

## Molecular Weight Control of Polyphenols by Enzymatic Copolymerization of Phenols

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(Received December 8, 2000; Accepted January 19, 2001)

KEY WORDS Enzymatic Polymerization / Phenol / Peroxidase / Molecular Weight Control / Oxidative Polymerization /

In the last decade, enzymatic syntheses of polyaromatics have been extensively investigated.<sup>1–9</sup> Oxidative polymerization of various phenol derivatives catalyzed by peroxidase produced a new class of polyphenols showing high thermal stability.<sup>10–19</sup> The enzymatic process is expected to be an alternative way for preparation of phenolic polymers without use of toxic formaldehyde, which is a monomer for production of conventional phenolic resins (phenol-formaldehyde resins). We have also found that iron-*N,N'*-ethylenebis(salicylideneamine) complex (Fe-salen complex), a model complex of peroxidase, efficiently catalyzed an oxidative polymerization of phenol derivatives.<sup>20,21</sup>

We have employed phenol, the simplest and most important phenolic compound in industry, as monomer for the enzymatic oxidative polymerization. The resulting polymer was a powdery material, consisting of a mixture of phenylene and oxyphenylene units (Scheme 1).<sup>12,14</sup> In using aqueous 1,4-dioxane as solvent, the polymer was produced in a good yield, however, the solubility of the polyphenol was low; it was partly soluble in *N,N*-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO). Very recently, it was found that the polymer structure and solubility were much influenced by the solvent composition.<sup>22,23</sup> In the polymerization in a mixture of methanol and buffer, the resulting polyphenol was completely soluble in DMF and DMSO, but insoluble in other organic solvents. Since polymer solubility is crucial for industrial applications, the solubility improvement is strongly desired. This study deals with the molecular weight control of the polyphenols by the enzymatic copolymerization of phenol with substituted phe-

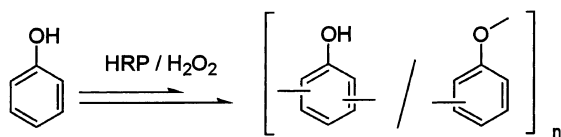
nols, leading to the solubility improvement of the enzymatically obtained polyphenols.

### RESULTS AND DISCUSSION

The copolymerization of phenol with a comonomer was performed by using horseradish peroxidase (HRP) and hydrogen peroxide as catalyst and oxidizing agent, respectively, in a mixture of methanol and phosphate buffer (pH 7) at room temperature under air. Hydrogen peroxide (30%) was added dropwise to the reaction mixture for 3 h. By the addition of hydrogen peroxide, the reaction mixture turned dark-brown, afterwards the powdery precipitates were formed. After 1 h of the addition of hydrogen peroxide, the polymer was isolated by filtration.

First, we examined the copolymerization with mono-, di-, and tri-substituted phenols: 4-ethylphenol, six dimethylphenols, and 2,4,6-trimethylphenol. In all cases, the comonomer was quantitatively consumed. Except the copolymerization with 2,4,6-trimethylphenol, more than 90% conversion of phenol was found. Copolymerization results are summarized in Table I. The copolymerization with 2,4-dimethylphenol produced the polyphenol with lower molecular weight than that by the homopolymerization of phenol under the similar reaction conditions (entries 1 and 4). The decrease of the molecular weight was observed in using 2,4,6-trimethylphenol (entry 9), however, the polymer yield also decreased. The low yield was due to the low conversion of phenol (67%). In the copolymerization with other substituted phenols, the molecular weight slightly decreased or did not change. From these data, 2,4-dimethylphenol was found to be the most suitable as comonomer to produce the polyphenol with lower molecular weight.

Liquid chromatography-mass (LC-MS) and matrix-assisted laser desorption/ionization-time of flight mass (MALDI-TOF MS) spectroscopic analysis of the copolymer from phenol and 2,4-dimethylphenol showed the formation of the polymeric product consisting of both monomers; peaks due to the copolymers were observed in MS spectra. The copolymer was readily soluble in



Scheme 1.

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**Table I.** HRP-Catalyzed Oxidative Copolymerization of Phenol and Substituted Phenols<sup>a</sup>

Entry	Comonomer	Yield /%	$M_n^b$	$M_w/M_n^b$
1	—	85	2200	3.0
2	4-Ethylphenol	88	1900	3.4
3	2,3-Dimethylphenol	88	2200	3.5
4	2,4-Dimethylphenol	94	1100	5.2
5	2,5-Dimethylphenol	85	2100	3.3
6	2,6-Dimethylphenol	79	1900	3.5
7	3,4-Dimethylphenol	91	1700	3.4
8	3,5-Dimethylphenol	91	2200	3.0
9	2,4,6-Trimethylphenol	65	1300	3.1

<sup>a</sup> Copolymerization of monomers (10.6 mmol, 10 mol% of comonomer) using HRP catalyst in an equivolume mixture of methanol and pH 7 phosphate buffer (20 mL) at room temperature under air.

<sup>b</sup> Determined by SEC using DMF as eluent with polystyrene standards.

**Table II.** HRP-Catalyzed Oxidative Copolymerization of Phenol and 2,4-Disubstituted Phenols<sup>a</sup>

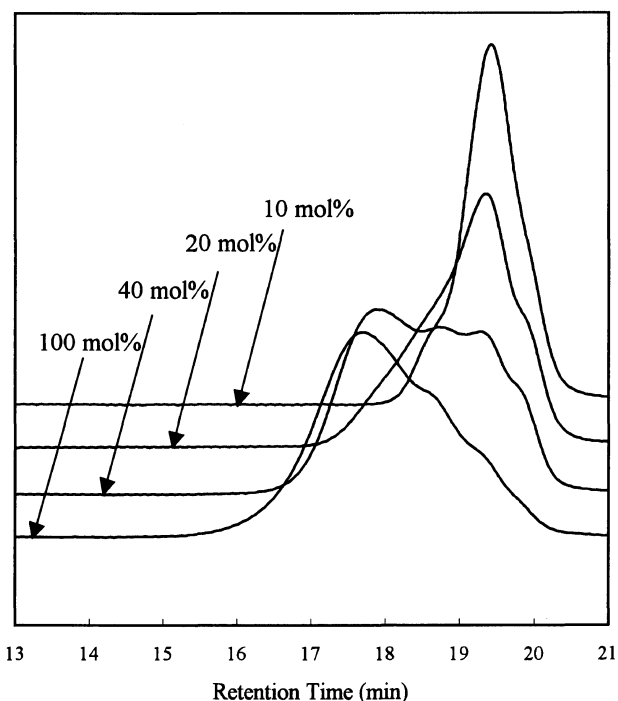
Entry	Comonomer <sup>b</sup>	Yield /%	$M_n^c$	$M_w/M_n^c$
1	—	85	2200	3.0
2	2,4-Dimethylphenol (5)	84	1800	3.6
3	2,4-Dimethylphenol (10)	94	1100	5.2
4	2,4-Dimethylphenol (20)	82	900	
5	2,4-Dimethylphenol (20) <sup>d</sup>	92	760	
6	2,4-Dimethylphenol (30)	88	370	
7	2,4-Dimethylphenol (40)	81	340	
8	2,4-Dimethylphenol (50)	92	190	
9	2,4-Dimethylphenol (100)	85	200	
10	2,4-Di- <i>tert</i> -butylphenol (10)	85	1600	3.9
11	2,4-Di- <i>tert</i> -butylphenol (20)	96	1100	3.9

<sup>a</sup> Copolymerization of monomers (10.6 mmol) using HRP catalyst in an equivolume mixture of methanol and pH 7 phosphate buffer (20 mL) at room temperature under air. <sup>b</sup> In parentheses: comonomer feed ratio for monomers (mol%). <sup>c</sup> Determined by SEC using DMF as eluent with polystyrene standards. <sup>d</sup> Comonomer was added dropwise.

acetone, ethyl acetate, pyridine, and 1,3-dimethyl-2-imidazolidinone as well as DMF and DMSO, supporting the copolymer formation from phenol and 2,4-dimethylphenol. <sup>1</sup>H NMR and IR analyses showed that the polymer structure was of a mixture of phenylene and oxyphenylene units.<sup>14</sup>

Table II shows the copolymerization results using 2,4-disubstituted phenols as comonomer. As the feed ratio of 2,4-dimethylphenol increased, the molecular weight decreased (entries 2–4 and 6–8), indicating the strong dependence of the molecular weight on the feed ratio of 2,4-dimethylphenol. In most cases, the formation of the copolymer with broad molecular weight distribution was observed. LC-MS analysis showed that the dimer was mainly obtained in the homopolymerization of 2,4-dimethylphenol. This may be related to the formation of the low molecular weight polyphenol in the copolymerization with 2,4-dimethylphenol. In using 2,4-di-*tert*-butylphenol as comonomer, the decrease of the molecular weight was also observed (entries 10 and 11).

The copolymerization of phenol with 2,4-dimethylphenol (feed ratio of 80:20 mol%) was monitored by using size exclusion chromatography (SEC) (Figure 1). At the initial stage of the copolymerization, the product of the low molecular weight was formed. A similar SEC trace was observed in the homopolymeriza-



**Figure 1.** SEC traces of the product obtained by the copolymerization of phenol and 2,4-dimethylphenol (80:20 mol%) with different amount of hydrogen peroxide.

tion of 2,4-dimethylphenol, suggesting that the product is the oligomer mainly derived from 2,4-dimethylphenol. This may be because the oxidative reactivity of 2,4-dimethylphenol is larger than that of phenol, and hence, 2,4-dimethylphenol was mainly reacted at the initial reaction stage. As a function of the added amount of hydrogen peroxide, the molecular weight increased and the peak intensity of the oligomer became smaller. The final product still contained a small amount of oligomers, resulting in the wide molecular weight distribution.

In order to inhibit the homopolymerization of 2,4-dimethylphenol at the initial stage, it was added dropwise for 1.5 h (entry 5). The molecular weight distribution became narrower and the polymer yield improved in comparison with the copolymerization in the all loading of the monomers (entry 4).

## CONCLUSIONS

The peroxidase-catalyzed copolymerization of phenol with 2,4-disubstituted phenols produced the polyphenols with low molecular weight, which were readily soluble in polar organic solvents. The molecular weight could be controlled by changing the feed ratio of the comonomer. The resulting polyphenols showing high solubility are highly expected to possess potential applications in industrial fields.

## EXPERIMENTAL

HRP was obtained from Toyobo Co. and used without further purification. Other reagents and solvents were commercially available and were used as received.

A typical run was as follows (entry 4 in Table II). Phenol (0.80 g, 8.6 mmol), 2,4-dimethylphenol (0.26 g, 2.1

mmol) and HRP (24 mg, 240 unit) in an equivolume mixture of methanol and 0.05 M phosphate buffer (pH 7) (20 mL) were placed in a 50 mL of flask. To the mixture, 1.5 mL of 30% hydrogen peroxide (14.7 mmol) was added dropwise for 3 h. The mixture was stirred at room temperature under air. After 1 h, the precipitated materials were collected by filtration and washed with water, followed by drying *in vacuo* to give 0.88 g of the polymer (yield 82%).  $^1\text{H NMR}$  ( $\text{DMSO-}d_6$ ):  $\delta$  1.9–2.4 (br,  $\text{CH}_3$ ), 6.2–7.7 (br, Ar); IR (KBr) 3400 ( $\nu$  O–H), 1588, 1489 ( $\nu$  C=C of Ar), 1208 ( $\nu$  C(Ar)–O–C(Ar) and C(Ar)–OH), 1102  $\text{cm}^{-1}$  ( $\nu$  C(Ar)–O–C(Ar)).

For SEC and HPLC measurement, a Tosoh SC8020 apparatus was used. SEC analysis was carried out by using a refractive index (RI) detector at 60°C under the following conditions: two TSKgel  $\alpha$ -M columns and DMF containing 0.09 M LiCl eluent at a flow rate of 1.0  $\text{mL min}^{-1}$ . The calibration curves were obtained using polystyrene standards. HPLC analysis was performed using a UV monitor (278 nm) at 40°C under the following conditions: two YMC-Pack ODS AM-312 columns and methanol/17 mM phosphoric acid eluent at a flow rate of 1.8  $\text{mL min}^{-1}$ . LC-MS measurement was carried out on a Waters 2690 chromatography equipped with a Waters 996 photodiode array detector (200–400 nm) and a micromass ZMD spectrometer under the following conditions: two YMC-Pack ODS AM-312 columns and methanol/water eluent. The mass spectrum was recorded by scanning in the mass range from  $m/z$  100 to 2000 with an electrospray ionization interface in the negative mode at a cone voltage of 60 V. MALDI-TOF MS measurement was carried out using a JEOL JMS-Elite mass spectrometer. Molecular weights were recorded using dithranol as matrix in a linear mode. Mass spectra were calibrated with substance P before measurement.  $^1\text{H NMR}$  spectra were recorded on a Jeol JNM-LA 600 spectrometer. FT-IR measurements were carried out with a Perkin-Elmer Paragon 1000 spectrometer.

*Acknowledgments.* This work was supported by NEDO for the project on Technology for Novel High-Functional Materials in Industrial Science and Technology Frontier Program, AIST.

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