

COMPARISON OF BAMBOO NATIVE LIGNIN AND ALKALINE LIGNIN MODIFIED BY PHASE-SEPARATION METHOD

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In this study, native lignins were separated from bamboo using a phase separation system. Three kinds of alkaline lignins were isolated from cooking black liquor; also three kinds of alkaline lignins were modified using the phase separation system. The characteristics of bamboo native lignin and alkaline lignins were compared using Fourier Transform Infrared spectroscopy (FT-IR), Gel Permeation Chromatography (GPC), Ultraviolet Spectrometry (UV), Atomic Force Microscopy (AFM) and Dynamic Light Scattering (DLS). The solubility of lignophenols derived from bamboo and from alkaline lignins is better than that of alkaline lignins. All materials were soluble in common organic solvents and showed similar groups in FTIR spectra. GPC data indicated that the molecular weight of bamboo lignophenols (circa 4000) was larger than that of alkaline lignophenols (circa 2000). AFM images taken from the same smooth plane showed that the molecular heights of bamboo lignophenols were higher than those of alkaline lignophenols, and the aggregation of bamboo lignophenols appeared to be very uniform. DLS results indicated that the average dimension of lignophenol molecular and its aggregates ranged from 115 nm to 1560 nm, depending on the pH of the solution and the type of material examined.

Keywords: lignin, phase separation system, characterization, aggregation, Atomic Force Microscopy, Dynamic Light Scattering Spectrometer

INTRODUCTION

Lignocellulose refers to plant biomass. It is the most abundant renewable raw material on the Earth for the production of bio-fuels. Lignocellulose is composed of carbohydrate polymers (cellulose, hemicellulose), and an aromatic polymer (lignin).

These carbohydrate polymers contain different sugar monomers (six and five carbon sugars) and they are tightly bound to lignin.

Lignin is one of the main components of plant cell walls, along with cellulose and hemicelluloses. Lignin can be defined as an amorphous material containing three phenyl-propane monomers: guaiacyl, syringyl and *p*-hydroxyphenyl, which are cross-linked by a large variety of bonds, including several types of ether and carbon-carbon linkages.¹⁻³ Because of the difficulties involved in the isolation of lignin without its degradation, and in the modification or

aggregation of lignin in most solvents, the data regarding molecular weight and polydispersity are often unreliable.⁴ Small differences in the isolation techniques can result in significant changes in the molecular structure and configuration. The amount of guaiacyl, syringyl, and *p*-hydroxyphenyl units present in the lignin structure can vary significantly depending on lignin source and lignin isolation technique. This variation and the inherent complexity of the lignin molecule itself have made it very difficult to characterize lignin completely. Though general structures for lignins from various sources are available and widely accepted, specific details about the nature of linkages and monomer components are still not clear. Lignin conversion involves complex reactions of bond scission, demethylations, hydroxylations, aromatic ring fission, and side chain modifications.⁵⁻⁷ The

process is mechanistically unusual because of lignin's chemical recalcitrance, heterogeneous structure and large molecular weight, and must begin with steps that are oxidative, non-specific, and extracellular.⁸⁻⁹ Although tracking these alterations is important, the lack of well-characterized model substrates, not to mention the unclear details of the native lignin structure itself, has made it a longstanding problem.¹⁰

The phase separation system developed at Mie University Japan is a process in which acid is used to hydrolyze carbohydrates and cresol is used to dissolve lignin, causing a selective separation of lignocellulosics under normal temperature and pressure.¹¹ The product is named "lignophenol" and its structure was characterized by FT-IR, UV, and GPC. Until now, the detailed aggregation structure in most solvents has never been reported. Small differences in the isolation techniques of lignin can result in significant changes in the detected molecular structure and configuration of lignin. Atomic Force Microscopy (AFM) and Dynamics Light Scattering (DLS) analysis were found to be very effective in evaluating the characteristics of lignin.¹² In this study, bamboo native lignin and alkaline lignin obtained using three sets of different cooking conditions were treated with the phase separation system, and the resulting lignophenols were characterized by FT-IR, UV, GPC, AFM and DLS.

EXPERIMENTAL

Raw material

Air dried bamboo (CiZhu, *Neosinocalamus affinis*) collected from JiangXi Province in China was used. The compositions of all raw materials are shown in Table 1. All tests were carried out according to TAPPI Standards. Prior to composition analysis, the biomass was ground using a Willey mill, and the fraction that passed through a 40 mesh screen, but was retained at 80 mesh, was collected. The dry content of the bamboo meals was determined after drying the samples at 105 °C to constant weight.

Pulp cooking conditions

CiZhu (moisture content 10%) was chopped to 2-3 cm in length and width, and then cooked using the three sets of conditions described below. The amount of bamboo used in each run was 150 g on oven-dried (D) basis. The three alkali cooking procedures used the same cooking conditions with respect to alkali charge (EA 16%), solid/liquid ratio (1:4 w/v), heating up time (120 min), soaking time (60 min), and maximum

temperature (160 °C). However the sulfidity used was

0, 25% and 0 for condition sets 1, 2 and 3, respectively. Also, 0.05-0.07% anthraquinone was only added in the experiment under condition set 3. The lignins produced under conditions 1, 2 and 3 are further referred to as alkaline lignin1, alkaline lignin2 and alkaline lignin3, respectively.

Table 1
Main chemical compositions of bamboo

Benzene-ethanol extractives (%)	Lignin (%)			Sugar (%)				
	KL	ASL	Total	Glucan	Xylan	Arabinan	Others	Total
4.5±0.1	24.3±0.1	2.3±0.0	26.6±0.1	42.2±0.1	15.2±0.2	0.6±0.1	4.4±0.0	62.4±0.4

KL: Klason lignin, ASL: Acid-soluble lignin

Separation of alkaline lignins from black liquors

After cooling the cooked slurry, the pulp (solid part) was filtrated and washed with hot water three times. The filtrate and washing liquor were acidified to pH 3.5-4 and allowed to stand for 12 h. Then the mixture was centrifuged to remove the supernatant liquid. The collected precipitates were washed by distilled water several times until the pH of the supernatant reached 7. After removing the supernatant, freeze-drying and then vacuum-drying, the products were used as crude alkaline lignin in the treatments described below.

Synthesis of lignophenols

Synthesis and isolation of lignophenols were carried out by the method reported by Funaoka and Abe.¹¹ A typical treatment is as follows. An excess amount of cresol was added to milled wood, followed by the addition of 72% sulphuric acid. The reaction mixture turned light green and its viscosity began to increase after 30 s, reaching a maximum after 2-3 min, after which it decreased rapidly to its original value. The reaction mixture formed an apparently homogeneous, viscous solution at the early stage, and then became heterogeneous: dark green cresol particles

were suspended in the grey aqueous phase. When the stirring of the reaction mixture was stopped, the system was separated quickly into the organic phase, containing the resulting lignophenol derivatives, and the aqueous phase, containing partially hydrolysed carbohydrates. (Cresol and sulphuric acid are immiscible at room temperature and their specific gravities are quite different). To obtain natural lignin based lignophenols from bamboo meal raw materials, the following reaction conditions were used: 72% sulfuric acid, normal temperature, stirring for 1 h. To obtain alkaline lignins based lignophenols, 60% sulfuric acid was used at 50 °C with stirring for 30 min, 60 min, or 120 min.¹³

Separation of organic solvent soluble alkaline lignin

Crude alkaline lignin (0.5 g) was extracted by 100 mL of dioxane: H₂O mixture (9:1) at 110 °C for 2 h. The solution was evaporated and the residues were dissolved in 50 mL of acetone. The solution was concentrated to 5-10 mL. The acetone solution was then mixed with 100 mL diethyl-ether, and the insoluble fraction was collected by centrifuging as the organic solvent soluble alkaline lignin (S-Lignin).

Elemental analysis and methoxyl groups content

Carbon, hydrogen, sulfur and nitrogen contents were determined using a Perkin Elmer 640-C Analyzer. The percentage of oxygen was calculated by subtracting the C, H, S and N contents from 100%. The methoxyl group was determined using the methods in literatures.¹⁴⁻¹⁶ Lignin (0.15 g) was treated with refluxing concentrated sulfuric acid (10 mL) for 10 min; the reaction mixture was cooled, 70 mL of distilled water was added, and the methanol produced in the reaction was distilled off under vacuum and quantified by gas chromatography.

Gel Permeation Chromatography (GPC)

Gel permeation chromatography (GPC) was carried out using PL-GPC 50 plus Integrated GPC System (Varian Inc. Company) equipped with PD-2000 detector. Pgel 10 µm MIXED-BLS 300×7.5 mm (Agilent Technologies, Inc.) was connected in series and tetrahydrofuran (THF) used as an eluent [flow rate: 1.0 mL/min]. Calibration for weight-average molecular weight (M_w), number-average molecular weight (M_n) and polydispersity (M_w/M_n) was performed using standard polystyrene.

Proton Nuclear Magnetic Resonance (¹H-NMR)

¹H-NMR spectra of lingo-*p*-cresols were acquired with a Bruker Avance 300 MHz NMR system. The amounts of combined cresols were calculated from the signal intensity of cresolic methyl protons (1.6-2.4 ppm) compared to aromatic protons (7.8-8.4 ppm) of internal standard (*p*-nitrobenzaldehyde) on ¹H-NMR spectra using the published method.¹⁷

Fourier Transform Infrared (FTIR) Spectroscopy

The chemical structure of lignophenols was evaluated by FTIR spectroscopy. FTIR spectra were obtained on an FTIR spectrophotometer (Tensor 27) using a KBr disc containing 1% finely ground samples. The spectra were recorded in the range from 400 to 4000 cm⁻¹ with a resolution of 4 cm⁻¹ over 32 scans.

Ultraviolet Spectrophotometry (UV)

Ultraviolet and Visible spectra of lignophenols were determined on a TU-1810 UV-Vis Spectrophotometer (BeiJing Purkinje General Instrument Co., Ltd.). They were measured continuously in the wavelength region of 200-600 nm.

Atomic Force Microscopy (AFM)

Atomic force imaging of the sample was carried out with an AFM-SPM9600 (SHIMADZU Instruments Inc., Japan) under ambient conditions, using the phase mode and pulsed-force modulation modes of operation. Silicon cantilevers were used for the imaging experiments.

Dynamics light scattering (DLS) analysis

DLS measurements were performed at 298 K using a Malvern Laser Light Scattering spectrometer (Malvern Instruments Ltd.) with a scattering angle of 90°. The light source had the power of 35 mW and a wavelength of 659 nm. The software for data analysis was provided by the supplier. Before analysis, the solutions were filtered through a 0.22 µm filter to remove any dust and samples were allowed to stand for 12 h before data collection. Each measurement was repeated three times, and the average result was accepted as the final hydrodynamic diameters (D_h) when all the values fluctuated within reasonable experimental errors. All solutions were prepared in distilled water and the pH of solutions was adjusted by 1 mol/L NaOH and HCl.

RESULTS AND DISCUSSION

Characterization of bamboo raw materials and pulp samples

Different growing location, season, age, storage condition, as well as different analytical procedures result in different chemical compositions of the biomass. In this study, bamboo was provided from the southern city of Jiangxi province, China. The optimal cooking conditions usually depend on the species of the lignocellulosic materials.¹³ The main chemical components, including reducing sugars, are presented in Table 1. Three mild cooking condition sets were used during the cooking process in order to protect lignin. As the aim of this study was to investigate the modification potential of alkaline lignins through the phase

separation system, the yields and kappa numbers of the pulps were higher than those normally obtained in commercial pulping (Table 2).¹⁸

Yield of lignophenols

The yields of lignophenols from bamboo increased as the reaction time increased from 10 min to 1 hour, however when the time was further extended to 2 hours, the yield was slightly decreased. It was considered that the amount of low molecular weight lignophenols increased with reaction time, and some of them were lost during the refining process in organic solvent.¹⁹ The yields of alkaline lignophenols also increased with the reaction time increasing from 30 min to 2 hours. The absolute yields of crude alkaline lignins were higher than those obtained in the bamboo meal. This was due to the high content of Klason lignin in crude alkaline lignins, namely 59.9%, 73.3%, and 74.2%. These data indicate that alkaline lignins have high potential for modification into lignophenols.

Elemental analysis and methoxyl content

Table 3 shows the elementary composition and methoxyl content obtained for various technical

lignins, together with the approximate C₉ formulae derived from these data. The MWL is also given for comparison.²⁰

The elementary analysis of technical lignins revealed that S-KL (Soluble kraft lignin) had higher carbon content than other lignins. The carbon content of S-KL lignins was 62.3% compared to 59.9% for lignophenol. This could be rationalized by assuming more extensive condensation of soluble kraft lignin when compared to lignophenols. S-KL has lower methoxyl contents than lignophenols. This indicates that the extracted lignins have a high content of *p*-hydroxyl phenyl propane units, which probably came from the cleavage of the end groups of bamboo lignins.

Table 3 also shows the average molecular weights of the lignin C₉ unit. The highest C₉ molecular weight was found for kraft lignin based lignophenols (KLignophenols), whose Mw reported in Table 4 was half as high as that of lignophenols, yet the molecular weights of their C₉ contents were similar.

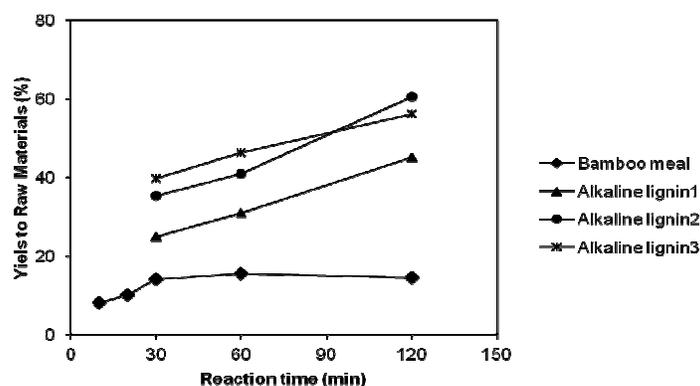


Figure 1: Yields of lignophenols

Table 2
Alkaline lignin yields and pulp features

Conditions	Crude lignin yields to raw materials (%)	Klason lignin in crude lignin (%)	Pulp yields (%)	Kappa number
NaOH	38.9	59.9	58.9	34.8
NaOH+Na ₂ S	33.2	73.3	54.2	32.2
NaOH+Na ₂ S+AQ	30.7	74.2	57.1	40.5

Table 3
Elemental composition, methoxyl contents, C₉ formulae, molecular weight (M_w) of different lignins
(%(w/w) on dry matter)

	%C	%H	%N	%S	%O	%OCH ₃	C ₉ formulae	M _w of C ₉ formulae (g/mol)
Lignophenol	59.95	5.78	0.61	0.09	33.57	17.67	C ₉ H _{8.27} O _{3.11} N _{0.088} S _{0.0006} (OCH ₃) _{1.16}	203.35
S-KL	62.29	5.87	0.70	1.17	29.97	12.32	C ₉ H _{8.77} O _{2.77} N _{0.093} S _{0.16} (OCH ₃) _{0.75}	187.76
KLignophenol	58.14	5.73	0.27	1.72	34.14	16.21	C ₉ H _{8.67} O _{3.36} N _{0.040} S _{0.11} (OCH ₃) _{1.09}	208.45
MWL	57.90	5.50	0.00	0.00	39.20	16.60	C ₉ H _{7.73} O _{3.81} (OCH ₃) _{1.24}	215.13

MWL – Milled bamboo lignin from *Phyllostachys makinoi* (from Fengel *et al.*²⁰)

The high molecular weight of these lignophenols indicated that the modified kraft lignins probably contained some condensed structures. S-KL had the lowest molecular weight, because the fraction directly soluble in organic solvent consists of small molecular weight components. This result is consistent with the GPC data described below.

Molecular weights and amounts of combined cresols in bamboo lignophenols and alkaline lignophenols

As shown in Table 4, the weight-averaged molecular weight (M_w) of bamboo mill lignophenols is about 4000 (M_w/M_n=1.55-1.97), the weight-averaged molecular weights (M_w) of the three alkaline lignophenols are in the range of 2000 to 3000 (M_w/M_n=1.63-2.01), while the M_w of the three organic solvent soluble lignins are close to 2000 (M_w/M_n=1.55-1.97). It can be observed that the dispersion (M_w/M_n) decreased with the reaction time for the bamboo mill lignin and increased with the reaction time for the alkaline lignins. This could be rationalized by degradation of alkaline lignin macromolecules and the loss of small molecules from the bamboo meal lignin in the organic solvent during the purification process. It is well known that most of alkaline lignins are insoluble in common solvents, so that industrial application of lignin is difficult. Through phase separation, it was found that a large proportion of alkaline lignins can be converted into lignophenols.

Table 4
Molecular weights and introduced cresol amounts of modified lignins

Raw material	Treatment time (min)	M _w	M _n	D=M _w /M _n	Introduced cresol amounts (mol/C ₉)
Bamboo meal	10	4083	2077	1.97	0.73
Bamboo meal	20	3560	2125	1.68	0.76
Bamboo meal	30	4155	2605	1.59	0.76
Bamboo meal	60	3939	2535	1.55	0.78
NaOH	30	2310	1414	1.63	0.36
(Alkaline lignin1)	60	2067	1136	1.82	0.41
	120	2222	1110	2.00	0.39
NaOH+Na ₂ S	30	2733	1360	2.01	0.23
(Alkaline lignin2)	60	2225	1265	1.76	0.26
	120	2007	1104	1.82	0.26
NaOH+Na ₂ S+AQ	30	2362	1394	1.69	0.41
(Alkaline lignin3)	60	2285	1292	1.77	0.45
	120	2002	1079	1.85	0.43
S-Lignin1	-	2141	1107	1.93	-
S-Lignin2	-	1954	1406	1.39	-
S-Lignin3	-	2276	1562	1.46	-

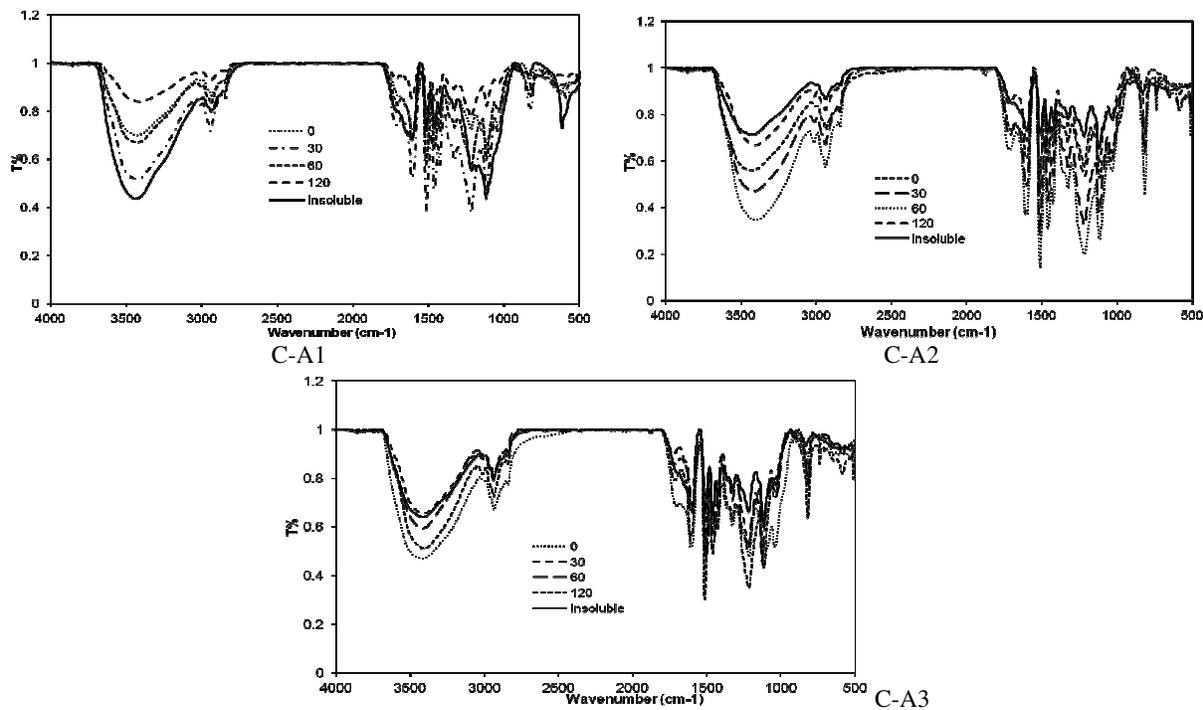


Figure 2: FT-IR spectra of alkaline lignophenols and alkaline lignins

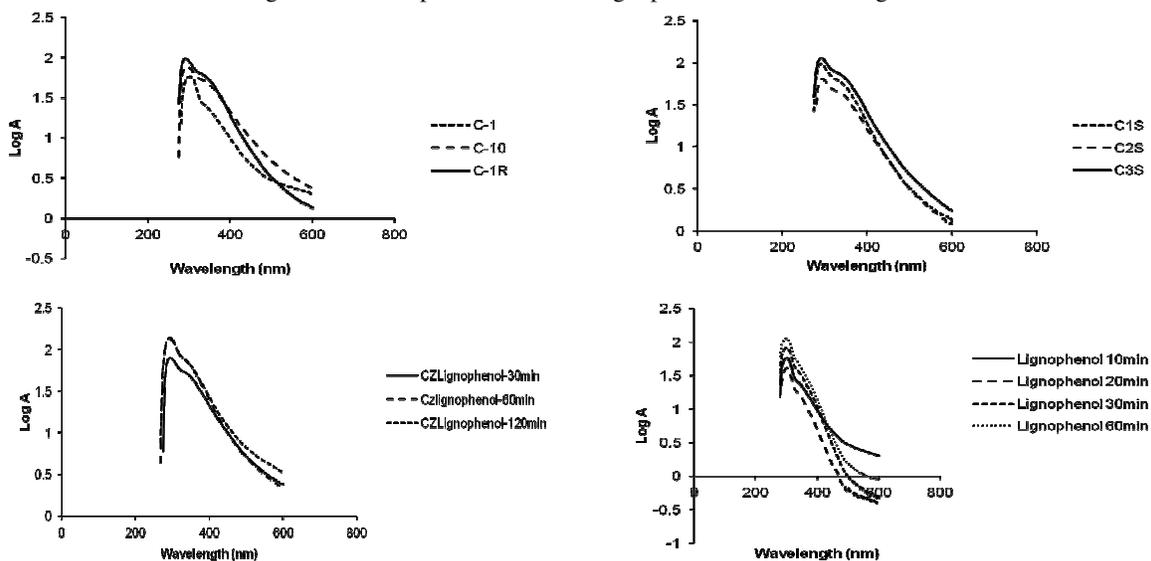


Figure 3: UV spectra of lignophenols and alkaline-soluble lignins under alkaline conditions

The content of combined cresols in bamboo lignophenols is in the range of 0.75-0.8 mol/C₉, whereas alkaline lignophenols contain 0.3-0.4 mol/C₉ (Table 4). These results also indicate that some active positions still remained in the alkaline lignins prepared by the phase separation process, but they were fewer than in the native lignins, probably because during the pulp cooking

procedure some of them were destroyed. For this reason, lignins separated from caustic pulping liquor had lower potential for conversion into lignophenols.

Fourier Transform Infrared spectra

The FT-IR spectra of three alkaline lignophenols and alkaline lignins (whole lignin

and insoluble lignin) are shown in Fig. 2, the alkaline lignophenols show bands at 1325, 1220, 1130 cm^{-1} , which belong to vibrations of syringyl group, at 1270, 1040 cm^{-1} , which belong to vibrations of guaiacyl group, and at 815 cm^{-1} , which probably resulted from combined cresols. These spectra are not much different from those

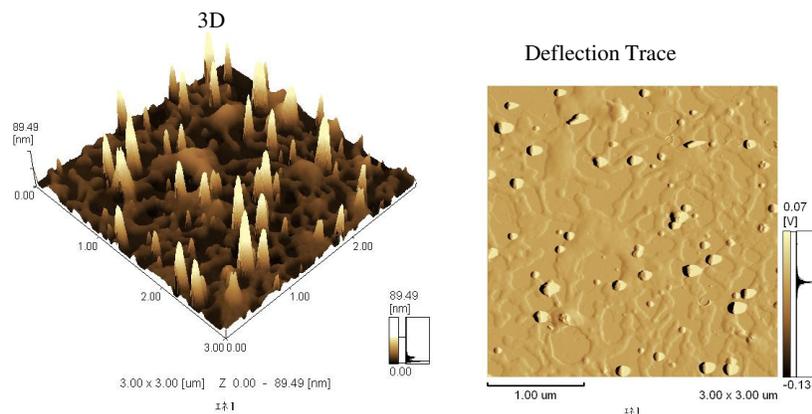
Ultraviolet spectrum

UV-visible spectra of bamboo lignins are characterized by a sharp band at 300 nm under alkaline conditions, and a small shoulder at 305 to 370 nm (Fig. 3). No large differences have been found among the bamboo lignophenols, bamboo alkaline lignophenols and insoluble alkaline lignins. The similarity of ultraviolet spectra is consistent with a similar skeletal structure of the three lignins.

Atomic force microscopy (AFM)

In this study, the atomic force microscopy (AFM) has been used for the first time to visualize the molecular conformation of lignophenols and modified kraft lignins. Fig. 4 shows AFM images of bamboo lignophenols obtained from bamboo mills, organic solvent soluble fractions of kraft lignins (S-Lignin), and alkaline lignophenols (kraft lignins modified through the phase separation system). From the

of bamboo lignophenols. The spectra of untreated alkaline lignins and insoluble lignins show broader shoulder type absorption than modified alkaline lignophenols, which was probably caused by sugars and other impurities. This means that through the phase separation system, alkaline lignins can be modified and purified concurrently. 3D images and deflection trace images, it is clear that the molecular conformation of lignophenol is unique, as its AFM image is spread out and more homogeneous than those of the other two samples. The heights of Z-axis in 3D photos are 89.49 nm, 30.79 nm and 70.96 nm for bamboo lignophenols (directly from bamboo mills), organic solvent soluble fractions of kraft lignins (S-Lignin), alkaline lignophenols (modified kraft lignins through phase separation system). These dimensions are proportional to the molecular weights of the three samples. Although among the examined materials, lignophenols have the largest molecular weight, their molecule can assume a flat configuration. The other two samples have smaller molecular weight, but, as has been reported before,¹⁸ they form agglomerates. From their rheological properties, it has been confirmed that the kraft lignins and the modified kraft lignins form layered supramolecular assemblies.



Lignophenol

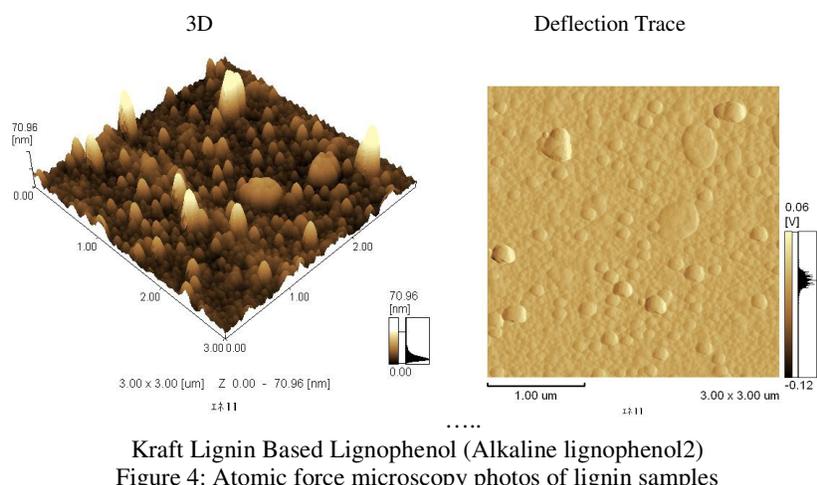
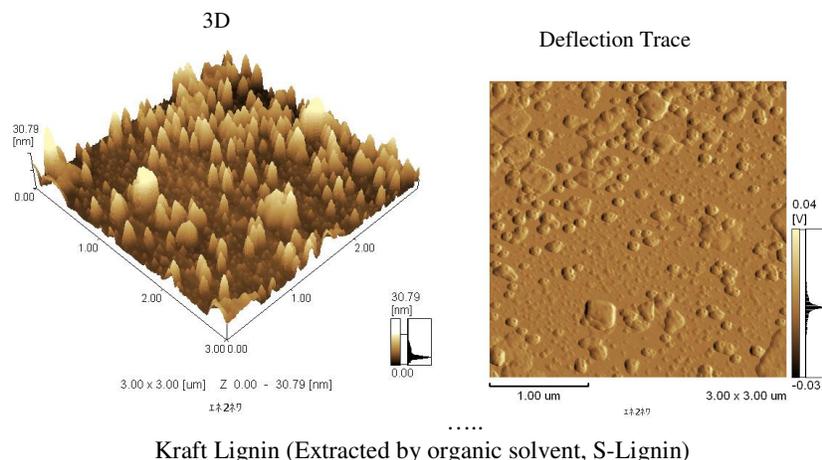
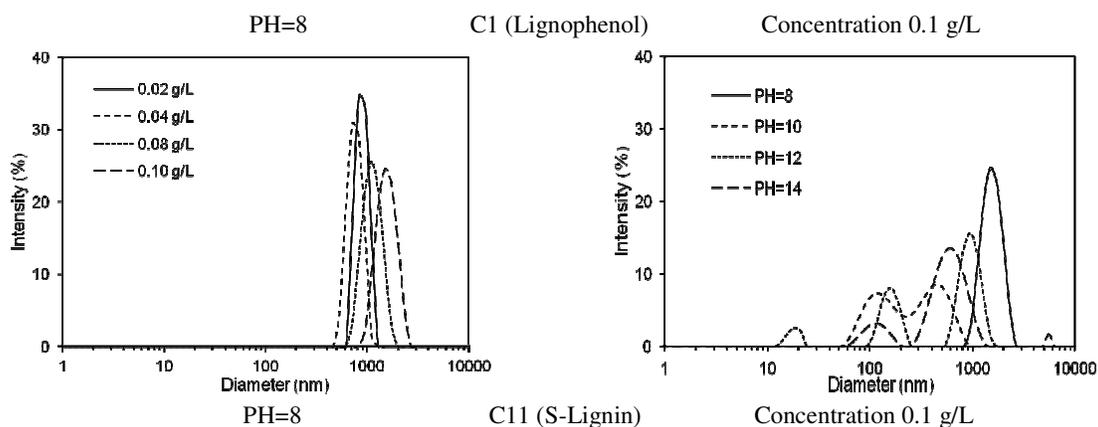


Figure 4: Atomic force microscopy photos of lignin samples

These data indicate that, although a large amount of kraft lignin has potential for conversion into lignophenols, the main spatial structures of kraft lignin have not been changed, and only a part of the active functional groups that are stretched outside the spherical structure are

modified during the pulping process. This result confirmed that the phase separation operation maintained the original spatial structure of lignin and produced relatively linear type lignin based polymer through its precise molecular design.²¹



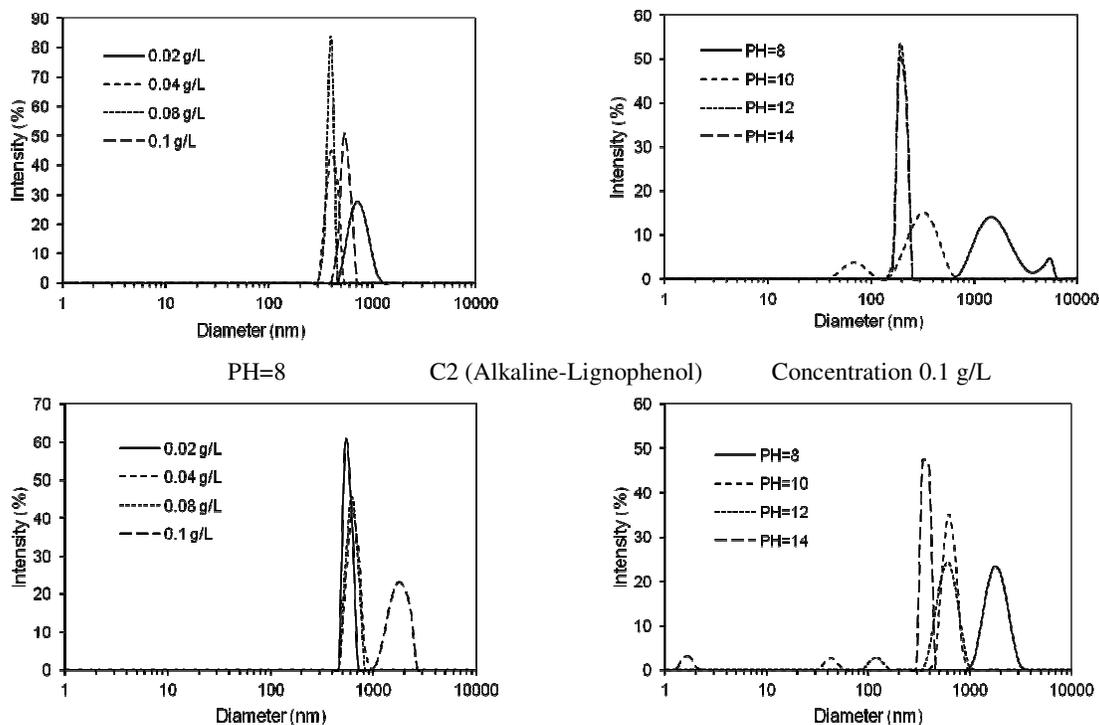


Figure 5: Influence of concentration and pH on the particle size distribution of three kinds of lignins at 298 K

Size of lignin aggregates

The DLS was used to determine the size of the aggregates, and the effect of pH on the aggregation of the three lignins is shown in Fig. 5. At pH 8 and 10, the molecular size distribution of all tested lignins appeared as a single, narrow peak, which moved to higher size and became larger at higher concentrations. When the pH value was increased to 12 and 14, the molecular size distribution peaks split into two or three wider peaks. The average dimension of the lignophenols aggregates, alkaline lignophenols and S-Lignin was 1000 nm, 600 nm and 500 nm, respectively. Since the molecular weights of lignophenols are larger than the molecular weights of the other examined materials, their aggregates are also larger. The splitting of the molecular size peaks observed at high pH values can be explained by the ionization of phenolic hydroxyl groups, or other functional groups, as the electrostatic repulsion of negatively charged molecules would prevent their agglomeration. In summary, the increase of lignin concentration favors its aggregation, while an increase in the pH limits the aggregation, because it causes ionization of functional groups. In contrast with that, under acidic conditions, an increase in the

pH of the solution makes the aggregate cores expand.¹²

CONCLUSION

Alkaline lignins can be collected from black liquor and modified through a phase separation system. Compared with the lignophenols obtained directly from bamboo meal, the lignins obtained in this manner have lower molecular weights, similar UV and FTIR spectra, but their spatial structure estimated by AFM is clearly different. The modification occurred on the surface of the macromolecular structure of alkaline lignins, the product's AFM micrograph presents similar spatial features with those of the alkaline lignin itself. The AFM micrographs of the lignophenols that were derived from bamboo meal were significantly different from those derived from kraft lignin, which once again confirmed that the pretreatment conditions are very important for the lignin structure. DLS results indicated that the average dimension of the lignophenols aggregates, alkaline lignophenols and S-Lignin were 1000 nm, 600 nm and 500 nm, respectively. The aggregation degrees of the three kinds of lignins decreased with the increasing of pH due to the ionization of phenolic hydroxyl groups. Although alkaline lignins have the potential to be further

modified, the amount of active positions and the features of the products are limited.

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