Molecular Weight and Distribution of Copolymer of Lignin-Phenol in Copolymerization Catalyzed by Peroxidase

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ABSTRACT: The molecular weight and distribution of lignin-cresol polymer in peroxidase-catalyzed copolymerization of lignin with cresol in the reversed micellar system can be controlled by adjusting the surfactant concentration and other factors. The maximal mean molecular weight of copolymer obtained is 1890 kDa. The surfactant sustains chain growth. The cresol and/or polycresol is incorporated into the copolymer, and the thermal property of the copolymer is improved. A correlation for copolymer mean molecular weight is developed. The synthesized copolymer drops out of solution and can be easily recovered. The materials appear to act as thermosets and may have applications as replacements of conventional phenolic resins. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 81: 2408–2418, 2001

Key words: lignin; reversed micelles; enzyme catalyzed copolymerization; peroxidase; phenolic polymer

INTRODUCTION

Lignin is the second most abundant biopolymer, after cellulose, on earth. Despite its abundance and availability as a byproduct of the pulp and paper industry, most lignin is burned for energy. Application of this raw material as a template for phenolic polymer synthesis has shown potential as polymeric dispersants, soil conditioning agents, phenolic resins or adhesives, and laminates, among others. Lignin is a polydisperse, irregular polymer of methoxyl- and hydroxyl-substituted phenyl propane units that is difficult to modify in a controlled manner, either chemically or biologically. Several methods have been developed for the production of lignin-containing phenolic resins,[1,2] many of which employ formaldehyde to methylolate the lignin and/or phenol in order to crosslink the polymer. However, the same problems of formaldehyde toxicity noted above apply to these lignin phenol resins.

The enzymatic polymerization of phenols has proven to be an attractive alternative to the synthesis of phenolic resins. In the presence of H_2O_2, peroxidases catalyze the oxidation of phenols, which eventually give rise to higher molecular weight polymer.[3,4] The copolymerization of phenols with kraft lignin was performed in aqueous-organic solvent mixtures catalyzed by horseradish peroxidase.[5,6] Thermal analysis shows that the copolymerization of phenols with lignin results in a material with markedly lower glass transition temperatures and higher curing exotherms. The materials appear to act as thermosets and may have applications as replacements of conventional phenolic resins. The results of the thermal analysis show that the progress of copolymerization can be controlled via the nature and concentration of organic solvent in the reaction mixture.

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Reversed micelles have been of much research interest recently due to their remarkable ability to solubilize macromolecular polyelectrolytes such as proteins without destroying catalytic activity. In addition, the fact that protein occupancy per micelle is seldom greater than unity indicates that the catalyst dispersion is extremely high, leading to efficient utilization. The observation that enzyme activity is retained in reversed micelles has led to a variety of synthetic applications. The enzyme horseradish peroxidase, when encapsulated in reversed micelles, is capable of catalyzing the polymerization of phenolic and aromatic amine.[7] The polymerization of ethylogenik is found to be extremely feasible in the micellar system. Polymer chain growth can be controlled to some degree by manipulating the ability of the solvent to sustain chain solubility by adjusting the surfactant concentration. This results in a degree of control of polymer molecular weight.

Our rationale for conducting copolymerization in reversed micelles is based on much more than the exploratory objective of determining whether the reaction is feasible in media other than aqueous-organic solvent mixtures. Our article deals with the copolymerization of lignin with cresol in revered micelles. The objective here is not so much to examine the application potential of the polymer, but rather to quantify the feasibility of the reaction in terms of kinetics and monomer conversions and to measure gross characteristics of the polymer, especially its molecular weight and distribution.

EXPERIMENTAL

Materials

Horseradish peroxidase (EC 1.11.1.7) was purchased from Shanghai Lizu Orient Technology Corp. Ltd., and it was a type II with activity 250 units/mg, assuming an enzyme molecular weight of 40,000. The buffer used was Na_2HPO_4–—NaH_2PO_4 buffer. The surfactant cetytrimethyl ammonium bromide (CTAB) was purchased from Jilin Institute of Chemical Engineering. Isooctane, *n*-butanol, hydrogen peroxide (30%), *p*-cresol, dimethylformamide (DMF), potassium dihydrogen phosphate, sodium dibasic phosphate, oxammonium hydrochloride, and other chemicals used in this work were obtained from various commercial suppliers and were of the highest purity commercially available. Polystyrene molecular weight standards were obtained from Polysciences, Inc. (Warington, PA).

The lignin used in this work was the straw-pulp-lignin, obtained from black liquor or alkali liquor. The straw-pulp-lignin was used as it is more abundant in China, and because black liquor is a huge source of pollution produced from many small paper mills. Using the lignin as a raw material of lignin-phenol resin will promote the recovery of the lignin from the black liquor, and hence, pollution resulting from black liquor will be minimized, which is very important for the environment.

Analytical Methods

For DMF-soluble fractions, the molecular weight of resulting polymer and lignin was determined by gel permeation chromatography (GPC, C-R7A, Warters, Milford, MA). The temperature of the column was 30°C, and a mobile phase was tetrahydrofuran with a flow rate of 1 mL/min. DMF was added to the resulting polymer to result in 1% liquid. After the liquid was at 80°C for 1 h, it was filtered, and the filtrate was used with GPC to measure the molecular weight and distribution of the resulting polymer.

The concentrations of unreacted phenols were measured by reverse phase high performance liquid chromatography (HPLC). A C_{18} reversed-column (Waters, Milford, MA) was used with a mobile phase with a ratio of 65 : 35 of methanol to water, and the flow rate of mobile was 0.6 mL/min. Detection was achieved at 254 nm using a UV spectrometer (Waters). The phenyl hydroxide (HPLC grade) was used as the internal standard. The sample was filtered by polytetrafluoroethylene film with 0.45 μm hole radius. The concentrations of lignin in reversed micelles were measured using a spectrophotometer at 640 nm, type 751 (Shanghai Optics Instrument Factory).

Fourier transform infrared spectroscopy (FTIR) was performed using a Nicolet Magna-IR 750 instrument with the sample as KBr pellet. Thermal analyses were conducted using a differential scanning calorimeter (DSC) SR-1 (Beijing Analysis instrument Factory). For thermal analyses the flow rate of nitrogen gas was 100 mL/min, the sample size was 10 mg, and the heating rate was 10°C/min.

Copolymerization Procedure

Preparation of straw-pulp-lignin (SPL) was conducted as follows. First, the black liquor of straw-

Table I Data of Concentration of Lignin Versus Absorbance of Lignin-Reversed Micelles

Concentration of Lignin, mg/mL	0.069	0.055	0.041	0.028	0.014	0.007	0.000
Absorbance	0.745	0.605	0.450	0.298	0.150	0.091	0.000

pulp, with pH 10–11, was filtered to remove solid contamination. Then, the pH of liquor was adjusted to about 5 by adding sulfuric acid after the liquor had been heated to 70–80°C. Thirdly, the temperature of the liquor was cooled to 60°C and was incubated for 2 h at 60°C, when brown precipitate appeared. Finally, the precipitate was collected by centrifugation with a speed of 3,500 rpm, the mud cake was washed several times with still water and separated by centrifugation, and the precipitate was dried at 60°C for 6 h. The resulting lignin was brown.

A typical preparation of reversed micelles was carried out as follows: 5.46 g of CTAB was added into a beaker, then 8 mL of *n*-butanol and 2 mL of isooctane was added into the beaker, respectively. Then, 21.6 mL of a buffer with pH 6.2 was added into the beaker, and finally, the mixture was vibrated until a uniform solution was observed, which was made up of the reversed micelles.

The preparation of lignin-reversed micelles was as follows. The lignin was added into the reversed micelles, the liquor obtained was filtrated to remove solid, and a brown uniform solution was obtained, which was made up of the lignin-reversed micelles. The absorbance of the solution was proportional to the concentration of lignin at some wavenumber, therefore, the concentration of lignin in reversed micelles could be determined using a spectrophotometer. With a reference of the reversed micelles that contained no lignin, an absorption curve was obtained via absorbance at 640 nm, and Table I shows the data. Using the curve, the concentration of lignin in reversed micelles in this work can be determined.

With the data, an equation was obtained via linear optimal estimation. The equation is as follows:

$$Y = 0.00916X \quad (1)$$

Where Y is the concentration of lignin in reversed micelles, and X the absorbance of lignin-reversed micelles. The concentration of lignin can be estimated conveniently via the equation.

The buffer used was Michaelis buffer, 0.1*M*. A stock solution of enzyme buffer was made up as follows: 10 mg of HRP was added to 10 mL of deionized water, and the concentration of the resulting solution was 2.5×10^{-5} mol/L, assuming an enzyme molecular weight of 40,000 and full enzyme content in the material. The enzyme-in-buffer solution was stored at 4°C.

Enzymatic polymerization was carried out in reversed micelles and a type reaction was carried out as follows. First, to a solution of lignin-reversed micelles we directly added phenol and the solution of enzyme-in-buffer, and the solution obtained was incubated in a constant temperature bath for 10–20 min at a designed temperature, e.g., 25°C. Using a microinjector, a designed size of hydrogen peroxide, e.g., 200 μm of hydrogen peroxide, was injected to initiate the copolymerization of lignin with phenol. The range of parameters used in this work was as follows. The range of concentrations of enzyme was from 1.0025×10^{-7} to 8.7525×10^{-7} mol/L; W0 was a molar ratio of water to surfactant, from 29.06 to 90.94; the concentration of phenol, from 62.04 to 141.05 mg/mL; the concentration of lignin, from 46.4 to 135.6 mg/mL; the concentration of surfactant, CTAB, from 0.863 to 1.637 mol/L; the ratio of *n*-butanol to isooctane, from 2.0 to 4.0.

We have found that the copolymerization of lignin with *p*-cresol in reversed micelles is extremely rapid and occurs with the formation of a dark precipitate within a minute of reaction initiation, and turbidness of reaction liquid appears. Accordingly, the precipitate was easily isolated from the supernatant by centrifugation or filtration, washed with water to remove any traces of enzyme if present, and washed repeatedly with isooctane to remove any residual surfactant. The final precipitate was dried free of both the solvent and water at 60°C and stored for subsequent polymer characterization. Micellar integrity was retained subsequent to reaction, and the filtrate, i.e., reversed micelles, could be reused because the enzyme activity was retained.

RESULTS AND DISCUSSION

The oxidation of phenols by peroxidase in the presence of H_2O_2 gives rise to phenoxy radicals

Table II Rate Data of Copolymerization

No.	Reaction Temperature (°C)	Concentration of Enzyme HRP (mol/L)	Reaction Time (s)	Conversion of *p*-Cresol (%)
1	20	5×10^{-7}	150	76.5
1	20	5×10^{-7}	300	91.9
35	35	1.33×10^{-7}	30	81.9
35	35	1.33×10^{-7}	60	94.4

that are subject to a number of nonenzymatic fates. In the absence of other phenols, or other hydrogen donors, phenoxy radicals can undergo radical transfer or termination (coupling) reactions to give phenolic homopolymers. In the presence of other phenols, however, radical transfer and coupling reactions can occur between different phenolic groups. One can expect three possible reactions to be catalyzed by peroxidase in the presence of a monomeric phenol[5]: phenol-phenol interactions, lignin-phenol interactions, and lignin-lignin interactions. The first case is undesirable in view of modifying lignin properties. The second is the most desirable. The third possibility is less likely than the other two cases, as peroxidase prefers low molecular weight substrates,[8] although in the absence of phenols, the enzyme will catalyze lignin oxidation.[9] The goal of our research was to insight the controllability of molecular weight and distribution of copolymer resulting from reversed micelles, in other words, to optimize the copolymerization of lignin with monomeric phenols.

Rate of Copolymerization

The data of copolymerization rates are given in Table II. The analysis procedure was as follows. Samples were taken out from the reacting liquor at interval after the reaction was initiated. Oxammonium hydrochloride was added into the sample to cease the reaction immediately, then, a size of the sample was used for concentration measuring by HPLC. The sample was put into a measuring flask of 10 mL, then, a size of internal standard phenyl hydroxide was added into the sample. The volume of the mixture was diluted to 10 mL; then, the diluent sample was filtered by polytetrafluoroethylene film with 0.45 μm hole radius. The size of designed filtrate was injected into HPLC for concentration measuring. The concentration of *p*-cresol was a relative value, not an absolute one; however, it could be used for an estimation of conversion.

As shown in Table II, *p*-cresol conversion was 94.4% at 60 s in run No. 35 with an enzyme concentration of 1.33×10^{-7} mol/L, which indicates that the reaction rate was very rapid. Comparing run 35 with run 1, one finds that the effect of temperature on reaction rate was significant: at 60 s run 35 had a conversion of 94.5% with a higher temperature, 35°C, which was much higher than 88.2% in run 1 at 150 s with a lower temperature, 20°C, although the enzyme of run 35 was much lower than that of run 1. Copolymerization did not occur when an enzyme concentration was reduced to 2.5×10^{-8} mol/L, and the reason for this might be an inhibition of hydrogen peroxide. The first observation in our work was that the reaction is feasible and rapid in the reversed micellar system, and Rao et al.[7] also found that the polymerization of phenols in a reversed micellar system of dioctyl sodium sulfosuccinate (AOT)-water-isooctane is very rapid and feasible, and there is a significant difference between polymerization in reversed micelles and in other monophasic organic systems. The fact that enzyme molecule occupancy per micelle is seldom greater than unity indicates that the catalyst dispersion is extremely high, leading to efficient utilization. The reversed micellar system may sustain the growing chain in solution very well, and it has a significant effect on keeping the growing chain in solution and in promoting continued polymerization. In addition to providing a high degree of dispersion to the enzyme, the surfactant does appear to interact with the monomer and with polymeric species, promoting the reaction. Perhaps this is a consequence of the surfactant (CTAB)-induced positioning of the substrate (cresol) at the interface and the easier accessibility of the enzyme active site.

Table III Factor Levels

Factor	Zero Level	Interval	Five Levels				
			−1.547	1.0	0	1.0	1.547
Y1 × 10^7	5.0	2.5	1.0225	2.5	5.0	7.5	8.7525
W0	60	20	29.06	40	60	80	90.94
Y2	1.25	0.25	0.863	1.0	1.25	1.5	1.637
Y3	90.0	30.0	45.07	60.0	90.0	120.0	141.05
Y5	3.0	1.0	1.453	2.0	3.0	4.0	5.547

Molecular Weight and Distribution of Copolymer

The properties of a copolymer, such as strength, significantly depend on its molecular weight and distribution, which are very important parameters in polymer manufacturing and processing, and therefore numerous works have focused on the control of the size of the molecule and the distribution. It is desirable to produce a polymer or copolymer with the expected molecular weight and distribution.

To our best knowledge, however, no report has been published on the control of molecular weight and distribution of lignin-phenol copolymer in the reversed micellar system. In the reversed micellar system, both the molecular weight and distribution may be related to the concentration of surfactant, enzyme, cresol, and lignin, and the ratio of alcohol to hydrocarbon in the organic phase. Here, we deal with the control of molecular weight and distribution.

Design of Experimental Program

Quadratic regression orthogonal design was employed for design of the experimental program in this work, and this method may decrease considerably the quantity of experiments. It has been used extensively for program designs in many disciplines. Five factors were considered: concentration of enzyme, mol/L, represented by Y1; W0, $[H_2O]/[CTAB]$; concentration of surfactant, mol/L, Y2; concentration of *p*-cresol, mg/mL, Y3; and ratio of *n*-butanol/isooctane, Y5, which succeeded the concentration of lignin represented by Y4 because it is difficult to make up exactly a designed concentration of lignin. Hence, a quadratic regression orthogonal design with five factors was used, and when a $\frac{1}{2}$ partial implement of the design was employed, the number of experimental runs was as follows:

$$N = m_c + 2P + m_0 \tag{2}$$

where P is the number of factors employed, $P = 5$ in this work. m_c is 2^{P-1} for $\frac{1}{2}$ partial implement. m_0 is a number of repeated runs at zero level.

Therefore, the number of runs was

$$N = 2^4 + 2 \times 5 + 1 = 27 \tag{3}$$

The asterisk arm represented by γ is 1.547 for five factor quadratic regression orthogonal design. The design of factor level is listed in Table III.

Experimental Results and Discussion

Table IV gives the molecular weights and dispersities of both native straw-pulp-lignin (lignin 1) and the straw-pulp-lignin in reversed micelles (lignin 2). Lignin 1 is the native straw-pulp-lignin soluble in DMF. As shown in Table IV, the M_W of lignin 1 is 3,111, which is slightly higher than that of kraft-lignin, which is 2,900. However, this value is much higher than that of lignin 2, 1,151. Lignin 1 also has a higher dispersity, 3.641, while the dispersity of lignin 2 is only 1.30. This indicates that the solubility of lignin in DMF is better than in reversed micelles. The small M_W of lignin 2 implies that a great molecule of lignin is not soluble in reversed micelles, and the solubility of

Table IV Molecular Weight and Dispersity of Lignin

Type of Lignin	M_W (Da)	M_N (Da)	Dispersity
Lignin 1	3,111	854	3.641
Lignin 2	1,151	885	1.30

M_W is weight-average molecular weight, and M_N, number-average molecular weight.

Table V Data of Molecular Weight and Dispersity

No.	Y1 $\times 10^7$ mol/L	W0 mol/L	Y2 mg/mL	Y3 mg/mL	Y4 mg/mL	Y5 mg/mL	M_W kDa	M_W Dispersity	T °C of Peak	Modal
1	7.5	80.0	1.5	124.8	77.9	4.0	2.066	1.318	25	Single
2	7.5	80.0	1.5	62.04	82.7	2.0	242.98	46.77	25	Bimodal
3	7.5	80.0	1.0	124.8	74.5	2.0	254.13	121.6	25	Bimodal
4	7.5	80.0	1.0	62.04	46.4	4.0	2.19	1.287	25	Single
5	7.5	40.0	1.5	124.8	87.6	2.0	5.00	1.885	25	Single
6	7.5	40.0	1.5	62.04	75.0	4.0	444.56	76.32	25	Bimodal
7	7.5	40.0	1.0	124.8	105.0	4.0	1890.00	2.49	25	Single
8	7.5	40.0	1.0	62.04	135.6	2.0	892.72	3.01	25	Single
9	2.5	80.0	1.5	124.8	82.5	2.0	526.20	70.38	25	Bimodal
10	2.5	80.0	1.5	62.04	68.2	4.0	431.85	59.40	25	Bimodal
11	2.5	80.0	1.0	124.8	60.4	4.0	532.10	91.10	25	Bimodal
14	2.5	40.0	1.5	62.04	87.6	2.0	557.63	38.22	25	Bimodal
15	2.5	40.0	1.0	124.8	78.6	2.0	698.25	65.65	25	Bimodal
16	2.5	40.0	1.0	62.04	97.5	4.0	1230.87	50.50	25	Bimodal
17	8.753	60.0	1.25	93.06	50.1	3.0	160.69	71.76	25	Bimodal
18	1.003	60.0	1.25	93.06	65.3	3.0	577.69	38.16	25	Bimodal
19	5.0	90.94	1.25	93.06	88.1	3.0	5561.87	39.17	25	Bimodal
20	5.0	29.06	1.25	93.06	78.3	3.0	841.67	59.96	25	Bimodal
21	5.0	60.0	1.63	93.06	79.7	3.0	712.74	65.46	25	Bimodal
22	5.556	60.0	0.86	93.06	112.1	3.0	1113.99	4.194	25	Single
23	5.0	60.0	1.25	141.05	65.2	3.0	45.75	78.11	25	Bimodal
27	5.0	60.0	1.25	93.06	75.2	3.0	581.58	53.06	25	Bimodal
31	5.0	60.0	1.25	93.06	70.4	3.0	1026.04	71.40	45	Bimodal
32	5.0	60.0	1.25	93.06	70.4	3.0	680.31	71.40	35	Bimodal
33	5.0	60.0	1.25	93.06	70.4	3.0	326.80	71.40	20	Bimodal

lignin in reversed micelles is not good. The dispersity of lignin 2 indicates that the molecular weight distribution of lignin in reversed micelles is even.

The experimental results for the control of molecular weight and distribution are listed in Table V, and several runs lack data because several experiments failed to collect data. As seen in Table V, the M_W change from 2,066 to 1.89×10^7 DA is very large. This indicates that the molecular weight of copolymer can be controlled by simply changing the concentration of surfactant, enzyme, cresol, and lignin, and the ratio of alcohol to hydrocarbon in the organic phase. These factors can be controlled easily, thus, copolymerization of phenol with lignin appears to be feasible in the reversed micellar system. In aqueous-organic solvent mixtures, lignin-cresol copolymerization formation can be controlled by changing the nature and content of organic solvent.[6] However, the maximal M_W in that previous work was only about 10,000. This value is much lower than that in our work, and the range of M_W change is much smaller than that in our work, which implies that the capacity to control M_W by changing the nature and content of organic solvent in aqueous-organic solvent mixtures is much smaller than that in the reversed micellar system. Moreover, changing the nature and content is less convenient than changing the concentrations of enzyme, surfactant, phenol, and lignin, and the ratio of alcohol to hydrocarbon in the organic phase. The M_W obtained is 1.89×10^7 Da, which is very high compared to about 10,000 Da synthesized in aqueous-organic solvent mixtures. A copolymer of high M_W can be obtained in a reversed micellar system. This observation appears to validate the hypothesis that chain growth in reversed micelles is strongly influenced by the ability of the surfactant to sustain the growing chain in solution, and that there is a rather sharp loss of solubility leading to growth cutoff.[7] The M_W of polyethylphenol is up to 400,000 Da in a reversed micellar system.[7] The importance of the above observation from a design point of view is the fact that surfactant concentration can be used to control the copolymer molecular weight. This is a much more feasible design variable than the choice of solvent

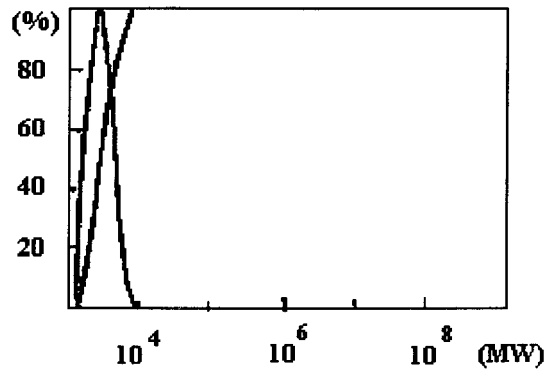

Figure 1 M_W distribution of copolymer of run 4 (differential and intergral curves of GPC). Vertical coordinate: absorbance. Abscissa: molecular weight.

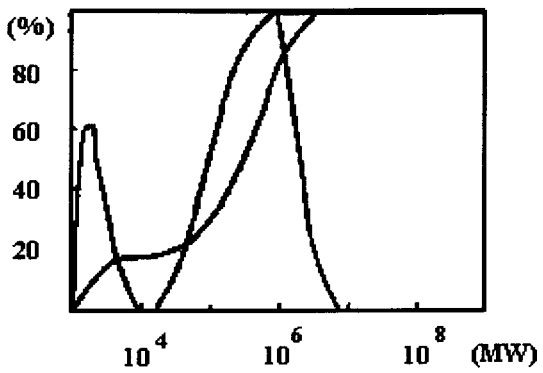

Figure 3 M_W distribution of copolymer of run 27 (differential and intergral curves of GPC). Vertical coordinate: absorbance. Abscissa: molecular weight.

in an organic solvent system; in such systems, the solvent has to be optimized not only to sustain chain growth, but also to maintain enzyme activity. Figures 1, 2, and 3 give the distributions of run 4, 7, and 27, respectively. Figure 2 illustrates the molecular weight distributions of copolymer of run 7; the M_W is 1,890 kDa, and dispersity is 2.49. This is a narrow distribution for such a high M_W. The M_W distribution of run 4 is shown in Figure 1, with an M_W of 2,190 Da and a dispersity of 1.287 the dispersity is rather narrow. The above observation indicates that a mean M_W distribution can be obtained in reversed micellar systems. On the other hand, the GPC of run 27 shown in Figure 3 has a bimodal with an M_W of 582 kDa and a dispersity of 53.06, which is very broad. The bimodal distribution indicates that there are two competitive initiating mechanisms. In an emulsion system, bimodal distribution may result from the initiation of monomer droplets and monomer-swollen micelles.[10] These two competitive initiating mechanisms may be adequate for our work, and may explain the broad and sometimes bimodal distribution obtained in the reversed micellar system. However, we cannot prove these speculations at this point. In the reversed micellar system the diameters of micelles only range from 10 to 80 nm, hence, the diameter of monomer droplets is smaller than that of micelles, and the droplet has a very large specific area. Consequently, the initiation of monomer droplets may become important. Therefore, aspects of the design of the reversed micelle, such as the diameter, have a strong influence on the mean molecular weight and distribution. The lower and middle molecular weight polymer or copolymer may be mainly the contribution of *p*-cresol homopolymers, due to the grafting of the cresol copolymer with cresol, while the high molecular weight polymer perhaps is primarily a bridge copolymer that forms between individual lignin molecules. In sum, the M_W and dispersity of copolymers can be controlled by simply changing the concentration of surfactant, enzyme, cresol, etc. In other words, copolymerization of lignin with cresol appears to be feasible in reversed micellar systems.

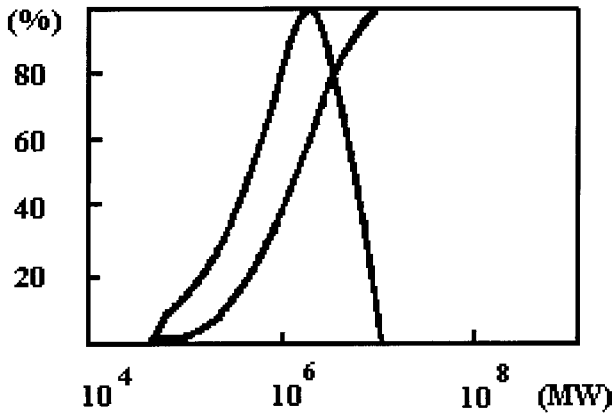

Figure 2 M_W distribution of copolymer of run 7 (differential and intergral curves of GPC). Vertical coordinate: absorbance. Abscissa: molecular weight.

Development of a Correlation of Molecular Weight

On the basis of the above discussion, a correlation of molecular weight was developed using a nonlinear optimal method. The factors considered in this work were: the concentration of enzyme, surfactant, cresol, and lignin, and the ratio of butanol to isooctane. The final form of the correlation ob-

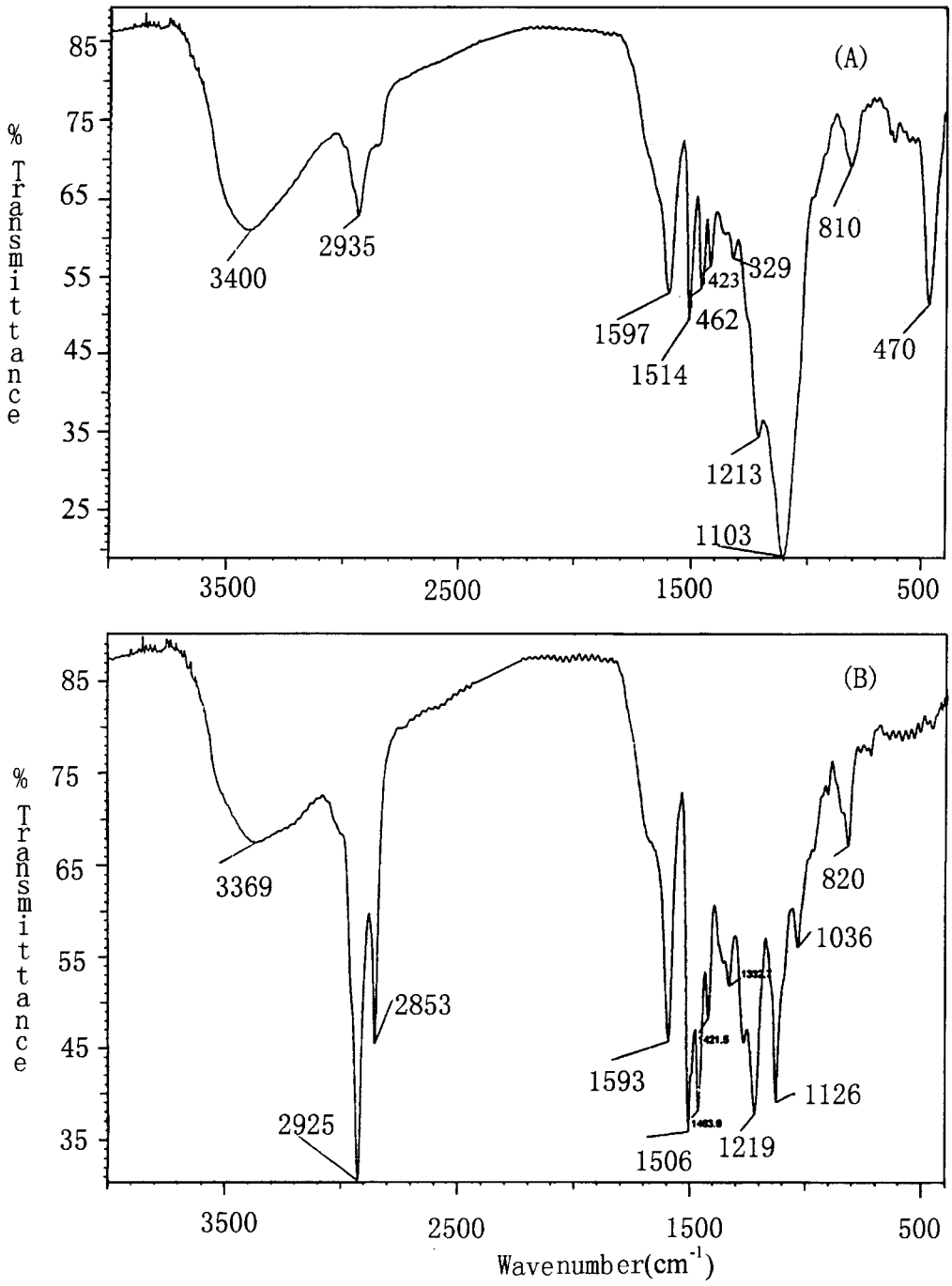

Figure 4 (A) FTIR spectrum of lignin, (B) FTIR spectrum of copolymer. Vertical coordinate: relative transmittance. Abscissa: wavenumber (cm^{-1}).

tained by employing a nonlinear optimal method is written as:

$$M_W = 6.271 \times 10^4 (\mathrm{Y1} \times 10^7)^{-0.34} \times \mathrm{Y2}^{.98} \times \mathrm{Y3}^{80} \times \mathrm{Y4}^{2.46} \times \mathrm{Y5}^{1.05} \quad (4)$$

The average deviation between the predictions of the correlation and the experiments was 9.40%, thus, the accuracy of the correlation developed was acceptable. Several run data, runs 1, 4, 5, were not included in the correlation because the molecular weight of these runs was too small. In addition, runs 31, 32, and 33 were not employed in developing the correlation because the temperature of copolymerization was not at 25°C. As shown in eq. (4), the exponent of Y1 (the concen-

tration of enzyme) is −0.34, which indicates that the molecular weight decreases with the increase of the concentration of enzyme. Phenolic units of lignin are oxidized by HRP/H_2O_2 to phenoxy radicals, which undergo subsequent radical transfer and radical coupling, and then form a bridge between individual lignin molecules. When a concentration of enzyme is low, the concentration of radical is also low. This is favorable to radical transfer and decreases the probability of a low molecular weight coupling, which results in a high molecular weight copolymer. On the contrary, the concentration of radical is high with a high concentration of enzyme. This increases the probability of coupling termination, which results in a low molecular weight copolymer.

The exponent of Y2 (the concentration of surfactant) is 0.98. Thus, the molecular weight is proportional to a concentration of enzyme, and the maximal molecular weight is up to 1,890 kDa. Such high molecular weights obtained in a reversed micellar system indicate that the addition of surfactant to isooctane-water-butanol mixtures has a significant effect on keeping the growing chain in reversed micelles and promoting continued copolymerization. A similar result was obtained in polymerization of entylphenol in reversed micelles.[7] The molecular weight increased with an increase in surfactant concentration, and the maximal molecular weight was up to 400 kDa. The concentration of surfactant affects the number and size of micelles, and accordingly, the probability of micelles colliding may be changed. Consequently, the transfer in both inter- and intramicelle may be changed. In addition, the sustenance of reversed micelles to copolymer molecules may change with the change in size of reversed micelles. As a result, the molecular weight of the copolymer may be changed. The molecular weight is proportional to the concentration, i.e., it is in inverse proportion to W0. This is consistent with a previous report,[7] but is not consistent with several reports in which the catalysis of enzymes in reversed micelles shows a bell-shaped dependency on W0.[11] Several different simplified models have recently been proposed in order to explain this phenomenon.[12] Surprisingly, although the physical bases of all these models are rather different, in each case, the authors claim that their theoretical treatment is adequate for explaining experimental results. We do not speculate on the rationale for the observed trend, and simply state that efficient operation can be achieved at some higher W0 value, e.g., 10.

The exponents of Y3 and Y4 are positive, which indicates that the size of a copolymer increases with the concentrations of cresol and lignin. In other words, the concentration of the substrate can be increased without sacrificing chain growth characteristics. This is according an implied feasibility to process high concentrations of the monomer, but the problem of removing the heat generated by the exothermic reaction should be considered when a high substrate is employed. With an exponent of 2.46, the influence of the concentration of lignin on the molecular weight of the copolymer is stronger than cresol, with a smaller exponent of 0.800. The reason for this may be that the molecular weight of lignin is higher than that of cresol, and the high molecular weight may be favorable to forming a high molecular weight copolymer.

Finally, the effect of Y5, e.g., the ratio of butanol to isooctane, on molecular weight is discussed. Butanol is a good solvent for lignin, but isooctane is not. The solubility of cresol in butanol is also better than that in isooctane. Thus, a change of the ratio of butanol to isooctane will result in a change of solubility of reversed micelles to substrates, i.e., cresol and lignin. As a consequence of the change of solubility, the copolymer molecular weight may increase with an increase of the ratio, and decrease with the decrease.

Effect of Temperature on Molecular Weight

As shown in Table V, the temperatures of copolymerization of runs 1–27 were 25°C, while runs 31, 32, and 33 were conducted at 45, 35, and 20°C, respectively. The data show that the molecular weight of copolymers increases considerably with an increase of the reaction temperature. The effect of reaction temperature on molecular weight may be complex. In the range of temperature employed, with an increase of the temperature, the activity became larger, which accelerated the copolymerization. The decrease of the viscosity of reversed micellar system leads to an increase of probability of colloid of micelles and promotes mass transfer of substrates and radicals. However, why the molecular weight is positive proportionally to the reaction is unclear. We simply state that a higher reaction temperature is favorable for a high molecular weight.

Infrared Spectroscopy

Figure 4 shows the FTIR spectra of the free (or native) lignin and lignin-*p*-cresol resin. From the

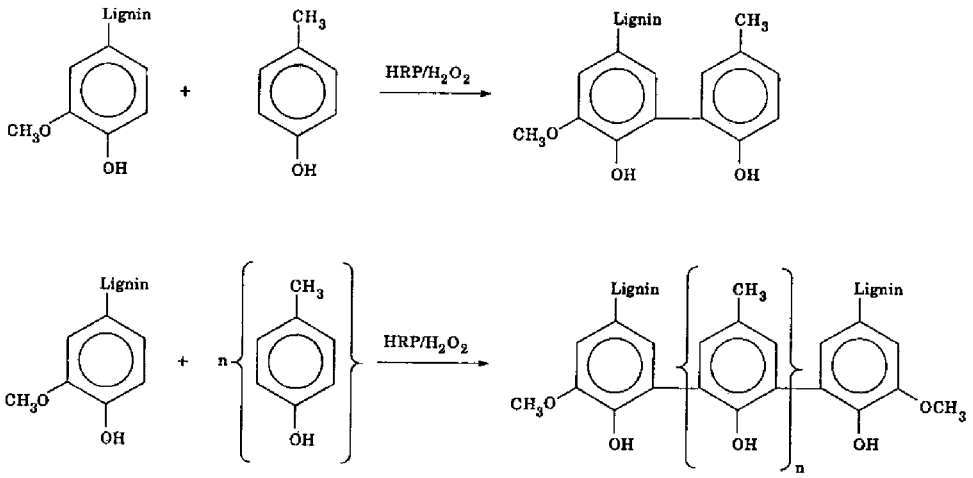

Figure 5 Reaction for HRP-catalyzed copolymerization of lignin with *p*-cresol.

two parts of the figure, strong differences were observed in the 470 to 3300 cm^{-1} range. For example, there is a strong absorbance peak at 2850 cm^{-1} in part (B) of Figure 4, but no peak in (A). The peak at 2850 cm^{-1} is a benzene ring CH_3 asymmetric stretch; the peak at 2925 cm^{-1} in (B) is bigger than that in (A). The peak is a benzene ring CH_3 symmetric stretch. Finally, in (B), the sample exhibits significant peak differences at 1593, 1506, 1463, 1218, 1126, 1101, 1035, and 470 cm^{-1}. The peak of 3400 cm^{-1} shifts to lower wavenumber, it may be a distribution of the hydrogen bond between polycresol. Hence, significant differences between the lignin-cresol copolymer and free lignin are most likely due to the distributions of incorporation of cresol into lignin and/or polycresol segments in the copolymer. Blinkovsky et al.[5] observed that the FTIR spectrum of lignin-cresol is different from that of polycresol obtained in aqueous-organic solvent mixtures, but not from polycresol obtained in reversed micelles.

Figure 5 shows a mechanism of copolymerization of lignin with cresol.[6] As seen in the figure, *p*-cresol and units of lignin are oxidized by HRP/H_2O_2 to phenoxy radicals, which undergo subsequent radical coupling and postulated radical transfer. However, cresol is a better substrate for HRP than is lignin, because of its size and phenolic hydroxyl accessibility, and because it is more readily oxidized. Thus, it is possible that phenoxy radicals of cresol also oxidize lignin subunits via a radical transfer reaction. The grafting of *p*-cresol onto lignin is not complete.

Another possible structure of the copolymers is phenol/polyphenol "bridges" that form between individual lignin molecules.[5] The bridges would give more degrees of rotational freedom to the copolymer as compared to native lignin or lignin-lignin polymer. Further reaction between different phenolic groups in the lignin and phenol results in additional bridges and a crosslinked lignin polymer. It is probable that both intra- and interlignin bridges are formed. Either would be expected to modify lignin sufficiently to alter its solubility and thermal properties.

CONCLUSIONS

The results of peroxidase-catalyzed copolymerization of straw-pulp-lignin with cresol in reversed micellar systems indicate the following conclusions. The surfactant sustains remarkable chain solubility and promotes copolymerization; the maximal molecular weight is up to 1,890 kDa. The reaction is very rapid. The copolymerization is attractive because it is possible to use the surfactant, enzyme, cresol, and lignin concentration, and the ratio of butanol to isooctane as design variables in manipulating the mean molecular weight of the copolymer. A correlation was developed for mean molecular weight, which considers five factors. The copolymer has some vastly different properties than native lignin, which include a lower glass transition temperature and higher curing exotherm. Potential applications of these materials include adhesives, bonding agents, laminates, and polymeric dispersants. The potential as thermosetting resins may also be examined in future works. Bimodal distribution appeared in many runs, which implies that there are two competitive initiating mechanisms, but

the exact mechanism is unclear. The mean molecular weight increases with the increase of copolymerization temperature, and a higher temperature is favorable for a higher molecular weight.

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