hexane (125 ml) was



Scheme 1: HWKL fractional precipitation procedure.

slowly added into the acetone solution to adjust the concentration to 20% (v/v) of hexane in acetone, and the mixture was stirred for 1 h. The lignin precipitated as a gel-like material and spontaneously adhered to the inner surface of the beaker. The supernatant was removed by decantation, and the precipitate was washed with 20% (v/v) hexane in acetone solution to yield an acetone-soluble HWKL fraction (AS-HWKL 0.2, with a ratio of hexane to the acetone solution [20% = 0.2]). Then, hexane (209 ml) was slowly added to the supernatant after decantation to obtain 40% (v/v) hexane in acetone, and then, the mixture was stirred. Another lignin fraction (AS-HWKL 0.4) precipitated during stirring and was collected by decanting the supernatant. The precipitate was washed with the corresponding solvent mixture. All HWKL fractions were dried *in vacuo* at 50°C for 24 h, and then, all samples, including unfractionated HWKL, were acetylated with acetic anhydride and pyridine at room temperature for 48 h. The acetylated samples (Ac-HWKL, Ac-AI-HWKL, Ac-AS-HWKL 0.2 and Ac-AS-HWKL 0.4) were precipitated by pouring the mixture into diethyl ether. The precipitates were filtered and washed with diethyl ether.

***dn/dc* measurements:** Specific refractive index increments (*dn/dc*) of different samples were determined on an interferometric refractometer (Optilab DSP, Wyatt Technology, Santa Barbara, CA, USA) at 658 nm (polarized light). Samples with different concentrations in THF were loaded into a flow cell at 25°C with a flow rate of 0.5 ml min⁻¹. All the collected data were processed using the ASTRA 5.3 software.

Determination of MM and PDI by SEC-MALLS: One measurement series was performed in an HPLC system of Agilent 1100 (Palo Alto, CA, USA) equipped with MALLS (DAWN HELEOS-II, Wyatt Technology, Santa Barbara, CA, USA) at LL_{658 nm} (25°C), while 6 of the 18 detectors were covered with band filters to diminish sample fluorescence. The HPLC system was also equipped with an RI detector. THF was the eluent (1.0 ml min⁻¹); the injection volume was 25 µl. Two ultra-styrigel™ linear columns (PL 1110–6300 and PL 1110–6530; Agilent 1100, Palo Alto, CA, USA) were connected in series. A conventional calibration curve was obtained from the retention volumes of 16 authentic polystyrene (PS) standards with MM values in the range of 510–4 226 000 g mol⁻¹. The other HPLC system (Shimadzu LC-10, Kyoto, Japan) was similar to the first one, but an LL_{785 nm} detector (35°C) was used in combination with filters for all 8 detectors. The THF flow rate was 0.5 ml min⁻¹; the injection volume was 100 µl. The same linear columns (Shodex GPC KF-804; Showa Denko Co., Ltd., Tokyo, Japan) were connected in series. A conventional calibration curve was created from the retention volumes of 18 authentic PS standards with MM values in the range of 580–4 226 000 g mol⁻¹ purchased from four suppliers (Agilent, USA; Waters, USA; Gasukuro Kogyo, Japan; GL Sciences, Japan). The Mn and Mw values from the MALLS at LL_{785 nm} were calculated in the retention time (Rt) range of 24–36 min using the ASTRA 6.1 software.

Intrinsic viscosity determination: The specific viscosity (η_{sp}) and relative viscosity (η_{rel}) of Ac-HWKL fractions at different concentrations (2.01–8.08 mg ml⁻¹) were measured in both THF and dimethyl sulfoxide (DMSO) in a thermostatic water bath at 25°C using an Ubbelohde viscometer with a viscometer factor of 0.00205 cSt/s (SU-1140, SIBATA). The $[\eta]$ values were obtained by extrapolating the linear line created by plotting η_{sp}/C against C, where C is the sample concentration.

Results and discussion

Fractional precipitation of HWKL

Figure 1 shows the RI-SEC profiles of the Ac-HWKL and its fractions, where the intensities are related to the highest value being 2.0 or 1.0. As is visible, the fractions obtained by solvent precipitation with hexane have a narrow dispersity.

Determination of *dn/dc*

Figure 2 shows the RI values vs. the concentration of Ac-HWKL and its fractions. Linear fitting has a

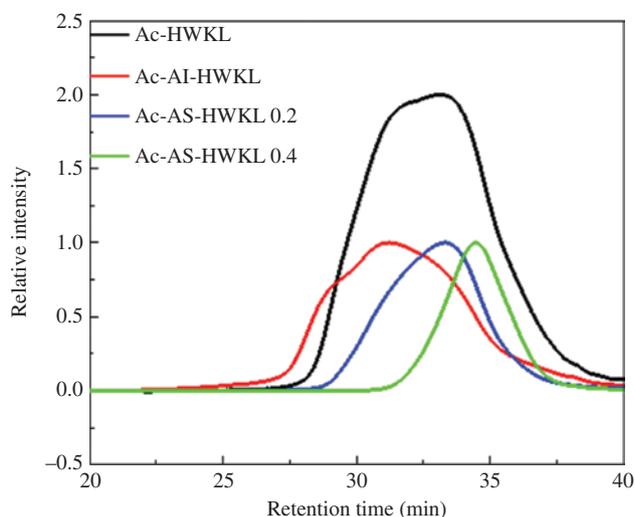


Figure 1: Size-exclusion chromatograms of acetylated HWKL and its fractions monitored by an RI detector in the Shimadzu LC system.

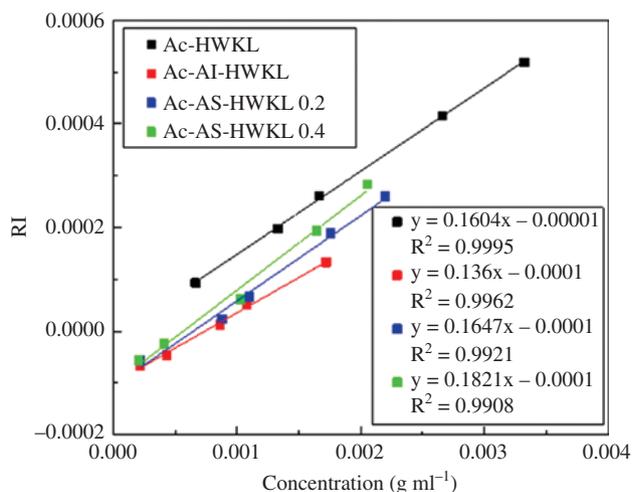


Figure 2: RI increments of acetylated HWKL and its fractions.

determination coefficient, $R^2 > 0.99$. The dn/dc value of each sample was calculated from the slope of the fitting line and resulted in values of 0.160 (Ac-HWKL), 0.136 (Ac-AI-HWKL), 0.165 (Ac-AS-HWKL 0.2) and 0.182 (Ac-AS-HWKL 0.4). That is, the dn/dc value is MM dependent. In the case of homopolymers, the dn/dc is in general independent from the MM (Huglin 1965; Michielsen 1999), but the dn/dc of copolymers, as not otherwise expected, is influenced by the chemical composition of its fractions (Pierre et al. 2003). Probably, the dn/dc of the HWKL MM is related to the relative moieties of the syringyl (S) and guaiacyl (G) units in the fractions.

SEC-MALLS analysis at 658 and 785 nm

Figure 3 presents the chromatograms of Ac-HWKL monitored by MALLS and RI detectors with the output voltage as the y-axis response. The chromatograms contain a conventional calibration curve derived from authentic PSs and the absolute MM value for each retention time is estimated by MALLS (with $LL_{658\text{ nm}}$ and $LL_{785\text{ nm}}$) with and without combination with band filters. Prior to the MM determination of Ac-HWKL, absolute MM of PS standards (with PDI of 1.01–1.05) was measured by the SEC-MALLS at $LL_{785\text{ nm}}$ to check the accuracy of the system. The data were found to be nearly identical to those reported by the suppliers. Thus, it can be safely concluded that the tested SEC-MALLS system is reliable.

The SEC-MALLS-RI chromatograms of the unfractionated Ac-HWKL with $LL_{658\text{ nm}}$ and $LL_{785\text{ nm}}$ are presented in Figure 3a and b, respectively. The SEC-MALLS-RI

chromatograms of the corresponding fraction are presented in Figure S1 for comparison. With $LL_{658\text{ nm}}$ in combination with filters, the absolute MM value is one order of magnitude smaller than in the case of unfiltered detection, i.e. the self-fluorescence of lignin was significantly affected by the filter. Both absolute MM curves are flat, and no relationship is seen between MM and Rt, which contradicts the principle of the SEC method. Thus, MALLS with $LL_{658\text{ nm}}$ is not suitable for measuring the absolute MM of Ac-HWKL, not even in combination with band filters.

On the other hand, in the case of MALLS with $LL_{785\text{ nm}}$ in combination with band filters, the absolute MM value decreased with Rt up to 36 min, but later the MM data increased and the reliability was not afforded. The MM detraction beyond 36-min Rt is due to the low LS intensity of small molecules. Therefore, MALLS $LL_{785\text{ nm}}$ is suitable for measuring the absolute MM value of Ac-HWKL.

MM and PDI of Ac-HWKL and its fractions

Table 1 shows the Mn, Mw and PDI data of Ac-HWKL and its fractions determined by SEC-MALLS in combination with $LL_{785\text{ nm}}$ and SEC-RI in combination with a conventional PS calibration. In both cases, the Mn and Mw data were calculated beginning with 24-min (void volume of the used column is $233\,000\text{ g mol}^{-1}$ of PS) up to 36-min Rt (corresponding to 1700 g mol^{-1} of PS). The Mn and Mw data obtained by SEC-MALLS are 6.2 and 6.5 times higher, respectively, than those by SEC-RI, i.e. the conventional calibration underestimates the MM data.

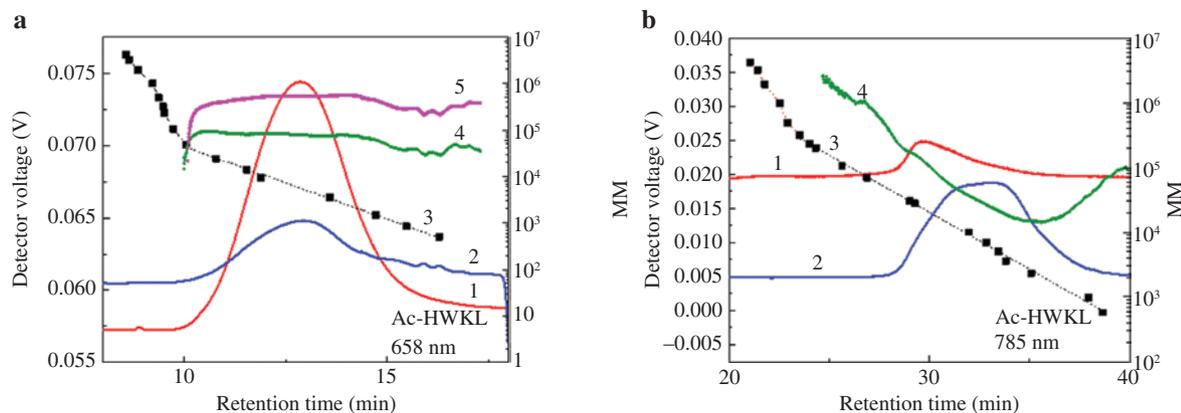


Figure 3: SEC-MALLS-RI chromatograms of Ac-HWKL obtained at 658 nm (a) and 785 nm (b).

In each figure, the left Y axis represents the detector voltage for the RI and MALLS responses, and the right Y axis represents the MM. Red line 1 is for chromatograms monitored by MALLS, and the detector was located at an angle of 90° from the LL. Blue line 2 shows the chromatograms monitored by RI. Black dashed lines 3 show conventional calibration curves of polystyrene. Green lines 4 show absolute MM obtained by MALLS with band filters. Pink line 5 shows absolute MM obtained by MALLS without band filter.

Table 1: MM and PDI values determined by SEC-MALLS.

Method		Ac-HWKL	Ac-AI-HWKL	Ac-AS-HWKL 0.2	Ac-AS-HWKL-0.4
LL _{785,3 nm} (with filters)	^a Mp (g mol ⁻¹)	27.8 × 10 ³	77.9 × 10 ³	16.6 × 10 ³	7.5 × 10 ³
	Mn (g mol ⁻¹)	31.7 × 10 ³	59.5 × 10 ³	18.9 × 10 ³	8.2 × 10 ³
	Mw (g mol ⁻¹)	55.5 × 10 ³	135.1 × 10 ³	29.7 × 10 ³	12.5 × 10 ³
	PDI	1.75	2.27	1.57	1.52
MM related to PS std.	^a Mp (g mol ⁻¹)	4.8 × 10 ³	10.3 × 10 ³	4.4 × 10 ³	2.7 × 10 ³
	Mn (g mol ⁻¹)	5.1 × 10 ³	6.9 × 10 ³	4.7 × 10 ³	3.0 × 10 ³
	Mw (g mol ⁻¹)	8.5 × 10 ³	13.6 × 10 ³	7.1 × 10 ³	3.4 × 10 ³
	PDI	1.68	1.98	1.49	1.14

^aMp is the MM at the highest peak of the RI chromatogram.

Table 1 shows that the PDI of the samples calculated from SEC-MALLS are slightly larger than those from SEC-RI because of the larger MM values of the former than the latter at low Rts. For example, the MM values of Ac-HWKL at 24-min Rt from SEC-MALLS and SEC-RI were 3.7×10^6 and 2.4×10^5 g mol⁻¹, respectively, which corresponds to a 15.4 ratio. This ratio is 10.6 at 36-min Rt. Accordingly, the Mw differences greatly affect the PDI.

The PDI of Ac-AI-HWKL calculated from both methods was larger than that of Ac-HWKL. The RI response of Ac-AI-HWKL is larger at shorter Rts in the range of 24–27 min, than that of Ac-HWKL. The larger MM region of Ac-AI-HWKL is more pronounced due to the removal of a small MM region

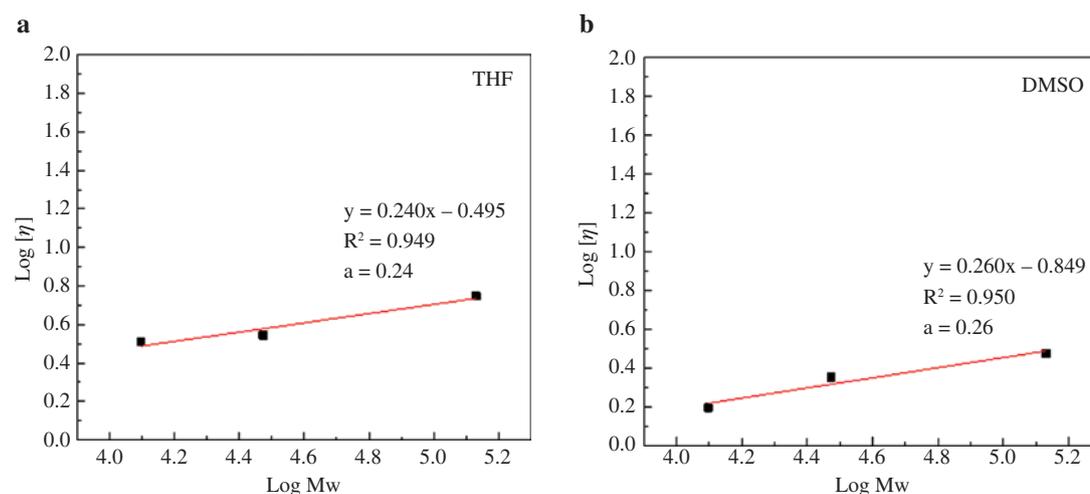
via solvent fractionation. As a result, the Mw of AI-HWKL was much larger than that of Ac-HWKL, and concomitantly, the PDI of Ac-AI-HWKL is also larger.

Mark-Houwink-Sakurada equation for Ac-HWKL

The power of the Mark-Houwink-Sakurada (MHS) equation ($[\eta] = KM^a$, $\log [\eta] = \log K + a \cdot \log M$) of Ac-HWKL was tested for lignin preparations. Figure S2 shows the Huggins plot (η_{sp}/C vs. C) and Mead-Fuoss plot ($[(\ln \eta_{rel})/C]$ vs. C) for three fractions in THF and DMSO, and linear fitting lines are inserted. Both plots are almost horizontal and overlapping, because the measured time of sample solution is only a little longer than that of the corresponding solvent, as shown in Tables S1 and S2. These plots can be interpreted to show that the viscosity of Ac-HWKL depends on the specific volume, like the Einstein's sphere. $[\eta]$ was obtained by extrapolating the Huggins plots to $C=0$ and is listed in Table 2. Figure 4

Table 2: Intrinsic viscosity of the HWKL fractions in THF and DMSO.

Method	Ac-AI-HWKL	Ac-AS-HWKL 0.2	Ac-AS-HWKL 0.4
$[\eta]$ in THF (ml g ⁻¹)	5.60	3.50	3.23
$[\eta]$ in DMSO (ml g ⁻¹)	2.98	2.24	1.57

**Figure 4:** Logarithmic plot of $[\eta]$ versus the absolute Mw for Ac-HWKL fractions in (a) THF and (b) DMSO.

shows the plots of $\log [\eta]$ against $\log Mw$, and the K and exponent a values in the MHS equation were calculated from the intercept and slope of the linear fitting line. The following equations were obtained: $[\eta]/\text{ml g}^{-1} = 0.320 M^{0.24}$ ($R^2 = 0.949$) in THF and $[\eta]/\text{ml g}^{-1} = 0.142 M^{0.26}$ ($R^2 = 0.950$) in DMSO. The a values of Ac-HWKL in THF and DMSO were in the range of 0.12–0.32, which is in agreement with the early reported data of several isolated lignins in alkaline aqueous solutions and organic solvents (Goring 1971). The a value 0.24 in THF was slightly smaller than that in DMSO (0.26), indicating the presence of more compact molecules in THF than in DMSO. However, the a values in both THF and DMSO are smaller than the value of a compact coil (0.5). Thus, the Ac-HWKL molecule likely has a shape similar to that of an Einstein's sphere. In addition, the a in THF is remarkably smaller than the value of 0.725 for PS in THF (Alliet and Pacco 1968; Sparatorico and Coulter 1973). Thus, the more compact conformation of the Ac-HWKL is indicative of its highly branched structure. This explains the smaller MM data of Ac-HWKL calculated via SEC-RI than those obtained via SEC-MALLS.

Conclusions

A retention time dependency was observed in the measurement of MM by SEC-MALLS at $LL_{785\text{ nm}}$ combined with band filters. This approach is more adequate for absolute MM determination of Ac-HWKL. The Mw and Mn data of this lignin derivative calculated from the SEC-MALLS results were much larger than those calculated from the traditional SEC-RI results based on calibration by polystyrene standards, and this was interpreted as a result of the more compact conformation of the Ac-HWKL molecule in THF. The very low value of a in the MHS equation for Ac-HWKL in THF ($[\eta]/\text{ml g}^{-1} = 0.320 M^{0.24}$) is also indicative of a compact conformation due to its highly branched structure. Our future investigation will focus on the influence of branched structures in lignin, such as 5-5 and 4-0-5 interunit linkages, and exponent a in the MHS equation.

Supporting information

SEC-MALLS-RI chromatograms of Ac-HWKL fractions (Ac-AI-HWKL, Ac-AS-HWKL 0.2 and Ac-AS-HWKL 0.4) obtained at 658 nm and 785 nm, raw data of viscosity measurement and Huggins plots and Mead-Fuoss plots for Ac-HWKL fractions in THF and DMSO.

Acknowledgment: We are very grateful to Dr. Antje Potthast for giving us useful suggestions and discussions to prepare this paper.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: Our research work was financially supported by JSPS KAKENHI (Grant-in-Aid for Scientific Research (A)) Funder Id: 10.13039/501100001691, Grant Numbers 26252022 and 18H03954.

Employment or leadership: None declared.

Honorarium: None declared.

References

- Alekhina, M., Ershova, O., Ebert, A., Heikkinen, S., Sixta, H. (2015) Softwood kraft lignin for value-added applications: fractionation and structural characterization. *Ind. Crops Prod.* 66: 220–228.
- Alliet, D.F., Pacco, J.M. (1968) The investigation of parallel-column systems in gel permeation chromatography. *J. Polym. Sci.* 21:199–213.
- Andrianova, A.A., Yeudakimenka, N.A., Lilak, S.L., Kozliaka, E.I., Ugrinob, A., Sibib, M.P., Kubátová, A. (2018) Size exclusion chromatography of lignin: the mechanistic aspects and elimination of undesired secondary interactions. *J. Chromatogr. A* 1534:101–110.
- Baumberger, S., Abaecherli, A., Fasching, M., Gellerstedt, G., Gosselink, R., Hortling, B., Li, J., Saake, B., de Jong, E. (2007) Molar mass determination of lignins by size-exclusion chromatography: towards standardisation of the method. *Holzforschung* 61:459–468.
- Brown, W. (1967) Solution properties of lignin. Thermodynamic properties and molecular weight determinations. *J. Appl. Polym. Sci.* 11:2381–2396.
- Cathala, B., Saake, B., Faix, O., Monties, B. (2003) Association behaviour of lignins and lignin model compounds studied by multidetector size-exclusion chromatography. *J. Chromatogr. A* 1020:229–239.
- Chee, K. (1987) Novel approach to Mark-Houwink-Sakurada constants and related parameters of polystyrene solutions. *Polymer* 28:977–979.
- Chemin, M., Rakotoveloa, A., Ham-Pichavant, F., Chollet, G., da Silva Perez, D., Petit-Conil, M., Cramail, H., Grelier, S. (2015) Synthesis and characterization of functionalized 4-o-methylglucuronoxylan derivatives. *Holzforschung* 69:713–720.
- Chuksanova, A., Grushnikov, O., Shorygina, N. (1961) Heterogeneity of nitrolignin. *Russ. Chem. Bull.* 10:1688–1690.
- Contreras, S., Gaspar, A.R., Guerra, A., Lucia, L.A., Argyropoulos, D.S. (2008) Propensity of lignin to associate: light scattering photometry study with native lignins. *Biomacromolecules* 9:3362–3369.
- Cui, C., Sun, R., Argyropoulos, D.S. (2014) Fractional precipitation of softwood kraft lignin: isolation of narrow fractions common to a variety of lignins. *ACS Sustainable Chem. Eng.* 2:959–968.

- Dolk, M., Pla, F., Yan, J.F., McCarthy, J.L. (1986) Lignin. 22. Macromolecular characteristics of alkali lignin from western hemlock wood. *Macromolecules* 19:1464–1470.
- Dong, D., Fricke, A.L. (1993) Investigation of optical effect of lignin solution and determination of Mw of kraft lignin by LALLS. *J. Appl. Polym. Sci.* 50:1131–1140.
- Fredheim, G.E., Braaten, S.M., Christensen, B.E. (2002) Molecular weight determination of lignosulfonates by size-exclusion chromatography and multi-angle laser light scattering. *J. Chromatogr. A* 942:191–199.
- Gang, D.R., Costa, M.A., Fujita, M., Dinkova-kostova, A.T., Wang, H., Burlat, V., Martin, W., Sarkanen, S., Davin, L.B., Lewis, N.G. (1999) Regiochemical control of monolignol radical coupling: a new paradigm for lignin and lignan biosynthesis. *Chem. Biol.* 6:143–151.
- Gidh, A.V., Decker, S.R., Vinzant, T.B., Himmel, M.E., Williford, C. (2006) Determination of lignin by size exclusion chromatography using multi angle laser light scattering. *J. Chromatogr. A* 1114:10–102.
- Glasser, W.G., Dave, V., Frazier, C.E. (1993) Molecular weight distribution of (semi-) commercial lignin derivatives. *J. Wood Chem. Technol.* 13:545–559.
- Goring, D.A.I. (1971) Polymer properties of lignin and lignin derivatives. In: *Lignins. Occurrence, Formation, Structure, and Reactions*. Eds. Sarkanen, K.V., Ludwig, C.H. Wiley-Interscience, New York. pp. 695–768.
- Gross, S., Sarkanen, K., Schuerch, C. (1958) Determinations of molecular weight of lignin degradation products by three methods. *Anal. Chem.* 30:518–521.
- Grubisic, Z., Rempp, P., Benoit, H. (1967) A universal calibration for gel permeation chromatography. *J. Polym. Sci. B.* 5:753–759.
- Guo, X., Condra, M., Kimura, K., Berth, G., Dautzenberg, H., Dubin, P. (2003) Determination of molecular weight of heparin by size exclusion chromatography with universal calibration. *Anal. Biochem.* 312:33–39.
- Han, J., Lim, S. (2004) Structural changes of corn starches by heating and stirring in DMSO measured by SEC-MALLS-RI system. *Carbohydr. Polym.* 55:265–272.
- Higuchi, T. (1990) Lignin biochemistry: biosynthesis and biodegradation. *Wood Sci. Technol.* 24:23–63.
- Hokputsa, S., Jumel, K., Alexander, C., Harding, S.E. (2003) A comparison of molecular mass determination of hyaluronic acid using SEC/MALLS and sedimentation equilibrium. *Eur. Biophys. J.* 32:450–456.
- Huglin, M. (1965) Specific refractive index increments of polymer solutions. Part I. Literature values. *J. Appl. Polym. Sci.* 9:3963–4001.
- Jacobs, A., Dahlman, O. (2000) Absolute molar mass of lignins by size exclusion chromatography and MALDI-TOF mass spectroscopy. *Nord Pulp Pap Res J.* 15:120–127.
- Kleen, M., Pranovich, A., Willför, S. (2016) Statistical modeling of pressurized hot-water batch extraction (PHWE) to produce hemicelluloses with desired properties. *Holzforschung* 70:633–640.
- Lindberg, J.J., Tylli, H., Majani, C. (1964) Notes on the molecular weight and the fractionation of lignins with organic solvents. *Pap. Puu* 46:521–526.
- Lourencon, T.V., Hansel, F.A., Silva, T.A.D., Ramos, L.P., Muniz, G.I.B.D., Magalhães, W.L.E. (2015) Hardwood and softwood kraft lignins fractionation by simple sequential acid precipitation. *Sep. Purif. Technol.* 154:82–88.
- Michielsen, S. (1999) Specific refractive index increments of polymers in dilute solution. In: *Polymer Handbook*, 4th ed. Eds. Brandrup, J., Immergut, E., Grulke, E. Wiley-Interscience, New York. pp. VII/547–628.
- Mikame, K., Funaoka, M. (2006) Polymer structure of lignophenol II—comparison of molecular morphology of lignophenol and conventional lignins. *Polym. J.* 38:592–596.
- Moacanin, J., Felicetta, V.F., Haller, W., McCarthy, J.L. (1955) Lignin. VI. Molecular weights of lignin sulfonates by light scattering. *J. Am. Chem. Soc.* 77:3470–3475.
- Mourey, T., Miller, S., Balke, S. (1990) Size exclusion chromatography calibration assessment utilizing coupled molecular weight detectors. *J. Liq. Chromatogr.* 13:435–452.
- Olivares, M., Guzman, J., Natho, A., Saavedra, A. (1988) Kraft lignin utilization in adhesives. *Wood Sci. Technol.* 22:157–165.
- Pierre, S., Cyrille, R., Isabelle, M., Christophe, V.A., Alain, D. (2003) Light scattering studies of the solution properties of chitosans of varying degrees of acetylation. *Biomacromolecules* 4:1034–1040.
- Powell, W.J., Whittaker, H. (1924) XLII. The chemistry of lignin. Part I. Flax lignin and some derivatives. *J. Chem. Soc.* 125:357–364.
- Rönnols, J., Jacobs, A., Aldaeus, F. (2017) Consecutive determination of softwood kraft lignin structure and molar mass from NMR measurements. *Holzforschung* 71:563–570.
- Saito, T., Yanagisawa, M., Isogai, A. (2005) Tempo-mediated oxidation of native cellulose: SEC-MALLS analysis of water-soluble and insoluble fractions in the oxidized products. *Cellulose* 12:305–315.
- Siochi, E.J., Ward, T.C., Haney, M.A., Mahn, B. (1990) The absolute molecular weight distribution of hydroxypropylated lignins. *Macromolecules* 23:1420–1429.
- Sparatorico, A., Coulter, B. (1973) Molecular weight determinations by gel-permeation chromatography and viscometry. *J. Polym. Sci. Part B: Polym. Phys.* 11:1139–1150.
- Tomani, P. (2010) The lignoboost process. *Cellul. Chem. Technol.* 44:53.
- Weiss, A.R., Cohn-Ginsberg, E. (1969) A note on the universal calibration curve for gel permeation chromatography. *J. Polym. Sci. Part C: Polym. Lett.* 7:379–381.
- Woerner, D.L., McCarthy, J.L. (1988) Lignin. 24. Ultrafiltration and light-scattering evidence for association of kraft lignins in aqueous solutions. *Macromolecules* 21:2160–2166.
- Yamamoto, E., Bokelman, G.H., Lewis, N.G. (1989) Phenylpropanoid metabolism in cell walls. In: *Plant Cell Wall Polymers: biogenesis and biodegradation*. Eds. Lewis, N.G., Michael G.P. American Chemical Society, Washington, DC. pp. 68–88.
- Yamamoto, Y., Cheng, N., Koda, K., Igarashi, K., Tamai, Y., Uraki, Y. (2017) Association of amphipathic lignin derivatives with cellobiohydrolase groups improves enzymatic saccharification of lignocellulosics. *Cellulose* 24:1849–1862.

Supplementary Material: The online version of this article offers supplementary material (<https://doi.org/10.1515/hf-2018-0119>).