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Enzymatic grafting modification of polyethylene film in nonaqueous solvents

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Summary

Modification of the surface of polyethylene film was performed in aqueous organic solvents in the presence of horseradish peroxidase. The affecting factors such as solvents composition, initiator, reaction time and reaction temperature were investigated. It was found that the grafting efficiency is higher in dioxane than that in the other solvents used and the initiators substituted by electron donating group facilitates the grafting reaction. And the optimum reaction condition was determined to be reacting in the 40% dioxane solution at 40°C for 2 hours. The treated film was characterized with scanning electron microscopy (SEM), UV-Vis spectra, FT-IR, and contact angle measurements. Polar groups such as –CO and $–NH_2$ were found to appear on the treated surface and the surface of treated film become rougher than the untreated film. The contact angle with water decreased from 80 to 50 and the absorption of the treated film in ultraviolet area increased significantly, which indicates that the hydrophilicity of PE film had been increased.

Introduction

Polyethylene is an engineering plastic and because of its favorable properties like low specific weight, high chemical resistance and mechanical flexibility, it is widely used in industrial application such as automobile, appliances and engineering components. Since the current approval of PE for use in the human body, it is also widely used in biomedical applications[1]. The limitation of polyethylene is the poor adhesion properties due to its non-polar nature, which leads to poor mechanical properties of their laminates. Various attempts such as plasma [2], corona discharge [3], radiation [4], melting [5] and chemical treatments [6] have been made to improve surface properties of polyethylene. Most of the conventional methods described in literature, used to alter the polymer surface, require strong chemical agents. As a consequence enormous quantities of environmentally hostile chemicals used daily in wet polymer processing at industrial level contribute to raise great concern about the ecological impact of industrial wastes and their treatment. Conversely, in order to lower the hazardous use of harsh chemical, new techniques exploiting the enzymatic catalysis was applied to polymer processing. The application of enzymes to modify the surface

of natural polymers, such as wool has been widely researched by industry [7]. Most of the industrial applications are aimed to improve surface properties by removing adsorbed components, such as fats, waxes, proteins, etc. Textiles processing areas, such as deseizing, scouring and bleaching of cellulose and woolen fabrics are some examples of successful bio-treatments of textiles. Not only unwanted adsorbed material may be removed but also chemical modification of the polymer surface may be accomplished by enzymes [8]. However, chemical modification of synthetic polymers using enzyme is quite new, only a few works have been dong on this subject. For example, Phenolic moieties of the synthetic polymer poly (4-hydroxystyrene) (PHS) were oxidized to reactive *O*-quinones catalyzed by mushroom tyrosinase [9]. Polyacrylonitrile (PAN) has been selectively modified by enzymatic attack, which led to the hydrolysis of the CN groups into the corresponding amides [10]. And the advantages of enzyme catalyzed surface modification over other methods are milder reaction conditions and highly specific nondestructive transformations targeted to surfaces. Further, the selectivity of enzyme offers the potential for better controlling macromolecular structure without the need for wasteful protection/deprotection steps.
Horseradish peroxidase is a heme-containing enzyme that utilizes hydrogen peroxide to oxidize a wide verity substrate. The oxidative coupling of phenol [11] and aromatic amines [12] catalyzed by HRP in the presence of hydrogen peroxide has been extensively studied. In addition, polymerization of vinyl monomers such as acrylamide [13], methyl methacrylate [14], and styrene [15] using β-diketones as initiators was also reported. Because horseradish peroxidase converts phenol substrates into free radical intermediates that undergo a complex set of non-enzymatic reactions, it was also exploited to graft phenols onto phenolic polymers [16]. However, little information has been available on the enzyme-initiated grafting of the mixture of phenols onto polymers to modify the surface properties of polymers. In our previous work, acrylamide was successfully grafted onto polyethylene by using horseradish peroxidase [17]. The purpose of the present paper 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

Polyethylene (PE) films (thick 0.07mm) were obtained from Hai Hong Polymer Manufactory. Horseradish peroxidase (HRP) used was purchased from Shang Hai Bio-chemical Company. The buffer and other chemical reagents used in this study are of analysis grades.

Enzymatic Modification of HDPE Film Surface

PE films were cut into rectangular pieces with a dimension of $120\times30\times0.1mm^3$ (length×width×thickness). Before surface modification by enzyme, the sample pieces

were washed with acetone and distilled water, then dried in vacuum oven for 1h at 70°C.
0.1 g PE films, 0.2mg of HRP in 20.0ml mixture of water and water-soluble organic solvents (pH7.0), and 1.0ml initiator (5% solution) were put into a 50ml flask. 0.1ml H_2O_2 (3.0% aqueous solution) was added in portion 20 times at 1h intervals at room temperature. Then the mixture was maintained for another 2h at certain temperature in a constant temperature shaker. After that the PE films were washed with water (three times), a mixture solvent of acetone and water (1:1 V/V, three times) sequentially, and finally dried under vacuum.

Staining Reaction

The modified PE film was stained with methyl violet. The enzyme-treated HDPE film was suspended in 50 ml flask with 30ml methyl violet solution (5% aqueous solution) for 2h at 60°C. Then the film was washed several times with water and acetone sequentially, and finally dried under reduced pressure. The absorbance of the methyl violet at 560nm was measured to characterize the surface changes.

Characterization

Scanning electron microscopy (SEM) micrographs were recorded using a HITACHI S-570 machine, with an acceleration voltage of 15kV. The surface of the PE sample was coated with gold by vacuum evaporation.
The IR analysis of the PE sample films were performed from 400 to 4000cm^{-1} with a Perkin-Elmer Spectrum 1000 IR spectrometer.
UV-Vis spectra were recorded on PERKIN-ELMER LAMBDA 17 UV-vis spectrophotometer.
Water contact angle of the films were evaluated using a face contact anglemeter (Kyowa Kaimen Kagaku CA-DP A type). Seven different spots were measured for each sample by reading the data within 20 s to ensure that the hydrophilic groups would not diffuse away from the surface.

Results and discussion

Effect of 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; 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 using different organic solvents and the concentration was also changed to examine the effect of solvents as well as solvent concentration. Figure 1 shows the effect of solvents on the absorbance (a) of the film after staining and its contact angle (b) with water. As can be seen, the absorbance increases with the increasing organic solvent concentration, attaining a maximum and then decreasing again. In consistent, the contact angles of the film with water decreased with the increasing solvents concentration, attaining a minimum and then increase again. This is because the solubility of the enzyme substrate, phenol, is better

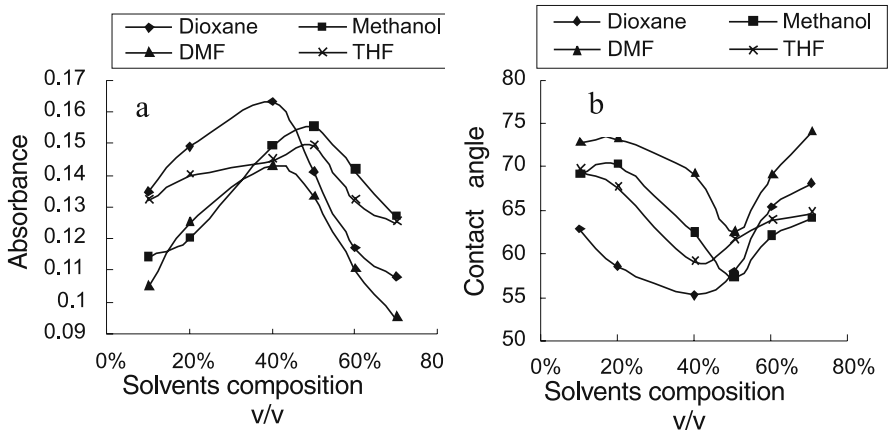

Figure 1. Effect of solvents on the absorbance (a) of film and the contact angle (b) with water

in organic solvents than that in water, which can increase the diffuse velocity of phenol, resulting increased phenol concentration in the vicinity of PE film, and thus enhancing the grafting effects. However, the catalytic activity of enzyme decreases at high nonaqueous solvent concentrations [18,19], which should be responsible for the subsequent decrease of grafting efficiency. Another reason may be that the increasing organic concentration changed the substrate partitioning between the solvent and the enzyme's active site. It can also be seen from figure 1 that in the case of dioxane, a high degree of grafting was obtained as compared with those obtained in other solvents used. This is because the catalytic activity of enzyme is higher in dioxane than that in the other organic solvents used.

Effect of reaction temperature

The temperature is one of the important factors that control the kinetics of graft co-Polymerization. In order to investigate the effect of reaction temperature, we carried

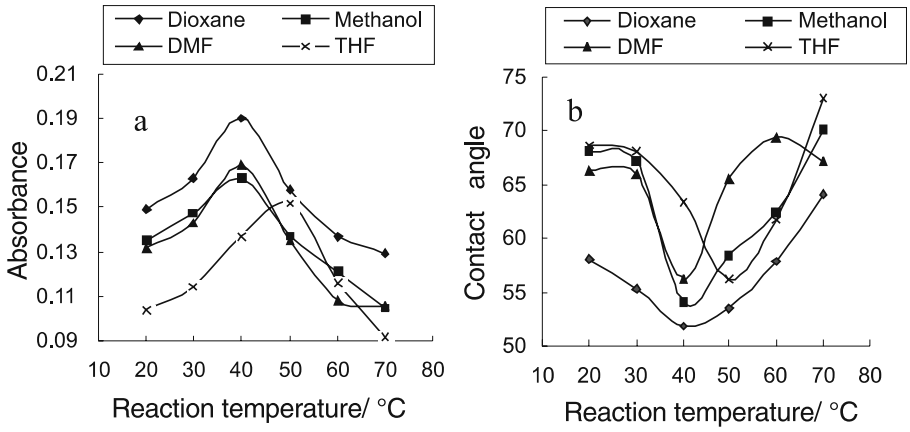

Figure 2. Effect of reaction temperature on the absorbance (a) of film and its contact angle (b) with water

out the experiments in the temperature range 20-70°C with the other factors in constant, the results were shown in figure 2, it can be seen from the figure that with the variation of reaction temperature the absorption got a maximum while the contact angle have a minimum value, which indicates that the grafting effect increases with the increasing temperature, until a limit is attained, and then decreased with the increasing temperature. The initial increase with increasing temperature in grafting is due to greater swelling of backbone [20], and a corresponding enhanced rate of diffusion of the monomers in the vicinity of PE. And the subsequent decrease is due to the increased molecular motion with increased temperature, resulting in increased radical decay. And according to the references, the loss of enzyme activity at higher temperature is another factor that should responsible for the decreased grafting efficiency.

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. In all processes for modifying PE film, 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 became red rapidly due to the oxidization of phenol by H_2O_2. The longer the reaction time, the slighter the color of the reaction solution, indicating the more polymerization took place. Variation of contact angle and absorbance with reaction time is shown in Figure 3, it can be seen that absorbance greatly increases and contact angle decreases with initially increasing the reaction time until a maximum was obtained at about 2 hours, and the continue increasing of reaction time have little effect on the grafting effect. This phenomenon may be explained by the slowly decay of the enzyme activity [21,10]. 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 because the catalytic activity of horseradish peroxidase is higher in dioxane than that in other solvents.

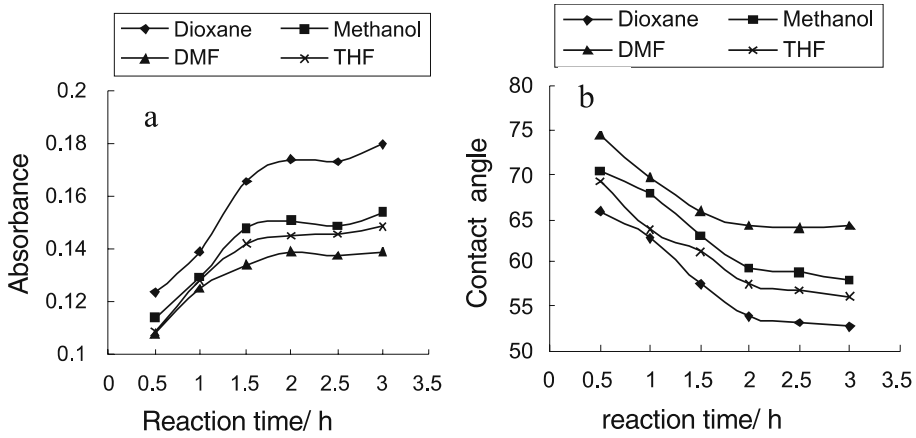

Figure 3. Effect of reaction time on the absorbance (a) of film and its contact angle (b) with water

Effect of initiator

The initiator is an important parameter in the grafting reaction since it is the true reactive species for the production of primary radicals, and its nature, concentration, and solubility need to be considered. In our experiment, the substate of enzyme was used as initiator, which is oxidized in the process of horseradish peroxidase catalyzed redox reaction and gives birth to the phenoxy radicals that subsequently induce the grafting reaction. In order 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 showed in table 1. 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 increasing of the concentration is accomplied by the increased absorption and decreased contact angle until an optimum is obtained, and the continual increase of concentration has little effect on the grafting. The table also indicates that the grafting order of all the initiators used is *O*-methoxyphenol > *p*-cresol > *O*-cresol. This is because the methoxy group is a electron donating group which makes the phenol easier to transfer a 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 attributed to steric effects. 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 effects should be considered.

Table 1. Effect of initiator on the absorbance and contact angle with water

Initiator	Contact angle			Absorbance		
m/v	*O*-cresol	*P*-cresol	*O*-methy-oxyphenol	*O*-cresol	*P*-cresol	*O*-methy-xyphenol
0.33	66	63	56	0.213	0.207	0.214
0.67	57	56	52	0.259	0.244	0.220
1.00	54	52	54	0.274	0.263	0.220
1.33	55	50	44	0262	0.281	0.299
1.67	66	65	54	0.261	0.280	0.257
2.00	64	62	54	0.258	0.243	0.216

SEM analysis

Figure 4 shows the SEM micrographs of acrylamide grafted PE and the untreated PE surface, in which a is the virgin film, the film that treated in 30% dioxane solution is defined as b, and the films treated in 40% and 60% dioxane solution are defined as c and d respectively. As can be seen, the grafted PE surface is highly rough and porous in comparison with the virgin film surface. Such porosity and roughness is attributed to a high graft density. Some researches have proved that the adhesion of the polymer film 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 [22], so the

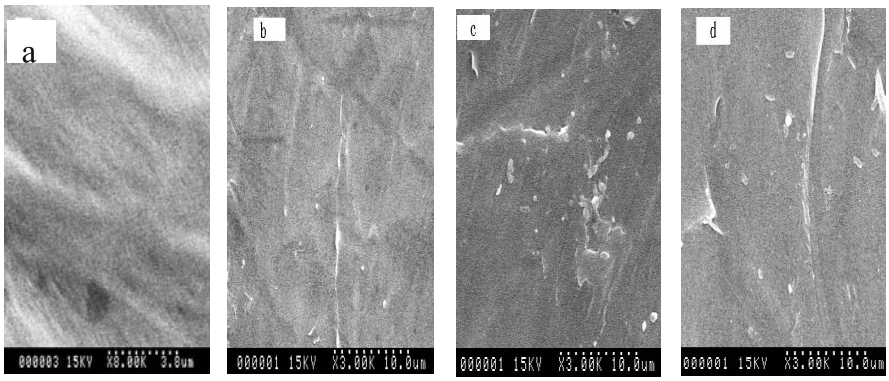

Figure 4. SEM micrographs of the origin film (a) and the treated films (b, c, d)

appearance of pits and roughen surface in the enzyme-treated PE film are expected to be benefit to improve the adhesion of PE film, and hence better mechanical performance of the laminates. The contact angle measurement also decreased significantly after grafting reaction, indicating that the hydrophilicity of the film has been increased. All of these indicate that the acrylamide have been grafted onto the PE film.

IR analysis

The IR spectra of the HRP treated and the virgin PE films were shown in figure 5.Where a is the spectrum of the untreated film while b, c, d, e, f and g refer to the Spectra of the film that were treated in 10%, 20%, 40%, 50%, 60%, 70% dioxane with the

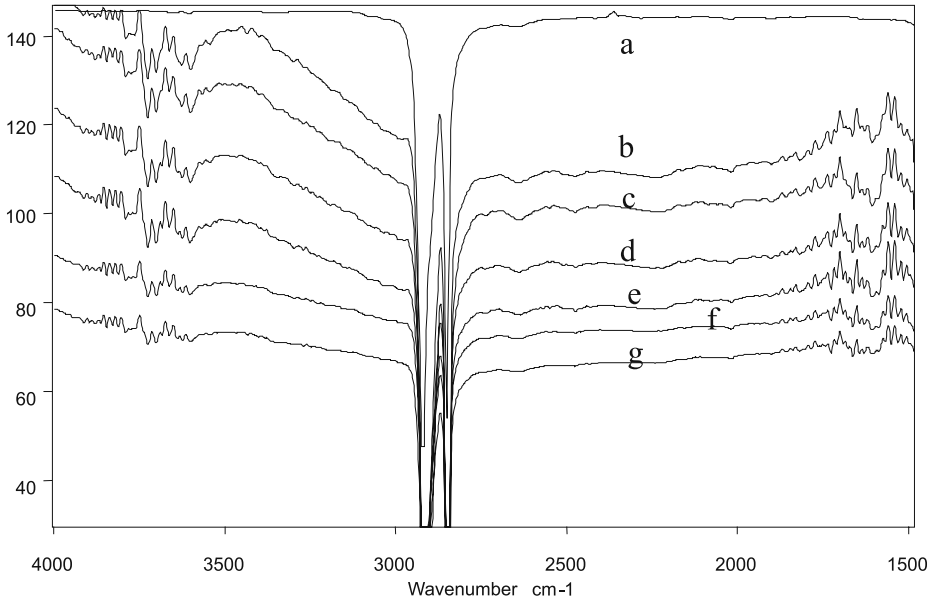

Figure 5. IR spectra of the virgin film (a) and the treated films (b, c, d, e, f, g)

monomer concentration at 15%. Compared to the spectra of virgin films, the AM grafted film surface has several new peaks at 1675cm^{-1}, 1603cm^{-1}, 3598cm^{-1}and 3637cm^{-1}, the broad band at 1675cm^{-1} corresponds to a >C=O group adjacent to an NH_2 group. The peak at 1603 cm^{-1} is assigned to the in-plane bending vibration of NH_2, and the two bands at 3598cm^{-1} and 3637cm^{-1} are ascribed to the stretching vibration of 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 contact angle measurement.

UV-vis analysis

Figure 6 shows UV-vis spectra of the virgin film and the treated films, where a is the virgin film, and b, c, d, e, refer to the films that were treated in 10%, 40%, 50%, 70% dioxane solution. Compared to the untreated film, absorption of the treated films showed a significantly increase at about 260 nm which may due to the introduction of –CO-NH_2 group as proved above by IR spectra. It also can be seen from the figure that the intensities of the peak increases with the increasing solvent concentration until a limit is attained at 40%, and the further increase of the solvent concentration caused a decrease in the absorption. This is in good agreement with the contact angle measurement.

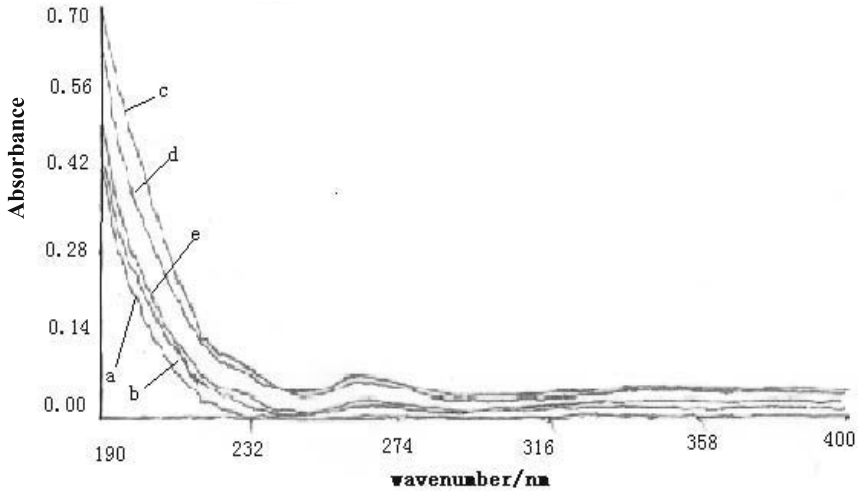

Figure 6. Uv-vis spectra of the virgin film (a) and the treated films (b,c,d,e)

Conclusion

In this paper, it was found that the nature of the solvents affects the grafting reaction as well as the solvent concentration. The grafting effect is higher in dioxane than that 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 sharply decline of the grafting effect.

The initiator affects the grafting reaction as well, in the initiators used; the best grafting effect in obtained by using *O*-methoxyphenol as initiator. Characterization of the treated film showed that The modified HDPE films has much improved hydrophilicity and lower surface energy, and hence are expected to have improved adhesion to other materials.

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