

# Solvents Effect in Horseradish Peroxidase Catalyzed Modification of UHMWPE Fiber

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**ABSTRACT:** Graft copolymerization of acrylamide onto ultrahigh molecular weight polyethylene (UHMWPE) using horseradish peroxidase as catalyst was performed in different reaction solution to study the solvent effect. The factors affecting the grafting effect such as the solvents composition, initiator, reaction temperature, and reaction time were investigated. The grafted UHMWPE fiber surface was characterized by IR spectrum, scanning electron microscopy, thermal analysis, contact angle measurement, and single fiber pull out test. Polar groups such as  $-\text{CO}$  and  $-\text{NH}_2$  are approved to appear on the surface after treatment. The contact angle with water decreased from 85 to 50, indicating the wettability of the fiber has been increased. The surface of

the grafted fiber became rougher and its adhesion to resin increased significantly, and hence is expected to produce reinforced plastics with resin. It was also found that the grafting effect was higher in dioxane than in other solvents used and the initiator substituted by electron donating group facilitates the grafting reaction. And the optimum reaction condition was determined to be reacting in 40% (v/v) dioxane solution at 30°C for 2 h. © 2006 Wiley Periodicals, Inc. *J Appl Polym Sci* 102: 674–678, 2006

**Key words:** horseradish peroxidase; surface modification; nonaqueous solvents; UHMWPE

## INTRODUCTION

The modification of polymers has received much attention recently. Among the methods of modification of polymers, grafting is one of the promising methods. In grafting mechanisms, solvent is the carrier by which monomers are transported to the vicinity of the backbone. The choice of solvent depends upon several parameters, including the solubility of monomer in solvent, the swelling properties of the backbone, the miscibility of the solvents if more than one is used, the generation of free radical in the presence of the solvents, etc.

Although enzyme catalysis has been known for well over a century,<sup>1</sup> it has been largely restricted to aqueous systems until the relatively recent development of nonaqueous enzymology.<sup>2</sup> From an industrial standpoint, nonaqueous enzymology has a significant impact on syntheses that are difficult, or even impossible, with conventional chemical catalysts. For example, the use of enzyme catalysis in organic solvents has enabled both the synthesis of polymers such as polyphe-

nylene<sup>3</sup> and polyaromatic amines<sup>4,5</sup> for their electro-optical properties. The effect of solvents composition on polymer molecular weight has been reported and it was shown that varying the solvents composition could control the polymer molecular weight. Madhu et al. found that as the amount of solvent in the reaction medium increased from 0 to 100%, the polymer molecular weight displayed a bell shaped dependency on the solvent composition and the polymer exhibited its highest molecular weight at an intermediate solvent concentration.<sup>6,7</sup> In addition to the enzymatic synthesis of polymers, enzyme catalyzed selective modification of polysaccharides for processability, and biodegradability has been studied.<sup>8</sup>

The enzymatic modification of synthetic materials has immense potential in the functionalization of bulk materials, such as polyacrylonitrile, polyamide, or polyester, and in the production of polymers for specialty applications.<sup>9</sup> The enzymatic grafting method is quite new. The principle involved is that an enzyme initiates the grafting reaction.<sup>10</sup> For example, tyrosinase is capable of converting phenol into reactive *o*-quinone, which undergoes subsequent nonenzymatic reaction with chitosan.<sup>11</sup> And enzymatic grafting on poly(dicarbazole-*N*-hydroxysuccinimide) film was reported by Cosnier et al.<sup>12</sup>

In our previous work, acrylamide was successfully grafted onto polyethylene using horseradish peroxi-

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dase.<sup>13</sup> The purpose of the present article is to examine the relationship between the grafting effect and the reaction media as well as initiator. The initiators used are *O*-cresol, *P*-cresol, and *O*-methoxyphenol; they are oxidized by hydrogen peroxide with horseradish peroxidase as catalysis to give birth to phenol radical, which subsequently initiated the grafting reaction of acrylamide. The results of experiments showed that dioxane is the best media in the solvents used to graft acrylamide onto polyethylene, and the initiator substituted by electron donating group facilitates the grafting reaction.

## EXPERIMENTAL

### Materials

Ultrahigh molecular weight polyethylene (UHMWPE) fibers were obtained from Da Cheng High Technology Manufactory. Horseradish peroxidase (HRP) used was purchased from Shang Hai Bio-chemical Company. *O*-Methoxyphenol was purchased from Shanghai Chemical Company. Hydrogen peroxide was bought from Xi'an Chemical Reagent Plant. The buffer and other chemical reagents used in this study were of analysis grades.

### Surface modification of UHMWPE fiber

Before surface modification by enzyme, the fibers were washed with acetone and distilled water, then dried in vacuum oven for 4 h at 70°C. UHMWPE fibers (0.1 g), 3 g acrylamide, 0.2 mg of HRP in 20.0 mL mixture of water and water-soluble organic solvents (pH 7.0), and 1.0 mL *O*-methoxyphenol (5% solution) were put into a 50 mL flask. H<sub>2</sub>O<sub>2</sub> (0.1 mL; 3.0% aqueous solution) was added in portion 20 times at 1 h intervals at room temperature. Then the mixture was maintained for another 2 h at certain temperature in a constant temperature shaker. After that the UHMWPE fibers were washed with water (three times), a mixture solvent of ethanol and water (1:1 (v/v), three times), and acetone (three times) sequentially, and finally dried under vacuum.

### Single fiber pullout test

A specially designed and homemade micro-tensile testing machine was used to perform single fiber pullout test. A single fiber was fixed at both ends, then embedded in an epoxy resin casting and cured at 70°C for 4 h. Upon curing, the fiber is pulled out with a homemade testing machine. The interfacial shear strength (ISS),  $\tau$ , of UHMWPE fiber can be calculated from eq. (1):

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

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

### Characterization

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

Thermal properties were characterized with a CDR-4p differential scanning calorimeter. Samples of 5.0 mg in aluminum cells were heated from 50 to 350°C at a rate of 10°C/min. Melting temperature,  $T_m$ , was identified at the peaks of melting endotherm.

The IR analysis of the PE sample fibers were performed from 400 to 4000 cm<sup>-1</sup> with a Perkin-Elmer Spectrum 1000 IR spectrometer.

Water contact angle of the fibers were evaluated using a face contact anglemeter (Kyowa Kaimen Kagaku CA-DP a type).

## RESULTS AND DISCUSSION

### Effect of the solvents

The effect of solvents on the grafting reaction is complicated because it interacts with almost every element in the reaction media. For example, the solvent is the carrier by which monomers are transported to the vicinity of the backbone, and the activity of enzyme as well as the solubility of the initiator depends upon the nature of the solvents. In addition, its swelling ability of the backbone also has a great influence on the grafting efficiency. So the selection of organic solvent is important. In our experiment the reaction was carried out in the mixed solvents of water and water soluble organic solvents, including methanol, dioxane, DMF, and THF, to study the solvent effect on the grafting reaction. Figure 1 shows the effect of solvents on grafting efficiency. As can be seen, the grafting order of the solvents used is dioxane > THF (methanol > DMF). This may be explained by its swelling ability of the backbone and the enzymatic activity in different solvents. The solubility parameter of polyethylene is 9.55, and the solubility parameters of the solvents used are 9.9 (dioxane), 9.5 (THF), 12.1 (DMF), 14.5 (methanol), respectively. It is well known that the solubility parameters  $\delta$  of the solvents should be close to the polymeric backbone so that there is necessary chemical energy to disrupt intermolecular cohesive forces between polymer chains and permit chain mobility. And from the solubility parameters we can see that the swelling ability of polyethylene is better in THF and dioxane, while the enzyme activity is higher in dioxane than in THF, and thus the grafting effi-

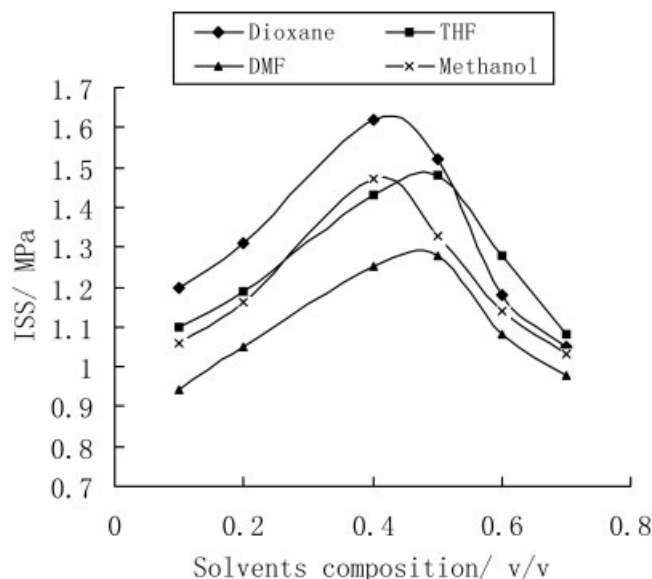


Figure 1 Effect of solvents on the ISS value.

ciency is higher in dioxane than that in THF. And for DMF and methanol, their swelling ability is not good, but the catalytic activity of HRP is higher in methanol, thus the grafting result is better in methanol. It also can be seen that the ISS value increases with the increase of organic solvent concentration, attaining a maximum and then decreasing again. This is because the solubility of the enzyme substrate, phenol, is better in organic solvents than that in water, which can increase the diffuse velocity of phenol. However, the catalytic activity of enzyme decreases at high non-aqueous solvent concentrations,<sup>14,15</sup> which should be responsible for the subsequent decrease of ISS value. Another reason may be that the increase in organic concentration changed the substrate partitioning between the solvent and the enzyme's active site.

#### Effect of reaction time

The graft reaction was carried out in the time range 0.5–3.0 h with the other factors in constant to study the influence of reaction time on the grafting reaction. The results are shown in Figure 2; it could be seen that the ISS value increases with the increase in reaction time until a maximum was obtained at about 2 h, and the continue increase of reaction time have little effect on the ISS value. This phenomenon may be explained by the slow decay of the enzyme activity.<sup>16,17</sup> The figure also indicates that the initial velocity of grafting is higher in dioxane than that in the other organic solvents used. This may be explained by the greater swelling ability of dioxane, and another reason may be because the catalytic activity of horseradish peroxidase is higher in dioxane than in other solvents.

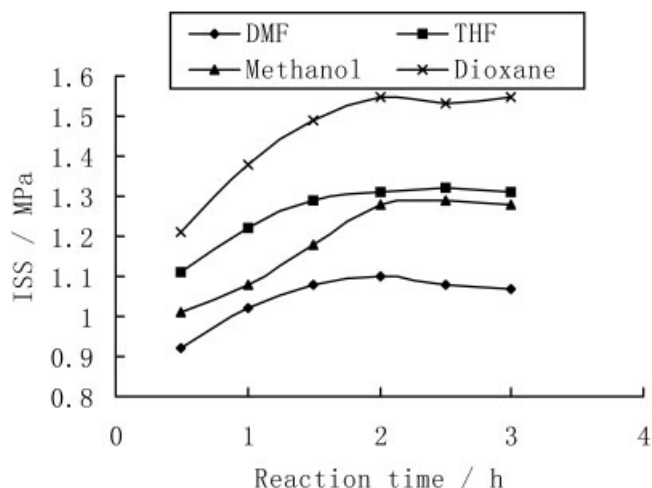


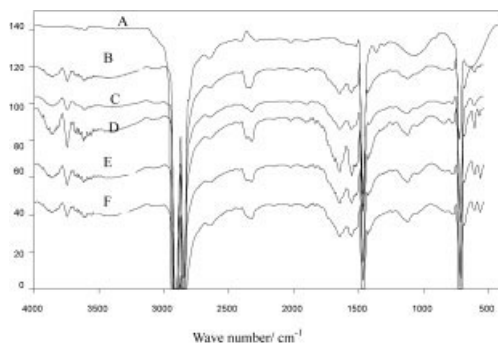
Figure 2 Effect of reaction time on the ISS value.

#### Effect of initiator

The grafting effect is dependent upon the properties of initiator as well as its concentration. In our experiment, the substrate of enzyme was used as initiator, which is oxidized in the process of grafting reaction and give birth to the phenol radicals, and subsequently induce the grafting reaction of acrylamide. To examine the effect of initiator on the grafting efficiency, the grafting reaction was carried out using different initiator with the other factors in constant. The results were shown in Table I. It can be seen from the table that the nature of the initiator affects the grafting reaction as well as the concentration of the initiator. The initial increase of the concentration is accompanied by the increased ISS value until an optimum is obtained, and the continual increase of concentration caused a little decrease on the ISS value. This may be because the initial increase of initiator concentration increased the concentration of free radicals in the system, and above a certain limit, the abundance of this radicals leads to their homopolymerization, thereby giving rise to decreased grafting. This is in good agreement with the result of Celik and Sacak.<sup>18</sup> The ISS value also indicates that the grafting order is *O*-methoxyphenol > *p*-cresol > *O*-cresol.

TABLE I  
Effect of Initiator on the Grafting

Initiator (m/v)	ISS (MPa)		
	<i>O</i> -Cresol	<i>P</i> -Cresol	<i>O</i> -Methyphenol
0.33	1.02	1.08	1.00
0.67	1.11	1.10	1.11
1.00	1.12	1.17	1.32
1.33	1.05	0.97	1.33
1.67	1.02	0.98	1.30
2.00	0.85	0.88	0.93



**Figure 3** IR spectrum of the virgin fiber (A) and the grafted fibers (B, C, D, E, F).

This may be because the methoxy group is an electron donating group, which makes the phenol easier to transfer an electron to the Fe(IV) in the active site of horseradish peroxidase, resulting in increased phenol free radical concentrations, and more active site on the PE surface, and thus enhanced the grafting efficiency. The difference of *p*-cresol and *O*-cresol on the grafting yield may be caused by steric effect. So we come to the conclusion that electron-donating group substituted on phenol can enhance the grafting efficiency and when the same group is substituted on different position, the steric effect should be considered.

### IR analysis

The IR spectra of the HRP-treated and the virgin PE fibers are shown in Figure 3, where A is the spectrum of the untreated fiber, while B, C, D, E, and F refer to the spectra of the fiber that were treated in 10, 20, 40, 50, and 70% dioxane solution with the monomer concentration at 15%. Compared to the spectra of virgin fibers, the AM grafted fiber surface has several new peaks at 1675, 1603, 3598, and 3637  $\text{cm}^{-1}$ , the broad band at 1675  $\text{cm}^{-1}$  corresponds to a C=O group adjacent to an  $\text{NH}_2$  group. The peak at 1603  $\text{cm}^{-1}$  is assigned to the in-plane bending vibration of  $\text{NH}_2$ , and the two bands at 3598 and 3637  $\text{cm}^{-1}$  are ascribed to the stretching vibration of  $\text{NH}_2$  group. All of these suggested that acrylamide has been grafted onto polyethylene. The intensities of these absorption peaks increased with the increasing solvent concentration and got a maximum at about 40%, and decreased at higher solvent concentration. This indicates that the grafting effect is dependent upon the solvents composition, which is in consistent with the results of single fiber pullout experiment.

### DSC and contact angle analysis

The melting point of the grafted PE fiber was determined from the peak in the endotherm. Table II shows

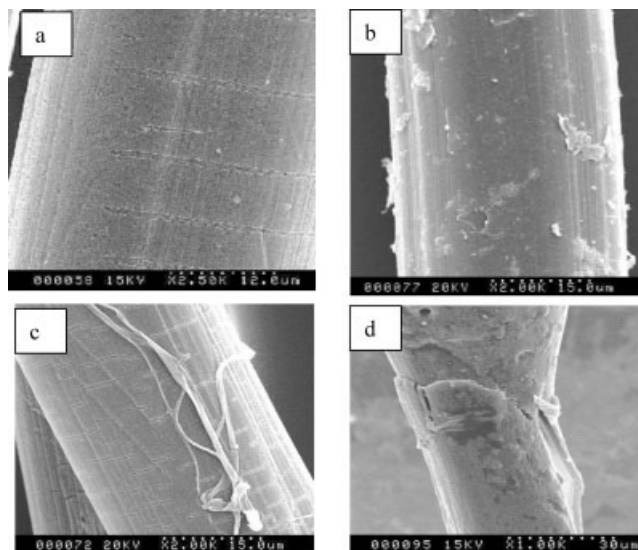
**TABLE II**  
Results of DSC Analysis and Contact Angle Measurement

Solvent	$T_m$ ( $^{\circ}\text{C}$ )	ISS (MPa)	Contact angle with water
PBS + methanol	148.5	1.34	68
PBS + DMF	147.9	1.30	76
PBS + dioxane	149	1.62	48
PBS + THF	148.5	1.23	64
Original fiber	146.5	0.89	85

the crystalline melting transition behavior of the fiber treated in different solvents and the untreated fiber. It can be seen that the melting point of the untreated fiber is 146.5 $^{\circ}\text{C}$ . However, because of the grafting reaction, an increase in the melting point was obtained, which indicate that grafting affect the crystalline structure of the fiber. And the contact angle decreased from 85 to 50, indicating that the wettability of the fibers has been increased significantly. It also can be seen from the result that the change of melting point and the decrease of contact angle are different in different solvents, and the biggest change occurs in dioxane, which is in agreement with the ISS value. This also proved that dioxane is the best solvent in the solvents used for PE surface modification.

### SEM analysis

Figure 4 shows the SEM micrographs of acrylamide grafted PE and the untreated PE surface. As can be seen, the grafted fiber shows roughen surface with pits compared to SEM micrograph of the virgin fiber.



**Figure 4** SEM micrographs of (A) untreated fiber, (B) the grafted fiber, (C) untreated fiber after single pullout, and (D) grafted fiber after pullout.

Moreover, after pullout test, many epoxy resin still remain on the surface of the grafted fiber, while almost no epoxy resin remained on the surface of untreated fiber, suggesting that the introduction of polar groups on the surface of PE fiber may improve the interfacial adhesion between the fiber and the matrix due to an increase in surface area for bonding and mechanical interlocking,<sup>19,20</sup> which is in good consistent with the result of ISS measurement.

### CONCLUSIONS

In this article, the effect of solvents and initiators on the grafting effect was investigated. The grafting effect is higher in dioxane than in the other solvents used. And in all the solvents used, the grafting effect increases with the increasing solvent concentration until a maximum is obtained, and the further increase of the solvent concentration caused a sharp decline of the grafting effect. The initiator affects the grafting reaction as well, in the initiators used, and the best grafting effect is obtained by using *O*-methoxyphenol as initiator. And it was also found that an electron-donating group facilitates the grafting reaction. Characterization found that the modified HDPE fibers has much improved hydrophilicity and higher surface energy, and thus is expected to produce reinforced plastics with resin.

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