Preparation of Polyphenol Particles by Dispersion Polymerization Using Enzyme as Catalyst

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(Received June 8, 1995)

Peroxidase-catalyzed dispersion polymerization of phenols has been performed in a mixture of 1,4-dioxane and phosphate buffer using a water-soluble polymer as stabilizer to produce relatively monodisperse polyphenol particles in the sub-micron range.

There have been many studies of dispersion polymerization of acrylic esters in non-polar media for the development of non-aqueous coating technology.[1] Until now, the addition polymerization of vinyl monomers such as styrene[2,3] and methyl methacrylate[4,5] and the oxidative polymerization of aniline[6] and pyrrole[7] in dispersion provided respective polymer particles. Recently, the dispersion polymerization of vinyl monomers in polar media has been extensively studied because this method affords monodisperse polymer particles in a single step of a convenient process.

An enzyme-catalyzed polymerization ("enzymatic polymerization") has received much attention as a new methodology for polymer synthesis.[8,9] Enzymatic synthesis of phenol and aniline polymers using peroxidase catalyst has been developed for the last several years.[10-15] The enzymatic polymerization of phenol derivatives has been performed in an aqueous organic solvent using horseradish peroxidase (HRP) and hydrogen peroxide as catalyst and source of oxidizing agent, respectively.[10-13] This system is initially homogeneous and during the polymerization the resulting polymer becomes insoluble, yielding powdery precipitates showing no definite morphology.

In this study, we have extended the scope of polyphenol synthesis through enzymatic catalysis to particle formation of such polymers by the polymerization in the presence of a polymeric stabilizer (dispersion polymerization). The physical adsorption of the stabilizer on the precipitated nuclei resulted in the formation of stable particle dispersion. So far, preparation of polyphenol particles using an enzyme as catalyst has been once claimed; the polymerization of *p*-ethylphenol in reversed micelles afforded sub-micron size particles.[16] However, the system needed to use a large amount (5 folds molar for the monomer) of an anionic surfactant for preparation of polymer particles.

The dispersion polymerization of phenol using HRP catalyst was carried out in a mixture of 1,4-dioxane and phosphate buffer (pH 7) (60:40 vol%) to produce a stable dispersion.[17] The polymer particles were separated by centrifugation of the dispersion and analyzed by scanning electron microscope (SEM).

At first, various water-soluble polymeric stabilizers were tested for preparation of polymer particles. The polymeric stabilizers used in this study were poly(vinyl methyl ether) (PVME, Mw=4.6x10^4), poly(ethylene glycol) (PEG, Mw=5.0x10^4), and poly(vinyl alcohol) (PVA, Mw=2.2x10^4).

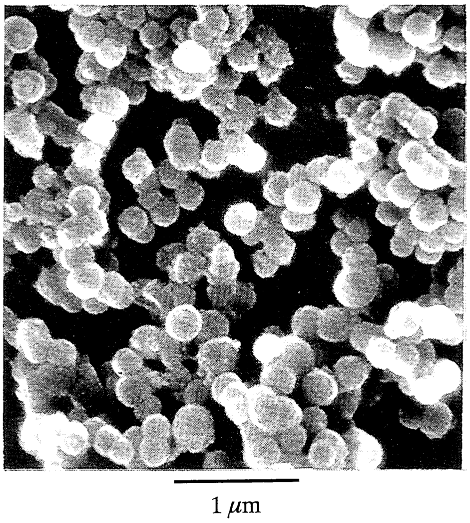

Figure 1. SEM photograph of polyphenol particles produced by dispersion polymerization using HRP catalyst in a mixture of 1,4-dioxane and phosphate buffer (entry 3).

Table 1. Preparation of polyphenol particles by HRP-catalyzed dispersion polymerization in an aqueous dioxane[a]

Entry	Monomer	HRP Amount/mg	Stabilizer	Stabilizer Conc./%[b]	Yield/%[c]	Dn/nm[d]
1	Phenol	10	PVME[e]	5	100	287 (239-327)
2	Phenol	10	PVME[e]	10	100	265 (200-307)
3	Phenol	10	PVME[e]	25	100	254 (228-278)
4	Phenol	20	PVME[e]	25	100	200 (164-243)
5	Phenol	10	PVME[e]	35	97	246 (213-286)
6	Phenol	10	PVME[e]	50	92	239 (210-262)
7	Phenol	10	PEG[f]	25	86	288 (201-384)
8	Phenol	10	PVA[g]	25	86	319 (241-437)
9	*p*-Phenylphenol	10	PVME[e]	25	85	318 (169-437)

[a]The polymerization was done as described in ref. 17. [b]Weight % for monomer. [c]Isolated yield. [d]Determined by SEM. In parenthesis, the range of particle size. [e]Poly(vinyl methyl ether). [f]Poly(ethylene glycol). [g]Poly(vinyl alcohol).

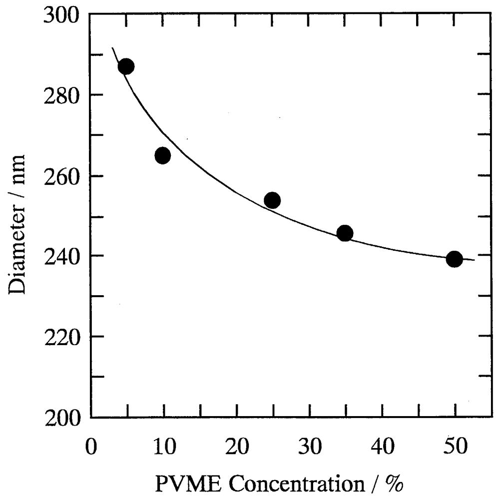

Figure 2. Effect of the PVME concentration on the particle size in the HRP-catalyzed dispersion polymerization of phenol in a mixture of 1,4-dioxane and phosphate buffer.

Figure 1 shows SEM photograph of polyphenol particles prepared by using 25 weight % of PVME for the monomer. The particles were relatively monodisperse in the sub-micron range. From PEG and PVA, particles in the sub-micron range were also obtained in high yields, and the size and size range were larger than those by using PVME (entries 3, 7, and 8 in Table 1). The polymerization without the stabilizer afforded the polymer with no morphology.

By changing the concentration of PVME, the particle size could be controlled; the diameter decreased from 287 nm to 239 nm as increasing the concentration of PVME from 5% to 50% (Figure 2). These data indicate that a higher concentration of PVME stabilized more efficiently to produce smaller particles. Similar behaviors were observed in the dispersion polymerization of vinyl monomers.[3-5]

The amount of the catalyst affected the particle size: smaller polyphenol particles were obtained by using more amount of HRP (entry 4). The dispersion polymerization of *p*-phenylphenol also produced particles in the sub-micron range (entry 9).

The particle polymer from phenol (entry 3) was not soluble in water as well as common organic solvents. Its IR spectrum pattern was very similar with that of the polymer obtained without PVME,[12] indicating that the resulting polymer was composed of a mixture of phenylene and oxyphenylene units.[18] TG analysis showed high thermal stability of the polyphenol particles (temperature of 10 weight % loss under nitrogen = 304°C). The glass transition point of the polymer was not observed under 300 °C by DSC.

In conclusion, relatively monodisperse polyphenol particles were prepared by the dispersion polymerization using HRP catalyst in polar media. The present dispersion polymerization by enzymatic catalysis provided a very convenient route for preparation of polyphenol particles. Conventional formaldehyde-based polyphenols (phenolic resins) are widely used in coating technology. Enzymatically synthesized polyphenols are known as a thermally very stable polymer.[19] Therefore, the present polyphenol particles have much potential for thermal stability-required uses. The more detailed studies including the control of particle size and the preparation of particles from wide variety of phenol derivatives are now in progress.

This work was partially supported by Grant-in-Aids for Scientific Research from the Ministry of Education, Science, and Culture, Japan (No. 06403026 and 07555289).

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17 A typical run was as follows (entry 3). Under air, 0.47 g of phenol (5 mmol), 10 mg of HRP, and 0.12 g of PVME were dissolved in a mixture of 15 ml of 1,4-dioxane and 10 ml of 0.1 M phosphate buffer (pH 7). 0.28 μL (0.25 mmol) of hydrogen peroxide (30%) was added to the mixture every 15 min for 20 times with vigorous stirring at room temperature. After 24 h, the resulting particles were isolated by centrifugation of the reaction mixture. Then, the particles were resuspended in water, followed by centrifugation (twice). Subsequently, the similar operation was conducted twice in 60% dioxane to give 0.47 g of the polymer particles (yield 100%). During the purification procedure, the particle size and size distribution was not changed.
18 During the dispersion polymerization of vinyl monomers, the monomer is known to be grafted on the stabilizer. Polyphenol synthesized by HRP catalyst was reported to contain proteins derived from the enzyme. Therefore, the incorporation of the stabilizer and/or HRP into the present polyphenol particles is not ruled out, however, they could not be detected by IR and elemental analyses.
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