

# Comparison of Chemical and Enzymatic Emulsion Polymerization of Styrene

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**ABSTRACT:** For the reasonable comparison of chemical and enzymatic polymerization, manganese(III) acetate and manganese peroxidase (MnP)-catalyzed synthesis of polystyrenes were chosen and studied with respect to the yield and the polydispersity of polymer particles. MnP allowed the production of polystyrene in a higher yield and higher polydispersity (82.34% and 2.61) than manganese(III) acetate (77.90% and 1.75). This is believed to be due to a gradual supply of initiator radicals via a cata-

lytic cycle in MnP-catalyzed polymerization, whereas radicals are generated all at once in Mn(III)-mediated polymerization. NMR spectroscopy revealed that atactic polystyrene was produced by emulsion polymerization using Mn(III) or MnP. © 2009 Wiley Periodicals, Inc. *J Appl Polym Sci* 112: 2935–2941, 2009

**Key words:** emulsion polymerization; enzymes; polystyrene; radical polymerization

## INTRODUCTION

As enzymes function under mild conditions such as ambient temperature and atmospheric pressure, enzymatic polymerization has been studied for the last two decades as an environmentally friendly method to synthesize polymers.<sup>1,2</sup> For example, peroxidases such as horseradish peroxidase (HRP), soybean peroxidase (SBP), and manganese peroxidase (MnP) are known to catalyze the polymerization of vinyl compounds,<sup>3–8</sup> phenolic compounds,<sup>8–11</sup> and aromatic amines.<sup>12–15</sup>

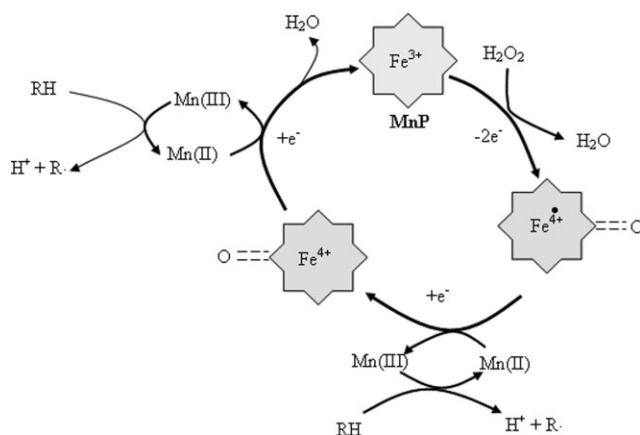
The generation of a primary radical was found to be indispensable for initiating radical polymerization of vinyl compounds, and an initiator such as acetylacetone or 2,4-pentanedione should be oxidized by peroxidases. In the case of HRP and SBP, binding to the initiator molecule should precede its oxidation. However, MnP catalyzes the oxidation of Mn(II) to Mn(III) using hydrogen peroxide as an oxidizing agent (see Fig. 1).<sup>16</sup> Mn(III) is a diffusible oxidant that can oxidize the initiator at a distance from the active site of MnP.<sup>17,18</sup> Unlike HRP and SBP, direct

contact between MnP and the initiator is not necessary for initiation of the polymerization reaction.<sup>19</sup> Hence, chemically prepared Mn(III), for example manganese(III) acetate, similarly acts as a mediator for MnP-catalyzed reactions. Glenn and Gold<sup>20</sup> reported that Mn(III) was capable of oxidizing substrates that were oxidized by MnP, and the rate of MnP-catalyzed oxidation was generally similar to that obtained with Mn(III).

The purpose of this study was to compare peroxidase-catalyzed polymerization with the chemical polymerization with respect to the polymer yield and polydispersity. In other studies with the same purpose, the average molecular weight and the polydispersity of the polymer were found to be higher in the enzymatic polymerization.<sup>3–5,7</sup> Iwahara et al.<sup>3</sup> suggested that this result was caused by the gradual supply of radicals in the enzymatic radical polymerization. In their study, the enzymatic polymerization of acrylamide was performed using HRP and MnP, and 2,4-pentadione was used as the initiator. For the chemical polymerization, ammonium persulfate and Fe<sup>2+</sup> were used as a redox initiator and an electron donor, respectively. The radicals generated in these two polymerization methods were different (e.g., radical of 2,4-pentadione in the HRP or MnP-catalyzed polymerization, and sulfate radical in the chemical polymerization). Hence, not only the rate of radical supply but also the type of radical was different. To explain the difference in enzymatic and chemical polymerization with the radical supply rate

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**Figure 1** Schematic diagram of MnP-catalyzed generation of a radical.<sup>16</sup> Mn(II) and Mn(III) denote  $\text{Mn}^{2+}$  and  $\text{Mn}^{3+}$  ions, respectively.

only, the same radical should have been used for initiating polymerization. In this study, polystyrene was chosen as the polymer to be synthesized. MnP and 2,4-pentanedione were chosen as the mediator and the initiator for enzymatic polymerization, respectively. HRP was also used as a comparative peroxidase. For the reasonable comparison with MnP-mediated polymerization, manganese(III) acetate and 2,4-pentanedione were used as the mediator and the initiator for chemical polymerization, respectively. As shown in Figure 1, the oxidation of Mn(II) to Mn(III) is the only difference between MnP-catalyzed and Mn(III)-mediated polymerization. In the enzymatic synthesis of polystyrene, a mixture of water and a water-miscible organic solvent<sup>4</sup> or a surfactant-aided emulsion<sup>5</sup> has been used to solve the problem of the low solubility of styrene in water. In this study, NP-40 and hexadecane were used as the emulsifying surfactant and cosurfactant, respectively. NP-40, nonylphenol polyethoxylate with an average of 40 ethylene oxide units per molecule, is one of the most commonly used nonionic surfactants.

## EXPERIMENTAL

### Materials

MnP was produced from an immobilized culture of *Phanerochaete chrysosporium* BKM-F-1767 (ATCC 24725) as previously described.<sup>21</sup> After concentration of the culture supernatant by ultrafiltration on an Amicon membrane (Amicon 8050 YM-10, 10 kDa cut-off), MnP was purified by fast-performance liquid chromatography (FPLC) using a Mono Q column (Amersham Pharmacia Biotech).<sup>22</sup> The activity of MnP and HRP was assayed using guaiacol as substrate.<sup>23</sup>

Styrene monomer (99%+, inhibited with 10–15 mg/L 4-*tert*-butylcatechol) was washed five times each with 10 wt % NaOH solution and distilled water, dried over calcium chloride, and passed through a column filled with inhibitor remover (to remove 4-*tert*-butylcatechol). Unless stated otherwise, chemicals used in this study were purchased from Sigma-Aldrich.

### Polymerization of styrene

Deionized water and 200-mM lactate (pH 4.5) and 200-mM phosphate (pH 6.0) buffers were used as solvents for styrene emulsion in Mn(III)-mediated and MnP- and HRP-catalyzed polymerization, respectively. Styrene miniemulsions were prepared by mixing 5 g (48 mmol) of styrene, 0.2 g (0.1 mmol) of NP-40, and 0.2 g (0.88 mmol) of hexadecane with 20 mL of the above solvents for 1 h, followed by 1 min of sonication (Sonoplus HD 2070; Bandelin Electronics, Germany) at maximum output with a 25% duty cycle. Mn(III)-mediated polymerization was initiated by the addition of 16 mg (0.16 mmol) of 2,4-pentanedione and manganese(III) acetate, whereas MnP-catalyzed polymerization was initiated by the addition of 16 mg of 2,4-pentanedione, 0.5 U of MnP,  $\text{MnSO}_4$ , and  $\text{H}_2\text{O}_2$ . HRP-catalyzed polymer-

**TABLE I**  
Effect of Oxidant Concentrations on the Yield and Size of Polystyrene

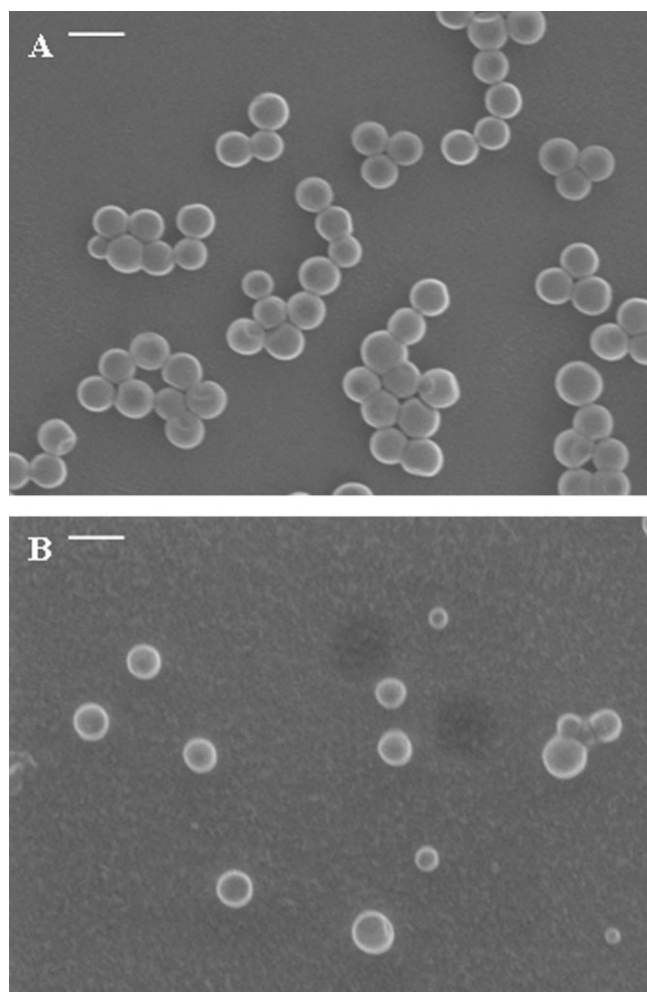
Entry <sup>a</sup>	Mn(III) (mmol) <sup>b</sup>	$\text{MnSO}_4$ (mmol) <sup>c</sup>	$\text{H}_2\text{O}_2$ (mmol) <sup>c</sup>	Yield (%)	Avg. size (nm) <sup>d</sup>	Std. dev. (nm) <sup>d</sup>
1	0.02	–	–	24.08	208	1.61
2	0.04	–	–	36.76	196	1.86
3	0.08	–	–	77.90	233	2.29
4	–	0.02	0.01	44.88	224	6.41
5	–	0.08	0.04	82.34	199	3.43

<sup>a</sup> Entries 1–3 are the results of the Mn(III)-mediated polymerization of styrene, whereas entries 4 and 5 are the results of the MnP-catalyzed polymerization of styrene.

<sup>b</sup> Number of mmol added to 20 mL of deionized water, a solvent of the styrene miniemulsion in the Mn(III)-mediated polymerization.

<sup>c</sup> Number of mmol added to 20 mL of 200-mM lactate buffer, a solvent of the styrene miniemulsion in the MnP-catalyzed polymerization.

<sup>d</sup> Avg. size and Std. dev. are the average particle size and its standard deviation.



**Figure 2** SEM photographs of polystyrene synthesized by Mn(III)-mediated polymerization (entry 3 of Table I) (A) and by MnP-catalyzed polymerization (entry 5 of Table I) (B). Scale bar: 100 nm.

ization was initiated by the addition of 16 mg of 2,4-pentanedione, 0.5 U HRP, and 0.04 mmol of  $\text{H}_2\text{O}_2$ . The reactions were conducted for 12 h with continuous stirring at 25°C. Then the polystyrene synthesized was collected by precipitation in excess methanol (HPLC grade). After evaporation of the supernatant, the precipitate was vacuum-dried.

### Analysis

The average molecular weight of the polystyrene particles was measured by gel permeation chromatography (Waters 515, Waters Corp.) relative to a polystyrene standard using tetrahydrofuran (THF) as the eluent at a flow rate of 1 mL/min. For the analysis of the particle size distribution, dynamic light scattering (DLS) measurements were conducted using a photon correlation spectrometer (Malvern Nano ZS System) equipped with an argon laser at a wavelength of 633 nm (25°C). The morphology of the polystyrene particles was determined by scanning electron microscopy (SEM; S-4200, Hitachi) and their chemical structure was analyzed by NMR spectroscopy (Bruker Advance 500-MHz nuclear magnetic resonance spectrometer, Bruker BioSpin Corp.).

## RESULTS AND DISCUSSION

### Comparison of Mn(III)-mediated and MnP-catalyzed synthesis of polystyrene

The polymerization condition (concentrations of NP-40 and 2,4-pentanedione) employed in this study was determined from the preliminary experiment. When increasing the amount of NP-40 in the 20 mL styrene emulsion from 0.04 to 0.1 mmol, the polymer yield increased and the particle size diminished as reported in other studies.<sup>6,24</sup> The activity of MnP and HRP was not affected by NP-40 in this concentration range. It has been reported that the rate of radical polymerization increases but the molecular weight of a polymer chain decreases as the concentration of an initiator increases.<sup>25</sup> In our preliminary experiment, the dosage of 2,4-pentanedione in the 20 mL styrene emulsion solution was varied between 0.04 and 1.44 mmol. The polymer yield, which was estimated with polystyrene molecules large enough to be precipitated in methanol, was maximum when 0.16 mmol of 2,4-pentanedione was added. Therefore, 0.1 mmol of NP-40 was added to prepare the 20-mL styrene emulsion solution, and 0.16 mmol of 2,4-pentanedione was added as the initiator in this study.

**TABLE II**  
Average Molecular Weights and Polydispersity Index ( $M_w/M_n$ ) Values of Polystyrene<sup>a</sup>

Entry	Polymerization method	$M_w$	$M_n$	$M_w/M_n$
1 <sup>b</sup>	Mn(III)-mediated	332,000	190,000	1.75
2 <sup>c</sup>	MnP-catalyzed	298,000	114,000	2.61

<sup>a</sup> The weight-average molecular weight and the number-average molecular weight of the polymer are denoted by  $M_w$  and  $M_n$ , respectively.

<sup>b</sup> Measured with the polystyrene particle in entry 3 of Table I.

<sup>c</sup> Measured with the polystyrene particle in entry 5 of Table I.

**TABLE III**  
Size Distribution of Emulsion Droplets Before Polymerization

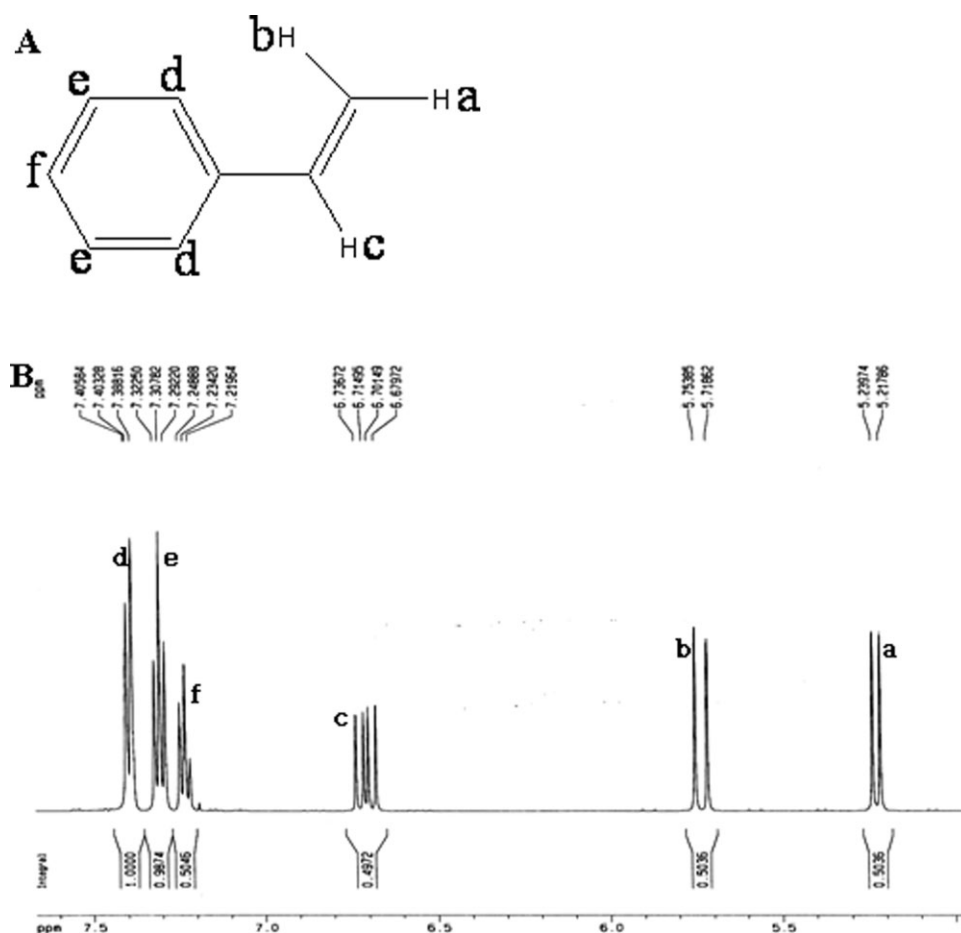
Polymerization method	Mean dia. (nm) <sup>a</sup>	Std. dev. (nm) <sup>a</sup>
Mn(III)-mediated	282	26.3
MnP-catalyzed	278	14.9
HRP-catalyzed	267	37.0

<sup>a</sup> Mean dia. and Std. dev. are the mean diameter of emulsion droplets and its standard deviation.

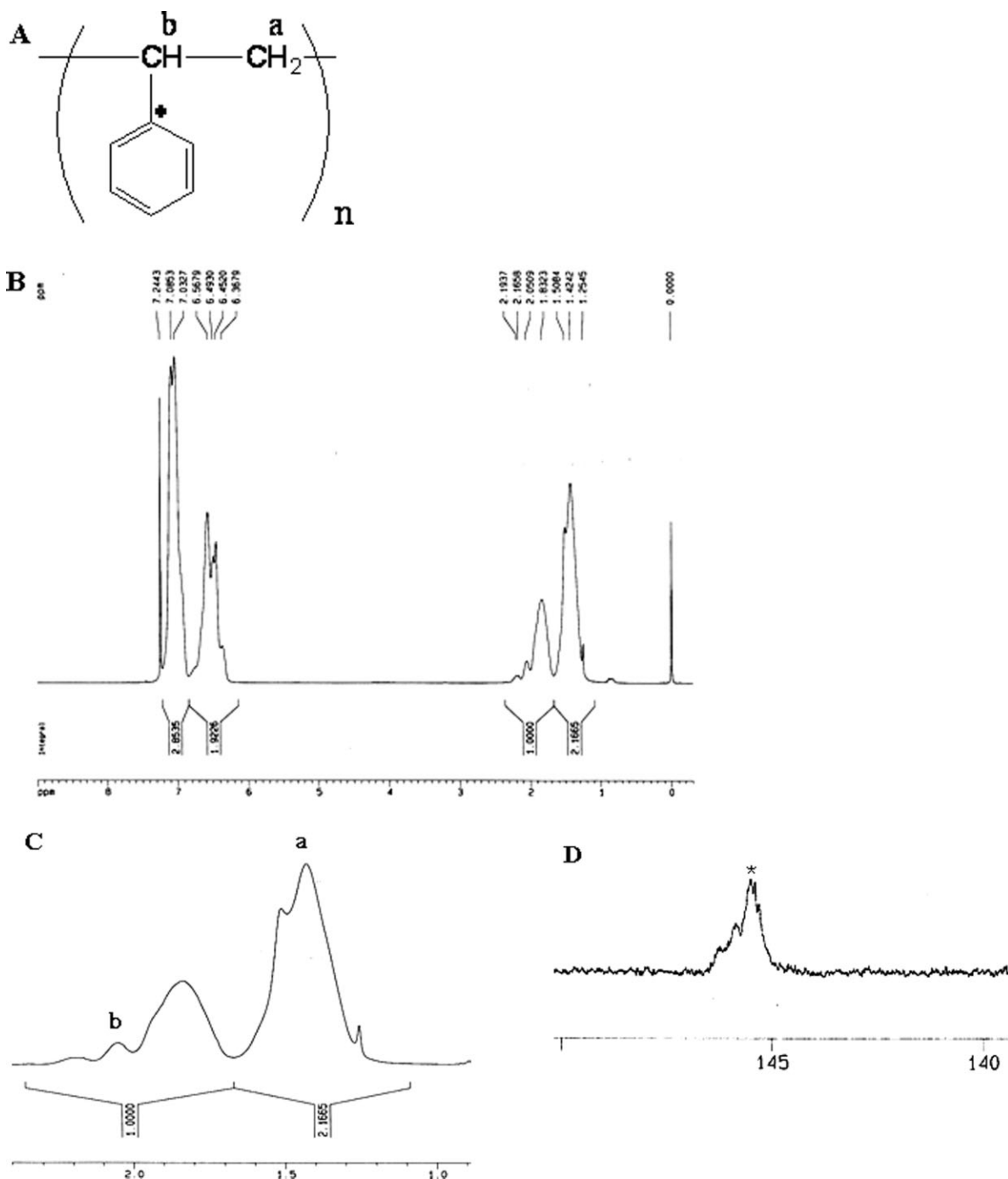
The yield and average size of the polystyrene particles are summarized in Table I. As hydrogen peroxide oxidizes Mn(II) to Mn(III) in the presence of MnP, the effect of manganese(III) acetate on Mn(III)-mediated polymerization corresponds to the effect of MnSO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub> on MnP-catalyzed polymerization. As expected, an increase in the level of Mn(III) or MnSO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub> resulted in an increased yield of polystyrene. Comparison of results obtained under similar reaction conditions (entries 1 and 4, and entries 3 and 5, Table I) reveals that the yield was higher for MnP-catalyzed polymerization than for Mn(III)-mediated polymerization. Iwahara et al.<sup>3</sup> also reported that a higher yield was obtained for

enzymatic radical polymerization. The particle size was more uniform for Mn(III)-mediated polymerization than for MnP-catalyzed polymerization, which was also evident in SEM images of polystyrene particles (Fig. 2).

The weight-average molecular weights ( $M_w$ ), the number-average molecular weights ( $M_n$ ) and their ratio ( $M_w/M_n$ ), usually referred to as the polymer polydispersity index, were measured and are summarized in Table II. A highly disperse polymer was synthesized with MnP, which agreed well with the results of other studies.<sup>3-5,7</sup> As emulsion polymerization of styrene involves radical generation followed by styrene addition at the interface between the aqueous phase and emulsion droplets, the yield and polydispersity of the polymer might be affected by the availability of initiator radicals and/or factors affecting the interface quality, such as the size distribution of emulsion droplets. The size distribution of emulsion droplets analyzed before polymerization is listed in Table III and shows little difference between the polymerization methods tested in this study. Initiator radicals are formed almost simultaneously during chemical polymerization such as Mn(III)-mediated polymerization, and the polydispersity



**Figure 3** Chemical structure (A) and <sup>1</sup>H-NMR spectrum (B) of styrene.



**Figure 4** Chemical structure (A) and  $^1\text{H-NMR}$  spectrum (B) of polystyrene, portion of  $^1\text{H-NMR}$  spectrum of polystyrene attributed to methine and methylene protons (C), and portion of  $^{13}\text{C-NMR}$  spectrum of polystyrene attributed to phenyl C-1 carbon (marked as \*) (D).

index ( $M_w/M_n$ ) is close to 1. During enzymatic radical polymerization, the enzyme oxidizes the initiator with the aid of an oxidant (e.g.,  $\text{H}_2\text{O}_2$  in this study) Especially in MnP-catalyzed polymerization, MnP-catalyzed oxidation of Mn(II) to Mn(III) is followed by the Mn(III)-mediated generation of initiator radicals (see Fig. 1). Hence, the rate of formation of 2,4-pentanedione radicals depends on the enzyme activity and  $\text{H}_2\text{O}_2$  concentration. As described in the Experimental

section, the activity of MnP and HRP was determined using guaiacol as substrate. At most, 0.5 U of peroxidase generates 2.0  $\mu\text{mol}$  of guaiacol radicals every minute.<sup>21,23</sup> Assuming the same reactivity for guaiacol and 2,4-pentanedione, the maximum rate of generation of 2,4-pentanedione radicals by 0.5 U of MnP and HRP would be 2.0  $\mu\text{mol}/\text{min}$ . As 20–80  $\mu\text{mol}$  of Mn(III) were initially present during Mn(III)-mediated polymerization (see entries 1–3, Table I), 20–80  $\mu\text{mol}$

of 2,4-pentanedione radicals must have been formed almost simultaneously. It is therefore believed that a gradual supply of radicals would lead to the higher polydispersity index of polystyrene for MnP-catalyzed polymerization compared with Mn(III)-mediated polymerization.

Interestingly, the average molecular weight and yield of polystyrene were lower for synthesis by HRP compared with MnP. Even though 0.5 U of HRP and 0.04 mmol of H<sub>2</sub>O<sub>2</sub> were added similar to entry 5 of Table I, the yield was 23.24%, and the weight and number average molecular weights were 34,849 and 9,043, which were all much less than those attained with MnP (82.34%, 298,000 and 114,000; see entry 5 of Table I and entry 2 of Table II). As HRP-catalyzed oxidation requires the enzyme binding to the substrate, the oxidation activity of HRP strongly depends on the substrate, and HRP is believed to exhibit higher activity for guaiacol than for 2,4-pentanedione. In the case of MnP, the oxidation of guaiacol and 2,4-pentanedione is conducted by diffusible MnP-generated Mn(III) and does not require direct binding of the substrate to MnP. Hence, MnP-generated Mn(III) exhibits lower substrate selectivity than HRP.

### Polymer characterization

Figures 3 and 4 show NMR spectra of styrene and polystyrene, respectively. <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of polystyrene synthesized by MnP were same as those by Mn(III). Peaks in the <sup>1</sup>H-NMR spectra of styrene and polystyrene are assigned as follows. Styrene: <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 6.70 (q, 1H, =CH), 5.22–5.73 (dd, 2H, =CH<sub>2</sub>), 7.21–7.40 (m, 5H, Ar-H). Polystyrene: <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 2.01 (s, 1H, -CH), 1.38 (s, 2H, -CH<sub>2</sub>), 6.36–7.24 (m, 5H, Ar-H). As polymerization occurred, the peaks at 6.70 (=CH) and 5.22–5.73 ppm (=CH<sub>2</sub>) in the <sup>1</sup>H-NMR spectrum of styrene disappeared, and broad peaks attributed to methine (-CH-) and methylene (-CH<sub>2</sub>-) protons appeared at 2.01 and 1.38 ppm, respectively, [Fig. 4(C)]. The chemical shift values and splitting patterns indicate that the polystyrene synthesized in this study was atactic.<sup>26</sup> Figure 4D shows the portion of the <sup>13</sup>C-NMR spectrum attributed to the phenyl C-1 carbon, which is the most important signal for determining the stereoregularity of polystyrene.<sup>26</sup> The appearance of multiple peaks in the range 145–146 ppm is further evidence that atactic polystyrene was prepared by emulsion polymerization using Mn(III) or MnP.

### CONCLUSIONS

Mn(III)-mediated and MnP-catalyzed emulsion polymerizations of styrene were performed to compare

chemical and enzymatic polymerization reactions in terms of polymer yield and polydispersity. The initial dosage of hydrogen peroxide was adjusted for MnP polymerization so that the amount of MnP-generated Mn(III) would be equal to the initial dosage of Mn(III) for Mn(III)-mediated polymerization. Under similar reaction conditions (same concentrations of styrene, emulsifiers, and initiator), the polymer yield and polydispersity were higher for MnP-catalyzed polymerization (82.34%, 2.61) than for Mn(III)-mediated polymerization (77.90%, 1.75). This is believed to be due to a gradual supply of initiator radicals during enzymatic polymerization. HRP, one of the most widely studied peroxidases for polymerization, was tested as a comparative peroxidase. The activity of the two enzymes was determined using guaiacol as substrate. When HRP was used at the same activity as MnP, it produced polystyrene in lower yield and with lower *M<sub>w</sub>* and *M<sub>n</sub>* values (23.24%, 34,849, and 9,043) compared with MnP-catalyzed styrene polymerization (82.34%, 298,000, and 114,000). HRP-catalyzed oxidation requires enzyme binding to the substrate, whereas such direct binding is not necessary for MnP. Hence, the oxidation activity of HRP strongly depends on the substrate and the enzyme exhibits higher activity for guaiacol than for 2,4-pentanedione oxidation. NMR spectroscopic analysis revealed that atactic polystyrene was prepared by emulsion polymerization using Mn(III) or MnP.

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