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SHORT COMMUNICATIONS

# Laccase-Catalyzed Curing of Vinyl Polymers Bearing a Phenol Moiety in the Side Chain

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Recently, environmentally benign process of polymer curing has been paid much attention in coating industry, since the conventional industrial method often involves severe curing conditions with a large amount use of energy.[1] Development of new curing methodology under ambient conditions is strongly desired.

Polymerizations catalyzed by enzymes (enzymatic polymerizations) have been investigated extensively as new methodology of polymer syntheses.[2—10] Characteristics of enzyme catalysis are expected to provide new polymeric materials, which are difficult to be obtained by conventional methods. Peroxidases induced the oxidative polymerization of phenol derivatives under mild reaction conditions to produce a new class of functional polyphenols in good yields. This process does not use toxic formaldehyde and their synthetic procedure is very facile. The polyphenols enzymatically obtained have structures normally composed of a mixture of phenylene and oxyphenylene units, which are formed by C–C and C–O couplings of phenols, respectively. Recent investigations revealed that the coupling selectivity (regioselectivity) could be controlled by changing the solvent composition, yielding a DMF-soluble polyphenol.[11, 12]

Very recently, we have created a novel system of enzymatic polymerization, *i.e.*, a laccase-catalyzed crosslinking reaction of new "urushiol analogues" for the preparation of "artificial urushi".[13—15] Single-step synthesis of the urushiol analogues was achieved by using lipase as catalyst. These compounds were cured in the presence of laccase catalyst under mild reaction conditions without use of organic solvents to produce the crosslinked polymeric film with high gloss surface and good elastic properties. This multienzymatic processes are highly significant as a fundamental study for an alternative of conventional commercial coatings utilizing much organic solvents and severe hardening conditions. In this study, we have demonstrated another approach of laccase-catalyzed curing system using vinyl polymers having a phenol moiety in the side chain, in which the enzymatic oxidative coupling of the phenol group proceeded to give the crosslinked polymeric materials (Scheme 1).

Peroxidases have been reported to be involved in the post-translational maturation of proteins, in which an oxidative coupling of tyrosine residue takes place to form a network through intermolecular dityrosine bonds.[16, 17] Recently, improvement of protein properties has been achieved by the peroxidase-catalyzed crosslinking using hydrogen peroxide as oxidizing agent.[18, 19] To our knowledge, there has been no report on laccase-catalyzed curing of synthetic vinyl polymers under air, in which oxygen in air acts as oxidizing agent.

## RESULTS AND DISCUSSION

We reported that peroxidase catalysis induced a chemoselective polymerization of 2-(4-hydroxyphenyl)-ethyl methacrylate (**1**), a phenol derivative, having methacryloyl group,[20] in which only the phenol moiety was polymerized without involving vinyl polymerization of methacryl to give a polymer having the methacryloyl group in the side chain. The resulting polymer was subjected to thermal and photochemical curings. In this study, the vinyl polymer from **1** was used as prepolymer for the laccase-catalyzed curing.

In the homopolymerization of **1** using 2,2′-azobis-(isobutyronitrile) (AIBN, 2 wt% for **1**) initiator in tetrahydrofuran (THF) at 70°C for 14 h, 80% of the monomer was consumed to give the powdery polymer,

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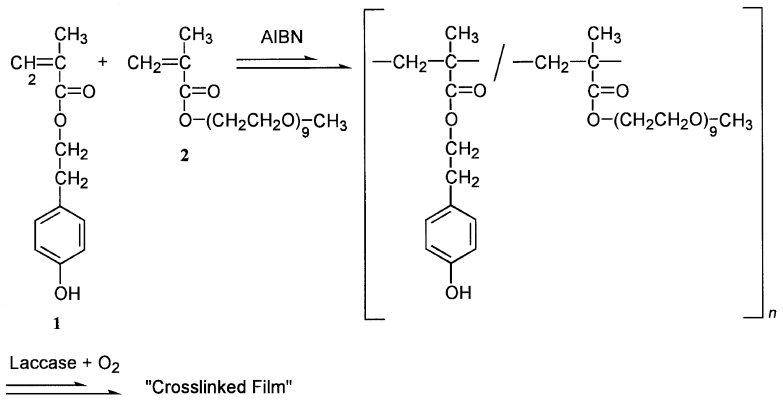


**Scheme 1.**

**Table I.** Synthesis of enzymatically crosslinkable copolymers from **1** and **2**[a]

| Entry | Feed Ratio of **1** / mol % | $M_n^b$ / $\times 10^{-4}$ | $M_w/M_n^b$ | $T_g^c$ / °C |
|---|---|---|---|---|
| 1 | 12 | 2.4 | 2.2 | −66 |
| 2 | 23 | 2.6 | 2.2 | −57 |
| 3 | 40 | 2.6 | 2.3 | −52 |
| 4[d] | 100 | 3.5 | 2.2 | 75 |

[a]Copolymerization using AIBN (1 wt% for monomers) in THF at 70°C for 6 h. [b]Determined by SEC. [c]Determined by DSC under nitrogen. [d]Homopolymerization of **1** using AIBN (2 wt% for **1**) in THF at 70°C for 14 h.

whose structure was confirmed by $^1$H NMR and IR spectroscopies. The number-average molecular weight ($M_n$) and its index ($M_w/M_n$) determined by size exclusion chromatography (SEC) were $3.5 \times 10^4$ and 2.2, respectively. Differential scanning calorimetric (DSC) analysis showed that the polymer possessed a clear glass transition temperature at 75°C. For coating use, however, the resulting powdery polymer was not suited; solid polymers cannot be coated directly on plates.

Next, the copolymerization of **1** with methacryloyl-type poly(ethylene glycol) macromonomer (**2**) with degree of polymerization = 9 was examined (Table I). In all cases, the conversion of the monomers was very high (more than 97%), suggesting the formation of the copolymer possessing with the structure close to the feed ratio. In all cases examined, the product was oily. $T_g$ values of the copolymer increased as a function of the content of **1**, whereas the molecular weight scarcely depended on the feed ratio.

For the curing, used was laccase derived from *Pycnoporus coccineus* as catalyst, which was highly active for the oxidative polymerization of 2, 6-dimethylphenol and syringic acid to give poly(1, 4-phenylene oxide).[21—23] Laccase belongs to an oxidoreductase having a copper-protein moiety as active site. The sample film prepared on a glass slide stood at 30°C under the humidity of 80% for 16 h.

The enzymatic curing of the samples of entries 1 and 2 took place to give the crosslinked polymeric films insoluble in any solvents. The UV-visible spectrum of the copolymer film (entry 2) showed a strong absorption at 278 nm. After the curing, the absorption shift to 296 nm was observed and the absorption became much broader, indicating the formation of the carbon–carbon oxidative coupling moiety of phenols. In the case of the sample containing larger content of **1** (entry 3), the homogeneous mixing of the copolymer and laccase was not observed. In using the deactivated laccase (preheated at 100°C before use), the curing did not occur. The homopolymer of **2** was not cured under the similar reaction conditions. These data clearly indicate that the curing of the present copolymer proceeded through the enzyme oxidation catalysis on the phenol moiety.

## CONCLUSIONS

The crosslinkable oily vinyl polymers bearing a phenol moiety in the side chain have been developed, which were subjected to the laccase-catalyzed curing *via* air-oxidation under mild reaction conditions. The present study is the first example that laccase catalysis enabled the curing of synthetic vinyl polymers, in which the crosslining takes place in the absence of organic solvents at an ambient temperature under air. Therefore, the present method can be regarded as an environmentally benign process of polymer coating, providing an example system of *green polymer chemistry*.[9] Further investigations on the evaluation of the enzymatically-cured film properties and development of other enzymatic curing systems are under way in our laboratory.

## EXPERIMENTAL

Monomer **1** was synthesized by a lipase-catalyzed regioselective esterification of 4-hydroxyphenetyl alcohol with methacrylic acid vinyl ester according to the

literture.[20] Laccase solution ($4 \times 10^4$ unit per mL) and **2** were purchased from Koken Co. and Shin-Nakamura Chemical Co., respectively. All the reagents and solvents were used as received.

The homopolymerization of **1** was performed as follows. Under nitrogen, a mixture of **1** (0.50 g) and AIBN (10 mg) in 3 mL of THF was placed in a dried test tube. The mixture kept at 70°C for 14 h under gentle stirring. The reaction mixture was poured into a large amount of petroleum ether. The resulting precipitates were collected by centrifugation, followed by drying *in vacuo* to give the polymer. $^1$H NMR (DMSO-$d_6$) $\delta$ 0.7 (3H, br, $CH_3$), 1.7 (2H, br, C$H_2$ C($CH_3$)), 2.7 (2H, br, $CH_2$Ar), 4.0 (2H, br, $CH_2$O), 6.8 (4H, d, Ar), 9.2 (1H, br, ArOH); FT-IR (KBr) 3407 ($\upsilon$ O–H), 2954 ($\upsilon$ C–H of $CH_3$), 1724 ($\upsilon$ C = O), 1597, 1516 cm$^{-1}$ ($\upsilon$ C = C of Ar).

A typical run of the copolymerization was as follows (entry 3). A mixture of **1** (10 g), **2** (40 g), and AIBN (0.50 g) in 100 mL of THF was heated at 70°C under nitrogen. After 6 h, the monomer consumption detected by SEC was almost quantitative. The copolymer was isolated by pouring the reaction mixture into a large amount of petroleum ether.

The cured film was prepared by coating a mixture of the copolymer (15 mg) and laccase solution (15 μL, 0.21 mg protein) on a glass slide. The sample stood with 80% humidity at 30°C for 16 h.

For SEC measurement, a Tosoh SC8010 apparatus was used. SEC analysis was carried out by using a refractive index (RI) detector at 40°C under the following conditions: Tosoh G5000 $H_{XL}$, G4000 $H_{XL}$, G3000 $H_{XL}$, and G2000 $H_{XL}$ columns and THF eluent at a flow rate of 1.0 mL min$^{-1}$. The calibration curves were obtained using polystyrene standards. $^1$H NMR, IR, and UV-visible spectra were recorded on a JEOL GSX270, JASCO FT/IR-300E, and Beckman DU-70 spectrometers, respectively. DSC measurement was made at a 10°C min$^{-1}$ heating rate under nitrogen using a TA Instruments DSC2910 differential scanning calorimeter calibrated with an indium reference standard.

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