Enzymatic synthesis of soluble phenol polymer in water using anionic surfactant as additive

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Abstract:

In phosphate–citric acid buffer (pH = 8.0) containing sodium dodecylbenzenesulfonate (SDBS) as a surfactant, enzymatic polymerization of phenol catalyzed by horseradish peroxidase (HRP) is efficiently performed. The as-synthesized phenol polymer is completely soluble in organic solvents such as dimethylformamide, dimethylsulfoxide, acetone and tetrahydrofuran. The aqueous micelle system provides a new environmentally friendly method for HRP-catalyzed phenol polymerization. The effects of the dosage of SDBS and reaction temperature on the yield of the target phenol polymer are investigated. The soluble phenol polymer is composed of phenylene and oxyphenylene units as determined using infrared and 1 H NMR spectral analyses. The number-average molecular weight calculated from gel permeation chromatography (GPC) is in the range 900–1500 g mol−1 and the dispersity in the range 3.4–6.3. In all cases, the values of weight-average molecular weight characterized using GPC–static light scattering are tens of thousands. It is concluded that a branched structure is produced in the aqueous micelle system. The phenol polymer prepared possesses good thermal stability for it is completely decomposed at a temperature as high as 628 °C in air.

INTRODUCTION

Polymerization catalyzed by an enzyme provides a new methodology for polymer synthesis.1–3 Enzymatic polymerizations are expected to be useful for the preparation of new polymeric materials which are difficult to obtain by conventional methods. In the past few decades, enzymatic synthesis of phenolic polymers has received much attention as an alternative process for preparing phenolic resins.4–6 It is well known that phenol–formaldehyde resin has excellent properties and is widely used in numerous areas. However, the toxic nature of formaldehyde and the extreme reaction conditions have limited its production and applications. The alternative route of enzymatic synthesis of phenolic resin has advantages such as mild reaction conditions and without the use of toxic formaldehyde. So far, many new useful phenolic polymers have been prepared by enzymatic catalysis, most of which cannot be obtained by conventional chemical methods.7–19

For phenol, the simplest and most important phenolic compound in industrial fields, its enzymatic polymerization in aqueous media without additives forms only dimers and trimers with extremely low yields. Since horseradish peroxidase (HRP) was reported to maintain its activity even in a mixture of buffer and water-miscible organic solvent.7,20 HRP-catalyzed phenol polymerizations have been extensively studied in such media.20–23 The polymer was produced in good yield in aqueous 1,4-dioxane; however, the resulting polymer was only partly soluble in N,N-dimethylformamide (DMF) and dimethylsulfoxide (DMSO).20,21 As polymer solubility is crucial for industrial applications, phenol polymers with good solubility are much desired. Kobayashi and co-workers found that soluble phenol polymer could be obtained on condition that the polymerization was carried out in a mixture of methanol and buffer.22,23 Nevertheless, the use of organic solvents is not preferred for establishing environmentally friendly processes for producing polymers. Poly(ethylene glycol) (PEG) and an amphiphilic triblock copolymer (PEG–poly(propylene glycol)–PEG) were adopted as additives to facilitate the enzymatic polymerization of phenol in water.24,25 In the research reported in the present paper, we selected HRP as an enzyme catalyst to conduct enzymatic oxidative polymerization of phenol in an aqueous micelle system. It is first reported that a soluble phenol polymer in moderate yield can be easily obtained in phosphate–citric acid buffer (pH = 8.0) containing sodium dodecylbenzenesulfonate (SDBS). The phenol polymer was obtained with a short reaction time using a simple method in this novel system and shows high solubility in common organic solvents. The effects of the dosage of SDBS and reaction temperature on the yield of the target phenol polymer were investigated. The structure and average molecular weight of the target polymer were also characterized systematically.

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**EXPERIMENTAL**

**Materials**

HRP (RZ = 2.5, activity = 200 U mg\(^{-1}\)) was purchased from Shanghai Guoyuan Biotechnology Co. Ltd and used without further purification. All other chemicals employed were obtained from various commercial suppliers and were used as received.

**Enzymatic polymerization of phenol in aqueous micelle system**

An appropriate amount of SDBS and 5 mmol of phenol were dissolved in 45 mL of phosphate–citric acid buffer (pH = 8), followed by addition of an enzyme solution of 0.5 mg of HRP in 5 mL of phosphate–citric acid buffer. To the resultant mixed solution, hydrogen peroxide (5% aqueous solution, 0.25 mL) was added every 5 min for a total of 14 times under mild magnetic stirring. The resultant black mixture was stirred for an additional 0.5 h, followed by vacuum-filtering and thoroughly washing with water to remove HRP, SDBS, residual hydrogen peroxide and phenol. The as-obtained black substance was dried in vacuum at 50 °C to give the desired phenol polymer.

**Titration of hydroxyl groups in phenol polymer**

The ratio of phenylene to oxyphenylene units (Ph/Ox) in the as-prepared phenol polymer was mainly determined using titration. Briefly, 0.10 g of as-prepared phenol polymer was dissolved in 5.0 mL of pyridine containing 2.5% acetic anhydride. The resultant solution was kept at 95 °C for 1 h under gentle magnetic stirring. After 10.0 mL of water was added, the mixed solution was titrated with 0.20 mol L\(^{-1}\) sodium hydroxide solution in the presence of phenolphthalein as an indicator.26

**Polymer characterization**

Fourier transform infrared (FTIR) spectra were obtained with an Avatar 360 FTIR spectrometer ( Nicolet, USA). \(^1\)H NMR spectra were recorded using a Bruker AV400 NMR spectrometer with tetramethylsilane as an internal standard using DMSO-\(d_6\) as solvent. TGA in nitrogen or air atmosphere was performed with a TGA/SDTA851e instrument (Mettler-Toledo, Switzerland) at a heating rate of 10 °C min\(^{-1}\). DSC was carried out with a DSC822e instrument (Mettler-Toledo, Switzerland) at a heating rate of 10 °C min\(^{-1}\) in nitrogen atmosphere. Gel permeation chromatography (GPC) was carried out (eluent: tetrahydrofuran (THF); flow rate: 1.0 mL min\(^{-1}\)) using a Waters 515 liquid chromatograph equipped with a refractive index detector.

**RESULTS AND DISCUSSION**

Table 1 summarizes the effects of SDBS dosage and reaction temperature on HRP-catalyzed phenol polymerization with respect to the yield and the molecular weight of the obtained polymer. It can be seen that the polymerization of phenol in pure buffer without SDBS proceeds inefficiently (entry 1 in Table 1) and the final yield is less than 5.0%. A similar result was also obtained by other groups.\(^7,\)\(^21,\)\(^27\) The encouraging thing is that the introduction of 0.3 g of SDBS into the buffer favors the polymerization of phenol and provides a yield of 54.5% (entry 2 in Table 1). The results indicate that the aqueous micelles play an important role in HRP-catalyzed phenol polymerization. The average size of the micelles in buffer containing phenol monomer before the polymerization determined using the laser particle size analyzer is 85 nm. After polymerization, the polymer particles exhibit an average size of 240 nm. The size distribution patterns are shown in Figs 1 and 2, respectively.

The low yield of enzymatic phenol polymerization in pure buffer is attributed to the insolubility of relevant oligomers in the buffer solution, thereby preventing the formation of a higher molecular weight polymer.\(^7,\)\(^21\) The solubility of a sparingly soluble substance in water increases when the concentration of surfactant solution is above the critical micelle concentration, which should be the reason for the enhanced yield of phenol polymer in the aqueous micelle system. In order to establish the optimal condition for HRP-catalyzed phenol polymerization in the aqueous micelle system, we further examined the polymerization with various dosages of SDBS. The results are summarized in Table 1. It is seen that the yield of the target polymer is highly dependent on the dosage of SDBS. Namely, the polymer yield increases when the dosage of SDBS in the buffer rises from 0.3 to 0.5 g (entries 2–4 in Table 1). The effect of reaction temperature on HRP-catalyzed phenol polymerization was also investigated. It is evident from Table 1 that, with the same dosage of SDBS, the yield of phenol polymer increases when the temperature rises from 0.3 to 0.5 g (entries 2–4 in Table 1).
reaction temperature rises from 20 to 40 °C (entries 3, 5 and 6 in Table 1). HRP maintains its activity in the aqueous micelle system even at 50 °C and gives a moderate polymer yield of 64.6%.

In all cases except entry 1 in Table 1, the resultant polymer is a black powdery material. In particular, the powdery material is completely soluble in conventional organic solvents, such as DMF, DMSO, acetone and THF. As far as we know, this is the first report concerning the synthesis of a soluble phenol polymer in an aqueous micelle system with moderate yield. Since no organic solvent is used, the aqueous micelle system used in the present work should be considered as an environmentally friendly system for producing soluble phenol polymers.

The good solubility of the resultant phenol polymer stimulated us to investigate the structure and molecular weight of the powdery material. Figure 3(a) shows the FTIR spectrum of the as-synthesized phenol polymer. The broad peak centered at 3400 cm⁻¹ is ascribed to the vibration of phenolic O–H bond. The peaks at 3058, 1585, 1489, 831, 754 and 692 cm⁻¹ are assigned to various vibration modes of C–H and C–C bonds of aromatic nuclei/rings. The peak at 1101 cm⁻¹ corresponds to the symmetric vibration of the ether bond. The strong peak at 1209 cm⁻¹ is due to the asymmetric stretching vibration of C–O–C and/or C–OH. These FTIR spectral data are very similar to those of phenol polymers obtained in aqueous organic solvent. 20,21 This means that the phenol polymer obtained in the aqueous micelle system is also composed of phenylene and oxyphenylene units. The ¹H NMR spectrum (Fig. 4(a)) of the phenol polymer shows two broad peaks: a multiplet around 6.0–8.1 ppm corresponding to aromatic protons, and a broad peak at 9.2–9.8 ppm assigned to the hydroxyl proton of the phenol group. The Ph/Ox ratio was determined using conventional titration methods. 26 Figure 3(b) shows the FTIR spectrum of the acetylated polymer. A characteristic broad peak at 3400 cm⁻¹ due to phenolic O–H bond has completely disappeared, indicating that the acetylation proceeds quantitatively. The ¹H NMR spectrum of the acetylated phenol polymer is shown in Fig. 4(b). After acetylation, the peak at 9.2–9.8 ppm assigned to the hydroxyl proton of the phenol group has disappeared. Meanwhile, new multiplet peaks around 1.4–2.4 ppm corresponding to protons of CH₃ are observed. The unit molar Ph/Ox ratios determined using titration are listed in Table 1.

The average molecular weight of the as-synthesized phenol polymer was first determined using GPC (Table 1). The elution traces are shown in Figs 5 and 6. The average molecular weight increases when the dosage of SDBS rises from 0.3 to 0.5 g. In addition, increasing the reaction temperature also results in an increase in the average molecular weight. As we know, increasing the concentration and temperature of an anion surfactant solution can lead to the enhanced capacity of the solubilization of the micelle system. As a result, the solubility of phenol oligomers is improved and a polymer with high molecular weight can be obtained finally. In all cases, the number-average molecular weight is in the range 900–1500 g mol⁻¹ (entries 2–7 in Table 1), which is less than that in a mixture of organic solvents and buffer. 20–23 It is also seen that the phenol polymers obtained in the aqueous micelle system have a broad molecular weight distribution, the
mass spectrum reported in another paper, 28 the spectrum of the units of the phenol polymer. Unlike the symmetric MALDI-TOF about 92 and exactly matches the molar mass of the repeat up to 4000 g mol−1 of phenol polymer from oligomers to the high molecular weight (entry 3 in Table 1). This spectrum gives the whole distribution MALDI-TOF mass spectrum of the as-synthesized phenol polymer. It is evident from Table 1 that the weight-average molecular weight is scores of time more than that obtained using GPC analysis alone. These properties are typical of hyperbranched polymers. It is well known that the first step of HRP-catalyzed oxidation of phenol is the formation of phenolic radicals. These free radicals then undergo coupling to produce dimers. When the concentration of free phenolic radicals is decreasing, electron transfer is more likely than further recombination. This leads to the formation of oligomer radicals which then form oligomers with high molecular weight by recombination. The radical transfer reaction of a phenolic monomer radical with an oligomer regenerates a phenol monomer which can be oxidized by HRP to initiate another new radical transfer reaction. The successive oxidation and coupling and transfer reactions eventually result in the formation of polymer. The chain propagation of enzymatic phenol polymerization has been described by Ritter. 6 Because of the resonance of the phenolic radical, there are four binding sites in a single phenol monomer. It is likely to form branched or crosslinked polymers due to the multiple binding sites occurring in phenol monomer and oligomers. The low solubility of phenol polymers described in the literature probably results from the formation of crosslinked structures. In our present work, HRP-catalyzed phenol polymerization in the aqueous micelle system (buffer pH = 8) may exactly lead to the formation of branched structures, and therefore leading to good solubility and broad distribution of molecular weight. The mechanism of the formation of a branched structure is described in Scheme 1.

To improve the solubility, Cui et al. 30 incorporated poly(ethylene oxide) into phenol polymer chains using living anionic polymerization to produce a soluble polyphenol-graft-poly(ethylene oxide) copolymer. This strategy not only involves complicated procedures but also affects the thermal properties of the final phenol polymer. In our studies, the thermal properties of the phenol polymer obtained using the aqueous micelle system (entry 3 in Table 1) were evaluated using TGA and DSC (Figs 8 and 9). The temperature at 10% weight loss of the polymer in nitrogen is 380 °C, which is higher than that of soluble phenol polymer 345 °C obtained in aqueous methanol.22 The temperature at 10% weight loss in air is as high as 376 °C. These data indicate that the phenol polymer prepared in the present work possesses good thermal stability. Moreover, the as-synthesized phenol polymer is completely decomposed at 628 °C in air, which is also much higher than that obtained in aqueous organic solvent.21 It retains a residue mass fraction of 42% after being calcined at 1000 °C in nitrogen atmosphere. The high residue content at 1000 °C of the as-synthesized phenol polymer in nitrogen atmosphere is significant for its potential applications, since phenol polymers are supposed to be precursors for carbonized products like polycenes. DSC measurements show that the as-synthesized phenol polymer has
a) Formation of phenolic radicals

\[
\text{PhOH} + \text{H}_{2}\text{O}_{2} \xrightarrow{\text{HRP}} \text{PhOOH} + \text{H}_{2}\text{O} + \text{OH}^-
\]

b) formation of the dimer

\[
2 \text{PhOOH} \rightarrow \text{PhOO} - \text{PhOH} + \text{PhOOH}^-\text{PhOH}^-\text{PhOH}^+
\]

c) radical transfer reaction (formation of oligomer radical)

\[
\text{PhOH} - \text{PhO}^- + \text{PhOH}^+ \rightarrow \text{PhO} - \text{PhO}^-\text{PhOH}^-\text{PhOH}^+
\]

d) combination of oligomer radical

\[
\text{oligomer radical} + \text{oligomer radical} \rightarrow \text{branched phenol polymer}
\]

e) radical transfer reaction and formation of the branched structure

\[
\text{PhOH} \rightarrow \text{PhO}^-\text{PhOH}^-\text{PhOH}^+\rightarrow \text{branched phenol polymer}
\]

**Scheme 1.** Mechanism of the formation of branched phenol polymer.

control the average molecular weight of the phenol polymer. The phenol polymer obtained in the aqueous micelle system (buffer pH = 8) has a branched structure, resulting in good solubility. HRP-catalyzed phenol polymerization in the aqueous micelle system provides a new environmentally friendly method for producing soluble phenol polymers and will greatly promote the application of phenol polymers obtained by enzymatic catalysis.

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**REFERENCES**


**CONCLUSIONS**

A soluble phenol polymer in moderate yield was synthesized using HRP-catalyzed polymerization of phenol in an aqueous micelle system. The addition of SDBS to the buffer facilitates greatly the enzymatic polymerization of phenol. Also, changing the dosage of SDBS and reaction temperature can be used to control the glass transition temperature of 87 °C in nitrogen atmosphere as measured in the second scan.

**Figure 8.** TGA curves of as-synthesized phenol polymer in (a) nitrogen and (b) air atmosphere.

**Figure 9.** DSC curves of as-synthesized phenol polymer: (a) first scan; (b) second scan.