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PEROXIDASE, HEMATIN, AND PEGYLATED-HEMATIN CATALYZED VINYL POLYMERIZATIONS IN WATER

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PEROXIDASE, HEMATIN, AND PEGYLATED- HEMATIN CATALYZED VINYL POLYMERIZATIONS IN WATER

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Dedicated to the memory of Professor Sukant K. Tripathy.

ABSTRACT

Horseradish peroxidase-, hematin- and pegylated-hematin mediated polymerization of sodium styrene sulfonate and sodium acrylate in water is reported. Molecular weight and yields were influenced by the concentrations of hydrogen peroxide and initiator, 2,4-pentanedione. Hematin and pegylated-hematin were studied in lieu of peroxidase at pH 11.0 and 7.0 in aqueous solution, respectively. Polymer with a high molecular weight ($M_n = 223,520$) was formed when the pegylated-hematin was used as the catalyst. The results demonstrate vinyl polymerizations in an all aqueous process in high yield and molecular weight catalyzed by peroxidase as well as biomimetic catalysts.

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Key Words: Vinyl polymers; Polystyrene; Peroxidase; Hematin; Polymerization

INTRODUCTION

Polymers of *p*-styrene sulfonic acid are used in many applications including ion exchange membranes and resins, [1-5] in biomaterials to influence cell adhesion [6-10] and as inhibitors of virus infections [11-18]. Resins of poly(sodium acrylate) are used as water absorbent materials for cleaning surfaces, in water and oil conditioning, personal care products, and disposable materials for medical applications [19-22]. Polymerization of sodium styrene sulfonate and copolymerization of styrene and sodium styrene sulfonate has been reported in organic solvents [23-25], emulsions [26-28], and emulsifier free emulsions [29-30]. Initiator, temperature, monomer concentration, and solvent have been investigated for their influence on the reactions.

In general, these synthetic routes used to synthesize polystyrenes with sulfonic acid are based on: a) direct aromatic sulfonation of pre-formed polystyrene or co-polymers of styrene; b) homopolymerization or co-polymerization of styrene sulfonic acid by conventional free radical polymerization; or c) chemical modification of a chloromethyl substituent of styrene by the Strecker reaction. Sulfonation of polystyrene is accomplished with anhydrous sulfur trioxide, triethylphosphate complexes of sulfur trioxide and chlorosulfonic acids. Free radical polymerization is initiated with AIBN-type initiators in solvents such as tetrahydrofuran, dimethylformamide and dioxane [31-32]. Styrene sulfonic acid can not be polymerized by anionic techniques while the sulfonate ester and sulfonamide are polymerized with organolithium and sodium methoxide initiators [23, 33]. *p*-Styrene sulfonate esters of perfume alcohols, herbicides alcohols, and sulfonamides have been synthesized using *p*-styrene sulfonylchloride in the presence of bases and polymerized by anionic and free radical mechanisms [31-32]. Acrylic acid esters are polymerized by anionic, cationic, and free radical mechanisms using chemical initiators in organic solvents [34].

Horseradish peroxidase (HRP) is an oxido-reductase isolated from horseradish roots that catalyzes the oxidation of many aromatic compounds, mediated by hydrogen peroxide and involving a cycle of changes in the oxidation state of an iron atom located at the catalytic site of enzyme. The oxidative coupling of a variety of substrates such as phenols and aromatic amines catalyzed by HRP in the presence of hydrogen peroxide has been extensively studied in aqueous, nonaqueous and interfacial systems [35-52]. The potential of using HRP and other oxidases to catalyze the free radical polymerization of vinyl monomers was first reported by Derango *et al.* [53]. The polymers were formed in the presence of a large excess of hydrogen peroxide [54-55]. More extensive studies have been conducted with respect to the polymerization of acrylamide, methyl methacrylate and styrene using β -diketones as initiators [56-58]. Recent studies on hematin

(hydroxyferritroporphyrin)-catalyzed polymerization of phenol demonstrated that the iron in hematin can undergo a cycle of oxido-reductive changes similar to HRP in the presence of hydrogen peroxide and is a suitable catalyst at pH 11.0 [59]. A major limitation of the enzymatic oxidation of styrene in aqueous solution, however, has been the insolubility of the styrene monomer which results in phase separation; the oligomers precipitate at lower molecular weight.

The objective of the present study was to explore all-aqueous, environmentally compatible, methods to prepare vinyl polymers. To accomplish this goal, we report the HRP-, hematin- and pegylated-hematin (PEG-hematin)-mediated polymerization of sodium styrene sulfonate and sodium acrylate in water. The PEG-hematin [60-61] was synthesized to maintain solubility and function at neutral pH, which was not possible with unmodified hematin due to the limitations in solubility at neutral pH. The results demonstrate for the first time that these types of enzymatic and biomimetic enzymatic reactions can be used to polymerize functionalized styrene monomers and other water soluble vinyl monomers like sodium acrylate in an all aqueous system, resulting in substantial yields and molecular weight for the vinyl polymers.

MATERIALS AND METHODS

Horseshoe peroxidase (Type II, 150-200 units/ mg solid) and hydrogen peroxide (30% w/w) were purchased from Sigma Chemical Co., St. Louis, MO. 2,4-pentanedione, styrene sulfonic acid sodium salt and sodium acrylate were purchased from Aldrich Chemical Co., Milwaukee, WI. Solvents used were high performance liquid chromatography grade and purchased from Fisher Scientific Co., Pittsburgh, PA. Polystyrene sulfonates-Na salt standards were purchased from American Polymer Standards Corporation, Mentor, Ohio. Hematin was purchased from Sigma Chemical Co., St. Louis, MO, and PEG-Hematin was prepared chemically by an esterification reaction of the hematin with poly(ethylene glycol) (MW 10 kD) in the presence of activators *N,N'*-carbonyldiimidazole and 1,8-diazabicyclo[5.4.0]undec-7-ene in DMF.

¹H NMR spectra were recorded using a Bruker DPX 300 spectrometer. Chemical shifts in parts per million (ppm) were referenced relative to 3-(trimethylsilyl) propionic-2,2,3,3-*d*₄ acid sodium salt (0.00 ppm) as internal reference. Molecular weight was determined by GPC, based on a calibration curve generated with narrow molecular weight distribution polystyrene sulfonates-Na salt standards. A Waters 2690 HPLC instrument with Shodex Asahipak GF-7M HQ with guard column was used with differential refractometer detection. GPC data were collected and processed by Millennium GPC software. Aqueous:acetonitrile, 60:40, with 50 mM lithium chloride was used as the mobile phase at a flow rate of 0.4 mL/min. The polymer samples were dissolved at 1-2 mg/mL and filtered prior to injection. The run time for each sample was 30 minutes.

2,4-Pentanedione was distilled under vacuum before use. In a general procedure for polymerization at room temperature, 2.0 mL of water and 4.6 mM (948 mg) of styrene sulfonic acid or acrylic acid sodium salt were mixed and flushed with nitrogen for 15 minutes. HRP (3.56×10^{-4} mmol, 2400 units, 16 mg) was dissolved in 200 mL of water. Hydrogen peroxide, 0.082 mM and 0.082 mM of initiator were added simultaneously after the addition of enzyme. Polymerization was conducted for 24 hours with continuous stirring. The reactions with hematin and PEG-hematin proceeded in a similar manner as with HRP, but with 2.5 or 1.0 mg/ml, of the two biomimetic catalysts, respectively. The reaction mixture was poured into 250 mL of methanol, kept overnight and filtered. The polymer precipitates were dried under vacuum.

RESULTS AND DISCUSSION

Horseradish peroxidase-mediated polymerization of sodium styrene sulfonate was conducted in water with 2,4-pentanedione as reducing substrate and hydrogen peroxide as oxidant (Figure 1). The possibility that the sodium styrene sulfonate polymerization did not proceed through the HRP-mediated initiation was rejected based on preliminary control experiments, since no polymer was detected in the absence of any one of the components (e.g., enzyme, hydrogen peroxide or 2,4-pentanedione). The effect of reaction time on poly(sodium styrene sulfonate) molecular weight, yield and polydispersity are shown in Table 1. The molecular weight of poly(sodium styrene sulfonate) increased (M_n 65,882 to 163,280) with reaction time while the polydispersity also increased (2.5 to 3.4), presumably due to the free radical process. More than 80% of the sodium styrene sulfonate was converted to poly(sodium styrene sulfonate) in 4 hours.

The effects of different ratios of initiator 2,4-pentanedione and hydrogen peroxide in the reaction on yield, molecular weight and polydispersity were deter-

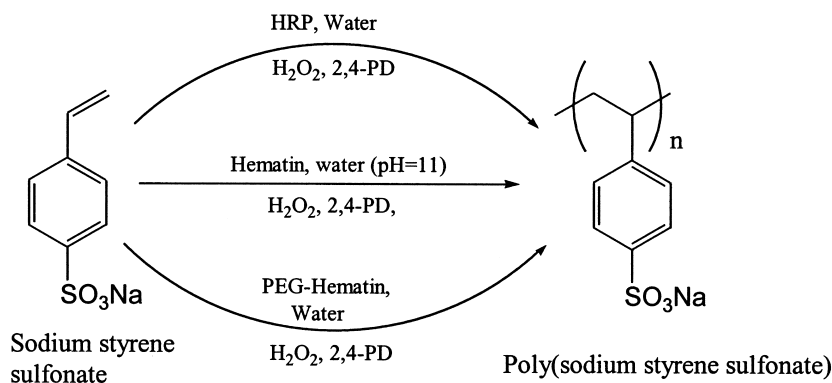


Figure 1. Schematic of sodium styrene sulfonate polymerization by the different catalysts. HRP (horseradish peroxidase), 2,4-PD (2,4-pentanedione), H_2O_2 (hydrogen peroxide).

Table 1. Changes in Poly(Sodium Styrene Sulfonate) Molecular Weight, Polydispersity, and Yield with Reaction Time: Reactions Catalyzed by Horseradish Peroxidase

Reaction time (h)	Mn	Mw	Polydispersity	Yield (%)
0.5	65,882	164,509	2.49	14.2
2	88,241	251,194	2.84	76.7
4	99,243	301,323	3.03	81.7
8	104,152	292,633	2.80	81.9
16	163,288	548,242	3.35	82.6

^aReaction conditions included 4.6 mmol sodium styrene sulfonate, 0.082 mmol 2,4-pentanedione, 0.082 mmol hydrogen peroxide, water 2.2 mL, and horseradish peroxidase 7-8 mg/mL. Reactions were run at room temperature. Mn=number average molecular weight, mw=weight average molecular weight, PD=polydispersity.

mined with time (Figure 2, Table 2). Experiments were run for 15, 30, 60, 120, and 240 minutes, and three different ratios of 2,4-pentanedione and hydrogen peroxide: a) $\text{H}_2\text{O}_2 = 0.056$ mmol:2,4-pentanedione 0.082 mmol; b) $\text{H}_2\text{O}_2 = 0.056$ mmol:2,4-pentanedione 0.224 mmol; and c) $\text{H}_2\text{O}_2 = 0.224$ mmol:2,4-pentanedione 0.082 mmol. When the ratio of 2,4-pentanedione was 1.46 times the molarity of hydrogen peroxide (condition a) a steady increase in molecular weight (from Mn 37,795 to Mn 90,389, Table 2) and yield (7.0% to 82.2%, Figure 2) was observed. When the concentration of 2,4-pentanedione was increased four times (condition b) a decrease in molecular weight (Mn 88,106 to Mn 49,407) and

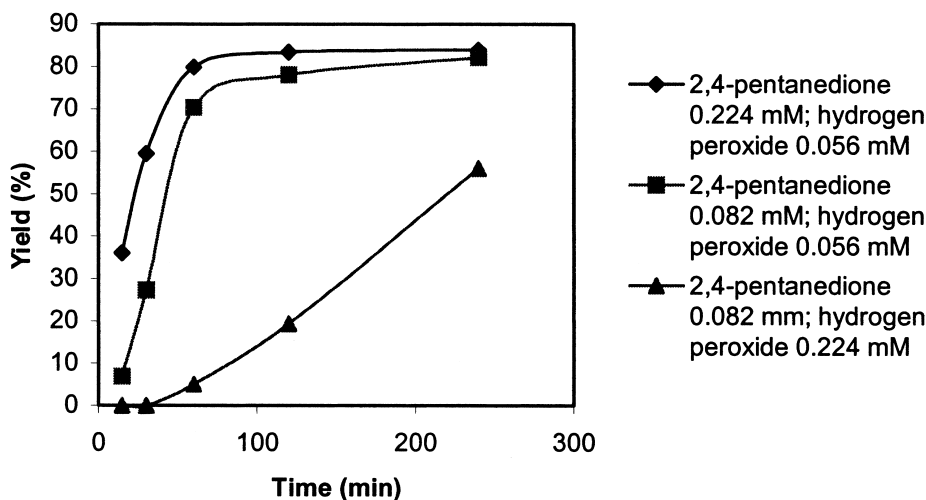


Figure 2. Effect of 2,4-pentanedione and hydrogen peroxide concentrations on polymer [poly(sodium styrene sulfonate)] yield with time. Reaction conditions included 4.6 mmol sodium styrene sulfonate, water 2.2 mL and horseradish peroxidase, 7-8 mg/mL. Reactions were run at room temperature under a nitrogen atmosphere.

Table 2. Effect of Ratio of Hydrogen Peroxide and 2,4-Pentanedione on Mn and Polydispersity with Respect to Reaction Time^a

Time (min)	Ratio of Hydrogen Peroxide and 2,4-Pentanedione in mmol					
	H ₂ O ₂ :2,4-PD 0.056:0.082		H ₂ O ₂ :2,4-PD 0.056:0.224		H ₂ O ₂ :2,4-PD 0.224:0.082	
	Mn	PD	Mn	PD	Mn	PD
15	37,795	2.31	88,106	2.25	ND ^b	ND ^b
30	63,870	2.68	121,233	2.18	ND ^b	ND ^b
60	85,864	2.73	82,981	2.63	65,785	3.21
120	85,543	2.79	64,848	2.82	91,854	2.44
240	90,389	2.70	49,407	3.82	137,098	2.76

^aReaction condition included horseradish peroxidase 7-8 mg/mL, water 2.2 mL, sodium styrene sulfonate 4.6 mmol, 948 mg. Reactions were run under nitrogen atmosphere.

^bNot detected.

increase in polydispersity (2.3 to 3.8) was observed. The reaction was slow initially (Figure 2) but yielded the same amount of polymer as in the case of 'a'. In comparison, with an increased ratio of hydrogen peroxide (condition c) no product was detected for the reaction run for 15 or 30 minutes, while the reaction run for 240 minutes with the same amount of hydrogen peroxide yielded polymer with a 56% yield (Mn = 137,098, PD = 2.8, Figure 2). Formation of higher molecular weight polymer, when the initiator was 1.46 times the hydrogen peroxide can be explained on the basis of the mechanistic cycle for HRP-catalyzed polymerization of acrylamide [56], phenol [62], methyl methacrylate [57], and styrene [58]. One molecule of hydrogen peroxide produces two catalytically active forms of HRP, each of which oxidizes the initiator to produce a free radical. The free initiator radical generates monomer radicals that react with other monomers to produce polymer. Therefore, based on the mechanism and optimal stoichiometry, one molecule of hydrogen peroxide would require two molecules of 2,4-pentanedione. When 2,4-pentanedione was present at four times the level of hydrogen peroxide, many initiator radicals were formed early in the reaction leading to the formation of many chains and thus lower molecular weight polymer. The higher concentration of hydrogen peroxide likely inhibited HRP activity in the short time frames (up to 30 minutes) leading to low overall yield after 240 minutes. To evaluate the influence of hydrogen peroxide and 2,4-pentanedione on molecular weight and polydispersity of poly(sodium styrene sulfonate), stepwise changes in the ratio of these two components were studied, with each reaction run for four hrs at room temperature (Table 3). The increase in hydrogen peroxide resulted in increased molecular weight of polymer, while the increase in 2,4-pentanedione resulted in decreased molecular weight and increased polydispersity (Table 3). The yield was influenced by the amount of water (Table 4) with the low water content yielding the most polymer (94%) and the highest water content yielding the lowest yield (58%).

Table 3. Changes in Mn, Polydispersity, and Yield with Different Ratios of Hydrogen Peroxide and 2,4-Pentanedione^a

H ₂ O ₂ :2,4-PD (mmol)	Mn	Polydispersity	Yield (%)
0.056:0.082	80,925	2.99	81.1
0.112:0.082	113,906	3.30	84.8
0.168:0.082	129,861	2.73	77.3
0.224:0.082	137,098	2.76	56.0
0.082:0.056	152,251	3.25	78.4
0.082:0.112	73,909	3.72	84.9
0.082:0.168	55,086	3.34	85.4
0.082:0.224	44,245	4.06	84.9

^aReaction conditions included sodium styrene sulfonate 4.6 mmol, 948 mg. Reactions were run for 4 hrs under nitrogen atmosphere.

The catalytic cycle of HRP has been thoroughly studied [56-58, 62-63]. The heme-iron group in HRP interacts with hydrogen peroxide, resulting in changes in the oxidation state of iron which in turn leads to the polymerization reaction. Hematin-iron undergoes similar changes in the presence of an oxidizing reagent and recently hematin-catalyzed polymerization of phenol at pH=11.0 buffer solution was reported [59]. The low solubility of hematin however, restricts its use at neutral pH and therefore, PEG-hematin, which is soluble in water at neutral pH was prepared. Hematin and PEG-Hematin were used to catalyze the polymerization of sodium styrene sulfonate in water at pH 11 and 7 (Table 4). PEG-Hematin yielded poly(sodium styrene sulfonate) with the highest molecular weight (Mn = 223,520, PD = 3.48) of all the reactions conducted (hematin, PEG-hematin, HRP).

Table 4. Horseradish Peroxidase, PEG-Hematin, and Hematin-Catalyzed Polymerization of Sodium Styrene Sulfonate in Water: Effect of Water Content and 2,4-Pentanedione on Reactions

Entry	Catalyst	Time	Mn	PD	2,4-Pentanedione (mmol)	Yield (%)
1	HRP	24	136,431	2.88	0.082	94.8 ^a
2	HRP	48	76,728	2.66	0.082	88.7 ^b
3	HRP	24	127,588	2.55	0.082	57.6 ^c
4	hematin	38	55,530	2.24	0.164	73.0 ^d
5	PEG-hematin	26	223,520	3.48	0.164	77.8 ^e
6	HRP	20	31,344	7.57	bulk (1 ml)	43.3 ^f

^aHorseradish peroxidase 7-8 mg/mL, hydrogen peroxide 0.082 mmol, water 1.2 mL.

^bHorseradish peroxidase 7-8 mg/mL, hydrogen peroxide 0.082 mmol, water 2.2 mL.

^cHorseradish peroxidase 7-8 mg/mL, hydrogen peroxide 0.082 mmol, water 4.7 mL.

^dHematin 2.5 mg/mL, hydrogen peroxide 0.082 mmol, water 2.0 mL.

^ePEG-Hematin 1mg/mL, hydrogen peroxide 0.082 mmol, water 2.0 mL.

^fHorseradish peroxidase 7-8mg/mL, hydrogen peroxide 0.082 mmol, water 1.0 mL. Reactions were run under nitrogen atmosphere at 20C.

In addition, >98% of sodium acrylate was polymerized by hematin at pH 11.0 to poly(sodium acrylate) at room temperature.

The ^1H NMR spectrum of poly(sodium styrene sulfonate) showed two peaks for aromatic protons with centers at δ 6.50 and δ 7.50, and methylene protons at δ 1.5. Initiators, 1,3-cyclopentanedione, and tetronic acid influenced the yield of HRP-catalyzed polystyrene in aqueous organic solvent [58], however, this influence was not evident in the polymerization of sodium styrene sulfonate.

CONCLUSION

The polymerization of sodium styrene sulfonate and sodium acrylate mediated by horseradish peroxidase, hematin and pegylated-hematin in the presence of hydrogen peroxide and 2,4-pentanedione was carried out in aqueous medium. Yields as high as 94% were found and molecular weights varied depending on the catalyst, with the highest molecular weight polymer produced with the hematins. The method provides an all aqueous green chemistry approach to vinyl polymerizations.

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