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ORIGINAL PAPER

Horseradish peroxidase (HRP) nanoflowers-mediated polymerization of vinyl monomers

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Abstract

The effects of flower-shaped hybrid nano biocatalyst (hFe-NFs) from coordination between horseradish peroxidase (HRP) enzyme and Fe^{2+} ions on the free-radical polymerization reactions of three different vinyl monomers (styrene, methylmethacrylate and acrylamide) were investigated. Polymerizations of styrene and methylmethacrylate (MMA) were performed under emulsion conditions using three different surfactants in the presence of acetylacetone (AcAc) and hydrogen peroxide (H_2O_2) initiator. Polymerization of water soluble acrylamide was accomplished under surfactant-free media. According to the obtained outcomes, hFe-NFs exhibited higher catalytic activity towards polymerization of vinyl monomers compared to the free-HRP enzyme in terms of yields and the number average molecular weights (M_n) of the synthesized polymers. hFe-NFs also demonstrated very high thermal stability. While optimum polymerization of styrene was achieved at room temperature (RT), the highest polymerization yields for acrylamide and MMA were respectively accomplished at 70 and 60 ℃ in which free-HRP enzyme loses its catalytic activity. Preparation of the flower-shaped hFe-NFs, therefore, enables inexpensive and stable catalyst system for free-radical polymerization of vinyl monomers compared to free-HRP enzyme. Increasing catalytic activity and stability of hFe-NFs at higher reaction temperatures are very crucial for utilization of these types of catalysts in both scientific and industrial purposes.

Keywords Horseradish peroxidase · Nanoflowers · Polymerization · Styrene · Methylmethacrylate · Acrylamide

Introduction

Enzyme mediated polymerizations have been extensively investigated for the synthesis of a variety of polymers including polyphenols, polyanilines etc [1]. High selectivity, mild and environmentally benign properties of enzymatic reactions provide alternative option for the synthesis of complex and stereoselective compounds which difficult to be synthesized by traditional synthetic pathways [2]. Peroxidase enzymes, such as horseradish peroxidase (HRP), generate radical-cation species in the presence of hydrogen peroxide (H_2O_2) and initiate free-radical polymerization [3]. Although enzymes show environmental friendly and simple procedure to be used, their high cost and denaturation under large scale applications including high temperatures and organic solvents limit their use in a various applications [4].

In order to enhance stability and reusability of enzymes, immobilization of enzymes onto solid supports was introduced, and extensive studies have been conducted in this field [5]. Interactions between enzyme and a support material through chemical bonding and physical interactions provide some specific chemical, biochemical and kinetic properties for immobilized enzyme [6, 7]. Even though immobilization allows increasing stability and recyclability of enzymes, catalytic activities of immobilized enzymes were reported to be lower compared to that of the free-enzymes due to the unsuitable conformation and mass-transfer limitations between enzyme and solid substance [5, 8].

Recently, organic-inorganic flower-shaped hybrid nanobiocatalysts were discovered using enzymes and

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metal ions (Cu^{2+}, Fe^{2+} etc.) [5, 9]. Coordination reactions between amide groups of the protein structure and metal ions provide appropriate nucleations for the formation of flower-shaped nano structures. Formation mechanism of hybrid nanoflowers was explained as nucleation and growth steps of three main components including HRP enzyme, metal ions (Fe (II) ions) and phosphate buffer. Interactions between phosphate and iron (II) ions generate iron phosphide complexes at the beginning of nucleation step. Then, binding HRP enzyme to iron phosphide nanocrystals starts nucleation, and generated hFe-NFs nanocrystals starts growing through assembly on the surface. Formation of hFe-NFs is completed after nanocrystal growth is attained to the saturation [10]. Although micrometer-sized particles were observed according to the SEM images, these catalysts are defined as nanoflowers due to their nano-scale properties. A variety of enzymes have been used to generate hybrid nanoflowers, and obtained biocatalysts exhibited much higher catalytic activity and stability compared to their free-enzymes. Investigation of very high catalytic activity and stability of the synthesized nanoflowers was defined as follows: (1) large surface area of the nanoflowers, (2) reduced mass-transfer limitations, (3) favorable conformation of the enzyme in the nanoflower, and (4) interactions between metal ions and protein molecules [5, 9, 11]. Ocsoy and coworkers recently discovered flower-shaped hybrid HRP-Fe^{2+} (hFe-NFs) nanobiocatalyst from the complexation between free-HRP enzyme and Fe^{2+} ions, and they investigated catalytic activity of the hFe-NFs on the oxidation of guaiacol [10]. According to the report, hFe-NFs showed considerably high catalytic activity and stability compared to the free-HRP enzyme. In the study, catalytic activity and stability of the nanoflowers were only investigated, and no information related to the oxidation product or polymerization behavior of guaiacol was given. As far as we know, there are also a limited number of studies existing in the literature about utilization of flower-shaped hybrid nano biocatalysts on the polymerization reactions [12].

In the present study, we demonstrate utilization of hybrid organic-inorganic flower-shaped nano biocatalyst (hFe-NFs) from coordination between HRP enzyme and Fe^{2+} ions for the polymerization of some vinyl monomers including styrene, acrylamide and MMA. hFe-NFs demonstrated very high catalytic activity towards polymerization of vinyl monomers. hFe-NFs also showed quite high thermal stability even at 70 °C reaction temperature in which free-HRP enzyme loses its catalytic activity [13]. Increasing stability at higher reaction temperatures is very beneficial for some polymerizations requiring high temperatures.

Materials and methods

Chemicals

Methanol (Isolab, catalog# 947046), styrene (Sigma Aldrich, catalog# 8076791000), acrylamide (Sigma Aldrich, catalog# 8008301000), MMA (Sigma Aldrich, catalog# 8005901000), AcAc (Sigma Aldrich, catalog# 8000231000), tween 40 (Sigma Aldrich, catalog# 8221851000), cetyltrimethylammonium bromide (Sigma Aldrich, catalog# 8141190500), sodiumdodecyl sulfate (Sigma Aldrich, catalog# 8220501000), pH 7.4 phosphate buffered saline (PBS, MP biomedicals, catalog# 2810305), hydrogen peroxide (Merck, catalog# 1.08597), iron (II) sulfate ($FeSO_4$, Sigma-Aldrich, catalog#F8263), horseradish peroxidase (HRP, Sigma-Aldrich, 77332, lyophilized, powder, beige, ~ 150 U/mg) were purchased and used for the synthesis of the nanoflowers and polymers.

Instrumentation

Scanning electron microscope (SEM) images were monitored using Zeiss Gemini 500 instrument. Bruker Avance III 400 MHz was used for ^{1}H and ^{13}C nuclear magnetic resonance (NMR) analyses of the polymers. Thermogravimetric analyses (TGA) of the obtained polymers were accomplished using Mettler-Toledo TGA/DSC 1 Star system thermal analyzer under nitrogen atmosphere (N_2). Approximately, 5–10 mg samples were taken for the analysis, and thermal decomposition temperatures were determined at a temperature range from room temperature (RT) to 600 °C with 10 °C/min heating rate. Differential scanning calorimetry (DSC) thermograms were recorded using a Mettler-Toledo DSC 1 Star system instrument at a heating rate of 10 °C/min from − 50 to 450 °C under N_2 atmosphere. Shimadzu LC-20AD instrument was used for gel permeation chromatography (GPC) analyses. GPC analyses were performed using Agilent PLgel mixed-B column with HPLC purity *N*, *N'*-dimethylformamide (DMF) mobile phase at a flow rate of 1.0 mL/min at 40 ºC. Polystyrene (PS) standards were used for calibration. GPC chromatograms of the synthesized polymers were given in Figs. S7-S20 (Supplementary information).

Synthesis of hFe-NFs nanoflowers

0.1 mg/mL Fe^{2+} solution was added to 0.8 mM HRP solution prepared in pH 7.4 PBS buffer. The obtained mixture was stirred at RT for 3 min and incubated at + 4 °C for 72 h. After that, the solution was centrifuged and obtained light blue precipitate was washed with pure water several times to remove impurities. Collected hFe-NFs nanoflowers was dried in an oven at 40 °C [10].

Polymerization of styrene

Before the polymerization, styrene was extracted with NaOH (10%) solution to remove inhibitors. Styrene (6.0 mmol, 0.69 mL) was put in a test tube with screw cap, and 10 mL of pure water was added in the tube. The obtained solution was purged with N_2 to remove dissolved oxygen while stirring. H_2O_2 (1.0 mmol), hFe-NFs (5.0 mg), surfactant (0.1 g Tween 40) and AcAc (0.05 mmol) were added to the mixture, and final solution was again purged with N_2 gas. The mixture was stirred at the room temperature for 24 h. Then, the mixture was poured into methanol, and obtained white precipitate was filtered, washed with methanol and dried in an oven [14]. Polystyrene formation (Table 1, entry 2) = ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.25–6.45 (m, 5 H, Ar-H), 1.83 (bs, -CH, 1H), 1.52–1.25 (m, $-CH_2$, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 145 (=C-), 128 (=C-), 127 (=C-), 126 (=C-), 43 (-CH), 40 ($-CH_2$).

Polymerization of other vinyl monomers

10 mL distilled water and 6.0 mmol monomer (acrylamide or MMA) were put in a test tube with screw cap, and the solution was purged with N_2 to remove dissolved oxygen. 5.0 mg hFe-NFs, H_2O_2 (1.0 mmol), 0.1 g of tween 40 and AcAc (0.05 mmol) were added to the solution, and final mixture was again purged with N_2. Tween 40 was not added to the polymerization of acrylamide due to solubility of acrylamide in water. The mixture was stirred at the desired reaction temperature for 24 h. Then, it was poured into methanol, and the obtained white precipitate was filtered, washed with methanol and dried in an oven [1, 15]. Polyacrylamide formation (Table 2, entry 14) = ^{1}H NMR (400 MHz, D_2O) δ ppm: 2.2 (-CH), 1.6 ($-CH_2$). ^{13}C NMR (100 MHz, D_2O) δ ppm: 179 (-C=O), 35 (CH), 34 (CH_2). Polymethylmethacrylate formation (Table 2, entry 16) = ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.58 (s, 3 H, -OMe), 1.79 (s, $-CH_2$, 2 H), 1.0–0.82 (s, $-CH_3$, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 177 (-C=O), 54 (CH_2), 51 ($-OCH_3$), 45 (-C), 19 ($-CH_3$).

Results and discussion

The SEM image of the flower-shaped hybrid hFe-NFs nano biocatalyst obtained from the coordination between HRP enzyme and Fe^{2+} ion is illustrated in Fig. 1. As can be seen from the SEM image, the hFe-NFs has flower-shaped and porous morphology with approximately 8.0

Table 2 Polymerization of other vinyl monomers by hFe-NFs in the presence of H_2O_2 and AcAc

Entry[a]	Monomer	T_p (°C)	Yield (%)	M_n (kDa)	Đ
12	Acrylamide	RT	0	-	-
13	Acrylamide	50	35	91	2.20
14	Acrylamide	70	92	100	1.57
15[b]	MMA	RT	15	14	1.30
16[b]	MMA	60	86	147	2.11

[a]all polymerizations were carried out with 6.0 mmol of monomer in 10 mL of water in the presence of 5.0 mg hFe-NFs, 0.05 mmol of AcAc and 1.0 mmol of H_2O_2 in 24 h, [b]0.1 g of tween 40 surfactant was added for the polymerization mixtures. T_p= polymerization temperature, M_n: the number average molecular weight, *Đ*: polydispersity index

Table 1 hFe-NFs mediated polymerization of styrene in the presence of AcAc and H_2O_2 initiators

Entry[a]	hFe-NFs (mg)	H_2O_2 (mmol)	Surfactant	T_p (°C)	Yield (%)	M_n (kDa)	Đ
1	2.5	1.0	0.1 g Tween40	RT	63	301	1.62
2	5.0	1.0	0.1 g Tween40	RT	94	450	2.06
3	7.5	1.0	0.1 g Tween40	RT	90	312	1.90
4	10.0	1.0	0.1 g Tween40	RT	83	200	1.58
5	5.0	1.0	0.1 g Tween40	40	90	210	1.71
6	5.0	1.0	0.1 g Tween40	50	63	114	1.90
7 [b]	5.0	1.0	0.1 g Tween40	RT	89	222	1.72
8	5.0	0.5	0.1 g Tween40	RT	60	207	1.75
9	5.0	2.0	0.1 g Tween40	RT	92	185	2.20
10	5.0	1.0	0.1 g SDS	RT	0	-	-
11	5.0	1.0	0.1 g CTAB	RT	70	112	2.38

[a]all polymerizations were carried out with 690 μL of styrene (6.0 mmol) in 10 mL water in the presence of 0.05 mmol of AcAc, tween 40 surfactant and H_2O_2 in 24 h; [b]The amount of AcAc was 0.1 mmol, other parameters were kept the same as condition a; SDS = sodiumdodecyl sulfate, CTAB = cetyltrimethylammonium bromide, T_p= reaction temperature, M_n: the number average molecular weight, *Đ*: polydispersity index

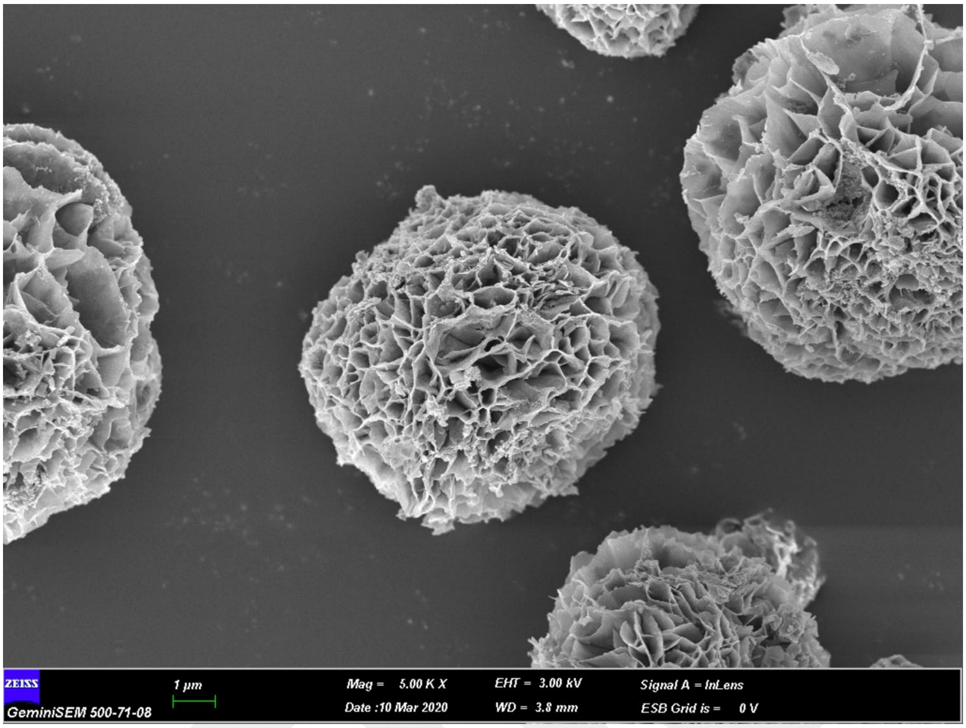

Fig. 1 SEM image of the hFe-NFs

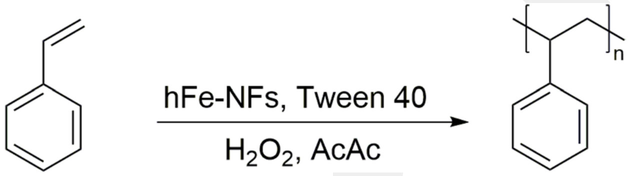

Fig. 2 hFe-NFs mediated polymerization of styrene in the presence of H_2O_2, AcAc and tween 40 surfactant

µm in size. Although micrometer-sized particles were observed according to the SEM images, hFe-NFs show nanoscale properties. Therefore, hFe-NFs nanobiocatalyst was defined as nanoflowers [5, 9]. According to previous report by Ocsoy and coworkers, the mass percentage of iron ion (Fe^{2+}) in the hFe-NFs was approximately 31%. The obtained nanoflowers were also reported to show about 512% higher catalytic activity compared to free-HRP enzyme on the oxidation of guaiacol. While free-HRP enzyme lost about 62% of its stability in 30 days, hFe-NFs hybrid nano biocatalyst lost only 2.5% of its stability in the same period [10].

In the polymerization of vinyl monomers using hFe-NFs, styrene was first polymerized to determine optimum condition (Fig. 2), and other monomers (acrylamide and MMA) were then polymerized under optimized polymerization condition of styrene. hFe-NFs mediated polymerization of styrene in the presence of AcAc and H_2O_2 initiators are given in Table 1. All polymerizations were carried out in emulsion medium using three different surfactants (cationic, anionic and non-ionic).

To study the effect of different reaction parameters, the amount of hFe-NFs was first optimized in the polymerization of styrene (Table 1, entries 1–4). According to Table 1, utilizing 5.0 mg of hFe-NFs provided the highest polymer yield with 94% (Table 1, entry 2). The number average molecular weight (M_n) of the polystyrene was 450 kDa with 2.06 polydispersity ($Đ$). Addition of higher (7.5 mg and 10.0 mg) or lower (2.5 mg) amount of hFe-NFs resulted in decreasing polymerization yield and M_n. Reaction temperature is also one of the important parameters for the vinyl polymerizations. We mainly performed three different reaction temperatures for styrene polymerization, RT, 40 and 50 ºC. Polymerization carried out at RT (Table 1, entry 2) resulted in the highest yield and molecular weight compared to those of carried out at 40 and 50 ºC (Table 1, entries 5 and 6).

Three different surfactants (anionic, cationic and non-ionic) were also used to detect the effects of the surfactant types. According to Table 1, non-ionic tween 40 (Table 1, entries 1–9) provided better polymerization media for styrene compared to cationic cetyltrimethylammonium bromide (CTAB) and anionic sodiumdodecyl sulfate (SDS) surfactants (Table 1, entries 10 and 11). No product formation was detected in the polymerization using SDS surfactant (Table 1, entry 10), whereas 70% of polymerization yield with 112 kDa molecular weight was observed with the utilization of CTAB surfactant in the polymerization of styrene (Table 1, entry 11). hFe-NFs was probably deactivated by negatively charged SDS through electrostatic interactions. Therefore, no catalytic activity was observed due to denaturation of hFe-NFs [16]. Polymerization performed with cationic CTAB significantly increased the conversion of styrene compared to the condition of SDS used. However, polymerization yield under CTAB media was not as high as the reaction carried out in non-ionic tween 40. Probably, CTAB surfactant desorption prevented achieving increasing polymerization yield [16].

The oxidant, H_2O_2, concentration plays important role in the hFe-NFs mediated polymerization of vinyl monomers. The highest polymerization yield, 94%, was achieved with addition of 1.0 mmol H_2O_2 in the reaction (Table 1, entry 2). Doubling the amount of peroxide to 2.0 mmol also gave 92% of polymer yield (Table 1, entry 9). However, the number average molecular weight (M_n= 185 kDa) of the synthesized polystyrene was significantly lower compared to that of adding 1.0 mmol H_2O_2. Increasing peroxide concentration probably resulted in increased number of active chains, and thus M_n decreased. Higher H_2O_2 concentration was also reported to be deactivating active sides of heme-enzymes [17]. Decreased peroxide concentration to 0.5 mmol in the solution resulted in both decreasing yield and M_n for the polystyrene (Table 1, entry 8).

Lastly, optimization of the amount of acetylacetone (AcAc) cocatalyst was determined, which is also an important parameter for enzyme catalyzed polymerization of vinyl monomers since β-diketones as reducing agents are key compounds to facilitate enzyme initiated free-radical polymerization of vinyl monomers [18]. Presence of AcAc in the enzyme mediated polymerization is reported to be responsible for the initiation of the reaction due to the formation of more stable keto-enoxy radical species. Initiation reaction probably occurs through this radical species [15]. Addition of 0.05 mmol of AcAc was sufficient for achieving polystyrene with the highest yield and molecular weight. Increasing AcAc concentration to 0.1 mmol was decided to have no significant effect on the polymerization yield, but caused observation of lower average molecular weight (Table 1, entry 7). According to these findings, optimal H_2O_2:AcAc ratio for the hFe-NFs mediated polymerization of styrene was decided to be 20:1.

According to the literature report, free-HRP enzyme mediated polymerization of styrene under mini-emulsion condition with AcAc and H_2O_2 initiator at RT resulted in up to 64.3% of yield. M_n of the synthesized polystyrene was 397 kDa. In this report, optimum HRP amount in the polymerization was 20 mg [19]. According to our findings, addition of only 5.0 mg of hFe-NFs gave upto 94% of polystyrene with 450 kDa molecular weight. Therefore, it can be concluded that hFe-NFs exhibited higher catalytic activity towards polymerization of vinyl monomers when compared to the free-HRP enzyme.

Thermogravimetric analysis of the synthesized polystyrene (Table 1, entry 2) showed that the polymer started to decompose around 300 °C, and 50% of weight loss occurred around 400 °C (Fig. S1, Supplementary information). From the DSC analysis, the glass transition temperature (T_g) of polystyrene appeared around 115 °C (Fig. S4, Supplementary information). No melting point (T_m) was detected, and polymer thermal decomposition temperature was observed around 430 °C. According to ^{1}H and ^{13}C NMR analyses, the synthesized polystyrene possessed atactic polymeric structure [14]. Aromatic protons were detected between 7.3 and 6.5 ppm from the ^{1}H NMR spectrum of polystyrene. Methylene (CH_2) and methine (CH) protons were respectively observed at 1.4 and 1.8 ppm (Fig. S21, Supplementary information). Aromatic carbons of the polystyrene were detected at 145, 127 and 125 ppm according to the ^{13}C NMR spectrum (Fig. S24, Supplementary information). The peaks appeared at 43 and 40 ppm were assigned as methylene (CH_2) and methine (CH) carbons, respectively.

After successful polymerization of styrene using hFe-NFs, polymerizations of other vinyl monomers (acrylamide and MMA) were also performed using the optimized reaction condition from the polymerization of styrene by changing only the temperature parameter (Table 1, entry 2). Polymerization of acrylamide was accomplished in surfactant-free media since both monomer and polymer are already soluble in water (Fig. 3). However, in contrast to styrene, polymerization of acrylamide at RT was not successful (Table 2, entry 12). Increasing polymerization temperature enabled observing polyacrylamide products (Table 2, entries 13 and 14). While polymerization carried out at 50 °C in the presence of hFe-NFs resulted in 35% yield, performing 70 °C reaction temperature gave the highest yield (92%) polyacrylamide (Table 2, entry 14). M_n of the polymer was 100 kDa, and $Đ$ was 1.57. Higher polymerization temperature was not carried out due to decomposition of hFe-NFs above 70 °C. On the contrary of acrylamide, polymerization of MMA at RT was successful (Fig. 4), however the yield was very low and only 15% of polymethylmethacrylate (PMMA) was observed (Table 2, entry 15). The highest polymerization yield (86%) was observed at 60 °C reaction temperature (Table 2, entry 16). M_n of this polymer was 147 kDa with

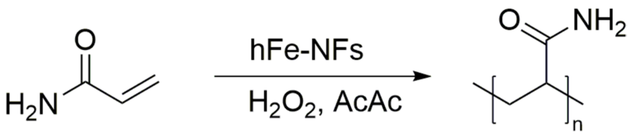

Fig. 3 hFe-NFs mediated polymerization of acrylamide in the presence of H_2O_2 and AcAc

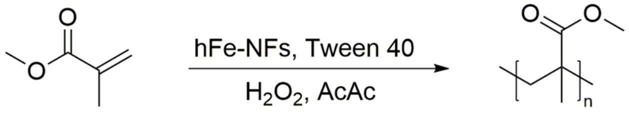

Fig. 4 hFe-NFs mediated polymerization of MMA in the presence of H_2O_2, AcAc and tween 40 surfactant

2.11 polydispersity. Higher polymerization temperatures above 60 ℃ were not tried due to thermal initiation of MMA in water without adding a catalyst and oxidizer [20].

According to ^1H and ^{13}C NMR analyses of polyacrylamide and PMMA, both polymers had atactic polymeric structures as expected. Methine (-CH) and methylene (-CH_2) protons of polyacrylamide (Table 2, entry 14) were observed at 2.20 and 1.51 ppm, respectively (Fig. S22, Supplementary information). According to the ^{13}C NMR analysis of polyacrylamide, carbonyl (-C = O), methine (-CH) and methylene (-CH_2) carbons were seen respectively at 179, 41 and 35 ppm (Fig. S25, Supplementary information). Polyacrylamide showed three stages of thermal decomposition according to the TGA analysis carried out between RT and 600 ℃ under N_2 atmosphere (Table 2, entry 14) [21]. About 17% mass loss occurred between RT-250 ℃, probably due to the loss of absorbed water. Approximately, 10% of weight loss appeared between 250 and 350 ℃ showing the removal of NH_3, H_2O and CO_2 by products. The final weight loss with 55% was observed between 350 and 450 ℃ indicating the removal of H_2O and CO_2 byproducts from the remaining residue (Fig. S2, Supplementary information). About 20% of carbonaceous residue remained at 600 ℃. From the DSC thermogram of polyacrylamide (Table 2, entry 14), T_g and T_m were detected at 160 and 300 ℃, respectively (Fig. S5, Supplementary information).

According to the ^1H NMR analysis of PMMA (Table 2, entry 16), methyl protons (-OCH_3) attached to the ester group was observed at 3.58 ppm. Methylene (-CH_2) and methyl (-CH_3) protons was respectively observed at 1.79 and 0.82 ppm (Fig. S23, Supplementary information). From the ^{13}C NMR analysis result of the PMMA, carbonyl (-C = O) carbon at 177 ppm, quaternary carbon (-C) attached to carbonyl at 54 ppm, methyl carbon (-OCH_3) attached to ester group at 51 ppm, methylene (-CH_2) carbon at 44 ppm and methyl (-CH_3) carbon attached to quaternary carbon at 16 ppm were observed (Fig. S26, Supplementary information). TGA analysis of PMMA (Table 2, entry 16) showed two stages decompositions occurred between RT and 600 ℃ under N_2 atmosphere. About 31% of mass loss was observed between 180 and 250 ℃ as a result of decomposition of ester group. The 2nd mass loss about 59% occurred between 250 and 430 ℃ due to the removal of by-products such as H_2O and CO_2 (Fig. S3, Supplementary information). PMMA (Table 2, entry 16) also showed T_g and T_d (decomposition temperature) observed respectively at 125 and 370 ℃ according to the DSC analysis (Fig. S6, Supplementary information).

Conclusion

The effects of flower-shaped hybrid nano biocatalyst hFe-NFs obtained by complexation between HRP enzyme and Fe^{2+} ions on free-radical polymerizations of some vinyl monomers (styrene, acrylamide and methylmethacrylate) were investigated. hFe-NFs mediated polymerization of vinyl monomers were successfully accomplished with very high yields and molecular weights. Cationic, anionic and non-ionic surfactants were used to create emulsion media in the polymerizations. Optimum polymerizations were obtained under conditions where non-ionic (tween 40) surfactant was used. Polymerization did not occur under conditions of anionic sodium dodecylsulfate surfactant used. Polymerization carried out with cationic cetyltrimethylammonium bromide surfactant resulted in lower yield and molecular weight compared to the conditions where non-ionic tween 40 was used. While hFe-NFs mediated polymerization of styrene was achieved at RT, acrylamide and methylmethacrylate monomers were polymerized respectively at 70 and 60 ℃ in which free-HRP enzyme loses its catalytic activity. Preparation of the flower-shaped hybrid hFe-NFs, therefore, enables inexpensive and stable catalyst system for free-radical polymerization of vinyl monomers compared to free-HRP enzyme. Increasing catalytic activity and stability of hFe-NFs at high reaction temperatures are very crucial for utilization of these types of catalysts in both scientific and industrial purposes.

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Data Availability The data related to the study is presented in the article.

Declarations

Competing interests The authors declare no conflict of interest.

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