

## Polymer Structure of Lignophenol II —Comparison of Molecular Morphology of Lignophenol and Conventional Lignins—

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**ABSTRACT:** The molecular morphology of lignophenol and conventional lignins were analyzed with SEC-MALLS system. MALLS analysis has been used as method for absolute molecular weight of polymers. However determination of absolute molecular weight of lignin is difficult due to divisively and complication of lignin structure. But the difference in the molecular weight of lignin determined by MALLS and conventional calibration methods using linear polystyrene standard can show the difference of molecular morphology of lignin. The molecular morphology of lignocresol was more spreading and linearity, compared with other lignins. It was suggested that this characteristic of lignophenol is concerned with thermoplasticity of lignophenol that other lignins do not have.

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Lignin is the most abundant natural aromatic network polymer and exists in plant cell walls as one of the major constituents. It serves as a cement between wood fibers, as a stiffening agent within fibers, and as a barrier to the enzymatic degradation of the cell wall, because pulp delignified pulp is easily resolved by the microorganism. But lignin-based products have scarcely been in human life in spite of the importance in nature. This phenomenon is due to complex structure of lignin. Lignins are three-dimensional network polymers of phenylpropane units with many different linkages between the monomers leading to a complicated structure that can only be defined by the frequency of occurrence of the various linkages. This random structure arises from an enzymatically initiated free radical polymerization of lignin precursors in the form of *p*-hydroxycinnamyl alcohols.<sup>1–3</sup> Furthermore, non-selective modifications of the lignin structure are caused by different ways due to the conditions for isolation from the cell wall. The control of structure is necessary to utilize lignin based polymer as functional polymer.

Recently Lignin-based functional polymer (lignophenol) were derived from native lignins through the phase-separation process.<sup>4–7</sup> This process includes a phase-separation reaction system composed of phenol derivatives and concentrated acid. In the process the concentrated acid is a solvent for carbohydrate and act as a catalyst for the hydrolysis of carbohydrate, but also works as a catalyst for the fragmentation and phenolation of lignin, whereas phenol derivatives

act as phenolation agents, a barrier to minimize the attack of acid on the lignin and a solvent for the lignin fractions. The lignin derivatives (lignophenol) have several unique functions, which conventional lignins do not have, in spite of retention of the original inter-unit linkages, for example, solid-liquid transformation and high immobilization capability for proteins (enzyme).<sup>7</sup> It is thought that the cause of these unique functions of lignophenol is attribute to molecular morphology of lignophenol. In the present paper, we attempted comparison of molecular morphology of lignophenol and conventional lignins by molecular weight measurement with the on-line combination analysis of size exclusion chromatography (SEC) and multi-angle laser light scattering (MALLS).

In an early review, Goring described the applicability of a number of different analytical methods employed to determine the average molecular weight of kraft lignins and other isolated lignin samples.<sup>8</sup> Gellestedt has discussed the advantages and limitations of SEC for lignin characterization in a review.<sup>9</sup> Complex interaction phenomena between lignin molecules or between lignin and the column gel can cause difficulties in SEC.<sup>10,11</sup> The choice of calibration substances is very important when using SEC for molecular weight analysis. Linear polystyrene standards with narrow molecular weight distribution are often used for apparent molecular weight calibration when THF is used as the mobile phase in SEC. A calibration has also been made using lignins with molecular weight determined by vapor pressure osmometry.<sup>12</sup> A

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good correlation up to a molecular weight of 10,000 was observed between calibrations using a set of polystyrene standards and calibrations using lignins characterized by vapor pressure osmometry.<sup>12</sup> Recently MALDI-TOF-MS is used as a new mass spectroscopy technique which has been employed in absolute molecular weight analysis of synthetic polymers and biopolymers etc. Jacobs *et al.* reported the absolute molecular weight of isolated lignins determined by SEC in combination with off-line MALDI-TOF-MS.<sup>13</sup> But to accurately determine the average molar mass of lignins, MALDI calibration methods for each type of lignin was needed and lignins that are difficult to ionize were not detected. On the other hands, the use of a low-angle laser light scattering detector coupled on-line with a SEC column (SEC-LALLS) has been suggested for lignin analysis.<sup>14</sup> The molecular weight distributions calibrated with lignins characterized by LALLS were found to deviate considerably from those obtained on the same THF-based SEC system calibrated with polystyrene standards.<sup>15</sup> However, the UV-absorptivity, fluorescence and anisotropy of lignins complicated the molecular weight determination by LALLS.<sup>14</sup> In this study, SEC-MALLS system equipped with narrow band-pass interference filters to eliminate fluorescence was utilized for solving these problems. However, since it is difficult to determine absolute molecular weight of complicate lignins by even this SEC-MALLS system, the measured value from this system expressed as 'MALLS index'.

## EXPERIMENTAL

### Lignin Preparations

Milled wood- and sulfuric acid lignins were prepared from extractive free spruce and birch wood meals, according to Björkman<sup>16</sup> and Tappi (Tappi Standard T222 om-83) methods, respectively. Tokai lignin (mixed soft wood) was used as a kraft lignin sample. This kraft lignin was fractionated by successive extraction with acetone. Explosion lignin was prepared with methanol extraction from birch explosion treatment (27 kgf/cm<sup>2</sup> for 4 min). Solvolysis lignin was prepared spruce wood treated with 70% *p*-cresol and 10% acetic acid solution at 170 °C for 120 min. Dioxane lignin was prepared from extractive free larch wood meal, according to extraction with dioxane:H<sub>2</sub>O:HCl = 90:8:1.8 mix solution under reflux for 1.5 h. This dioxane lignin was fractionated by successive extraction with same solvent. Hydro-tropic lignin was prepared from beech wood meal, according to Funaoka *et al.*<sup>17</sup> method.

### Preparation of Lignophenol

For solvation of lignin with phenol derivatives,

3 mol/C9 (phenyl propane unit of lignin) of *p*-cresol dissolved in acetone was added to wood meal and acetone was evaporated with stirring. Sulfuric acid (72% 10 mL/g wood) was added to the mixture and the vigorous stirring was continued at 30 °C for 60 min. The reaction mixture was rapidly poured to excess distilled water. The insoluble fraction was collected by centrifugation, washed with distilled water until neutral and lyophilized. The dried insoluble fraction was extracted with acetone, The acetone solution was then concentrated under reduced pressure and added dropwise to an excess amount of diethyl ether with stirring. The precipitated lignin derivative (lignocresol) was collected by centrifugation. Yields of lignocresol from spruce and birch were 27.5% of wood and 22.2% of wood respectively.

### Fractionation of Lignophenol

Lignocresol was fractionated by preparative size exclusion chromatography (SEC) on Shimadzu LC-8A recycle preparative system. The fractionated lignocresols solution were evaporated to dryness under reduced pressure and dissolved in acetone. The acetone solutions were added dropwise to an excess amount of *n*-hexane with stirring. The precipitates were collected by centrifugation.

Prep. SEC condition: Column; Shodex GPC KF-5002.5 (50 mm ID × 300 mm), Eluent; THF, Flow rate; 10 mL/min, Temp.; 23 °C, Detect; 280 nm.

### SEC-MALLS Analysis

SEC was performed using a JASCO PU-1580 High-Performance Liquid Chromatograph (HPLC).

SEC condition: Column; Shodex GPC KF-801,802, 803,804 (8 mm ID × 300 mm), Eluent; THF, Flow rate; 1.0 mL/min, Temp.; 40 °C, Standard; polystyrene standard.

MALLS analysis was performed on a DAWN-EOS multi-angle laser light scattering detector (Wyatt Technologies). The DAWN-EOS have 18 Detectors, detector number 18, 16, 14, 12, 10, 8, 6, 4 and 2 were equipped with narrow band-pass interference filters (Wyatt Technologies) to eliminate fluorescence. MALLS detector was combined SEC system. In addition, the SEC system was equipped with refractive index detector and photodiode array detector. Data acquisition and molecular weight calculation were performed using the ASTRA software (Wyatt Technologies). Molecular weight determination of lignins was calculated through the next equations.

$$(RI) = (dn/dc) \times C \times K_{RI} \quad (1)$$

$$(LS) = (dn/dc)^2 \times C \times M_w \times K_{LS} \quad (2)$$

$$M_w = (LS)/(RI) \times 1/(dn/dc) \times K_{RI}/K_{LS} \quad (3)$$

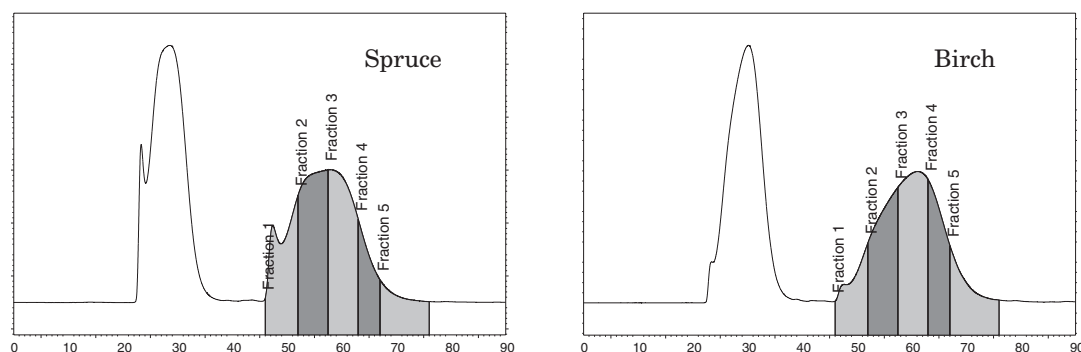


Figure 1. Preparative GPC profiles of lignocresols.

Table I. Molecular weights of fractionated lignocresol spruce by SEC/MALLS method

	PS* $M_w$	MALLS $M_w$ index	MALLS/PS	PS $M_w/M_n$	MALLS $M_w/M_n$	Yields
Fr-1	66714	630350	9.45	2.57	1.46	10.5
Fr-2	28513	88365	3.10	1.62	1.40	18.8
Fr-3	9592	22875	2.39	1.34	1.25	36.3
Fr-4	4034	9474	2.35	1.20	1.30	25.2
Fr-5	2556	8224	3.21	1.44	1.61	9.1
Lignocresol	12922	31940	2.47	3.08	1.83	

\*polystyrene standard method.

Table II. Molecular weights of fractionated lignocresol birch by SEC/MALLS method

	PS* $M_w$	MALLS $M_w$ index	MALLS/PS	PS $M_w/M_n$	MALLS $M_w/M_n$	Yields
Fr-1	47253	203600	4.31	3.34	1.91	9.6
Fr-2	13141	37045	2.82	1.42	1.55	24.9
Fr-3	5536	10325	1.87	1.20	1.24	35.9
Fr-4	2938	5549	1.89	1.14	1.24	19.1
Fr-5	2265	7193	3.16	1.53	2.04	10.5
Lignocresol	8196	17215	2.08	2.16	1.73	

\*polystyrene standard method.

LS: light scattering intensity

dn/dc: refractive index increment

C: concentration of sample

$K_{LS}$ : calibration constant of MALLS apparatus

RI: intensity of refractive index detector

$K_{RI}$ : calibration constant of refractive index detector

In the MALLS analysis, dn/dc is characteristic value of sample which has to be known from independent measurement. But dn/dc is widely variable in the same lignin, because lignin is amorphous polymer. In this study, molecular weight was calculated by Mass method. In the Mass method, dn/dc was calculated assuming that injected sample completely eluted on SEC analysis and the dn/dc was assigned to the described above equations. All samples for SEC-MALLS analysis were acetylated to prevent intermolecular association.

## RESULTS AND DISCUSSION

### SEC-MALLS Analysis of Fractionated Lignophenols

Lignocresols from spruce and birch were fractionated into five fractions at second elution by recycle preparative SEC (Figure 1). These fractions and original lignocresols were analyzed by SEC-MALLS. The MALLS index of original lignocresol from spruce and birch were 31,900 and 17,200, respectively (Table I, II), although weight average molecular weights of these lignocresols obtained on the same SEC system calculated with polystyrene standards were 12,900 and 8,200, respectively. The proportions of MALLS index and weight average molecular weights calculated with polystyrene standards (MALLS/PS) of these lignocresols were 2.5 and 2.1, respectively. It is known that same retention time in a SEC separation indicates the same hydrodynamic volume. Therefore,

**Table III.** Molecular weights of fractionated kraft lignin by SEC/MALLS method

	PS* $M_w$	MALLS $M_w$	MALLS/PS	PS $M_w/M_n$	MALLS $M_w/M_n$	Yields
Fr-2	13261	380200	28.7	5.71	1.50	41.7
Fr-3,4	3751	52625	14.0	2.40	1.51	4.8
Fr-5	2451	20830	8.5	2.42	1.92	53.8
Kraft lignin	7358	82640	11.3	5.35	1.98	

\*polystyrene standard method.

**Table IV.** Molecular weights of MWL and industrial lignins by SEC/MALLS method

	PS* $M_w$	MALLS Index	MALLS/PS	PS $M_w/M_n$	MALLS $M_w/M_n$
Solvolytic lignin	7600	29535	3.89	2.52	1.37
Explosion lignin	9645	135850	14.21	6.08	1.79
Dioxane lignin D1	7824	37775	4.83	3.52	2.25
Dioxane lignin D2	10387	110400	10.63	5.05	2.18
Hydrotropic lignin	6414	128900	20.09	3.41	1.71
MWL spruce	4632	19985	4.32	1.81	1.67
MWL birch	6925	30020	4.34	2.18	1.54

\*polystyrene standard method.

the same hydrodynamic volume with different molecular weight indicates a different density. Li *et al.* reported that molecular weights of phenolic resin determined by the SEC-MALLS analysis method were higher than that by polystyrene standard method. The difference in the molecular weight distribution determined by MALLS and conventional calibration methods can be explained by the phenolic compounds being more compact and dense than the polystyrene standard used.<sup>18</sup> Therefore spruce lignocresol was more compact and dense than birch lignocresol. Because hard wood lignin composed bifunctional syringyl unit and trifunctional guaiacyl unit has more linkage through  $\beta$ -ether bond than soft wood lignin composed only guaiacyl unit, molecular morphology of hard wood lignin was linear than soft wood lignin.<sup>8</sup>

The MALLS/PS values of fractionated lignocresol increased with increasing molecular weight (Table I, II). This results indicate that high molecular weight fraction of lignocresol is more compact and dense than low molecular weight fraction.

#### SEC-MALLS Analysis of Fractionated Kraft Lignin

The MALLS index of kraft lignin was much higher than weight average molecular weights calculated with polystyrene standards. The MALLS index was 82,640, the MALLS/PS value was 11.3 (Table III). These values may be overestimate. But the effect of narrow band-pass interference filters is defined; the malls index by detector not equipped with narrow band-pass interference filters was 349,000. This result indicates that the polymer structure of kraft lignin was more compact and dense than lignocresol. Because kraft lignin is derived through the cleavage of  $C_\beta$ -aryl

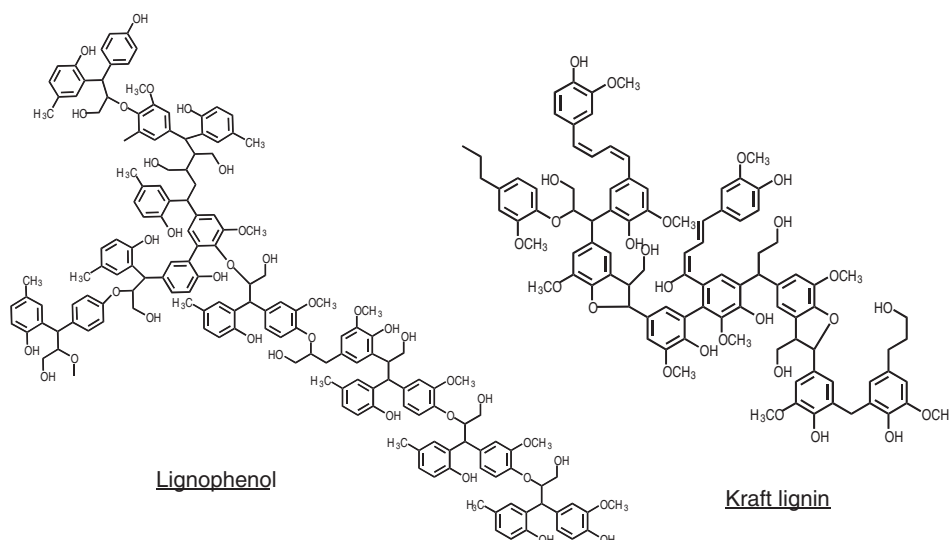
ether bond and rearrangement by formation many carbon-carbon linkage under high energy condition, its polymer structure becomes compact, whereas structure of lignocresol is branched linear type structure based on  $C_\beta$ -aryl ether bond.

The MALLS/PS values of fractionated kraft lignin increased with increasing molecular weight (Table III). Therefore the high molecular weight fraction of kraft lignin is more compact and dense than low molecular weight fraction.

#### SEC-MALLS Analysis of Industrial Lignins and MWL

Table IV is results of SEC-MALLS analysis of industrial lignins and MWL. The MALLS index of lignins derived under hard energy condition, for example explosion lignin and hydrotropic lignin, were higher than lignins derived under relatively mild condition, such as solvolytic lignin. The MALLS/PS value of explosion lignin and hydrotropic lignin were higher than solvolytic lignin. The MALLS/PS value of dioxane lignin low molecular weight fraction D1 was lower than high molecular weight fraction D2. These results indicate the lignins derived under high energy condition was converted to compact polymer structure because of cleavage of lignin interunit ether bond and rearrangement associated with formation of interunit carbon-carbon linkage.

The MALLS index and the MALLS/PS value of MWL are lower than industrial lignins. However MALLS/PS value of lignocresol were even lower than MWL, although it is said that MWL is most similar to native lignin. Therefore the molecular morphology of lignocresol was more spreading, compared with MWL. Because native lignin, three dimensional



**Figure 2.** Model polymer structure of lignophenol and kraft lignin.

network polymer, are converted to branched linear type polymers with high frequency of 1,1-bis(aryl)propane-2-O-aryl ethers during the phase-separation process.<sup>19</sup>

## CONCLUSIONS

The molecular morphology of lignophenol and conventional lignins were analyzed with SEC-MALLS system. MALLS analysis has been used as method for absolute molecular weight of polymers. However determination of absolute molecular weight of lignin is difficult due to divisively and complication of lignin structure. But the difference in the molecular weight of lignin determined by MALLS and conventional calibration methods using linear polystyrene standard can show the difference of molecular morphology of lignin. The proportions of MALLS index and weight average molecular weights calculated with polystyrene standards (MALLS/PS) of lignocresols from spruce and birch were 2.5 and 2.1, respectively. The MALLS/PS value of kraft lignin, representative industrial lignin, was 11.3. MALLS/PS value of lignocresol were lower than MWL. Therefore the molecular morphology of lignocresol was more spreading and linearity, compared with other lignins (Figure 2). It was suggested that this characteristic of lignophenol is concerned with thermoplasticity of lignophenol that native lignins do not have. That is native lignin, three dimensional network polymers, are converted to branched linear type polymers with high frequency of 1,1-bis(aryl)propane-2-O-aryl ethers during the phase separation process.

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