Enzyme-Mediated Grafting of Acrylamide to Ultrahigh Molecular Weight Polyethylene Fiber: A Novel Radical Initiation System

Jingchan Zhao,[1,2] Zhian Guo,[1] Guozheng Liang,[2] Junlong Wang,[2] Gangshen Zhang[1]

[1]*Department of Chemistry, Northwest University, Xi'an 710069, China*
[2]*Department of Chemical Engineering, Northwestern Polytechnical University, Xi'an 710072, China*

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ABSTRACT: The enzyme-mediated grafting of acrylamide (AM) to ultrahigh molecular weight polyethylene (UHMWPE) fibers using horseradish peroxidase (HRP) was demonstrated. To optimize the reaction condition, the concentrations of monomer, H_2O_2, the initiator, and time were varied. The grafting results were discussed and a reaction mechanism was proposed. Function groups and structural change of the graft copolymer were determined by FTIR spectroscopic and scanning electron microscopy micrographs for proof of grafting and the results were discussed. Results show that the surface of treated fiber becomes rougher than the untreated surface. Compared to unmodified fiber, modified fiber surface had significantly increased the interfacial shear strength, and carbonyl-stretching regions in the IR spectra. The interfacial shear strength of the UHMWPE fiber increased, clearly indicating that enzymatic-grafted acrylamide could significantly increase the hydrophilicity of the surfaces of UHMWPE fibers. Moreover, the hydrophilicity of treated fiber depends on the monomer concentration, the initiator concentration, and oxidizing agent concentration as well as the time of the reaction. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 96: 1011–1016, 2005

Key words: fibers; peroxidase; graft copolymers; acrylamide; enzymes

INTRODUCTION

High-performance fiber, such as carbon, aramid, and polyethylene (PE), have been developed and applied to various fields. Ultrahigh molecular weight polyethylene (UHMWPE) fiber was developed by a gel-spinning method in the 1980s. This highly oriented or drawn fiber has an extremely high tensile strength and elastic modulus (2.83 and 100 GPa, respectively) and has been applied in various ways, such as a nautical sea. The disadvantage of UHMWPE fiber is the poor adhesion to other materials, which results from the lack of chemical-bonding sites and smooth surface of the UHMWPE fiber. To improve the surface properties of UHMWPE, it is necessary to modify the surface of the UHMWPE fiber.

Chemical modification of polyethylene by radical mediated grafting chemistry is a robust method of generating added products from commodity materials. Conventional grafting processes are initiated by oxygen-centered radicals derived from the decomposition of organic peroxides.[1,2] Grafting polyethylene with acrylonitrile monomers by a free-radical reaction, by either chemical radical starters[3–7] or irradiation,[8–12] has already been performed to initiate the process.

It is now largely accepted that the redox system relies on the generation of free radicals through the horseradish peroxidase (HRP)–catalyzed oxidation of several organic substances (phenols, anima) by hydrogen peroxide. The generally accepted mechanism involves the production of free radicals.[13] These species are potential initiators for the polymerization of vinyl monomers. This property has widely been used in the case where phenol and aromatic amines are the reducing species[14]; in addition, the catalysis has been tested in nonaqueous and interfacial systems. The radicals produced in this way can initiate free-radical polymerization and cover the ternary mixture (HRP, H_2O_2, substrate) into an enzymatic catalyzed redox system. The polymerization of vinyl monomers in which HRP as a mediator was first reported by Derango et al.[15] with acrylic monomers like acrylamide (AM) and hydroxyethylmethacrylate.

This article reports on the development of a surface grafting onto UHMWPE fiber by using peroxidase production of free radicals, characterization of treated fiber, and investigation of the optimization of reaction parameters. The aim of the present study was to verify the mechanism underlying the enzymatically induced graft

Correspondence to: G.-S. Zhang (zhanggangzhui@163.com).
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copolymerization and exploration of the precipitation process as a new method for the treatment of aqueous phenols.

EXPERIMENTAL

Materials

Ultrahigh molecular weight polyethylene (UHMWPE) fibers were obtained from Da Cheng High Technology Manufactory (China). Horseradish peroxidase (HRP) was purchased from Shanghai Biochemical Co. (China). *O*-Methoxyphenol was purchased from Shanghai Chemical Co. (China). Hydrogen peroxide was bought from Xi'an Chemical Reagent Plant (China). The buffer and other chemical reagents used in this study were analytical grade. Acrylamide (AM) was bought from Xi'an Chemical Reagent Plant (China).

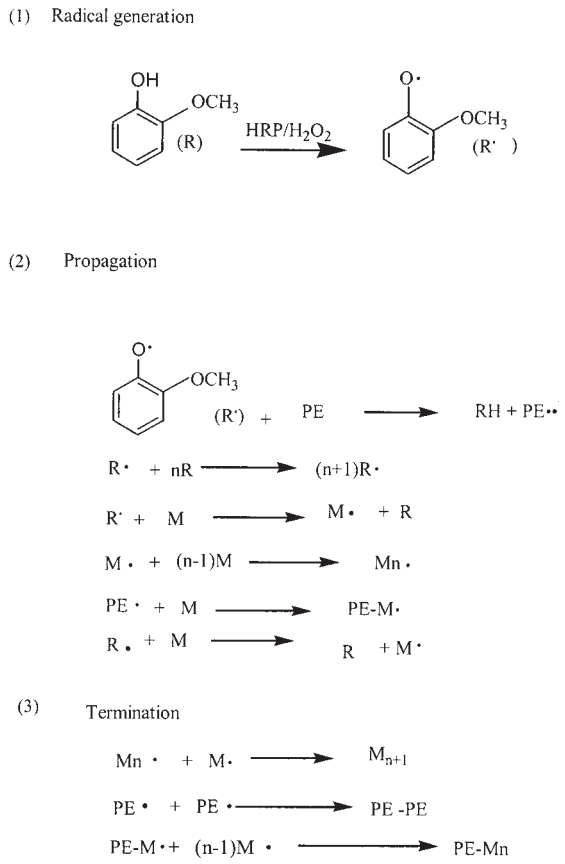
Surface grafting of UHMWPE fiber

Before surface modification by use of enzymes, the sample pieces were washed with acetone and distilled water, then dried in a vacuum oven for 4 h at 70°C. UHMWPE fibers (0.1 g), HRP (0.1 mg) in 2.0 mL phosphate buffer (100 m*M*, pH 7.0), and 1.0 mL *O*-methoxyphenol (5% acetone solution) and 15 mL acrylamide (5% water solution) Mohr's salt were charged to a 50-mL flask. The mixture was degassed for 30 min. Then 0.1 mL H_2O_2 (3.0% aqueous solution) was added in portions 20 times at 40-min intervals at room temperature. Then the mixture was maintained for another 2 h at 30°C in a constant temperature shaker. After that the UHMWPE fibers were washed sequentially with water (three times), a mixture solvent of ethanol and water (1 : 1 v/v, three times), and acetone (three times), and finally dried at 70°C under vacuum. The resultant fiber was designated as fiber A. Using inactive HRP solution that was heated, an HRP solution in boiling water for 30 min replaces 0.2 mg/mL of HRP in 2.0 mL phosphate buffer. The resultant fiber was designated as fiber B. Using 2.0 mL phosphate buffer replace to 0.2 mg/mL of HRP in 2.0 mL phosphate buffer. The resultant fiber was designated as fiber C.

Preparation of sample for pull-out test

A single fiber was fixed at both ends, then embedded in an epoxy resin casting and cured at 70°C for 4 h. A specially designed custom-made microtensile testing machine was used to perform the pull-out test. The interfacial shear strength (ISS), τ, of UHMWPE fiber can be calculated from the following equation:

$$\tau = F_{max}/\pi DL \quad (1)$$



Scheme 1

where F_{max} is the maximum tensile load, and D and L are the fiber diameter and the embedded fiber length, respectively.

Scanning electron microscopy (SEM) micrographs were recorded using a Hitachi S-570 SEM (Hitachi, Osaka, Japan) with an acceleration voltage of 15 kV. The surface of the UHMWPE fiber was coated with gold by vacuum evaporation.

An FTIR spectrum of UHMWPE fiber was recorded on a Perkin–Elmer Spectrum 1000 IR spectrometer (Perkin Elmer Cetus Instruments, Norwalk, CT).

RESULTS AND DISCUSSION

Catalysis grafting of AM onto the surface of UHMWPE fibers

Previous studies have found that the catalysis mechanism of most peroxidases contains three distinct steps, as shown in the following scheme.[16]

Because free-radical species are generated as intermediates in many peroxidase catalysis reactions,[17–19] it has been assumed that they could be used as primary radicals to initiate polyethylene free radicals. The enzyme-mediated graft copolymerization is assumed to follow the reaction, where M is the monomer, PE is the polymer chain, and R is the *O*-methoxyphenol. At the beginning

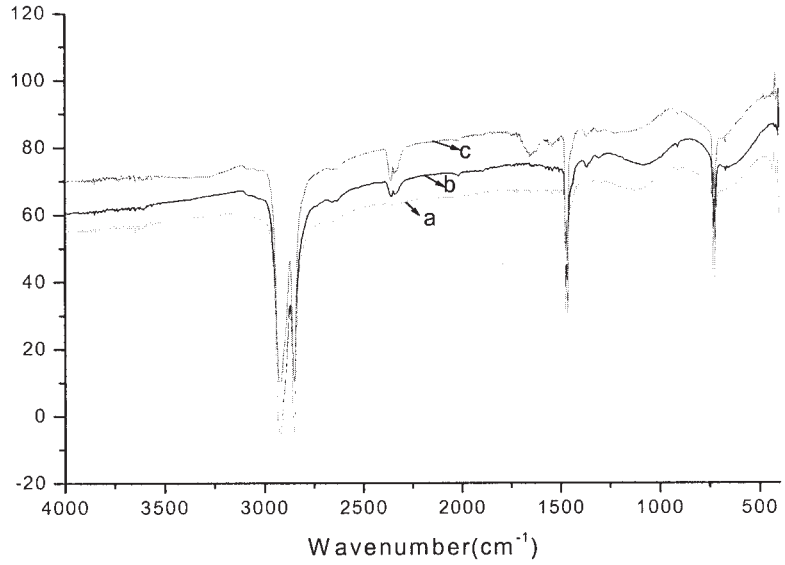


Figure 1 Infrared spectra of UHMWPE fiber (a) virgin; (b) fiber B; (c) fiber A.

of the reaction, phenols are preferentially oxidized and subsequently deprotonated to corresponding phenoxyradicals. The phenoxyradical abstracts hydrogen from the polyethylene chain and forms a surface radical on the PE chain. If the formed radicals (polyethylene radical) abstracts transfer an electron onto the AM, the latter is initiated to form radicals, which not only could copolymerize with the polyethylene radical, but also could form homopolymer (PAM). Mohr's salt was added often as the effective inhibitor to minimize this monomer homopolymerization[20] and its suitable concentration was found to be 2–3 wt %. Therefore, the grafted polymer is formed by termination between AM radical and PE radical, coupling the termination of PE-M with M radical, and disproportionate termination of PE with M_{n+1} radicals.

Characterization of AM-*g*-PE fiber

IR analysis

The main objective of this work was to improve the interfacial adhesion between UHMWPE fiber and a resin, such as epoxy, by imparting new functional groups to the surface of the fiber. Figure 1 shows the FTIR spectra of AM-*g*-fiber and virgin PE fiber. Compared to the spectra of virgin and inactive HRP-treated fiber, the spectrum of active HRP-treated fiber exhibits several new peaks at 1650 and 3400 cm^{-1}, which correspond to –CO– groups being adjacent to an olefin double bond, Ph—CO—R groups being adjacent to the olefin double bond, and –NH grafting groups, respectively, suggesting that functional groups were imparted to the fiber after surface grafting; in turn this may improve the interfacial adhesion between the fiber and the matrix, which could be proved by the topography and morphology of untreated and treated fiber surfaces [Fig. 2(a), (b)] as well as that after the pull-out test [Fig. 2(c), (d)].

SEM analysis

Compared to the SEM micrograph of the virgin fiber [Fig. 2(a)], AM-*g*-PE fiber shows a rough surface with pits [Fig. 2(b)]. Moreover, after the pull-out test, an abundance of epoxy resin still covered the surface of the AM-*g*-PE fiber, whereas almost no epoxy resin remained on the surface of untreated fiber, suggesting that the surface grafting of UHMWPE fiber by using enzyme as radical initiator substantially improved the interfacial adhesion between the fiber and the matrix, which could be further proved by ISS measurement presented in the following section.

Comparison of the results of different methods

The radical graft polymerization of AM was carried out under several conditions. The results are shown in Table I. Compared to fibers B and C, the AM-*g*-fiber was initiated in the presence of UHMWPE fiber A and the ISS is much higher than that of fibers B and C.

Optimization of reaction parameters

H_2O_2 concentration

Durand et al.[13] examined the reaction of HRP-catalyzed redox system involved in the ternary system, and found that the numerous chemical processes involved in the ternary system should be separated into two families: "initiation process" and "degradation process." The role of H_2O_2 was complicated because in the explored range of concentrations it was involved in three processes: (1) the catalytic oxidation of *O*-methoxyphenol; (2) degra-

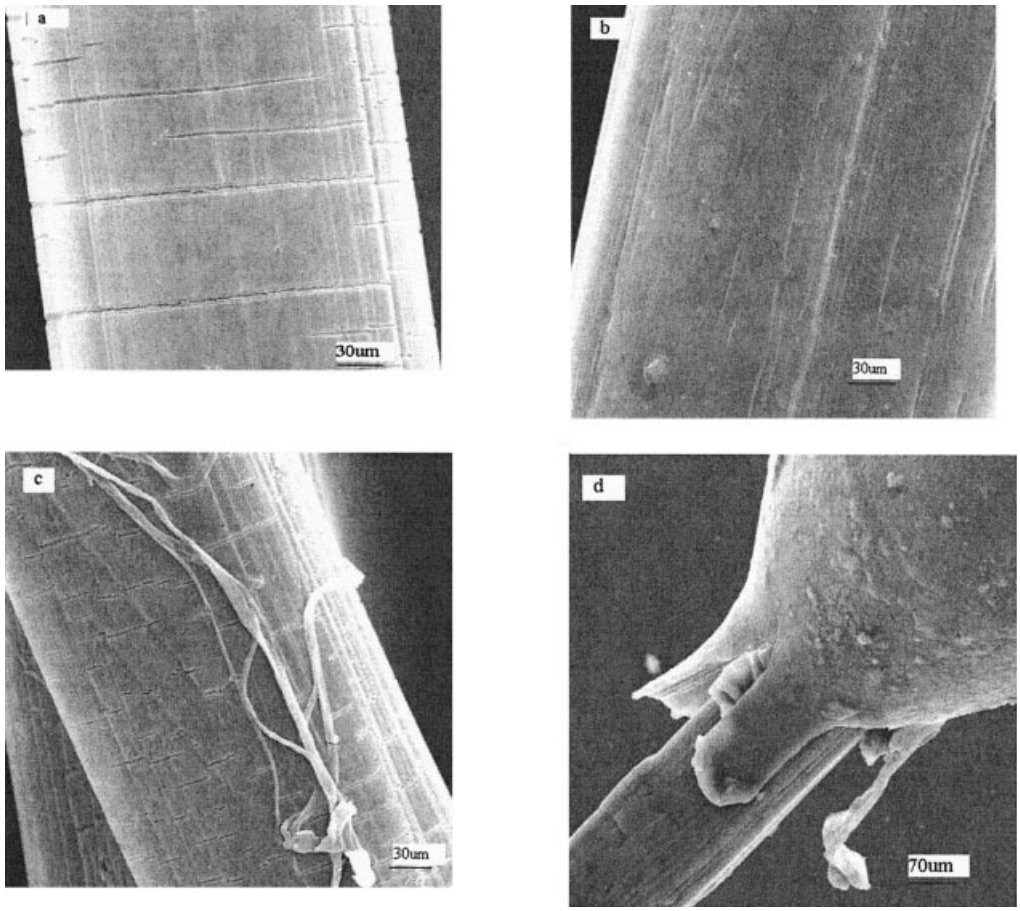


Figure 2 SEM micrographs of (a) virgin fiber, (b) fiber A, (c) virgin fiber after single pull-out, and (d) fiber A after pull-out.

dation reactions of HRP through the formation of the so-called compound II; and (3) the formation of quinone arising from the chemical oxidation of *O*-methoxyphenol. As a matter of fact, the choice of adequate concentration for H_2O_2 was shown to be a good way to limit the undesirable reactions (2) and (3), so the H_2O_2 solution should be added dropwise to the mixture for 40 min. The effect of H_2O_2 concentration on ISS of treated fiber was investigated by changing H_2O_2 concentration from 0.1 to $9.5 \times 10^{-3}M$ while keeping other parameters constant in the total volume of 20 mL (Fig. 3). The results show that ISS of treated fiber initially increases with the H_2O_2 concentration up to $4.0 \times 10^{-3}M$, indicating that ISS gradually increased with increasing extent of enzymatic treatment. By continually increasing H_2O_2 concentration to $6.0 \times 10^{-3}M$ or even more, the ISS almost remained constant or slightly decreased, probably because HRP was degraded by H_2O_2, and thus decreased the catalytic activity of the enzyme. Therefore the optimum H_2O_2 concentration was $4.0 \times 10^{-3}M$.

O-Methoxyphenol concentration

The effect of *O*-methoxyphenol concentration on ISS was studied at different initiator concentrations while keeping other parameters constant in a total volume of

TABLE I
Results of Different Methods[a]

	Sample fiber				
	Virgin	Fibers A_1	Fibers A_2	Fiber B	Fiber C
Pull-out adhesion (MPa)	0.86	1.46	1.32	0.87	0.85
RSD (%)	7.2	4.0	5.3	6.2	6.5

[a] Fibers A_1: [*O*-methoxyphenol] = $1.0 \times 10^{-2}M$, temperature 30°C, time of reaction 360 min, $[H_2O_2] = 5.0 \times 10^{-3}M$, [AM] = 0.15*M*, [HRP] = 0.020 mg/mL. Fibers A_2: [*O*-methoxyphenol] = $1.0 \times 10^{-2}M$, temperature 30°C, time of reaction 180 min, $[H_2O_2] = 5.0 \times 10^{-3}M$, [AM] = 0.15*M*, [HRP] = 0.020 mg/mL.

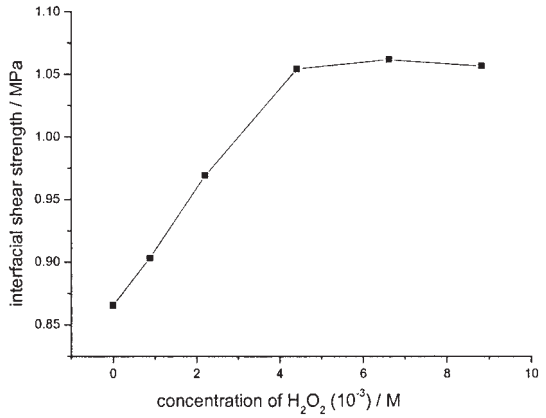


Figure 3 Variation of interfacial shear strength versus H_2O_2 concentration: [*O*-methoxyphenol] = $1.0 \times 10^{-2}M$; temperature 30°C; time of reaction 4 h.

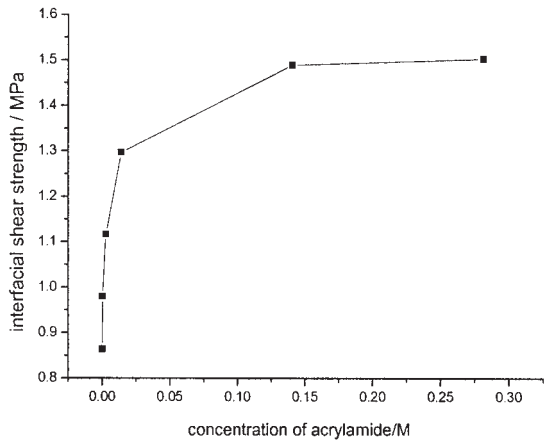


Figure 5 Variation of interfacial shear strength versus acrylamide concentration: [H_2O_2] = $6.0 \times 10^{-3}M$; [*O*-methoxyphenol] = $1.0 \times 10^{-2}M$; temperature 30°C; time of reaction 4 h.

20 mL. The results are shown in Figure 4. It can be seen that initially increasing the *O*-methoxyphenol concentration up to $2.0 \times 10^{-2}M$ is accompanied by a significant enhancement in ISS because of the increase of the reactive sites on the fiber surface, thus improving the interfacial adhesion between the fiber and the matrix. Increasing the initiator concentration increases the free radicals in the reaction medium, which in turn increases the number of active sites on the fiber. The free radicals can take part in many reactions; for example, they can directly interact with the fiber surface to form active sites and may also initiate the homopolymerization of *O*-methoxyphenol. Moreover, some active homopolymers may react with the UHMWPE surface by chain transfer reaction, thus creating additional active sites on the fiber surface. However, further increasing the concentration of initiators could not increase ISS any further because a large number of primary radicals were produced, which may interact with each other and terminate radicals. The appropriate concentration of *O*-methoxyphenol was selected as $3.0 \times 1.5^{-2}M$.

AM concentration

The effect of AM concentration on ISS of graft fiber is presented in Figure 5. The result shows that ISS of graft fiber concomitantly increases when the monomer concentration is increased to 0.15*M*. In fact increasing the monomer concentration increases the number of short chains and thus better diffusion of the polymer into the film occurs. However, we observed that grafting did not succeed, which is probably attributable to

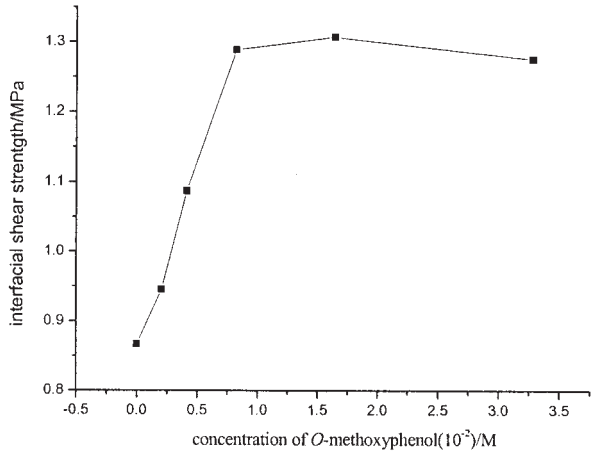


Figure 4 Variation of interfacial shear strength versus *O*-methoxyphenol concentration: [H_2O_2] = $5.0 \times 10^{-3}M$; temperature 30°C; time of reaction 4 h.

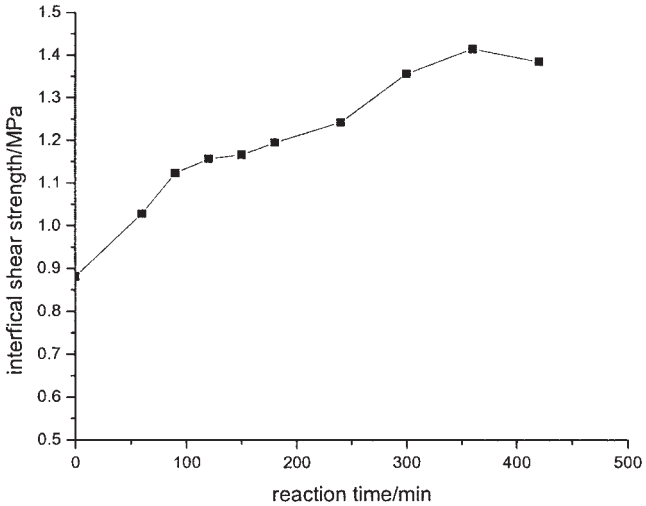


Figure 6 Variation of interfacial shear strength versus reaction time: [H_2O_2] = $5.0 \times 10^{-3}M$; [*O*-methoxyphenol] = $1.0 \times 10^{-2}M$; temperature 30°C.

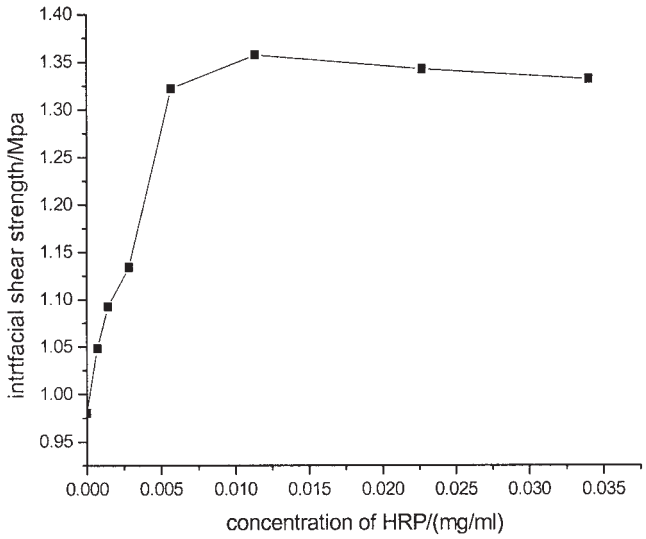


Figure 7 Variation of interfacial shear strength versus HRP concentration: $[H_2O_2] = 6.0 \times 10^{-3}M$; [*O*-methoxyphenol] = $1.0 \times 10^{-2}M$; temperature 30°C; time of reaction 4 h.

the saturation of radical sites present on the fiber by progressive growth of chains.

Reaction time

Because primary radicals should be produced even after a long inhibition period, to ensure that the reaction does not stop, a slow initiation step is consistent with this interpretation.[21] In all processes for grafting UHMWPE fiber, there was a long and engrafting inhibition period. This phenomenon was observed as the change of the color of the reaction solution. When H_2O_2 was added, the color of the solution immediately became red as a result of the oxidization of *O*-methoxyphenol by H_2O_2. The longer the reaction time, the slighter the color of the reaction solution, indicating more polymerization of quinone took place. Variation of ISS with reaction time is shown in Figure 6, where it can be seen that ISS was substantially increased by initially increasing the reaction time attributed to the increase in the initial stage of the reaction, and almost levels off at 360 min.

HRP concentration

Several experiments were carried out with enzyme concentrations ranging from 7×10^{-4} to 0.03 mg/mL. Figure 7 shows that initially, HPR concentration strongly influences the ISS: the higher the enzyme concentration, the higher the ISS. When the HRP concentration is greater than 0.005 mg/mL, ISS almost remains constant. This may be explained because the increase in the concentration of HRP results in the primary radical, UHMWPE radical, and grafting macroradicals of side chains, which may interact with each other, thus terminating the radicals.

CONCLUSIONS

In this study, grafting of UHMWPE fibers with AM monomer using peroxidase was performed. To prove that UHMWPE fibers were grafted, FTIR analysis and SEM scanning were used. By using SEM and FTIR, we noticed a change in topography and roughness, and hydrophilic groups such as –NH and C═O were introduced onto the treated fiber surface. The pull-out test proved that the interfacial adhesion between the fiber and epoxy was improved. The optimum reaction parameters were as follows: $4.0 \times 10^{-2}M$ initiator concentration, $4.0 \times 10^{-3}M$ H_2O_2 concentration, 0.15*M* monomer concentration, 0.005 mg/mL HRP concentration, and 360 min reaction time.

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