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Effect of Tetrabutylammonium Bromide on Enzymatic Polymerization of Phenol Catalyzed by Horseradish Peroxidase*

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Abstract In this article tetrabutylammonium bromide (TBAB) was first added in buffer to compose a convenient and environmentally friendly system, and enzymatic polymerization of phenol catalyzed by horseradish peroxidase (HRP) could proceed efficiently in this system. When TBAB was added, the most conversion of phenol could reach 99.1%. The phenol polymer was considered to consist of a mixture of phenylene (Ph) and oxyphenylene (Ox) units by IR analysis, and the ratio of phenylene to oxyphenylene units (Ph/Ox) was measured by titration. Moreover, the effects of the dosage of horseradish peroxidase (HRP) and pH value on the conversion of phenol were investigated. The reaction performed very effectively in this novel system when the addition of HRP was only 0.2 mg. In all cases, the weight-average molecular weight calculated by GPC-SLS was in a range from 12000 Da to 30000 Da. The phenol polymer prepared in the present research possessed good thermal stability shown by TG analysis.

Keywords: Horseradish peroxidase; Phenol; Polymerization; Tetrabutylammonium bromide.

INTRODUCTION

As a kind of highly effective catalyst, enzyme is often feasible for enzymatic reactions to take place in mild conditions such as room temperature and atmospheric pressure, and this is why enzymatic polymerization is highly significant in preparing novel polymeric materials which are difficult to obtain by conventional methods[1–7]. In the recent decades, enzymatic synthesis of phenol polymers has received much attention as an alternative process for preparing phenolic resins.

Among various polymers, phenol-formaldehyde resin plays an important role in our daily life and is widely used in manufacturing varnish, corrosion resistant coating, adhesive, sound insulation material and insulation material. Phenol-formaldehyde is obtained by the polymerization of phenol and formaldehyde. The application of this route, however, is limited, due to the toxicity of formaldehyde. Therefore, it is imperative to pursue new methods for fabricating phenolic resin. In this sense, the enzymatic polymerization of phenol and its derivatives is attractive and competitive owing to its benign reaction conditions[1, 3, 5, 8]. For example, various enzymes such as HRP and soybean peroxidase (SBP) can be adopted as catalysts to synthesize polymers possessing high thermal stability which is particularly interesting in engineering[5–12]. Moreover, the basic structure of phenol polymer and its derivatives comprises hydroxyl groups that are capable of adsorbing metal ions, so the polymers as adsorbents may find promising applications in sewage treatments[13, 14].

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Unfortunately, the enzymatic polymerization of phenol still faces some problems. Namely, the poor solubility of dimer and trimer in water system limits their further reaction[8, 15]. The mixture of buffer and water-miscible organic solvent has been used to increase the solubility of dimer and trimer, however that will cause environmental problems and reduce the activity of enzyme catalysts[8, 16–19]. Therefore, it is imperative to search new reaction systems which are applicable to the enzymatic polymerization of phenol so as to improve the solubility of dimer, trimer and yield while the activity of the enzyme catalysts remains unaltered. In this respect, cyclodextrin and polyethylene glycol (PEG) have been tried as templates in the aqueous system to promote the enzymatic polymerization of phenol without the organic solvent , but the product is found to be a complex of phenol polymer and the additives[8, 20–22]. Similarly, it has been found that sodium dodecyl benzene sulfonate (SDBS) and sodiumdodecyl sulfate (SDS) are able to form an aqueous micelle system thereby increasing the conversion of phenol and giving rise to polymerized phenol under the catalysis by horseradish peroxidase (HRP)[15, 23–25]. Enzymatic phenol polymerization performed in ionic liquid has also been investigated recently[26, 27].

In this paper, tetrabutylammonium bromide (TBAB) was selected to solve these problems. The phenol polymer was obtained with short reaction time by a simple method in this novel system. The effects of the dosage of TBAB and HRP on the conversion of phenol were investigated. The structure and weight-average molecular weight of the target polymer were also characterized systematically.

EXPERIMENTAL

Materials

Horseradish peroxidase (denoted as HRP; *RZ* = 2.5, activity = 200 U/mg) was purchased from Shanghai Guoyuan Biotechnology Company and used without further purification. Other chemicals were provided by various commercial suppliers and used as-received.

Characterization

Thermogravimetric (TG) analyses were conducted under nitrogen and air atmospheres with a TGA/SDTA 851e facility (Mettler-Toledo Company, Switzerland) at a heating rate of 10 K/min. Infrared (IR) spectra was recorded with an Avatar 360 Fourier transform infrared (FTIR) spectroscope (Nicolet Company, USA). The weight-average molecular weight (M_w) of as-synthesized polymers was measured with a gel permeation chromatography coupled with static laser light scattering (GPC-SLS; DAWN EOS and OPTILAB rEX, Wyatt Technology Corporation; flow rate of fluent tetrahydrofuran: 1.0 mL/min). The concentration of phenol during the enzymatic polymerization was monitored by high performance liquid chromatography (HPLC; Agilent 1100). ^{1}H-NMR spectra were measured with an AVANE 400 MHz spectrometer (Bruker Company, German).

Synthesis of Phenol polymer

HRP was used as the catalyst for the enzymatic polymerization of phenol separately conducted in three kinds of buffers, and hydrogen peroxide was used as the oxidizing agent. Phenol (0.47 g, 5.0 mmol) and TBAB (0.50 g) were dissolved in 45 mL of pre-selected buffer and mixed with the enzyme solution of HRP (0.50 mg) in the same buffer (5.0 mL). 0.25 mL of 5% hydrogen peroxide was added into the resultant mixed solution every 5 min up to a total of 14 times of addition. The mixture was then magnetically stirred at room temperature for 1.5 h to allow polymerization yielding a brown powdery precipitate as the crude product. The crude product was filtered, fully washed with water, and dried in a vacuum oven until its weight kept unchanged. As-dried product was finally used for analysis and characterization.

Titration of Hydroxyl Groups in the Polymer of Phenol

The unit ratio of phenylene (abbreviated as Ph) to oxyphenylene (abbreviated as Ox) in phenol polymer was tested based on acetylation. Briefly, the polymer of phenol (0.100 g) was added in 5.0 mL of pyridine containing 2.50% acetic anhydride. The mixed solution was magnetically stirred at 95 °C for 1 h before 45.0 mL of H_2O was added. The diluted mixed solution was then titrated by 0.20 mol/L NaOH in the presence of phenothalin as the indicator[3], and the product was collected by filtration and completely washed with water.

RESULTS AND DISCUSSION

Influence of TBAB on the Enzymatic Polymerization of Phenol

First of all, the polymerization with various dosages of TBAB was examined to establish optimized condition for HRP-catalyzed phenol polymerization in this system, and the results with those respects ae summarized in Table 1. The conversion of phenol is very low (4.0%) in the absence of TBAB, but it monotonically rises with increasing dosage of TBAB. Namely, the conversion rates of phenol at TBAB dosages of 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 g are 23.7%, 38.7%, 49.8%, 74.2%, 92.6% and 99.1%, respectively, indicating that use of TBAB with HRP catalysis is a very important factor to produce phenol polymer.

Table 1. Enzymatic polymerization of phenol with different amounts of TBAB

Entry [a]	TBAB (g)	Conversion (%)	Ph/Ox [b]	$M_w \times 10^{-4}$ (Da)
1	0	4.0	–	–
2	0.1	23.7	66:34	1.4
3	0.2	38.7	68:32	1.2
4	0.3	49.8	68:32	2.3
5	0.4	74.2	66:34	1.7
6	0.5	92.6	64:36	1.2
7	0.6	99.1	64:36	1.3

[a] Reaction condition: Citrate-phosphate and pH = 8, HRP (1 mg); [b] Measured by titration

To investigate the founction of TBAB, the ^{1}H-NMR spectrum of TBAB (Fig. 1, curve a) was measured. For comparison, the ^{1}H-NMR spectrum of the mixture of TBAB and phenol (Fig. 1, curve b) and the mixture of TBAB and benzene (Fig. 1, curve c) were also measured. The peaks A, B and C in curve (b) corresponded to the various resonances of protons in phenol, and the peak H in curve (c) corresponded to the protons in benzene. TBAB exhibited four kinds of protons in its ^{1}H-NMR spectrum, and these four kinds of protons were : terminal methyl, γ-methylene, β- and α-methylene protons, and they were marked as D, E, F and G, respectively, and the specific data have been presented in Table 2. It has been found that the chemical shifts of terminal methyl protons (D) and γ-methylene protons (E) show few changes in mixture of TBAB and phenol (Fig. 1, curve b). However, larger changes can be seen from the methane protons (F and G), and the chemical shift of F shifts from $\delta F_1 = 1.614$ to $\delta F_2 = 1.562$, and the chemical shift of G shifts from $\delta G_1 = 3.161$ to $\delta G_2 = 3.090$. However, the chemical shifts of the four protons in mixture of TBAB and benzene system are almost the same (the chemical shift of D shifts from$\delta D_1 = 0.911$ to $\delta D_3 = 0.877$, and the chemical shift of E shifts from $\delta E_1 = 1.315$ to $\delta E_3 = 1.279$ ppm, and the chemical shift of F shifts from $\delta F_1 = 1.614$ to $\delta F_3 = 1.576$, and the chemical shift of G shifts from $\delta G_1 = 3.161$ to $\delta G_3 = 3.122$). Above all, these results indicate that the hydroxyls of phenol monomers are close to the inner of TBAB because of electrostatic interaction, which are similar to the aqueous micelle system[28–30], so we consider that TBAB plays a role as the micelle to facilitate the dissolution of phenol because of its tetrahedron structure. Due to the solubilization effect, phenol monomers aggregate and the concentration of phenol is high. It is because of the high concentration of phenol that the enzymatic polymerization of phenol can be performed efficiently.

Table 2. The chemical shift of D, E, F and G

n	δD_n	δE_n	δF_n	δG_n
1	0.911	1.315	1.614	3.161
2	0.893	1.287	1.562	3.090
3	0.877	1.279	1.576	3.122

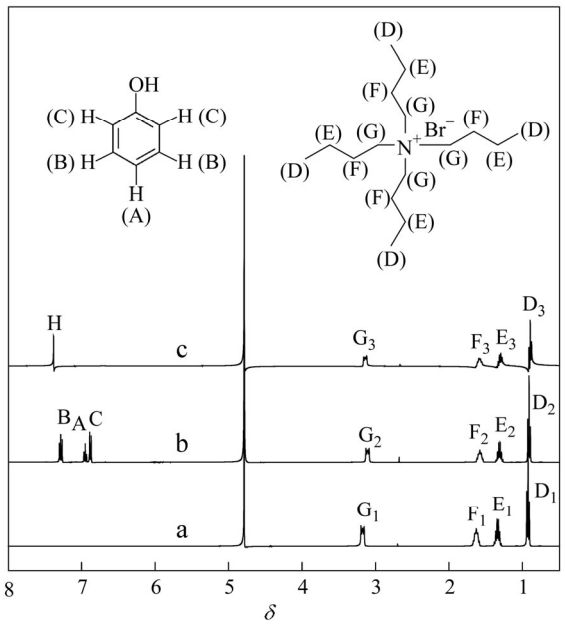


Fig. 1 ^{1}H-NMR spectra of TBAB (a), the mixture of TBAB and phenol (b) and the mixture of TBAB and phenol benzene (c) (solvent: D_2O)

The Influence of the Buffer Solution on the Enzymatic Polymerization of Phenol

Next, the effect of three different kinds of buffer on the polymerization was examined, and the results are summarized in Table 3. The polymerization produced polymers with high conversion in buffers with pH value ranging from 4 to 10, and the increasing pH value of the buffer solution (entry 1–8 in Table 3) resulted in phenol polymers with a reduced molecular weight and increasing conversion. Kobayashi found that the polymerization proceeded hardly with the yield of only 20% in the mixture of 80% 1, 4-dioxane and buffer of pH 10.0, and the polymerization in phosphate buffers of pH 6.0, 7.0 and 8.0 produced polymers with yield of 29%, 75% and 64%, respectively[11]. In contrast to these results, the addition of TBAB to buffer is an obvious advantage system compared with organic system in the polymerization. The gel permeation chromatography coupled with static laser light scattering (GPC-SLS) has received much attention in recent years because of its unique advantage, thus GPC-SLS is performed to determine the absolute molecular weight (M_w) of phenol polymers in this paper[25, 31–33]. In all the cases examined, the weight-average molecular weight is in the range of 12000–30000 Da.

Table 3. Enzymatic polymerization of phenol in different buffers

Entry [a]	pH	Buffer salt	Conversion (%)	Ph/Ox [b]	$M_w \times 10^{-4}$ (Da)
1	6	Phosphate	64.8	63:37	2.4
2	7	Phosphate	80.6	66:34	2.1
3	8	Phosphate	87.6	64:36	1.9
4	4	Citrate-phosphate	60.2	65:35	3.0
5	5	Citrate-phosphate	67.3	64:36	2.4
6	6	Citrate-phosphate	74.8	62:38	1.7
7	7	Citrate-phosphate	88.5	66:34	1.5
8	8	Citrate-phosphate	92.6	64:36	1.2
9	9	Carbonate	93.0	62:38	2.3
10	10	Carbonate	95.0	66:34	2.7

[a] Reaction condition: TBAB (0.5 g), HRP (1 mg); [b] Measured by titration

The Influence of Horseradish Peroxides on the Enzymatic Polymerization of Phenol

It is obvious that there is no reaction to take place in the absence of HRP from Table 4, and the introduction of a small amount of HRP results in a very high conversion rate (above 90%) of phenol (entry 2 in Table 4). In this paper, the amount of HRP is investigated no more than 1.0 mg, which is less than that used by others[11, 14, 19]. What is more, the amount of HRP reached 10 mg in some systems[11], and this also indicated the organic solvent would reduce the activity of enzyme catalysts again[8, 16–19]. The reduced consumption of HRP may be promisingly applied to produce the polymers of phenol and its derivatives in industry. In the meantime, the molecular weight of phenol polymer varies rarely with varying HRP dosage.

Table 4. Enzymatic polymerization of phenol with different additions of HRP

Entry [a]	HRP (mg)	Conversion (%)	Ph/Ox [b]	$M_w \times 10^{-4}$ (Da)
1[b]	0	–	–	–
2[b]	0.2	91.3	61:39	1.2
3[b]	0.4	90.0	62:38	1.5
4[b]	0.6	91.1	64:36	1.6
5[b]	0.8	91.4	65:35	1.2
6[b]	1.0	92.6	66:34	1.2

[a] Reaction condition: Citrate-phosphate and pH = 8, TBAB (0.5 g); [b] measured by titration

The Conversion of Phenol versus Reaction Time upon the Addition of H_2O_2

The curves of conversion rate of phenol versus reaction time upon the addition of H_2O_2 are showed in Fig. 2. The conversion rate of phenol is only about 4% when there is no TBAB introduced into the reaction system (curve a in Fig. 2), and Zhang has also found the same phenomenon without adding SDBS[23]. After 0.1 g of TBAB has been added into the reaction system, the reaction equilibrium can reached upon only two times addition of 5% H_2O_2, and the conversion rate rises to about 24% (curve b in Fig. 2). Further increase of TBAB dosage leads to monotonous rise of the conversion rate of phenol, and the maximum conversion rate reaches at a TBAB dosage of 0.6 g (curves c–g in Fig. 2), but it takes a longer time for the reaction to reach the equilibrium state.

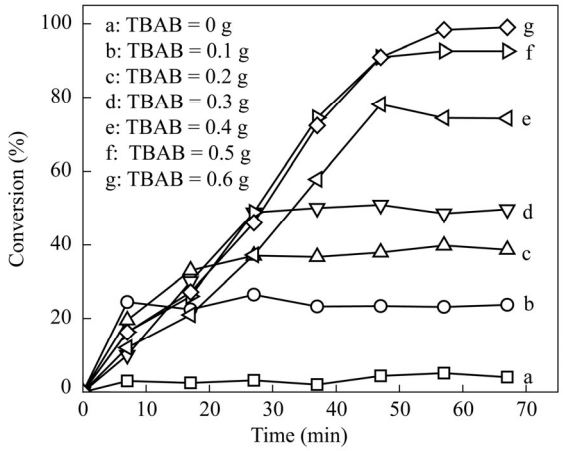


Fig. 2 Plots of conversion of phenol versus reaction time with the addition of H_2O_2

IR Analysis of the Phenol Polymer

Because of the poor solubility of phenol polymer and the polymer after acetylation, the structures were estimated mainly by IR analysis. As seen from Fig. 3 the broad peak at 3401 cm^{-1} is attributed to the vibration of phenolic O—H linkage, and those at 3056, 1589, 1485 and 694 cm^{-1} are assigned to benzene ring (curve a). Besides, the peaks at 1213 cm^{-1} and 1069 cm^{-1} are ascribed to the vibration of C—O—C linkage. Thus it can be inferred that as-synthesized polymer of phenol consists of phenylene units and oxyphenylene units. After acetylation of the phenol polymer, the peak of phenolic O—H bond at 3401 cm^{-1} disappears, and the strong peaks corresponding

to the C=O bond and C—O—C linkage of the ester group emerge at 1766 cm^{-1} and 1193 cm^{-1} (curve b), which indicates that the acetylation is successfully completed. In all cases the data are similar to IR spectra of phenol polymers obtained in other systems[5, 14, 23–25]. The structure of polymer obtained with TBAB is also composed of a mixture of phenylene (Ph) and oxyphenylene (Ox) units. The unit molar ratio of phenylene to oxyphenylene (Ph/Ox) was determined by titration of the hydroxyl group in the polymer, and the results are listed in the above table, and the ratio of the phenylene is in the range of 61%–68%. The FTIR spectra was consistent with the mechanism of the formation of phenol polymer shown in Scheme 1.

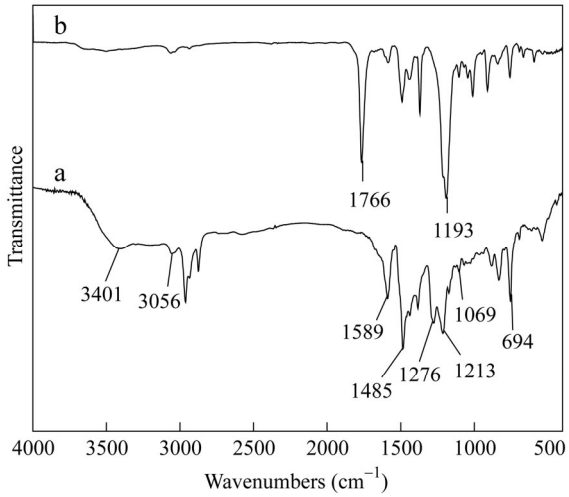
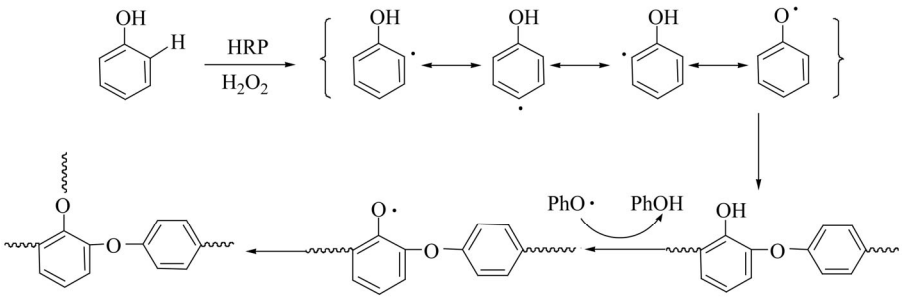


Fig. 3 FTIR spectra of phenol polymer (a) and the polymer after acetylation (b)



Scheme 1 The mechanism of the formation of phenol polymer

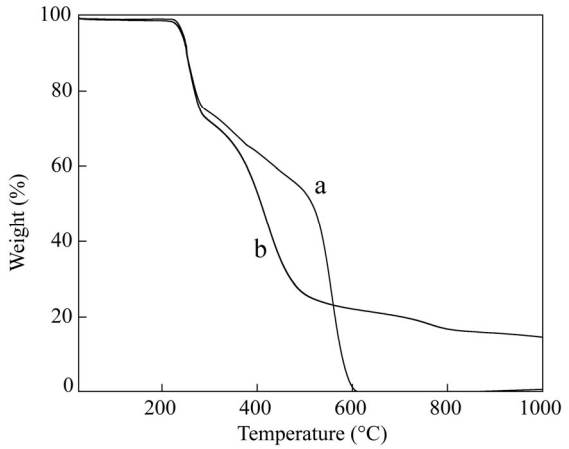


Fig. 4 TG curves of the phenol polymer (a) under air (b) under N_2

Thermal Stability of Phenol Polymer

Figure 4 shows the TG curves of the as-synthesized phenol polymer under air and N_2. It can be seen that under both air and N_2, the weight loss is about 25% when the temperature is around 300 °C. Besides, the as-synthesized phenol polymer retains about 50% of weight when it is heated at about 521 °C in air, and its complete decomposition in air occurs at about 600 °C. When phenol polymer is heated in N_2, it retains about 15% of weight even at 1000 °C. These thermal analysis data prove that the phenol polymer possesses good high thermal stability.

CONCLUSIONS

In this article the effect of TBAB on enzymatic polymerization of phenol catalyzed by horseradish peroxidase has been investigated, and the phenol polymerization catalyzed by HRP has been performed efficiently in this novel system. The addition of TBAB to buffer is an obvious advantage compared with organic system, and the maximum conversion rate of phenol reaches as much as 99.1% with short reaction time. The weight-average molecular weight is in the range of 12000–30000 Da measuresd by GPC-SLS. The polymerizations were carried out successfully in this system over a wide range of pH value from 4.0 to 10.0. The present synthetic strategy, with significantly reduced consumption of HRP and good environmental acceptability, may find application in the industrial production of the polymers of phenol and its derivatives.

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