

Peroxidase-Catalyzed Dispersion Polymerization of Phenol Derivatives

Hideharu KURIOKA, Hiroshi UYAMA,* and Shiro KOBAYASHI*[†]

*Department of Materials Chemistry, Graduate School of Engineering,
Tohoku University, Sendai 980-8579, Japan*

**Department of Materials Chemistry, Graduate School of Engineering,
Kyoto University, Kyoto 606-8501, Japan*

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ABSTRACT: Preparation of polyphenol particles has been carried out by dispersion polymerization using horseradish peroxidase as catalyst in an aqueous 1,4-dioxane. The polymerization of phenol using poly(vinyl methyl ether) as steric stabilizer in 40% phosphate buffer (pH 7) solution afforded relatively monodisperse particles in the sub-micron range. Effects of the concentration and molecular weight of the stabilizer on the particle size and size distribution have been investigated. The buffer pH and mixed ratio of the buffer and 1,4-dioxane also affected the particle formation. From *m*- and *p*-cresols and *p*-phenylphenol, sub-micron size microspheres were obtained. The thermal treatment of the polyphenol particles at 1000°C under nitrogen afforded the particles of carbonized polymer.

KEY WORDS Enzymatic Polymerization / Dispersion Polymerization / Polyphenol / Polymer Particle / Oxidative Polymerization / Carbonized Polymer /

Polymerization catalyzed by an enzyme (“enzymatic polymerization”) provides a new methodology for polymer syntheses.^{1–3} Recently, syntheses of new polyaromatics using oxidoreductase catalyst have been developed.^{4–11} Peroxidases or oxidases induced an oxidative polymerization of phenol derivatives, yielding a new class of polyphenols showing high thermal stability.^{7,8} Furthermore, this enzymatic process is expected to be an alternative way for preparation of phenol polymers without using toxic formaldehyde, which is a monomer component for production of conventional phenolic resins (phenol-formaldehyde resins).¹²

Dispersion polymerization is often used for preparation of polymer particles, since the process is very simple and convenient. The dispersion polymerization of vinyl monomers such as styrene^{13–15} and methacryl esters^{16–18} in polar media has been extensively studied for the last decade to produce micron-size monodisperse particles. The oxidative polymerization of aniline^{19,20} and pyrrole²¹ in the presence of steric stabilizer afforded relatively monodisperse particles in the sub-micron range.

In many cases, the enzymatic polymerization of phenols was carried out using horseradish peroxidase (HRP) as catalyst in a mixture of water-miscible solvent and buffer. The polymerization starts by the addition of hydrogen peroxide as oxidizing agent. Before the polymerization, the reaction mixture is homogeneous, whereas the polymeric precipitate is formed during the polymerization. Our previous communication showed that the enzymatic polymerization of phenol in the presence of a water-soluble polar polymer produced the monodisperse particles in the sub-micron range.²² The present paper describes comprehensive results of the dispersion polymerization of phenol derivatives and the conversion of the resulting particles to the carbonized polymer particles by the thermal treatment. In relevant to this study, polyphenol particles were enzymatically produced in a reverse-micellar system,^{23,24} which employed much amount of a detergent.

EXPERIMENTAL

Materials

Horseradish peroxidase (HRP) was purchased from Wako Pure Chemicals and used without further purification. Phenol was purified by distillation. Other reagents and solvents were commercially available and used as received.

Dispersion Polymerization

A typical run was as follows (entry 4 in Table I). A mixture of 0.47 g (5 mmol) of phenol, 10 mg of HRP, and 0.12 g of poly(vinyl methyl ether) was dissolved in 15 mL of 1,4-dioxane and 10 mL of 0.1 M phosphate buffer (pH 7). Hydrogen peroxide (30%) (0.28 μ L, 0.25 mmol) was added 20 times to the mixture every 15 min with vigorous stirring at room temperature under air. After 24 h, the particles were separated by centrifugation of the reaction mixture. The particles were twice subjected to the resuspension in water and the successive centrifugation. Subsequently, the particles were washed twice with a mixture of 1,4-dioxane and water (60:40 vol%) in the similar manner, followed by drying *in vacuo* to give 0.47 g (yield 100%) of the particles.

Measurements

Scanning electron micrographic (SEM) analysis was carried out on a Hitachi H-8010 electron microscope. IR spectra were recorded on Shimadzu IR-460 spectrometer. Thermal gravimetric (TG) analysis was performed using a Seiko SSC/5200 apparatus for thermogravimetry/differential thermal analysis at a heating rate of 10°C min⁻¹ in a gas flow rate of 300 mL min⁻¹.

RESULTS AND DISCUSSION

Dispersion Polymerization of Phenol

Polymeric stabilizer for the dispersion polymerization of phenol was screened (Table I). The polymerization was performed using HRP as catalyst in a mixture of 1,4-dioxane and phosphate buffer (pH 7) (60:40 vol%).

[†] To whom correspondence should be addressed.

Table I. Stabilizer screening for dispersion polymerization of phenol^a

Entry	Stabilizer	Yield of particles	D _n ^b
		%	nm
1	PAA	0	
2	PEG	86	288 (201—384)
3	PVA	86	319 (241—437)
4	PVME	100	254 (228—278)

^a Polymerization of phenol (5 mmol) using HRP catalyst (10 mg) in the presence of stabilizer (0.12 g, 25 wt% for phenol) in a mixture of 1,4-dioxane and phosphate buffer (pH 7) (60:40 vol%) at room temperature for 24 h. ^b Determined by SEM. In parenthesis, the range of particle size.

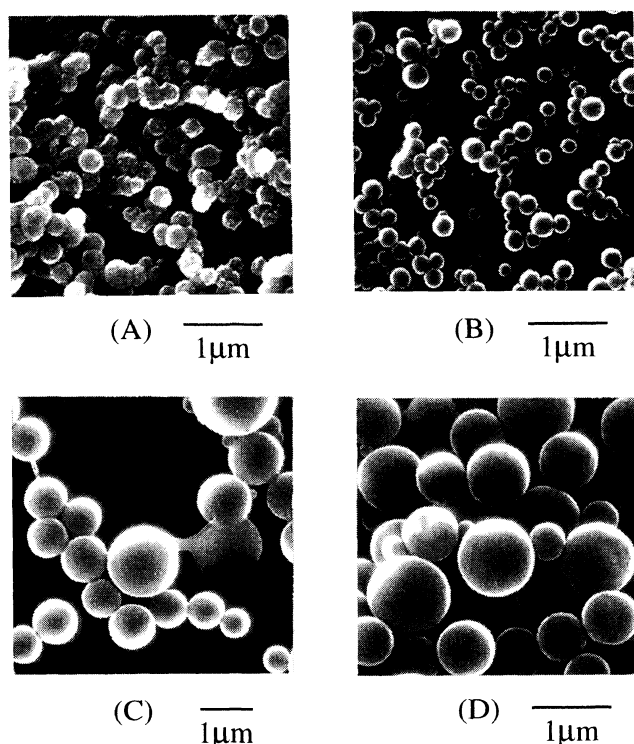


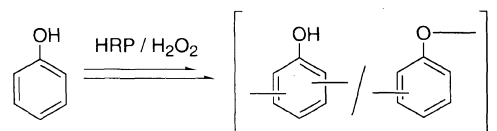
Figure 1. SEM photographs of polymer particles from (A) phenol, (B) *m*-cresol, (C) *p*-cresol, and (D) *p*-phenylphenol. Particles were obtained by the dispersion polymerization of phenol derivative (5 mmol) using HRP catalyst (10 mg) in the presence of PVME (25 wt% for the monomer) in a mixture of 1,4-dioxane and phosphate buffer (pH 7) (60:40 vol%) at room temperature for 24 h.

Water-soluble polar polymers, poly(acrylic acid) (PAA), poly(ethylene glycol) (PEG, $M_n = 5.0 \times 10^4$), poly(vinyl alcohol) (PVA, $M_n = 2.2 \times 10^4$), and poly(vinyl methyl ether) (PVME, $M_n = 4.6 \times 10^4$) were used as stabilizer. The resulting particles were analyzed by SEM.

In using PAA as stabilizer, polymeric precipitate was not formed during the polymerization (entry 1). This may be due to the loss of the HRP activity by the interaction with PAA. In case of other stabilizers, the polymerization took place to give a stable dispersion. The polymerization in the presence of PEG or PVA produced the sub-micron size particles in high yields, which were not monodisperse (entries 2 and 3). With PVME stabilizer, the polyphenol particles were quantitatively obtained (entry 4) and SEM analysis showed that the resulting particles were relatively monodisperse

(Figure 1(A)). In all cases, the particle diameter was *ca.* 300 nm.

The structure of the enzymatically synthesized polyphenols was reported to be complicated. We have estimated that the polymer from phenol was mainly composed of a mixture of phenylene and oxyphenylene units by IR and NMR analyses.^{6,8} The present polyphenol particles were insoluble in organic solvents. Therefore, the structural analysis of the polyphenol particles was performed by IR spectroscopy. The absorption peaks at 1608, 1489, 833, and 753 cm^{-1} were characteristic of the various vibration modes of the C–H and C–C bonds of aromatic nuclei. A peak due to the asymmetric vibrations of the C–O–C linkage and a peak ascribed to the C–OH vibration were overlapping at 1210 cm^{-1} . A peak at 1077 cm^{-1} corresponded to the symmetric vibration of the ether bond. The spectrum pattern of the particles was similar to that obtained without the polymeric stabilizer. These data indicate that the polyphenol particles were also composed of a mixture of phenylene and oxyphenylene units (Scheme 1).



Scheme 1.

Table II summarizes results of the dispersion polymerization of phenol using PVME stabilizer. Effects of the stabilizer concentration on the particle size have been investigated (entries 1, 2, 7, 19, and 20). As the concentration increased, the particle size decreased. This is probably because the larger amount of PVME stabilizes the particles more efficiently. A similar tendency was observed in the dispersion polymerization of vinyl monomers.^{15,16} In the low concentration of the stabilizer (less than 10%), the size dispersity was larger.

The buffer pH enormously affected the particle formation. In the pH range from 4 to 7 (entries 3–5 and 7), the polymer particles were obtained quantitatively, whereas the yield decreased in the alkaline region (pH 9–11) (entries 11–13). The polymerization in the buffer of pH 10 produced the particles with the size of less than 100 nm. The product obtained in the buffer of pH 11 showed no clear morphology. Except pH 7, monodispersity was not achieved.

The effects of the mixed ratio of the aqueous 1,4-dioxane solution have been examined by using phosphate buffer of pH 7 and distilled water. In using the phosphate buffer, the polymer particles were only obtained when the 1,4-dioxane content was 60% (entries 6, 7, and 9). In case of the polymerization in a mixture of 1,4-dioxane and distilled water (entries 14–18), the particle formation was observed in the range of 1,4-dioxane content from 40 to 60%. The amount of HRP also affected the particle size (entries 7 and 8); the larger amount afforded the smaller particles.

PEGs with different molecular weight are commercially available. Here, the effect of the stabilizer molecular weight was examined by using PEG with molecular weight from 6×10^2 to 4×10^5 (Table III). In all cases,

Table II. Dispersion polymerization of phenol using PVME stabilizer phenol^a

Entry	Stabilizer concn. ^b	Aqueous solution ^c	Content of 1,4-dioxane ^d	HRP amount	Yield	Dn ^e
	%		%	mg	%	nm
1	5	Phosphate buffer (7)	60	10	100	287 (239—327)
2	10	Phosphate buffer (7)	60	10	100	265 (200—307)
3	25	Acetate buffer (4)	60	10	100	467 (295—648)
4	25	Acetate buffer (5)	60	10	100	423 (345—537)
5	25	Phosphate buffer (6)	60	10	100	420 (343—583)
6	25	Phosphate buffer (7)	50	10	100	No morphology
7	25	Phosphate buffer (7)	60	10	100	254 (228—278)
8	25	Phosphate buffer (7)	60	20	100	200 (164—243)
9	25	Phosphate buffer (7)	80	10	53	No morphology
10	25	Phosphate buffer (8)	60	10	91	414 (287—557)
11	25	Borate buffer (9)	60	10	62	197 (170—253)
12	25	Carbonate buffer (10)	60	10	71	<100
13	25	Carbonate buffer (11)	60	10	62	No morphology
14	25	Distilled water	20	10	100	No morphology
15	25	Distilled water	40	10	100	482 (353—609)
16	25	Distilled water	50	10	100	417 (283—627)
17	25	Distilled water	60	10	100	380 (329—456)
18	25	Distilled water	80	10	51	No morphology
19	35	Phosphate buffer (7)	60	10	97	246 (213—286)
20	50	Phosphate buffer (7)	60	10	92	239 (210—262)

^a Polymerization of phenol (5 mmol) using HRP catalyst in the presence of PVME in an aqueous 1,4-dioxane at room temperature for 24 h.

^b Weight % based on monomer. ^c In parenthesis, value of buffer pH. ^d Volume % of the mixed solvent. ^e Determined by SEM. In parenthesis, the range of particle size.

Table III. Effect of stabilizer molecular weight in the dispersion polymerization of phenol^a

Entry	Molecular weight	Yield	Dn ^b
		%	nm
1	6.0×10^2	78	332 (245—504)
2	2.0×10^3	83	304 (210—414)
3	8.4×10^3	79	297 (230—338)
4	5.0×10^4	86	288 (201—384)
5	4.0×10^5	85	482 (343—757)

^a Polymerization of phenol (5 mmol) using HRP catalyst (10 mg) in the presence of PEG (0.12 g, 25 wt% for phenol) in a mixture of 1,4-dioxane and phosphate buffer (pH 7) (60:40 vol%) at room temperature for 24 h. ^b Determined by SEM. In parenthesis, the range of particle size.

the particle yield was *ca.* 80%. Except using the highest molecular weight stabilizer, the particle size slightly decreased with increasing the molecular weight of the stabilizer (entries 1—4). This is probably because the stabilizer of higher molecular weight has the more efficient stabilizing ability toward the particles. The polymerization using PEG with molecular weight of 4×10^5 gave the particles in the broader range of the size and the average size was larger than that using other PEGs (entry 5).

Dispersion Polymerization of Substituted Phenols

Not only phenol but various phenol derivatives have been subjected to the enzymatic oxidative polymerization.^{4–11} In this study, HRP-catalyzed dispersion polymerization of cresols and *p*-phenylphenol was examined using PVME stabilizer (25% for monomer) in a mixture of 1,4-dioxane and phosphate buffer (pH 7) (60:40 vol%). In the polymerization of *o*-cresol, the polymeric precipitate was obtained in 88% yield, however, SEM analysis showed the formation of the particles including

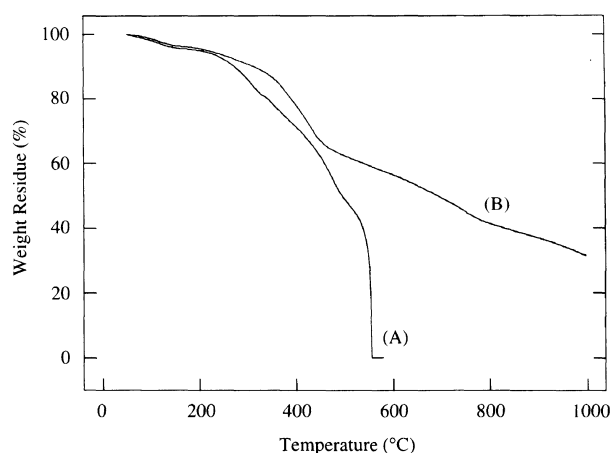


Figure 2. TG traces of polyphenol particles (entry 4 in Table I) measured at a heating rate of $10^\circ\text{C min}^{-1}$ under (A) air; (B) nitrogen.

the doublets. The polymerization of *m*- and *p*-cresols and *p*-phenylphenol afforded the particles in the yield of 100, 66, and 85%, respectively. SEM analysis showed that these particles were polydisperse in the sub-micron range (Figure 1(B)—(D)), whereas the particle surface was more smooth than that from phenol obtained under the similar polymerization conditions.

Thermal Treatment of Polyphenol Particles

Carbonized polymers, typically graphite and polyacene, are widely used as functional materials.²⁵ Particles of such polymers are expected to have potential applications in various fields. Our previous study⁸ showed that the enzymatically synthesized polyphenol was a good precursor for carbonized polymers; the carbonization of the polyphenol took place by heating at 1000°C under nitrogen to produce a graphite-like polymer.

The preparation of carbonized polymer particles was attempted by the thermal treatment of polyphenol

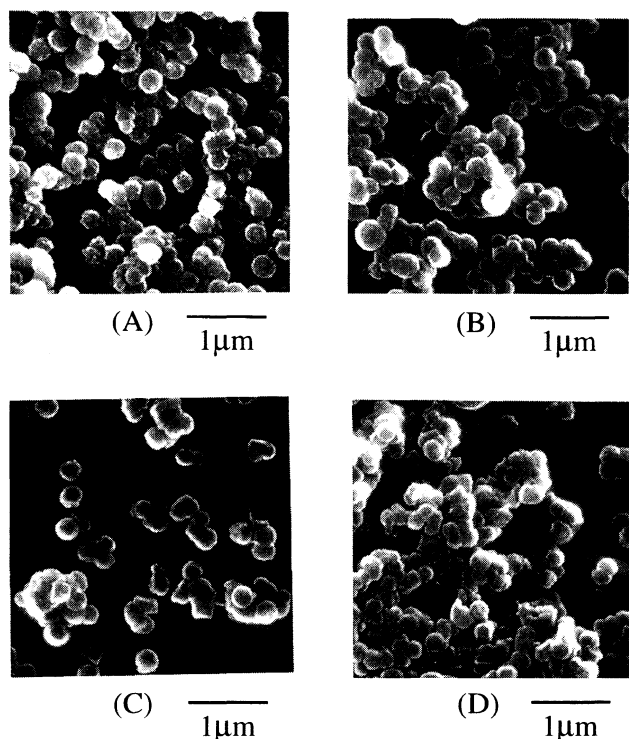


Figure 3. SEM photographs of particles of thermal treatment at different temperatures: (A) without thermal treatment (entry 4 in Table I); (B) 330°C; (C) 550°C; (D) 1000°C. The starting polymer particles were virtually kept at each temperature for 30 min under nitrogen.

particles. At first, the thermal property of the particles was evaluated by thermal gravimetric analysis (TGA). Figure 2 shows TG traces of the enzymatically obtained polyphenol particles (entry 4 in Table I). A slight gradual weight loss was observed below 250°C, which might be due to the evaporation and/or evolution of low molecular weight compounds. Under air, the decomposition started at lower temperature and the particles was completely decomposed at 560°C. At 1000°C under nitrogen, 32 wt% of the particles remained.

The thermal treatment of the particles was performed at three temperatures: 330, 500, and 1000°C. The sample was kept at each temperature for 30 min under nitrogen in TGA measurement apparatus. From SEM observation (Figure 3), the particle morphology was found to hardly change by the thermal treatment even at 1000°C, suggesting the formation of the carbonized polymer particles.

CONCLUSION

Relatively monodisperse polyphenol particles in the sub-micron range were prepared by HRP-catalyzed dispersion polymerization of phenol using PVME stabilizer in a mixture of 1,4-dioxane and phosphate buffer. The

solvent composition greatly affected the particle formation. From *m*- and *p*-cresols and *p*-phenylphenol, polymer particles were also formed. The present particles were converted to the carbonized polymer particles by thermal treatment at 1000°C under nitrogen.

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