

Jinyu Shan  
Yoshiro Kitamura  
Hidekazu Yoshizawa

## Emulsion polymerization of styrene by horseradish peroxidase-mediated initiation

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J. Shan · Y. Kitamura · H. Yoshizawa (✉)  
Department of Environmental Chemistry  
and Materials, Faculty of Environmental  
Science and Technology,  
Okayama University,  
3-1-1 Tsushima-Naka,  
Okayama 700-8530, Japan  
E-mail: yhide@cc.okayama-u.ac.jp  
Tel.: +81-86-251-8909  
Fax: +81-86-251-8909

**Abstract** A new horseradish peroxidase (HRP)-catalyzed redox initiating system was successfully applied in the emulsion polymerization of styrene, producing stable polymer colloids and nanospheres. The particle size was about 30–50 nm with a diameter distribution (CV) 14–20% obtained by SEM. The molecular weight,  $\bar{M}_n$ , was around  $10^5$ , and  $\bar{M}_w$  was around  $10^6$ . All the results were well consistent with the control, potassium persulfate (KPS)-initiating polymerization. The obtained

experimental results supported a micelle mechanism similar to that of conventional emulsion polymerization of styrene.

**Keywords** Horseradish peroxidase (HRP) · Enzyme-catalyzed redox initiation · Styrene · Emulsion polymerization · Nanospheres

### Introduction

During the last 2 decades, enzymatic polymerization, as a new method of polymer synthesis, has brought out a breakthrough for innovative materials [1–3]. The fundamental problem was the conflict between the aqueous environment required by enzymes and the hydrophobicity of most monomers. Many attempts to accommodate enzymes to an inveterate organic medium have been made, resulting in the new realization that many enzymes can work in organic solvents containing little or no added water [4–6]. Enzyme can be used in water–water-miscible organic solvent, anhydrous organic solvent (containing water below the solubility limit) and reverse micelles (enzymes are dissolved in micropools of water, which are emulsified in water-immiscible solvents).

Recently, a horseradish peroxidase (HRP)-catalyzed redox system was reported to initiate vinyl polymerization of acrylamide in water, in which  $\beta$ -diketone was adopted as the substrate of HRP to generate primary radicals in the presence of hydrogen peroxide [7–9]. The

representative  $\beta$ -diketone was acetylacetone (Acac). This is a “natural way” to generate primary radicals in water and at room temperature. It includes no organic peroxide or azo structure of conventional initiators with high risk in preparation and storage; the oxidant  $H_2O_2$  with high concentration is also a hazardous substance, but 30% solution used in the initiating system is enough safe in preparation, storage and handling. It also includes no metal salt associated with hazardous residue in the conventional redox initiating system used at room temperature. Therefore, it is a potential “natural initiating process” for radical polymerization. But it is water-soluble, while most of monomers of radical polymerization are hydrophobic. Initiation of the polymerization of hydrophobic methyl methacrylate (MMA) and styrene in a water-mixed organic solvent has been attempted, and the highly hydrophobic styrene yielded only 21.2% [10, 11].

It is well known that emulsion polymerization of hydrophobic monomers with a water-soluble initiator can produce polymer microspheres. Emulsion polymerization of phenol derivatives catalyzed by HRP in the

presence of H<sub>2</sub>O<sub>2</sub> has been reported and it was found that the molecular weight was controllable and molecular weight distribution was narrower [12]. And emulsifier-free emulsion polymerization of MMA initiated by HRP/H<sub>2</sub>O<sub>2</sub>/Acac was reported with a higher yield and faster reaction rate than in water–dioxane [13]. However, in these previous studies, there is no comment on the characterization of isolated product, such as morphology, diameter etc. Effect of various parameters has not been clarified yet. The above cited phenol, MMA is water-soluble in some extent, while research on enzymatic emulsion polymerization of higher hydrophobic monomers like styrene has not been found. Moreover, emulsion polymerization of hydrophilic monomer will have a more complicated mechanism. Therefore, we selected hydrophobic monomer, styrene, to study enzymatic emulsion polymerization initiated by HRP/H<sub>2</sub>O<sub>2</sub>/Acac, and successfully obtained stable colloids and isolated nanospheres.

## Materials and methods

### Reagent

HRP (100 unit/mg) was supplied by Wako. Styrene (99%, inhibited with 30 ppm 4-*t*-butylpyrocatechol), 2,4-pentanedione (Acac), H<sub>2</sub>O<sub>2</sub> (30% wt), sodium dodecyl sulfate (SDS), potassium persulfate (KPS) and methanol were obtained from Wako. Styrene monomer was purified by distillation under reduced pressure, and the distillate was stored in refrigerator at –30°C prior to use.

### Polymerization

Styrene emulsion (reagent grade water prepared with Millipore Milli-Q system) was made by homogenizer with the speed of 20,000 rpm/min for 2 min, which included 1% (0.11 g) SDS, 10 g H<sub>2</sub>O, and 12 mmol styrene. The obtained emulsion droplets were enough small to be stable in polymerization, therefore, mild mixing by electromagnetic stirring was exerted during polymerization. The obtained emulsion was introduced to a flask and deaerated by vacuum and supersonic wave, then flushed with nitrogen for 30 min. HRP (20 mg×100 units) was dissolved in 1 mL of water. Acac and 30% H<sub>2</sub>O<sub>2</sub> were added after the addition of HRP. After 24 h continuous stirring by electromagnetic stirring at room temperature, half of the reaction mixture (5 mL) was poured into methanol and filtered to obtain isolated nanospheres, another half (about 6 mL) was kept.

Conventional emulsion polymerization of styrene with 1 wt% KPS was also conducted for comparison, reaction temperature was 70 °C.

### Analysis

The molecular weight of isolated nanospheres was measured by gel permeation chromatography (Tosoh HLC8220 GPC) relatively to the polystyrene standard with THF as the eluent and the chemical structure was analyzed by FT-IR spectroscopy (Shimadzu, FTIR-8600PC, KBr method). Scanning electron microscope (SEM) was performed with Hitachi S-4700 FE-SEM, and the SEM photograph was analyzed by Winroof for particle size.

The particle size of obtained colloids was measured by a dynamic light scattering method (Otsuka electronics, DLS-4700). And the conversion of styrene was determined by high-pressure liquid chromatography (HPLC) method, the chromatographic system (Tosoh Co.) consisted of a UV spectrophotometric detector ( $\lambda = 254$  nm, UV-8010), a CCPM pump, a TSK-gel column (ODS-80Ts), a column oven (CO-8020) and a chromatopack recorder (SC-8010).

## Result and discussion

### Emulsion polymerization of styrene initiated by HRP/H<sub>2</sub>O<sub>2</sub>/Acac

From the previous work [14, 15], we found that the amount of H<sub>2</sub>O<sub>2</sub> was a major influence on polymerization. So first, we adjusted its amount, and obtained stable colloids in the range of 0.01–0.11 mmol. The main results are shown in Table 1.

Photographs of the polystyrene colloid obtained by enzyme-mediated (run 9) and KPS-initiating emulsion polymerization are shown in Fig. 1. The polystyrene colloid obtained by enzyme-mediated emulsion polymerization is very similar to that by KPS-initiating emulsion polymerization in appearance, but with a little color. The obtained colloid was very stable, and no obvious change was observed after half-year storage at room temperature. The yield of isolated nanospheres was about 40–60% except for entry 11, and the conversion of styrene was achieved at a rate of about 86–96%. The polymerization initiated by KPS achieved rates of 64% and 98%, respectively. The difference between yield and conversion in both systems were attributed to the difficulty in recovery of nanospheres product.

### Particle size and distribution of the prepared microspheres

Figure 2 shows SEM photographs of isolated nanospheres in entries 3 and 8. The particle size was about 30–50 nm, which is well consistent with the results

**Table 1** Emulsion polymerization of styrene initiated by HRP/H<sub>2</sub>O<sub>2</sub>/Acac\*: influence of the amount of H<sub>2</sub>O<sub>2</sub>

Entry	H <sub>2</sub> O <sub>2</sub> (mmol)	Yield (%)	Conversion (%)	$\bar{M}_n$ ( $\times 10^5$ )	$\bar{M}_w$ ( $\times 10^5$ )	$\bar{M}_w/\bar{M}_n$	d <sup>1</sup> (nm)	CV <sup>1</sup>	d <sup>2</sup> (nm)	CV <sup>2</sup>
1	0.01	59.8	90.3	1.73	14.5	8.34	39.3	10.7	35.8	20.6
2	0.02	51.3	89.1	2.20	11.7	5.30	35.1	7.89	35.7	17.5
3	0.03	43.4	85.9	2.02	7.54	3.73	34.0	8.47	34.0	15.9
4	0.04	59.5	92.9	3.35	12.1	3.61	39.6	9.74	37.9	14.8
5	0.05	64.3	93.5	3.97	12.0	3.04	42.8	8.42	40.9	17.6
6	0.06	49.4	95.1	3.60	11.9	3.29	45.9	5.96	42.9	15.1
7	0.07	45.2	91.2	5.16	16.4	3.19	52.8	9.76	50.5	20.2
8	0.08	55.4	92.8	5.06	15.0	2.96	47.9	9.88	46.1	16.1
9	0.09	41.8	95.2	3.94	13.6	3.46	47.9	6.13	44.7	16.5
10	0.10	53.8	93.1	4.75	14.8	3.11	51.3	7.91	46.3	16.2
11	0.11	27.7	96.0	2.44	8.47	3.47	41.0	9.89	38.6	14.2

\*12 mmol styrene, 1% SDS (0.11 g), 10 mL water, 20 mg HRP dissolved in 1 mL water, 0.12 mmol Acac, 24 h reaction at room temperature

d<sup>1</sup>, CV<sup>1</sup> is the average particle diameter and diameter distribution determined by DLS; d<sup>2</sup>, CV<sup>2</sup> is that analyzed from SEM photograph by Winroof

$\bar{M}_n$ , number average molecular weight;

$\bar{M}_w$ , weight average molecular weight;

$\bar{M}_w/\bar{M}_n$ , molecular weight distribution



**Fig. 1** Photographs of the polystyrene colloid obtained by enzyme-mediated (run 9) emulsion polymerization (left) and that by KPS-initiating emulsion polymerization (right)

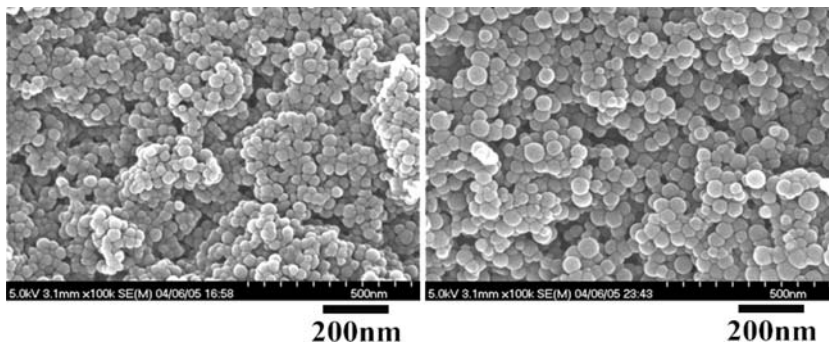
determined by DLS (see in Table 1, Fig. 3). In the polymerization initiated by KPS, the average diameter analyzed by SEM was 44.3 nm, and that determined by DLS was 48.3 nm. The diameter distribution (CV) in

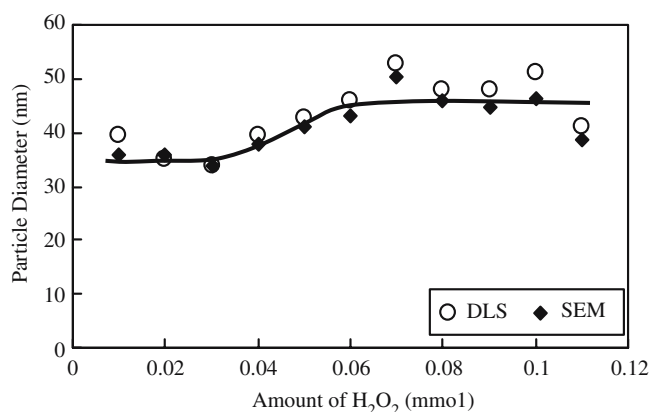
enzyme-initiating polymerization obtained by the two methods was in the range of 6–10 and 14–20, respectively, comparable to the values (7.9 and 16.1) obtained from the polymerization initiated by KPS. Figure 3 shows the effect of the oxidant amount on particle size. The average diameter plateaued at about 35 nm in the range of 0.01–0.03 mmol and 46 nm in the range of 0.06–0.10 mmol, and increased from 35 nm to 46 nm within the range 0.03–0.06 mmol.

#### Chemical analysis of the prepared microspheres

The molecular weight of the obtained polymer,  $\bar{M}_n$ , was around  $10^5$ , while  $\bar{M}_w$  was around  $10^6$ , values which were almost equal to those by KPS-initiating polymerization ( $\bar{M}_n$   $5.92 \times 10^4$ ,  $\bar{M}_w$   $7.13 \times 10^5$ ). We took a sample 30 min after H<sub>2</sub>O<sub>2</sub> was added, then analyzed it by GPC. The obtained molecular weight was very close to that of the isolated nanospheres. The phenomenon of quick reaction is a characteristic of emulsion polymerization, so that a micelle mechanism similar to that of conventional emulsion polymerization was believed to have

**Fig. 2** SEM photographs of isolated nanospheres obtained from HRP-mediated emulsion polymerization (entries 3 and 8)





**Fig. 3.** Effect of the oxidant amount on particle size of HRP-mediated emulsion polymerization

occurred. It also should be noticed that the molecular weights of entries 1 and 2 have double peaks, which leads to a wide distribution. Considering the phenomenon occurred only at the lower amount of H<sub>2</sub>O<sub>2</sub>, we guess that it was brought about by insufficiency of the primary radical.

In the IR spectrum of isolated nanosphere, the C–H stretch at 2,900~3100 cm<sup>-1</sup>, the aromatic ring C=C

stretch at 1,601, 1,493, 1,453 cm<sup>-1</sup>, and the absorption at 2,000~1,667 cm<sup>-1</sup>, and 500~800 cm<sup>-1</sup> are in a good accordance with the standard IR spectrum of polystyrene [16]. A new absorption at 1,640 cm<sup>-1</sup> was due to the C=O stretch of Acac, and the broad band at 3,300 cm<sup>-1</sup> was due to OH hydrogen bonding of an enol tautomer of Acac, indicating a polystyrene chain with an Acac unit as terminal group.

## Conclusions

In the present study, we applied HRP/H<sub>2</sub>O<sub>2</sub>/Acac to initiate the emulsion polymerization of styrene, and successfully obtained stable polymer colloids and isolated nanospheres. The obtained experimental results supported a micelle mechanism similar to that of conventional emulsion polymerization of styrene. By studying it, a general approach for enzymatic emulsion polymerization of hydrophobic monomer has been established, which provided a new enzymatic approach to prepare vinyl polymer colloid and nanospheres at room temperature. More detailed studies around the initiation activity of HRP/H<sub>2</sub>O<sub>2</sub>/Acac in emulsion polymerization are now in progress.

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