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Grafting modification of Kevlar fiber using horseradish peroxidase

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Summary

Horseradish peroxidase catalyzed grafting of acrylamide (AM) onto Kevlar fibers in mixed solvents of dioxane and water was studied. The factors affecting the grafting reaction, such as solvents composition, reaction time, temperature, pH of reaction media, concentration of acrylamide and hydrogen peroxide were examined. The modified fiber was characterized with IR, scanning electron microscopy (SEM), elemental analysis, XPS, contact angle measurement and the grafting yield. The O/C ratio and N/C ratio on the surface increased after treatment and the surface of the Kevlar fiber became rougher. The contact angle decreased from 80 to 45 after grafting, indicating the wettability of the fiber increased after modification. All the results suggested that AM have been grafted onto the Kevlar surface through HRP-mediated radical initiated grafting reaction. And the probably mechanism of HRP catalyzed grafting of AM onto Kevlar surface was proposed.

Instruction

Kevlar fiber exhibits an excellent thermal stability, as well as superior tensile strength and modulus [1]. And owing to its high tenacity, Kevlar fiber is widely used in manufacture of advanced composites. It is well known that the mechanical properties of fiber reinforced composites depend on the effectiveness of the interactions between the fiber and the matrix, however, the adhesion between Kevlar fiber and most matrices is poor as a result of the high crystallinity resulting in a chemical inertness and smooth surface of the fiber [2]. Therefore, to use Kevlar fiber as reinforcement, surface modification is essential to enhance its reinforcing effect, extensive studies have been performed on this subject [3,4]. Enzymatic technology has attracted much attention in recent years owning to its advantages such as milder reaction conditions and highly specific nondestructive transformations targeted to surfaces [5]. The application of enzymes to modify the surface of natural polymer, such as wool has been widely researched by industry [6]. However, enzymatic modification of synthetic polymer is quite a new technology, only a few works has been done on this subject [7,8].

Horseradish peroxidase is a heme-containing enzyme that utilizes hydrogen peroxide to oxidize a wide variety substrate. The oxidative coupling of phenol [9] and aromatic

amines [10] catalyzed by HRP in the presence of hydrogen peroxide has been extensively studied In addition; polymerization of vinyl monomers such as acrylamide [11], methyl methacrylate [12], and styrene [13] using β-diketones as initiators was also reported. And it was also exploited to graft phenols onto phenolic polymers [14]. Amine radical was generated in the process of HRP catalyzed oxidative coupling reaction according to reference [15], and hence we are encouraged to utilize the radicals to initiate the grafting reaction of acrylamide onto Kevlar fiber. The reaction was carried out in mix solvents of water and dioxane. The reaction condition was optimized. And the probably reaction mechanism was discussed.

Experimental

Materials

Kevlar 49 fibers (1.5denier, diameter 11.7μm) were obtained from Do Pont, Inc., USA. Horseradish peroxidase (HRP) used was purchased from Shang Hai Bio-chemical Company. Acrylamide was of analysis grades, which purchased from Shang Hai Chemical Company. The buffer and other chemical reagents used in this study were of analysis grades.

Enzymatic Grafting of AM onto Kevlar Fiber Surface

0.1 grams washed Kevlar fiber and 0.5mg HRP in 2.0ml phosphate butter (PBS) (100mM, pH7.0) were put in a 50ml flask. 0.75g acrylamide and certain volume of solvents were introduced into the flask. The mixture was degassed for 30 min. Then the required volume of the H_2O_2 solution was added dropwise to the mixture over 1 h at certain temperature in a constant temperature shaker, after that the mixture was maintained for required time. And then the fibers was refluxed in boiling acetone for 4 h. and finally dried under vacuum. The resultant fiber was designed as fiber A. The fiber using inactive HRP solution that was heated in boiling water for 30min replaces the 0.5mg/ml of HRP in 2.0ml phosphate butter was designed as fiber B. And that using 2.0ml phosphate butter replaces the 0.5mg/ml of HRP in 2.0ml phosphate butter was fiber C.

Characterization

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

Elemental analysis was performed in a GE instruments apparatus Mod. EA 1110 (ThermQuest Italia SPA)

The X-ray photoelectron spectroscopy experiment was performed using a PHI-5400 spectrometer equipped with an Mg-Kα X-ray source. The base pressure in the sample chamber was controlled in the range of 10^{-8} to 10^{-9} Torr.

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

Attenuated total reflectance-FTIR (ATR-IR) spectra were recorded using Tensor-27 Fourier transform Infrared spectrometer.

The Kevlar fibers after graft copolymerization were characterized by graft yield determination. The graft yield was calculated as follows:

$$\%\text{Graft yield} = 100\% \ (W_2 - W_1)/W_2 \qquad (\mathbf{1})$$

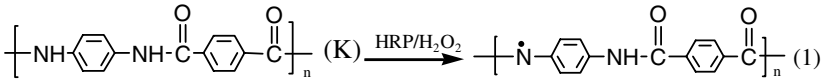
Where W_1 and W_2 are the weight of the initial fiber and the grafted fiber respectively.

Results and Discussion

Mechanism of Catalyzed Grafting of AM onto the Surface of Kevlar fiber

Previous studies have found that the catalysis mechanism of most peroxidase is similar, which contains three distinct steps [16]. The probably mechanism of horseradish peroxidase catalyzed grafting modification of Kevlar fiber in the presence of hydrogen peroxide is proposed based on our investigation. As shown in scheme 1, the aniline group of Kevlar fiber (K) first undergoes an oxidative reaction catalyzed by horseradish peroxidase in the presence of hydrogen peroxide and subsequently formed the corresponding radicals on the surface of Kevlar fiber [15]. Then the radical transfers an electron to the monomer AM (M) and forms the AM radicals that can be grafted onto the Kevlar surface to modify the Kevlar surface. The side reaction is the homopolymerization of the acrylamide. And the reaction is terminated by the copolymerization between Kevlar radicals and acrylamide radicals, polymerization of acrylamide, and the crosslinking of Kevlar fiber.

Radical generation



$$K\bullet + M \longrightarrow M\bullet + K \qquad (2)$$

Propagation

$$M\bullet + (n\text{-}2)M \longrightarrow M_{n+1}^{\bullet} \qquad (3)$$

$$K\bullet + M \longrightarrow K\text{—}M\bullet \qquad (4)$$

$$K\text{—}M\bullet + M \longrightarrow K\text{—}M_2^{\bullet} \qquad (5)$$

Termination

$$K\text{—}M\bullet + M_{n-1}^{\bullet} \longrightarrow K\text{—}M_n \qquad (6)$$

$$M\bullet + M_{n-1}^{\bullet} \longrightarrow M_n \qquad (7)$$

$$K\bullet + K\bullet \longrightarrow K\text{—}K \qquad (8)$$

Scheme 1. mechanism of the enzyme catalyzed grafting reaction

Elemental Analysis and contact angle mearament

The content of three elements on the fiber surface, that is, carbon, hydrogen, and nitrogen was estimated. The results were shown in Table 1. As can be seen, the

content of nitrogen atom increased in comparison with Kevlar-B and Kevlar-C. This may be caused by the introducing of acrylamide on the fiber surface. And the contact angle decreased greatly after modification, suggesting that the wettability of the treated fiber increased significantly. The grafting yield was different under different condition, which suggested that the grafting condition such as monomer concentration, pH of medium greatly affect the grafting yield. From the above results, it is clear that the grafting of AM onto Kevlar surface through HRP-mediated radical initiated reaction must have occurred.

Tab. 1 Results of elemental analysis and contact angle measurement

Polymer	AM%	pH	Carbon%	Hydrogen%	Nitrogen%	Contact angle	Grafting yield%
Kevlar			67.81	3.600	11.19	82	
Kevlar-B	10	7	67.75	3.623	11.20	79	0.14
Kevlar-C	10	7	67.80	3.558	11.19	80	
Kevlar-A1	10	7	68.24	3.567	11.34	54	2.52
Kevlar-A2	5	5	67.49	3.843	11.24	47	5.35
Kevlar-A3	3	9	68.57	3.478	11.37	62	1.72

IR analysis

The IR spectroscope was also applied to observe the effect of the grafting reaction on the Kevlar fiber surfaces .The IR spectrum of the virgin fiber (A) and AM grafted fiber (B, C, D) were shown in figure 1. As can be seen, the spectrum of the treated and untreated fiber has three peaks at 3300cm^{-1}(-NH group), 1640cm^{-1}(-C=O group), and 1540cm^{-1}(-NH group by bending). But we can see that the intensities of the treated

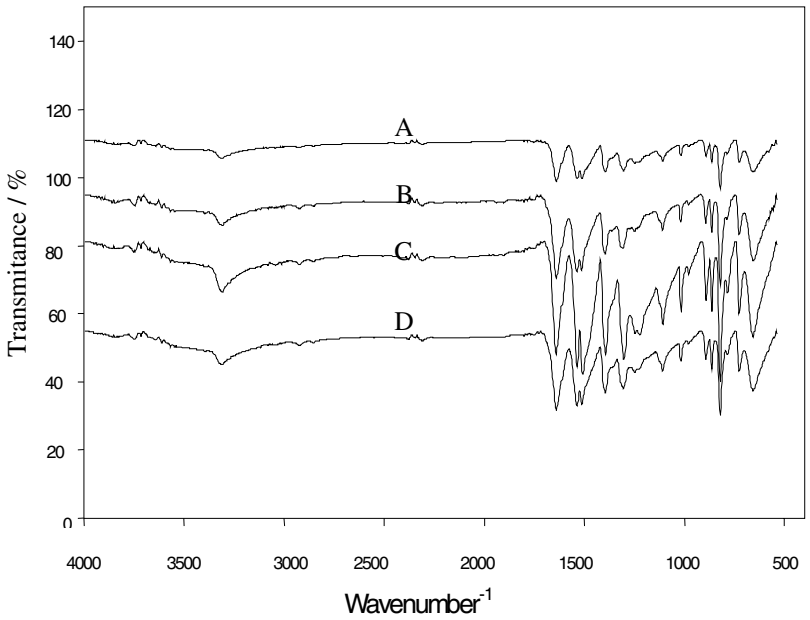


Fig. 1. IR spectra of the virgin fiber (A) and the treated fibers (B,C,D)

fiber improved significantly compared to the spectrum of the virgin fiber, which suggest that the monomer has been grafted onto the polymer surface. Therefore, we confirm that the surface properties of Kevlar fiber are increased, resulting in increasing the interfacial binding force with matrix [17].

XPS analysis

The surface composition of the Kevlar fiber was analyzed by XPS, and the result was shown in figure 2. Where A is the spectrum of the virgin fiber and B refers to the modified fiber. The N_{1s}/C_{1s} ratio and O_{1s}/C_{1s} ratio of the virgin fiber are 0.47 and 0.09 respectively, while after grafting reaction, the value increased to 0.485 and 0.14, indicating the increasing polar group on the fiber surface. Consequently, increasing the polar groups on the surface may promote the surface energy of the fibers as well as interfacial bonding by establishing secondary or van derwaals forcers at the interfaces between fibers and the matrix, resulting in increasing the mechanical interfacial properties of the composites.

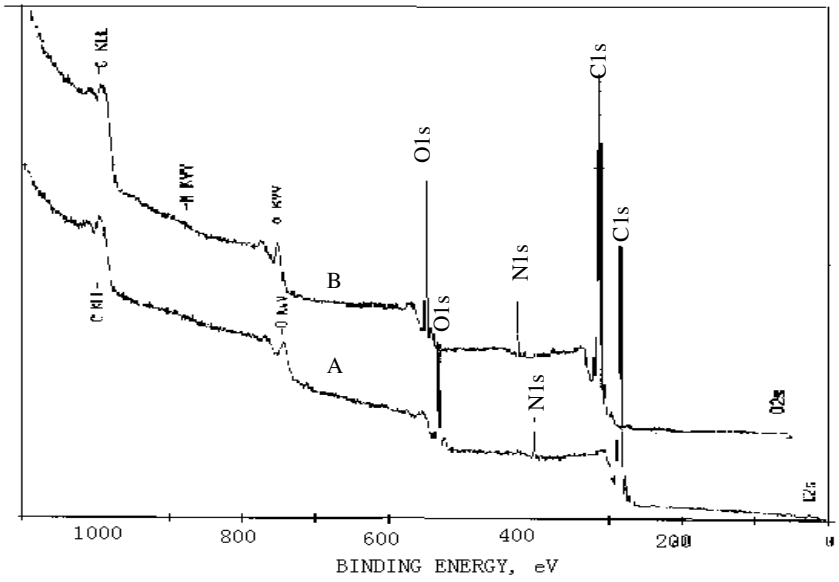


Fig. 2. X-ray photoelectron spectrum of Kevlar fiber (A) and AM grafted fiber (B)

SEM Analysis

The changes in the topography and morphology of fiber surfaces were studied by SEM; the result was shown in figure 3. It can be observed that the surface of the treated fibers is highly rough in comparison with the untreated fiber, which is attributed to the high graft density. Some researches have proved that the adhesion of the treated fiber to other materials was improved with an increase in the roughness of its surface due to an increase in surface area for bonding and mechanical interlocking [18]. So the rougher surface on the Kevlar is expected to be benefit to improve the adhesion of Kevlar fiber to other polymers, and hence improve the mechanical performance of composites.

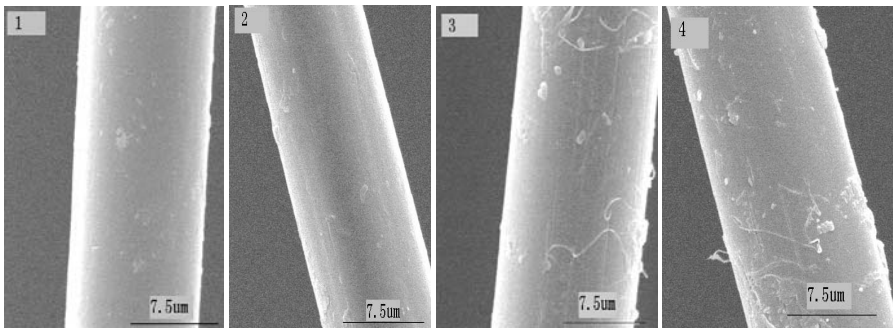


Fig. 3. SEM micrographs of the untreated fiber (1), the fiber treated with inactivate HRP (2) and the treated fibers (3,4)

Effect of the AM Concentration

The monomer concentration is an important factor that influences the grafting reaction. Since it is the real substance that was grafted onto the polymer surface. The effect of monomer concentration on grafting reaction is presented in Fig 4. As can be seen, the grafting yield increases with the increasing AM concentration until a maximum is attained at about 5.0%, and then decreases with the further increase in monomer concentration. This is in consistent with reference [19]. This behavior may reflect an initial increase of the monomer concentration in close proximity to the backbone. After a certain limit, the increase in monomer concentration accelerates the homopolymerization reaction rather than grafting, which lead to the decreased grafting yield.

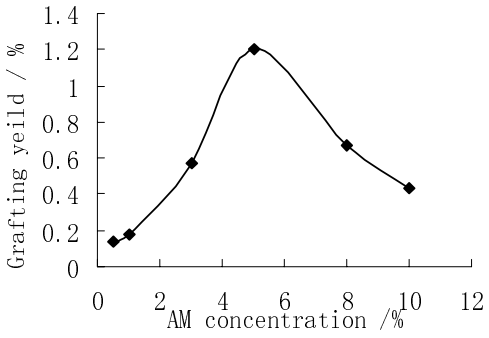


Fig. 4. Effect of AM concentration on the grafting yield

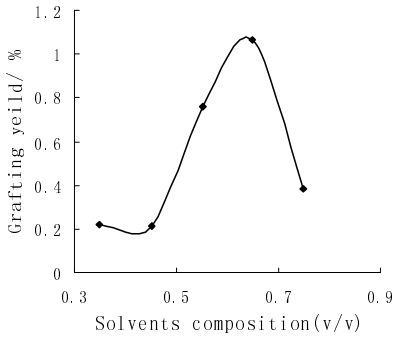


Fig. 5. Effect of solvents concentration on the grafting yield

Effect of solvents composition

The effect of solvents on the grafting reaction is complicated because it interacts with almost every element in the reaction media. In our experiment, the reaction was carried out in the mix solvents of water and dioxane. Figure 5 shows the effect of solvents concentration on grafting yield. As can be seen, the grafting yield increases with the increasing dioxane concentration, attaining a maximum at about 65% dioxane solution and then decreasing again. This may be because the catalytic activity of

enzyme decreases at high nonaqueous solvent concentrations [20,21]. Another reason may be that the increasing organic concentration changed the substrate partitioning between the solvent and the enzyme's active site.

Effect of H_2O_2 concentration

The effect of H_2O_2 concentration on Grafting yield is presented in figure 6. The results show that grafting yield increases with the H_2O_2 concentration. This indicates that the grafting yield gradually increased with the increase in the extent of enzymatic treatment. Beyond the value of 1.5 ml H_2O_2 we notice a decrease-grafting yield. This may be due to degrade of HRP by H_2O_2, which cancels out the catalytic activity of the enzyme [22].

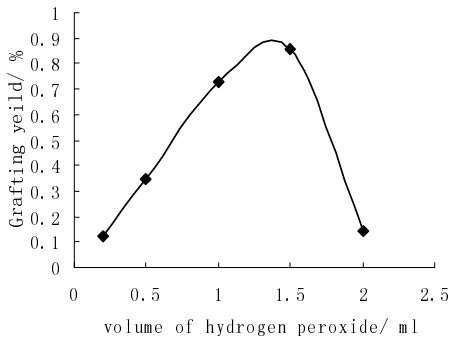


Fig. 6. Effect of H_2O_2 concentration on the grafting yield

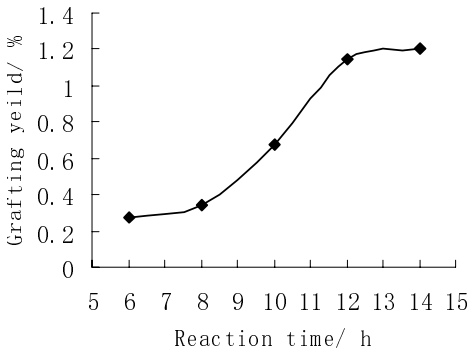


Fig. 7. Effect of reaction time on the grafting yield

Effect of reaction time

Because primary radicals should be produced even after a long inhibition period to ensure that the reaction do not stop, and a slow initiation step is consistent with this interpretation [23]. In all processes for grafting Kevlar fiber, there was a long and engrafting inhibition period. Variation of grafting yield with reaction time was shown in Fig 7, it could be seen that grafting yield was greatly increase with increasing of reaction time and almost levels off after 12 hours. This phenomenon may be explained by the slowly decay of the enzyme activity [8].

Effect of pH

The influence of pH on the grafting reaction was considered. Phosphate buffer was used to cover a pH range from 5 to 9. The result was shown in figure 8, as can be seen, the reaction media with pH between 5 and 9 all lead to grafting reaction, and the maximum grafting yield was obtained at 7. In order to explain this phenomenon, we examined the effect of pH on the catalysis activity of horseradish peroxidase according to reference [24]. It is found that the catalysis activity showed an inverse bell dependent on the pH in the range investigated, (data not shown) which was in good consistent with the phenomenon. So the grafting yield is influenced by the enzyme activity.

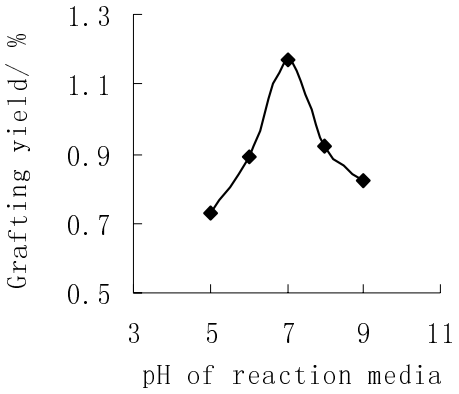


Fig. 8. Effect of reaction pH on grafting yield

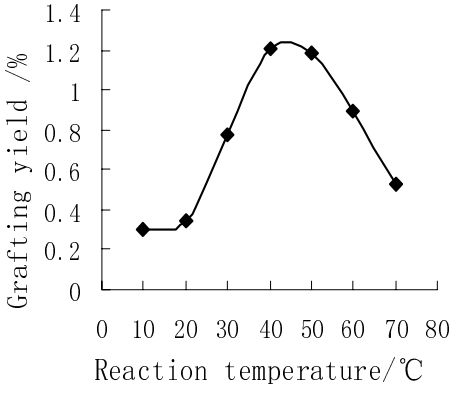


Fig. 9. Effect of reaction temperature on grafting yield

Effect of reaction temperature

The temperature is one of the important factors that control the kinetics of graft co-Polymerization. The effect of reaction temperature on the grafting reaction was shown in figure 9, it could be seen from the figure that with the variation of reaction temperature the grafting yield has a maximum value at about 40 ^{o}C. The initial increase with increasing temperature in grafting is due to greater swelling of backbone [25], and a corresponding enhanced rate of diffusion of the monomers in the vicinity of Kevlar. And the subsequent decrease is due to the increased molecular motion with increased temperature, resulting in increased radical decay. And the loss of enzyme activity at higher temperature may be another factor that should responsible for the decreased grafting efficiency.

Conclusions

In this study, grafting modification of Kevlar fibers with AM monomer using horseradish peroxidase as catalyst was performed. Several independent analytical approaches were used to provide evidence that AM could be grafted onto the surface of Kevlar fibers through horseradish peroxidase catalyzed reaction. Practically, peroxidase may offer an environmentally friendly method for conferring function properties to Kevlar surface. And the optimum reaction condition was determined to be reacting in 65% dioxane solution for 12 hours at 40^{o}C, with the monomer concentration at 5%, pH at 7. Characterization of the fiber found that the polar content of the surface increased significantly and the modified fiber become rougher compared to the untreated fiber, and thus are expected to improve the adhesion of Kevlar fiber to other polymers. The grafting mechanism was determined to be free radical grafting mechanism.

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