Characterization and Application of Grafted Polypropylene and Polystyrene Treated with Epichlorohydrin Coupled with Cellulose or Starch for Immobilization Processes

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ABSTRACT: The direct-irradiation-induced grafting of acrylamide onto polypropylene films and polystyrene films with crosslinking epichlorohydrin (Epi) as a spacer was studied. The reaction of polyamide–Epi with cellulose and starch was carried out. Fourier transform infrared spectroscopy, scanning electron microscopy, and thermogravimetric analysis were carried out before and after the enzyme was immobilized to confirm the characterization of the polymer and the α-amylase enzyme-immobilized one. The physicochemical parameters of the immobilized enzymes were measured, and the kinetics of the coenzyme–polymer-catalyzed reactions and stability were investigated. A direct comparison was made between the activities of the free and immobilized preparations. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 112: 629–638, 2009

Key words: radiation; grafting; epichlorohydrin; cellulose; starch; immobilization

INTRODUCTION

Radiation-induced graft copolymerization is a well-known method used to introduce functional groups into different polymer materials with specially selected monomers. There have been several reports on the use of radiation graft copolymerization of polar monomers onto polymer films to obtain hydrophilic properties for versatile applications.[1–5] Grafting copolymerization performed by the radiation method is one useful method because of its uniform and rapid creation of active radical sites.[6,7] It is known that both catalysis and enzymes are used to accelerate the chemical reactions and that enzymes are very efficient and advantageous catalysts. The selection of the appropriate support for enzyme immobilization is of the utmost importance because the efficiency of the enzyme depends largely on the nature of the support and its bonding with the enzyme. In addition to its high affinity or capacity for the enzyme, a suitable chemical structure of the support maximizes the degree of interaction between the enzyme and the substrate. The support should also be thermally stable, chemically durable, resistant to contamination, and available at a reasonable cost.[8] There are two basic advantages for the use of enzymes as catalysts in organic media instead of aqueous solutions. First, organic solvents favor the solubility of hydrophobic substrates, and second, the presence of such solvents shifts the thermodynamic equilibrium of condensation/hydrolysis reactions in favor of the desired product. In fact, there are enormous applications in organic synthesis and numerous examples also in the fields of food-related conversions and analysis.[9–11] However, enzymes show a lower catalytic efficiency (up to three or four orders of magnitude) when they are used in organic solvents rather than in aqueous buffers.[12,13] Moreover, the use of immobilized enzymes can lead to easier purification of the product; another concern is the running and investment costs, which if lowered, can provide the design of a more efficient process. *Immobilization* is defined as the physical confinement or localization of the enzyme molecule with the retention of its catalytic activity so that it can be used repeatedly or continuously.

α-Amylase (α-1,4-glucan-4-glucanohydrolase) is a glycoside hydrolase and catalyzes the hydrolysis of α-1,4-glycosidic linkages of starch. Glycosidic hydrolysis by α-amylase is a double-displacement mechanism involving the formation and hydrolysis of a covalent β-glycosyl enzyme intermediate. The formation of the intermediate involves attack at the sugar anomeric center by a nucleophilic amino acid (aspartate (Asp197)) assisted by general-acid catalysis (glutamate (Glu233)). The covalent glycosyl enzyme intermediate then undergoes general-base-catalyzed (Glu233) hydrolysis via the attack of water at the anomeric carbon (see Scheme 1).

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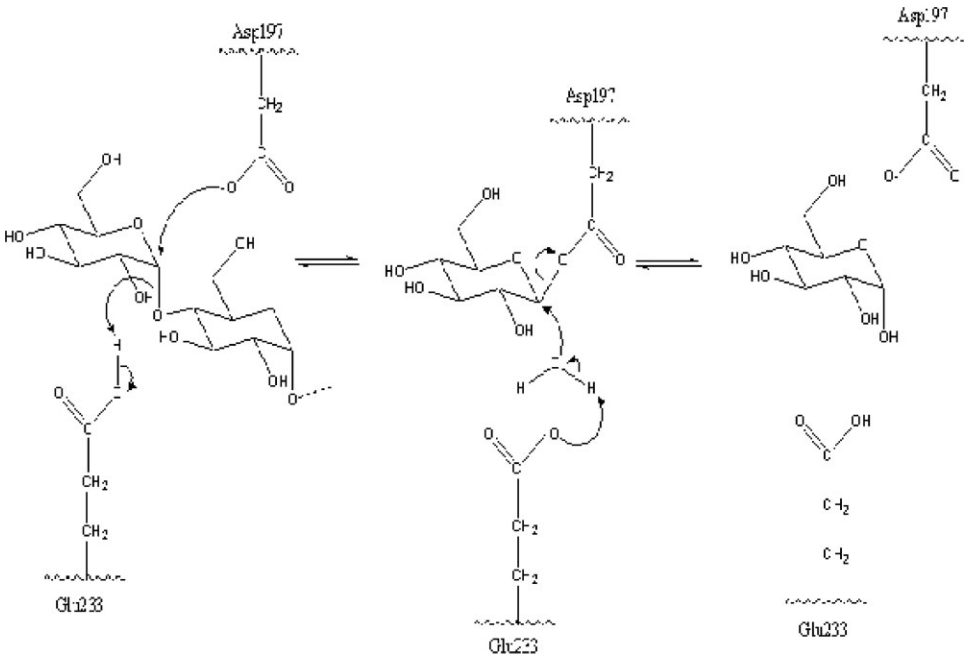


Scheme 1

As mentioned previously, the hydroxyl groups of the starch molecule play the main role in their reaction with the α-amylase. So, the modification of starch (support) through the reaction of these groups (hydroxyl groups) with the polymer inhibit the reaction of the α-amylase with the modified starch and, thus, eliminate the undesired digestion of the support.[14]

In this study, the preparation of polyamide–epichlorohydrin (Epi)–cellulose (Cell) and polyamide–epichlorohydrin–starch was carried out to study the effect of the grafting percentage, crosslinking of Epi used as a spacer, and Cell or starch on the activity and stability of the resulting immobilized enzyme film.

EXPERIMENTAL

Materials

The Epi was an extrapure reagent supplied by Japan Hayashi Chemical Co. The α-Cell was supplied by Sigma, Ltd. The starch was supplied by VEB Jenapharm-Laborchemie-Apolda (Germany). α-Amylase of *Bacillus subtilis* (EC 3.2.1.1) was purchased from ICN Biomedicals, Inc. (Aurora, OH). Polypropylene (PP) with a thickness of 30 μm and polystyrene (PSty) films with a thickness of 70 μm were purchased from Techoback Co. (Egypt). Acrylamide (AAm) was from Merck-Schuchardt (purity = 99%) and was used without further purification.

The other chemicals and reagents were used as received.

Grafted copolymerization

The blank (PP and PSty) films were washed with acetone, dried in a vacuum oven at 40°C, weighed, immersed in solutions with different concentrations of monomer (AAm) and solvent (butanone; 10/90 and 20/80) in glass ampules[15–17] deaerated by bubbling with nitrogen gas for 5 min, sealed, and then subjected to γ irradiation at a dose rate of 1.19 Gy/s. After irradiation, the grafted samples were washed thoroughly with a suitable solvent to get rid of the residual monomer and the homopolymer included in the films. The films were then dried in a vacuum oven at 50–60°C for 24 h and weighed. The grafting percentage was determined by the percentage increase in weight as follows:

$$\text{Grafting } (\%) = [(W_g - W_o)/W_o] \times 100 \qquad (1)$$

where W_o and W_g are the weights of the initial and grafting films, respectively.

Preparation of the crosslinking polypropylene-*g*-polyacrylamide–epichlorohydrin (PAE)–Cell, PAE–starch, polystyrene-*g*-polyacrylamide–epichlorohydrin (SAE)–Cell, and SAE–starch copolymers

The grafted copolymers PAE and SAE were prepared in two steps. The first step for the preparation of PAE and SAE was the synthesis of the grafted copolymers p(AAm) and PSty-*g*-p(AAm). The second preparation step for the PAE and SAE was to place 0.018 g of the grafted PP-*g*-p(AAm) and

0.084 g of the grafted PSty-*g*-p(AAm) in 200 mL of distilled water and then add 60% of Epi/g of the grafted films. The solution was heated to 60°C for 1 h, which resulted in PAE or SAE.[18] The PAE and SAE were based on an α-Cell weight ratio of 60%, and other PAE–starch compounds were based on a starch weight ratio of 60%. Next, the PAE–cell, PAE–starch and SAE–cell, SAE–starch compounds were soaked in distilled water at 25°C for 72 h; then, the activated carrier was dried and stored at 4°C for later use.

Immobilization of α-amylase onto the activated carrier

α-Amylase (1 g of protein, i.e., 300 g in 1 mL of phosphate buffer at pH 7) and 1 cm^2 of activated carriers were suspended in 30 mL of phosphate buffer at pH 7. After 48 h of stirring at room temperature, the sample was separated from the solution and washed with 0.1*M* phosphate buffer at pH 7 to remove the other byproducts. The final products were stored at 4°C after drying.

Protein determination

The concentration of immobilized enzymes was determined from the difference between the amount of protein introduced to the coupling reaction mixture and the amount of protein present in the filtrate and washings after immobilization. The amount of protein was determined by a modification of the method of Lowry et al.[19]

α-Amylase assay

The activity of α-amylase was assayed by the method of Bernfield and Colowick.[20] The reaction mixture (1 mL) containing equal volumes of soluble amylose [0.5% (w/v)] and enzyme solution (90 mg of protein) properly diluted with 50 mmol/L phosphate buffer (pH 7) was incubated at 25°C for 5 min. To assay the immobilized enzyme activity, an appropriate weight of biopolymer containing approximately 90 mg of protein was suspended in 1 mL (50 mmol) phosphate buffer at pH 7 containing 1% starch. The reaction mixture was incubated at 60°C for 5 min with shaking. The reaction was stopped with 1 mL of 3,5-dinitrosalicylate reagent. The amount of reducing sugar (maltose) produced was determined spectrophotometrically with a double-beam UV–visible spectronic 200 instrument (Pye-Unicam, England) at 540 nm.

Fourier transform infrared (FTIR) measurement

FTIR spectra were determined for the grafted films and PAE–Cell compounds with a FTIR spectrometer (Pye-Unicam Sp 2000 type, England).

Scanning electron microscopy (SEM) measurement

The surface topography of the grafted and PAE–Cell compounds films was studied with a Jeol ISM 5400 scanning microscope (Jeol, Japan).

Thermogravimetric analysis (TGA)

The thermal properties of the PP and PSty were investigated with a Shimadzu TGA system (type TGA-50, Japan) under a nitrogen atmosphere (20 mL/min). The temperature ranged from ambient temperature to 600°C at a heating rate of 10°C/min.

RESULTS AND DISCUSSION

FTIR

The IR spectra of the original films, grafted PP films, and PAE films with and without Cell and starch and the IR spectra of the PSty films were also determined. The physical and chemical changes in the grafted and crosslinking starch and Cell in both polymers were investigated. The IR spectra of the immobilized enzyme were studied to characterize the structure of the films. Figure 1 shows the IR spectra of the blank PP [Fig. 1(a)], grafted PP-*g*-p(AAm) [Fig. 1 (b)], PAE [Fig. 1 (c)], PAE–Cell [Fig. 1 (d)], and PAE–starch [Fig. 1 (e)] films. As shown in Figure 1(b), new broad bands appeared around 3200–3300 cm^{-1}, which were characteristic for the structure of AAm, which indicated the presence of the NH_2 group, and at 1660 cm^{-1}, which indicated the presence of the carbonyl group (C=O) due to the grafting of PP with AAm. The peak at 1640–1400 cm^{-1} present in Figure 1(c) was due to the N—H bending of the PAE film secondary amide band.[21,22] The peaks at 1170–1040 and 3500–3300 cm^{-1} in Figure 1(d,e) were due to the ether linkage and hydroxyl group of Cell (PAE–Cell) and starch (PAE–starch).

Figure 2 shows the IR spectra of the PSty film and its grafted counterpart. Figure 2(a) shows the spectra of the blank PSty film, which showed characteristic bands out of plane at 720–600 cm^{-1}, a stretching band at 3050 cm^{-1} assigned to the aromatic =C—H, and bands at 1700–1500 cm^{-1} assigned to the conjugated C=C aromatic stretching. The characteristic band at 950–890 cm^{-1} was due to =C—H aliphatic out-of-plane bending. The band at 2900 cm^{-1} was characteristic for the CH_2 aliphatic bending, and the band at 2950 cm^{-1} was due to the CH_3 aliphatic

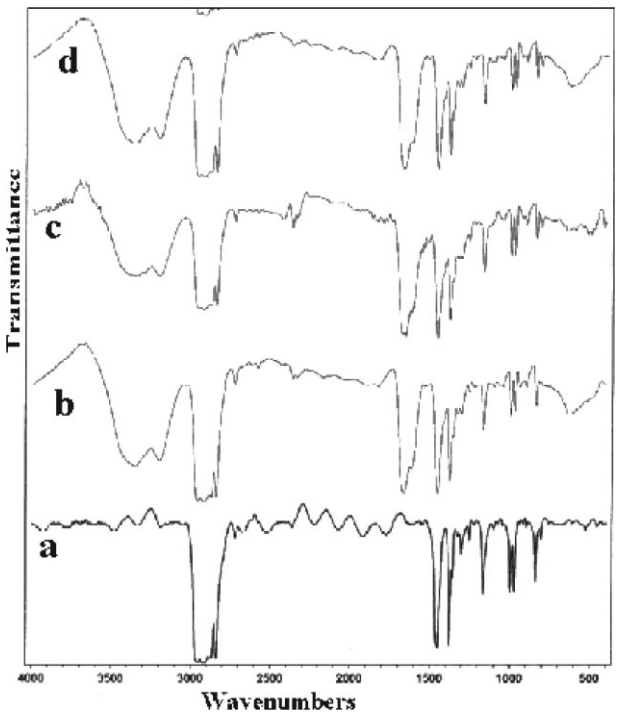


Figure 1 FTIR spectra of the PP films: (a) blank, (b) PP-*g*-P(AAm), (c) PP-*g*-P(AAm)–Epi, (d) PP-*g*-P(AAm)–Epi–Cell, and (e) PP-*g*-P(AAm)–Epi–starch.

bending. Certain changes in Figure 2(b) of the PSty-*g*-p(AAm) films appeared in the presence of new bands at 2600–2400 cm^{-1} because of the grafting with AAm.[22,23] The other changes in the IR spectra of Figure 2(c–e) corresponded to similar changes in Figure 1(c–e) because of the same treatments of the PP film. Schemes 2 and 3 show an approximate structure of the PP grafted with AAm and the crosslinking of the grafted PP with Epi. Figures 3 and 4(a–e) show the IR spectra of the blank, grafted, grafted Epi, grafted Epi–Cell, and grafted Epi–starch samples for both the PP and PSty films with immobilized enzyme, respectively. We noted that a change in the intensity of the bands due to the immobilized enzyme and a shift in the bands at 2000–2900 cm^{-1} for the enzyme indicated the presence of CH_2 groups and NH groups for both polymers.[18,24] Also, the blank polymers showed no significant change.

SEM measurements

SEM measurements were carried out to investigate the surface morphology of the copolymer samples. Figure 5(a–e) shows the morphology of the original PP, 36.8% PP-*g*-p(AAm), 40.6% PAE, 40.6% PAE–Cell, and 36.8% PAE–starch films. As shown in Figure 5(b), the grafted p(AAm) on the surface of PP film was shown as bright hills in contrast to the uniform texture of the original PP film. The surface of PAE became coarse and not smooth but covered with Epi molecules [Fig. 3(c)]. The Cell and starch of the PAE–Cell and PAE–starch films were still granular in structure and agglomerated on the film surfaces [Fig. 5(d,c)].[25] A similar appearance in the morphology of the PSty film surface is shown in Figure 6(a–e) due to the similar treatments.

The results obtained by SEM enabled us to observe the physical characterization of the external morphology of the PP and PSty immobilized samples. SEM of the enzyme-immobilized PP samples, grafted, grafted Epi, grafted Epi–Cell, and grafted Epi–starch, are given in Figure 7(a–d), respectively. The morphology of the PP samples was not smooth but covered by enzyme because of the crosslinks formed between PP grafted with AAm and the enzyme molecules. The surface morphology of the me-immobilized PSty samples was shown as small bright hills; this was expected for the enzyme reaction with the surface of PSty because of the low grafting percentage [Fig. 8(a–d)].

TGA

The TGA thermograms of the original and grafted PP and PAE films with and without Cell and starch and of the PSty films also were studied. Figure 9(a) shows that the ungrafted PP films had stable

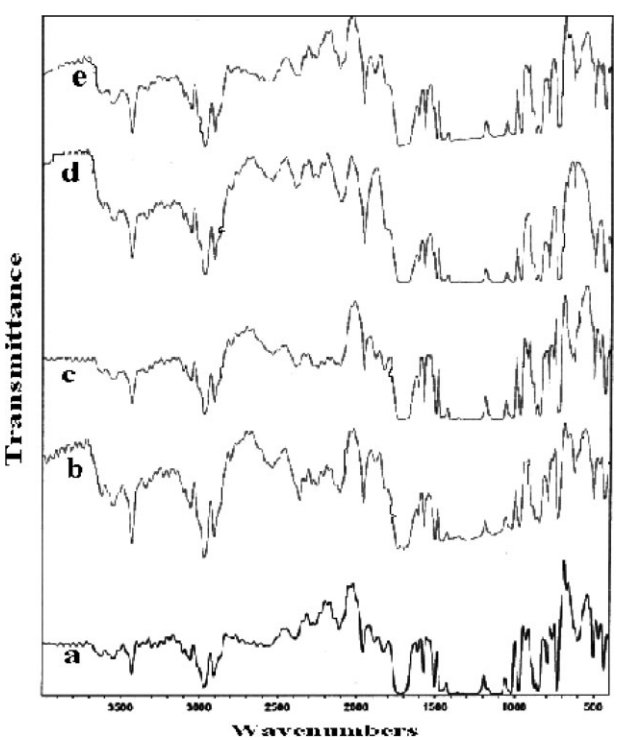
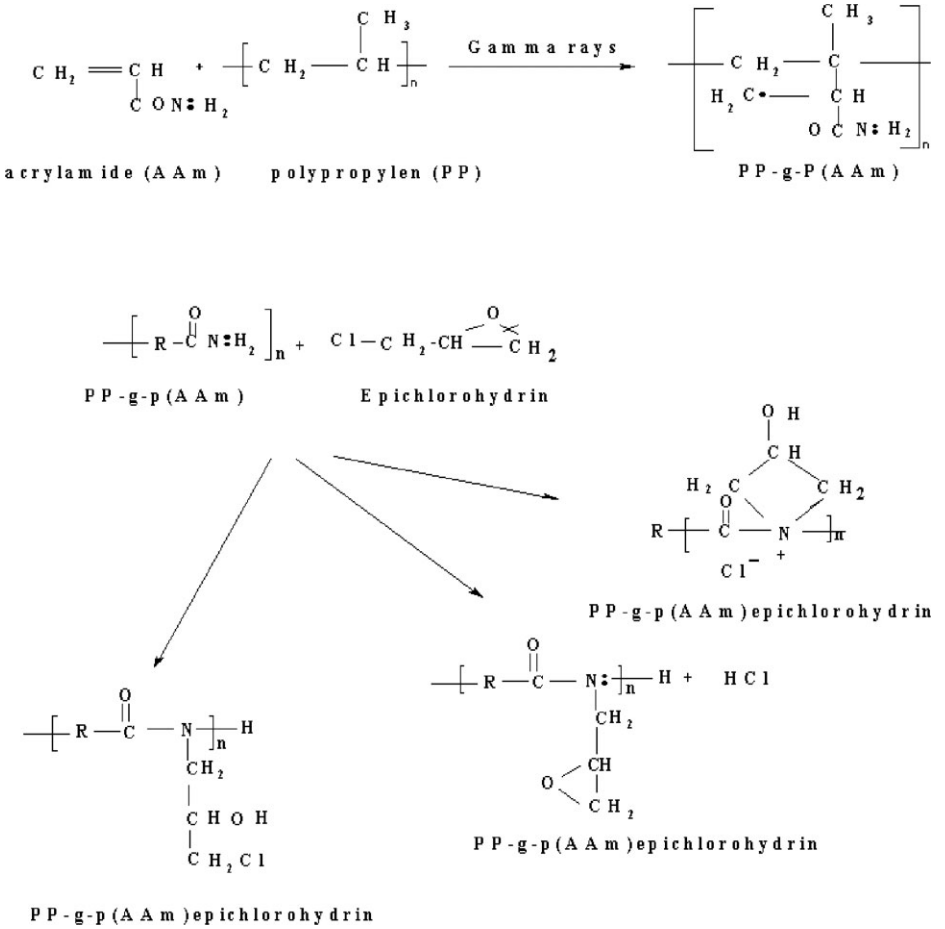
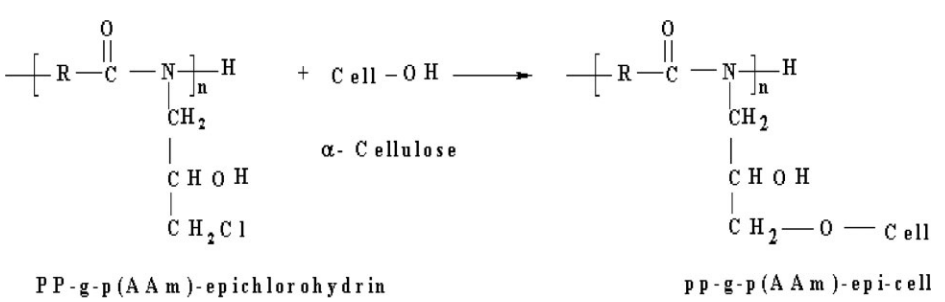


Figure 2 FTIR spectra of the PSty films: (a) blank, (b) PSty-*g*-P(AAm), (c) PSty-*g*-P(AAm)–Epi, (d) PSty-*g*-P(AAm)–Epi–Cell, and (e) PSty-*g*-P(AAm)–Epi–starch.



Scheme 2

thermograms up to a temperature of 350°C, beyond which a smooth decrease in weight and complete depolymerization with main-chain scission were observed. The TGA curves of the grafted PP-*g*-p(AAm) are shown in Figure 9(b). The thermal degradation occurred via a three-stage process; the first stage, from 100 to 200°C, was related to the elimination of water adsorbed,[26] and the second, major degradation at temperatures from 200 to 380°C, due to the thermal degradation of p(AAm), corresponded to the loss of ammonia. The latest decomposition stage observed at temperatures up to 380°C was due to the weight loss with the extensive degradation of the polymer backbone chain. From these results, we noticed that the grafting process decreased the thermal stability of the PP film. Figure 9(c) shows that the thermal degradation processes of the PAE films were in the temperature range 100–420°C; the first process, from 100 to 220°C, was related to the elimination of water adsorbed, and the second, major degradation step was from 220 to 420°C. We noticed that Epi increased the thermal stability of the grafted



Scheme 3

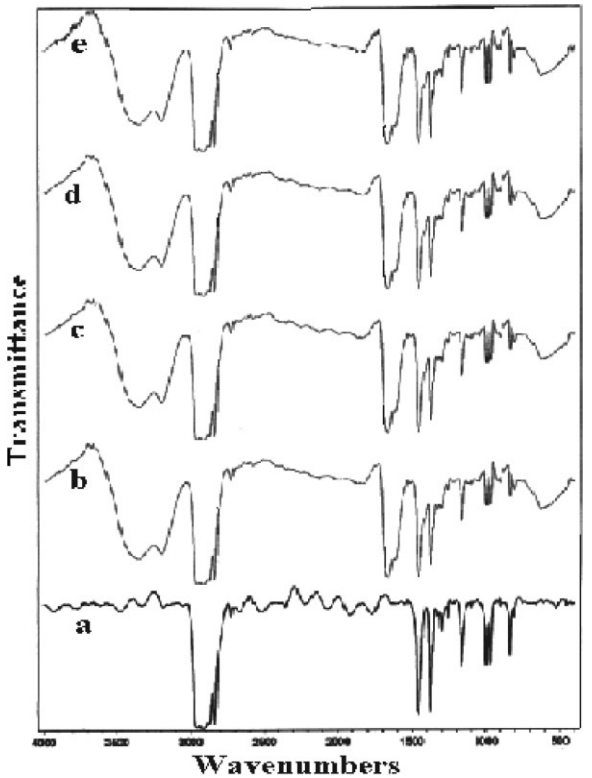


Figure 3 FTIR spectra of the PP immobilized enzyme films: (a) blank, (b) PP-*g*-P(AAm), (c) PP-*g*-P(AAm)–Epi, (d) PP-*g*-P(AAm)–Epi–Cell, and (e) PP-*g*-P(AAm)–Epi–starch.

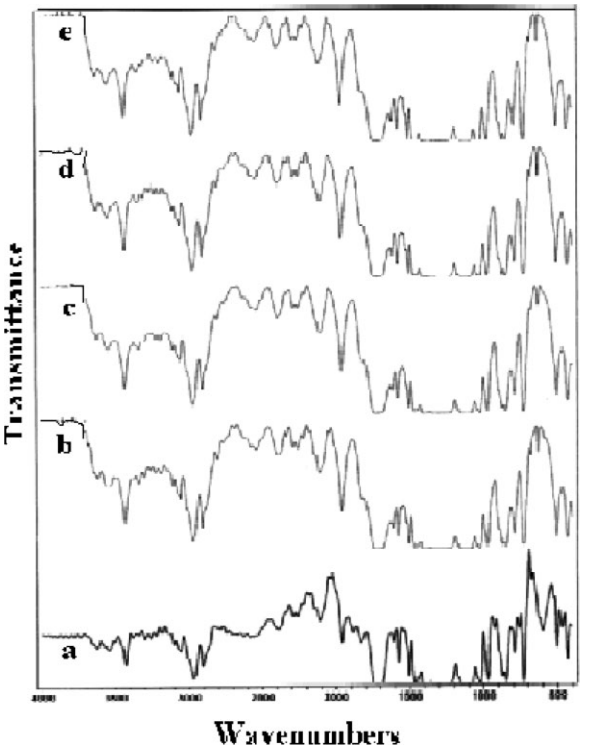


Figure 4 FTIR spectra of the PSty immobilized enzyme films: (a) blank, (b) PSty-*g*-P(AAm), (c) PSty-*g*-P(AAm)–Epi, (d) PSty-*g*-P(AAm)–Epi–Cell, and (e) PSty-*g*-P(AAm)–Epi–starch.

films, which may have been due to the formation of the polyamide–Epi structure. Figure 9(d,e) shows the thermal degradation processes of both PAE–Cell and PAE–starch grafted film components, respectively. The treatment of the grafted films of PAE with Cell and starch increased the thermal stability. The weight loss starting at 100°C and continuing up to about 220°C was due to dehydration. The degradation starting at about 300 and continuing to 450°C is shown in Figure 9(d), but the degradation starting at 350–480°C is shown in Figure 9(e) and was due to the preparation of both components of PAE–Cell or PAE–starch to form an interpolymer crosslinked by both ionic and covalent bonds.[17] As also shown in Figure 9(d,e), the thermal stability of PAE–starch was higher than that of PAE–Cell; this may have because the starch had a higher molecular weight (ca. 1 million) than Cell (ca. 0.5 million).

Figure 10 shows the TGA thermograms of the original PSty film [Fig. 10(a)], the grafted PSty-*g*-p(AAm) film [Fig. 10(b)], the SAE film [Fig. 10(c)], the SAE–Cell film [Fig. 10(d)], and the SAE–starch film [Fig. 10(e)]. The blank PSty matrix had thermal stability up to 380°C, which was followed by a one-step degradation, as depicted by the thermogram shown in Figure 10(a). In the grafting of PSty by AAm, the initial weight loss in the thermogram shown in Figure 10(b) was ascribed to the loss of water from the membranes. There was continuous

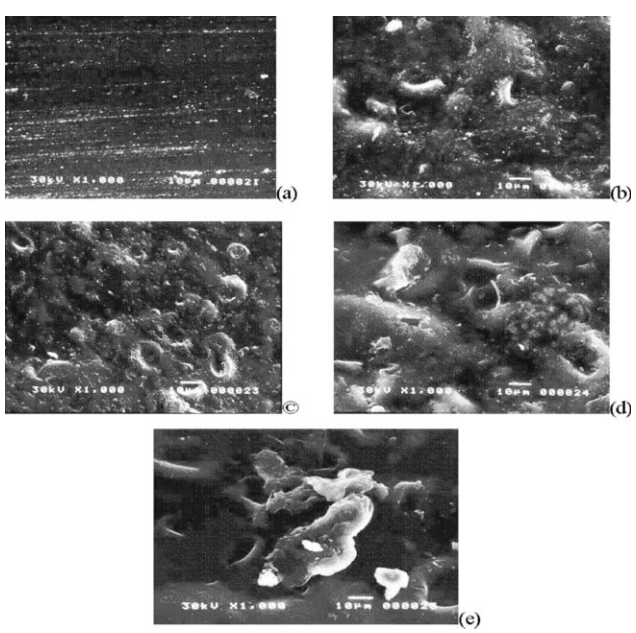


Figure 5 Scanning electron micrographs of the (a) blank PP film and (b) 36.8% PP-*g*-p(AAm), (c) 40.6% PAE, (d) 40.6% PAE–Cell, and (e) 36.8% PAE–starch films.

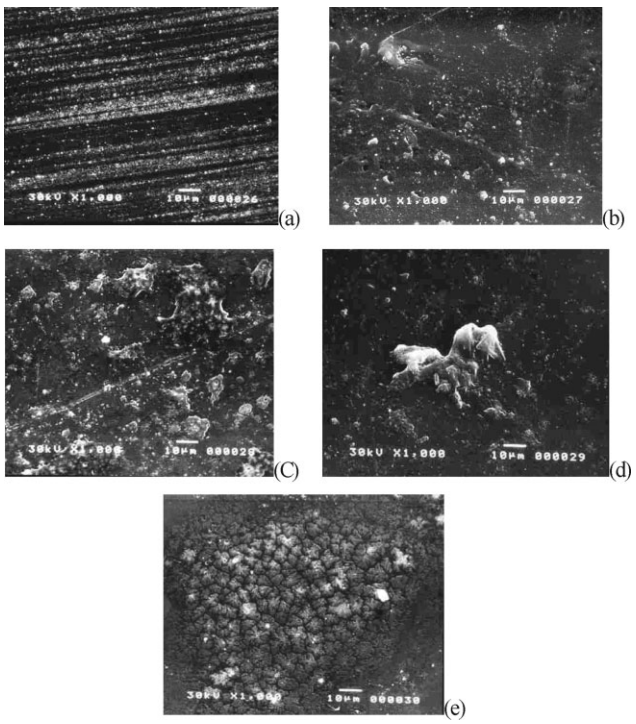


Figure 6 Scanning electron micrographs of the (a) blank PSty film and (b) 36.8% PSty-*g*-p(AAm), (c) 40.6% SAE, (d) 40.6% SAE–Cell, and (d) 36.8% SAE–starch films.

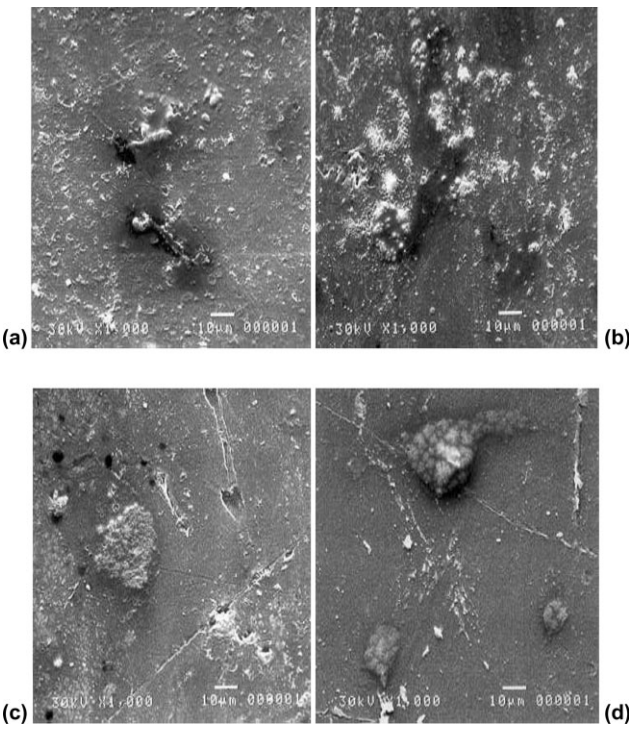


Figure 8 PSty–enzyme samples: (a) PSty-*g*-p(AAm), (b) SAE, (c) SAE–Cell, and (d) SAE–starch.

water loss up to 200°C. The grafting of PSty that led to the formation of the poly(styrene-*g*-P(AAm) matrix introduced a two-degradation pattern. The PSty graft started to degrade at 400°C and continued up to 460°C. This was followed by the degradation of the PSty backbone up to 520°C. The TGA thermograms of the SAE, SAE–Cell, and SAE–starch films are presented in Figure 10(c–e), respectively. All of

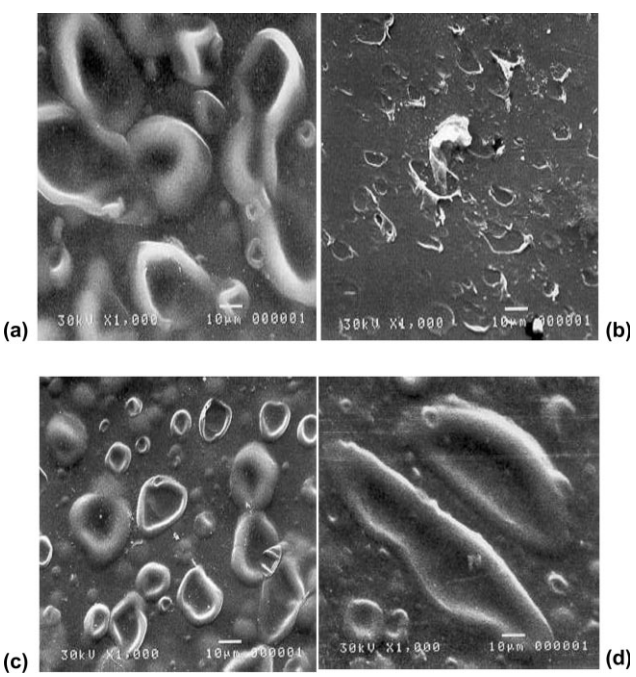


Figure 7 PP–enzyme samples: (a) PP-*g*-p(AAm), (b) PAE, (c) PAE–Cell, and (d) PAE–starch.

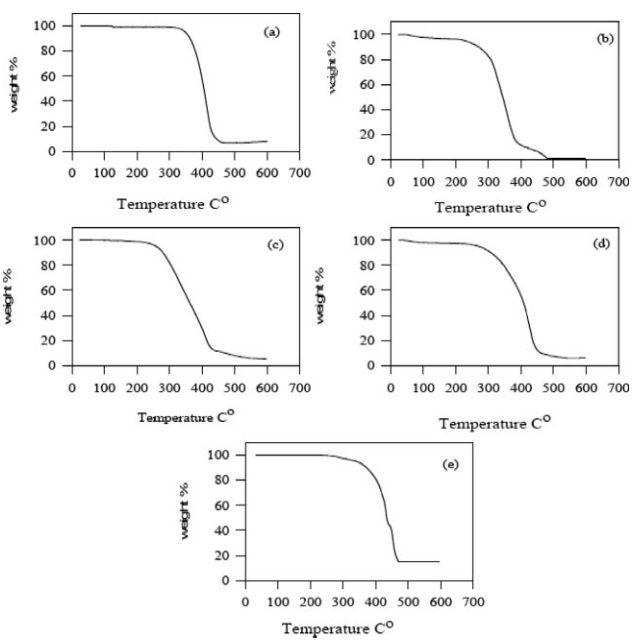


Figure 9 TGA thermograms of the (a) original PP, (b) grafted PP, (c) PAE, (d) PAE–Cell, and (e) PAE–starch films.

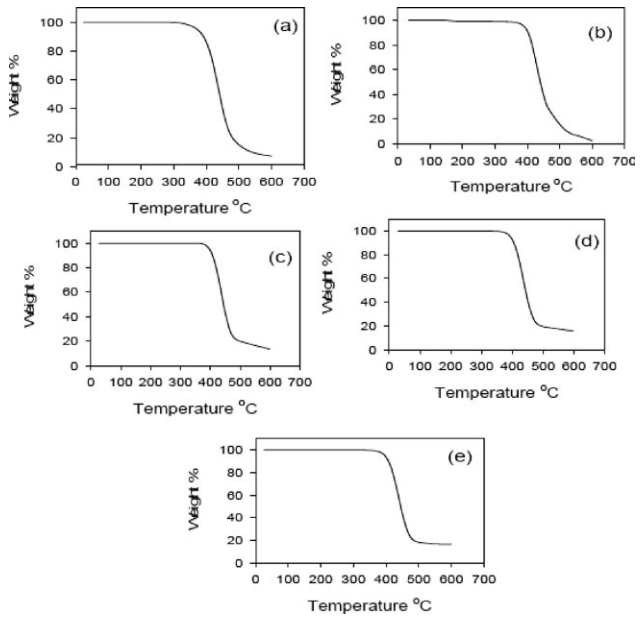


Figure 10 TGA thermograms of the (a) original PSty, (b) grafted PSty, (c) SAE, (d) SAE–Cell, and (e) SAE–starch films.

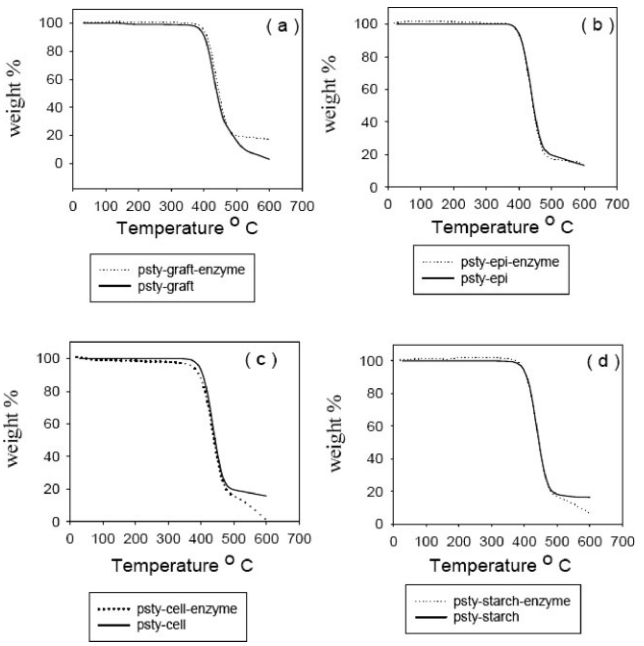


Figure 12 TGA thermograms of the enzyme-immobilized PSty films: (a) grafted PSty, (b) SAE, (c) SAE–Cell, and (d) SAE–starch.

the membrane showed a degradation starting at about 420°C, which continued up to 480°C; this may have been due to the PSty grafts, which had pure hydrocarbon structures that were incompatible with Epi, Cell, and starch.[27]

The TGAs of the immobilized enzyme membranes are shown in Figures 11 and 12. Figure 11 shows the effect of the immobilized enzyme on the PP films with different treatments. The immobilized enzyme process increased the thermal stability of the grafted PP films [Fig. 11(a)] and the thermal stability of the PAE film [Fig. 11(b)]; the other treatment, for the PAE–Cell and PAE–starch films, caused no significant change in the degradation processes from the immobilized one [Fig. 11(a)]. The major degradation of the immobilized grafted PP film was in the temperature range from 300 to 450°C. This may have been due to the crosslinking between the enzyme and the membranes. Also, Figure 11(b) shows the thermal stability of the PAE immobilized films, in which the major degradation was in the temperature range from 300 to 500°C. Figure 12 shows that the

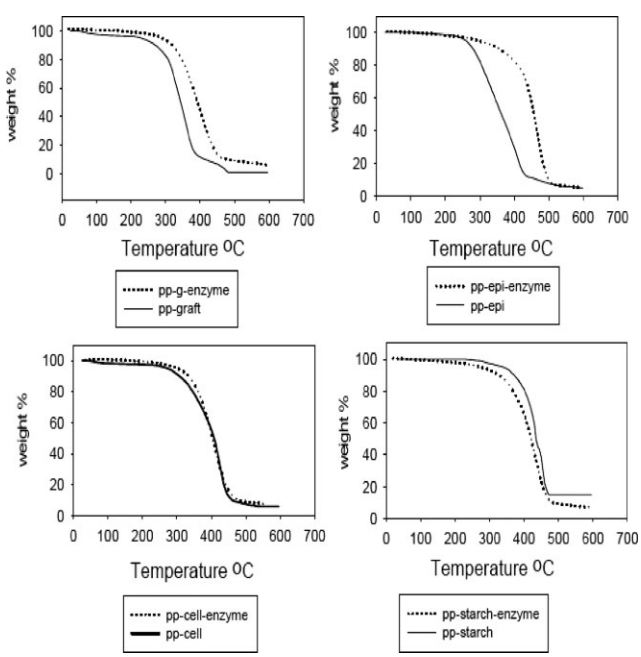


Figure 11 TGA thermograms of the enzyme-immobilized PP films: (a) grafted PP, (b) PAE, (c) PAE–Cell, and (d) PAE–starch.

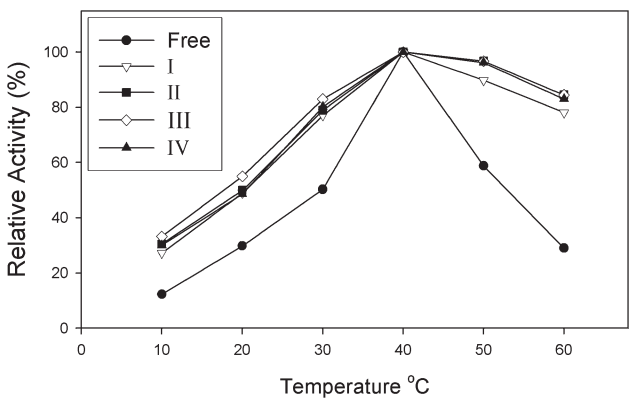


Figure 13 Effect of the temperature on the activity of the free and immobilized α-amylase: (I) grafted, (II) grafted Epi, (III) grafted Epi–Cell, and (IV) grafted Epi–starch.

immobilized enzyme in the PSty films with different treatments caused no significant change from the unimmobilized membranes; this may have been due to the very low degree of grafting, so the immobilized enzyme ratio was also small.

Effect of the temperature on the activity of the free and immobilized α-amylase

Figure 13 shows the effect of the temperature on the relative reactivity of the free and immobilized α-amylase. As expected, the free enzyme showed a severe loss in its activity at higher temperatures, whereas the temperature profile of the immobilized enzyme was much broader at higher temperatures, which demonstrated the effectiveness of the carrier protecting the enzyme activity under higher temperature conditions.[9]

Effect of pH on the activity of the free and immobilized α-amylase

Figure 14 shows the maximum enzymatic activity at different pH values (2–9). The activity of both the free and immobilized enzyme was found to be sensitive to pH variations. However, the immobilized α-amylase was found to be more stable than free one. Also, the pH optimum of α-amylase did not change on immobilization. This implied that the immobilization of α-amylase did not affect the enzyme-active site.[2]

Storage stability

The storage stability of the free and immobilized enzyme at 4°C was investigated. The results, presented in Figure 15, show the relative activity after up to 60 days of storage. As shown in Figure 15, the

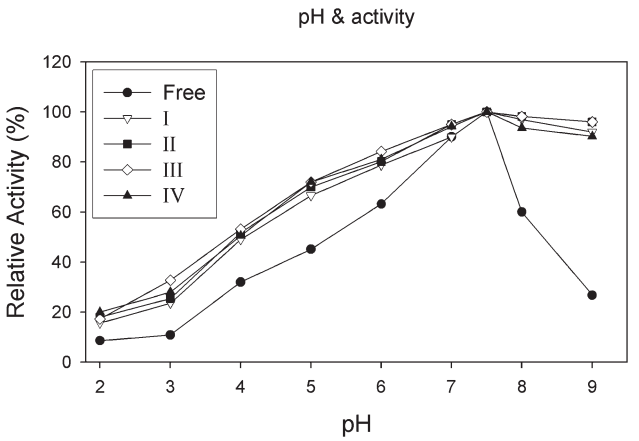


Figure 14 Effect of pH on the activity of the free and immobilized α-amylase: (I) grafted, (II) grafted Epi, (III) grafted Epi–Cell, and (IV) grafted Epi–starch.

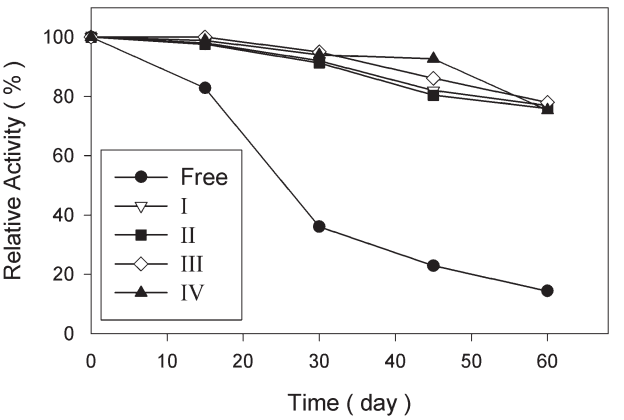


Figure 15 Storage stability of both the free and immobilized α-amylase: (I) grafted, (II) grafted Epi, (III) grafted Epi–Cell, and (IV) grafted Epi–starch.

immobilized α-amylase was much more stable than the free enzyme. This confirmed that the immobilized enzymes could be stored over extended periods without any loss in their enzymatic activity. The prevention of thermal denaturation resulting from the immobilization of the enzyme may reflect the relatively high storage stability of the immobilized α-amylase.[13]

CONCLUSIONS

Films were prepared by the direct-irradiation-induced grafting of AAm onto PP films and PSty films with a crosslinker as a spacer. Crosslinking Cell and starch with Epi is the most common method used in polysaccharide chemistry. The α-amylase enzyme was immobilized onto the polysaccharide films. A direct comparison was made between the activity of the free and the immobilized preparations. We noticed that

1. The activities of both the free and immobilized enzyme were found to be sensitive to pH variations. However, the immobilized α-amylase was found to be more stable than the free one.
2. The immobilized enzyme had a higher thermal stability than the free one, which was due to the carrier protecting the enzyme activity under higher temperature conditions.
3. The immobilized enzyme could be stored at 4°C over extend periods without any loss in its enzymatic activity.

These films could be used as biocatalysts because of their important and multiple applications. Thus, the immobilized films may be an economically viable alternative to currently available technologies.

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