

Adsorption of Urokinase by Polypropylene Films with Various Amine Groups

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ABSTRACT: Polypropylene films with various amine groups were prepared by radiation-induced grafting of glycidyl methacrylate (GMA) onto polypropylene (PP) films and the subsequent amination of poly-GMA graft chains were examined. The physical and chemical properties of the GMA-grafted PP film and the aminated PP film were investigated by IR, SEM, XPS, and TGA. The adsorption of urokinase for the PP films modified with various amine groups was examined under various conditions of different amine group contents and pH values. The adsorption of urokinase increased by increasing the amine group content. In the range of amine group content from 1.0 to 2.7 mmol/g, the adsorption of urokinase by the PP films modified with six kinds of amines was in the following order: trimethylamine > diethylenetriamine > triethylenetetraamine > ethylenediamine > dimethylamine > diethyleneamine. The adsorption amounts of urokinase by the PP film with the trimethylamine and triethylenetetraamine groups at pH 7.4 were higher than those at pH 9. © 2001 John Wiley & Sons, Inc. *J Appl Polym Sci* 80: 2851–2858, 2001

Key words: polypropylene films; urokinase; radiation-induced graft copolymerization; glycidyl methacrylate; amination; adsorption characterization

INTRODUCTION

Urokinase is a plasminogen activator which is found in human urine in trace amounts. It is a potent, blood-clot lysing agent and has been used clinically for the treatment of thromboembolic disorders. To recover urokinase from an urokinase-containing solution such as urine, several methods such as ultrafiltration,¹ precipitation with a heavy metal compound,² adsorption with silica gel,³ ion exchange,⁴ and so forth^{5–7} have been used. However, the adsorption method of urokinase by the amine group-containing an ad-

sorbent has not been reported yet, to our knowledge.

In recent years, radiation-induced graft polymerization has become an important research subject, because it has been known as a good method for the modification of physical and chemical properties of polymeric material. To obtain various kinds of functional polymers, monomers which are easily modified to functional groups can be grafted to the polymer substrate. Glycidyl methacrylate (GMA) is one of the monomers which is easily modified into various functional groups. As GMA is polymerized, the epoxy groups in poly-GMA can be modified to alcohol,⁸ amine,⁹ phosphonic acid,¹⁰ sulfonic acid,¹¹ etc.¹² In our previous works,¹³ we chose several amine groups among the many chelating groups for the removal of heavy metal ions such as Pb²⁺ and Pd²⁺. The

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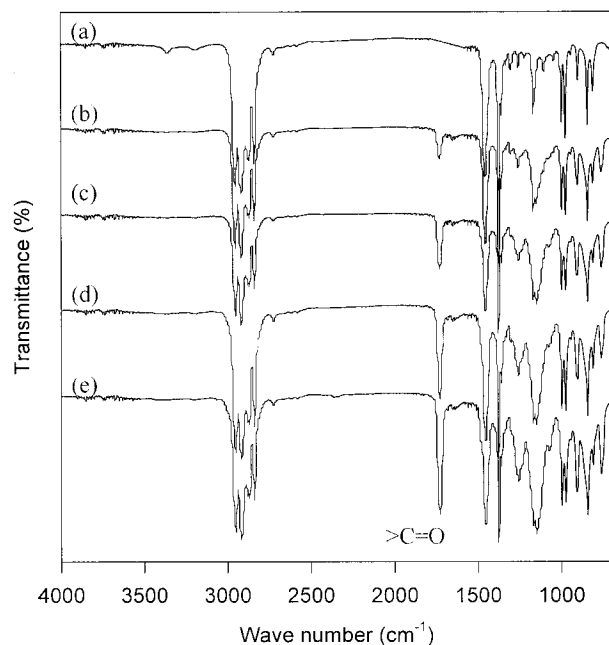


Figure 1 FTIR-ATR spectra of (a) original PP film and (b) 25%, (c) 35%, (d) 55%, and (e) 75% GMA-grafted PP film.

membranes with amine groups showed a high adsorption efficiency for Pb^{2+} and Pd^{2+} .

Polyolefin nonwoven fabric is repeatedly used as one among polymer substrates for grafting because of its beneficial properties such as a large of surface area and easy treatment. However, polyolefin nonwoven fabric was difficult to be characterized by spectroscopy analysis. For these reasons, polypropylene (PP) film was used in this study.

To recover urokinase, the adsorbents with various amine groups were prepared by radiation-induced grafting of glycidyl methacrylate (GMA), and the subsequent amination in poly-GMA graft chains was examined. The adsorption of urokinase for PP films with various amine groups was examined under various conditions of different amine group contents and pH values.

EXPERIMENTAL

Materials

PP films (Tae-Syeng Chemistry Co., Taegu, South Korea) with a thickness of 0.03 mm were washed with methanol and dried in a vacuum oven at 50°C for 8 h prior to use. Urokinase of 20,000 IU (Korea Green Cross Corp., Seoul, South Korea) and reagent-grade GMA [$\text{CH}_2=\text{C}(\text{CH}_3)\text{COOCH}_2$ -

CHOCH_2 , GMA; Aldrich], trimethylamine [$\text{N}(\text{CH}_3)_3$, TMA, Showa, Osaka, Japan], triethylamine [$\text{N}(\text{CH}_2\text{CH}_3)_3$, TEA; Showa], and dimethylamine [$\text{N}(\text{CH}_3)_2$, DMA; Showa] were used without further purification. Diethylamine [$\text{N}(\text{CH}_2\text{CH}_3)_2$, DEA; Duksan, Taegu, South Korea] and ethylenediamine [$\text{NH}_2(\text{CH}_2)_2\text{NH}_2$, EDA; Duksan], 1,6-hexanediamine [$\text{NH}_2(\text{CH}_2)_6\text{NH}_2$, HAD; Aldrich], diethylenetriamine [$\text{NH}_2(\text{CH}_2)_2\text{NH}_2(\text{CH}_2)_2\text{NH}_2$, DETA; Aldrich], triethylenetetramine [$\text{NH}_2(\text{CH}_2)_2\text{NH}_2(\text{CH}_2)_2\text{NH}_2(\text{CH}_2)_2\text{NH}_2$, TETA; Aldrich, St. Louis, MO, USA], and all the other chemicals of reagent grade were also used without further purification.

Grafting Procedure

The PP film was used as the base polymer for the grafting polymerization. The 4×6 -cm PP film was irradiated by γ -ray from Co-60 (Co-60 gamma-ray irradiator IR-79) under atmospheric pressure and ambient temperatures. The irradiated PP film was immediately reacted with the monomer. The unreacted monomer and homopolymer after the grafting of GMA were removed with tetrahydrofuran (THF). The grafted PP film was dried in a vacuum oven at 60°C for 7 h. For the studies of the effects of various parameters on the grafting copolymerization, the degree of grafting is defined as

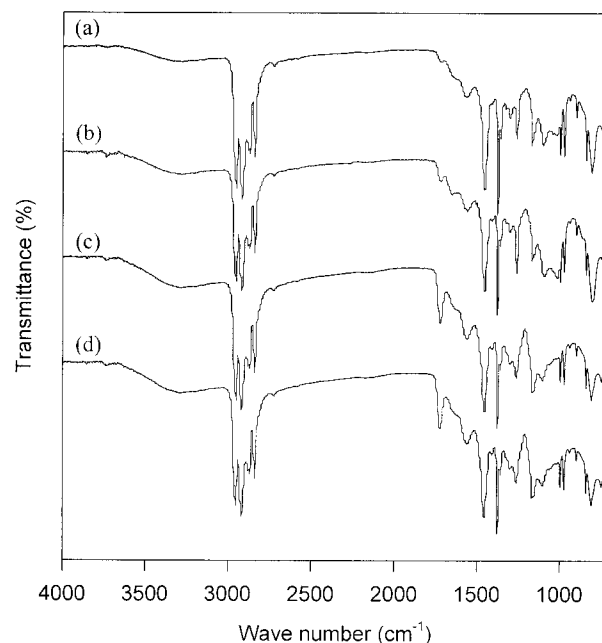


Figure 2 FTIR-ATR spectra of PP film modified with TETA groups: (a) 0.95 mmol/g; (b) 1.12 mmol/g; (c) 1.18 mmol/g; (d) 1.95 mmol/g.

were repeatedly washed with deionized water. Then, the PP films with various amine groups were dried in a vacuum oven at 50°C for 8 h. The individual contents (mmol/g) of TMA, TEA, DMA, DEA, EDA, HAD, DETA, and TETA were determined by the following equation:

$$R = [(W_s - W_g)/W_s] \times (1000/MW) \quad (2)$$

where R is the various amine groups; W_s , the weight of the aminated PP adsorbents; and MW , the molecular weight of TMA, TEA, DMA, DEA, EDA, HAD, DETA, and TETA.

Adsorption of Urokinase

Urokinase (30 mg) was dissolved in a phosphate-buffered solution (PBS, 50 mL, pH 7.4). The aminated PP films (1 × 2 cm) were immersed into a 2-mL urokinase solution at 37.3°C and rinsed using 2 mL of PBS, prior to the absorbance measurements.

Characterizations

IR spectra were recorded using a Nicolet Model 205 FTIR spectrometer. UV/vis spectra were

measured with UV-vis-NIR scanning spectrophotometer (Shimazu Co.). For scanning electron microscopy (SEM), a sample of 0.5 × 0.5-cm size was coated with a gold-palladium alloy prior to the measurement. A sputtered sample was then scanned by an electron beam in a scanning electron microscope (JSM-840A, JEOL Co., Japan).

The X-ray photoelectron spectra were obtained using an ESCALab 220i (VG Scientific) equipped with a full 180° hemispherical electrostatic analyzer to examine the chemical state of the constituent elements. As a photon source, AlK α radiation (1486.6 eV) was used. The half-width at half-maximum of the 4f7/2 line in the XPS spectrum of gold obtained with our XPS spectrometer was smaller than 1.0 eV. The energy scale of the spectrometer was calibrated using the lowest BE component of the C 1s peak (285.0 eV). The C 1s spectra were deconvoluted using a Gaussian-Lorentzian model to obtain the best binding energy values. Thermogravimetric analyses (TGA) of the GMA-grafted PP film were made on a TA instrument (TGA 2950 Model, DuPont Co.), with a heating rate of 10°/min in the temperature range of 50–700°C.

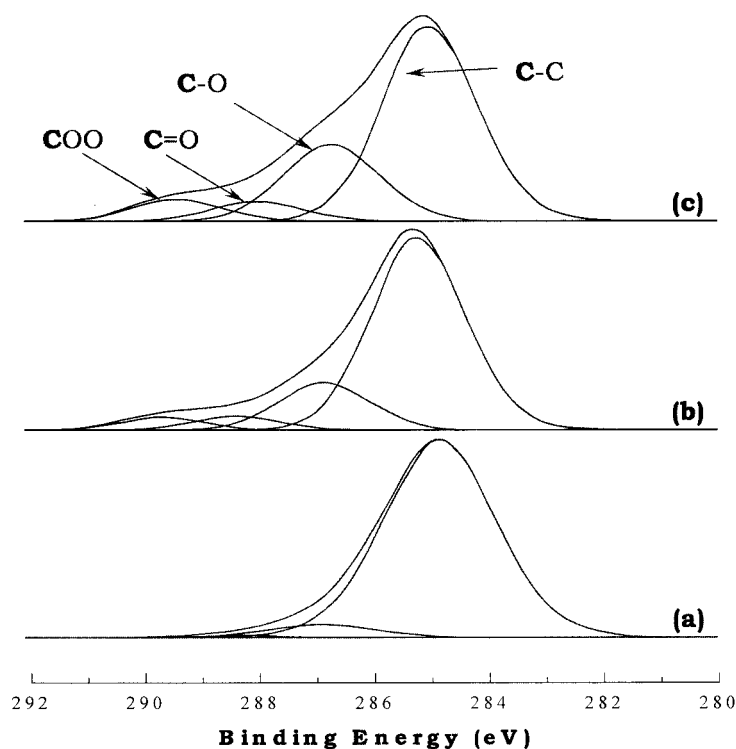


Figure 4 XPS spectra of C 1s of (a) original PP film and (b) 25% and (c) 55% GMA-grafted PP film.

RESULTS AND DISCUSSION

Properties of GMA-grafted PP Film and the Aminated PP Film

In previous articles,^{12,13} we examined reaction conditions such as the irradiation dose, solvents, reaction temperature, and reaction time on the grafting of GMA onto PE film and hollow fiber. It was found that the grafting yield increased with an increasing reaction temperature, reaction time, and irradiation dose. Furthermore, the maximum grafting was obtained in dioxane among the many organic solvents such as MeOH, THF, and acetone. However, little has been studied on the physical and chemical properties of GMA-g-PP film and the aminated PP film. The GMA-grafted PP films in this experiment were obtained in dioxane at 60°C by varying the reaction time.

Figure 1 shows the IR spectra of (a) the original PP film and (b) the 25%, (c) 35%, (d) 55%, and (e) 75% GMA-grafted PP film. The prominent bands of the original PP film at 1471 and 1351 cm^{-1} are shown for the $-\text{CH}_2-$ group in the chain. As the PP film was grafted with GMA, the strong absorption at about 1730 cm^{-1} (C=O stretching) and the weak broad band at about 1272 and 1250 cm^{-1} (C—O— stretching) were observed. The characteristic vibrations of the carbonyl group increased by increasing the grafting yield. On the other hand, no carbonyl peak was observed in the IR spectrum of the original PP film. These results indicate that the introduction of poly-GMA onto PP film is clearly produced.

Figure 2 shows the infrared spectra of the PP film with different TETA group contents. A strong absorption at about 1730 cm^{-1} (C=O stretching), a weak broad band at about 1250 cm^{-1} (coupling of C—O— stretching), and a broad band at about 3450 cm^{-1} (NH_2 stretching) appeared for the TETA group-modified PP film. From the results, we could see that the amine group was introduced into grafted poly-GMA as shown in Scheme 1.

Figure 3 shows the scanning electron micrographs of the cross section of (a) the original PP film and (b) the 25%, (c) 55%, and 75% GMA-grafted PP film. The cross-sectional view of the original PP film was shown as a uniform texture in Figure 3(c), while the cross-sectional view of the GMA-grafted PP film was shown as the bright hill in Figure 3(d). In Figure 3(b), the bright hill in the upper field was observed due to GMA grafting. On the other hand, the bright spot of the PP film appeared in the lower field, due to degrada-

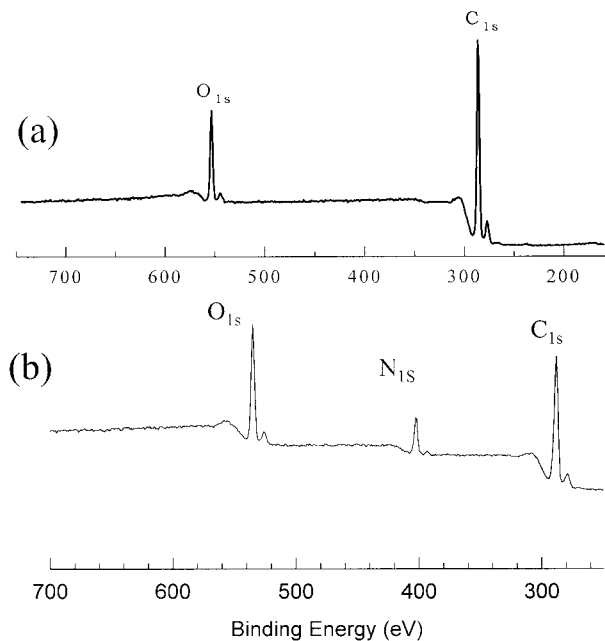


Figure 5 XPS survey scan spectra of (a) 55% GMA-grafted PP film and (b) the 1.95 mmol/g TETA group-modified PP film with the same grafting yield.

tion which is caused by gamma irradiation. These results indicated that the GMA grafting onto the PP film propagated from the surface into the inside of it.

Figure 4 shows the XPS spectra of C 1s of (a) the original PP film and (b) the 25% and (c) 55% GMA-grafted PP film. The core-level binding energy of the C 1s of the PP film was calculated to be 285.1 eV. As the polymer film was grafted with GMA, an additional peak was observed at 288.7–289.5 eV because of the carboxylic group at the polymer chains. Furthermore, the C 1s spectrum was resolved into four characteristic peaks: The peaks at 288.6, 287.3, 286.3, and 284.6 eV on the surface indicate the functional groups of O—C=O, C=O, C—O, and C—C, respectively. With an increased grafting yield, the peak intensity of three functional groups, O—C=O, C=O, and C—O, increased, while that of the C—C group decreased. The original PP film has no peak of the O—C=O functional group.

Figure 5 shows XPS spectra of (a) the 55% GMA-grafted PP film and (b) the 1.95 mmol/g TETA-modified PP film with the same grafting yield. In Figure 5(a), two characteristic peaks corresponding to C 1s at 285.1 eV and O 1s at 533.5 eV were observed. In Figure 5(b), an additional peak was observed at 399.0 eV due to N 1s. These results also clearly indicated the introduction of

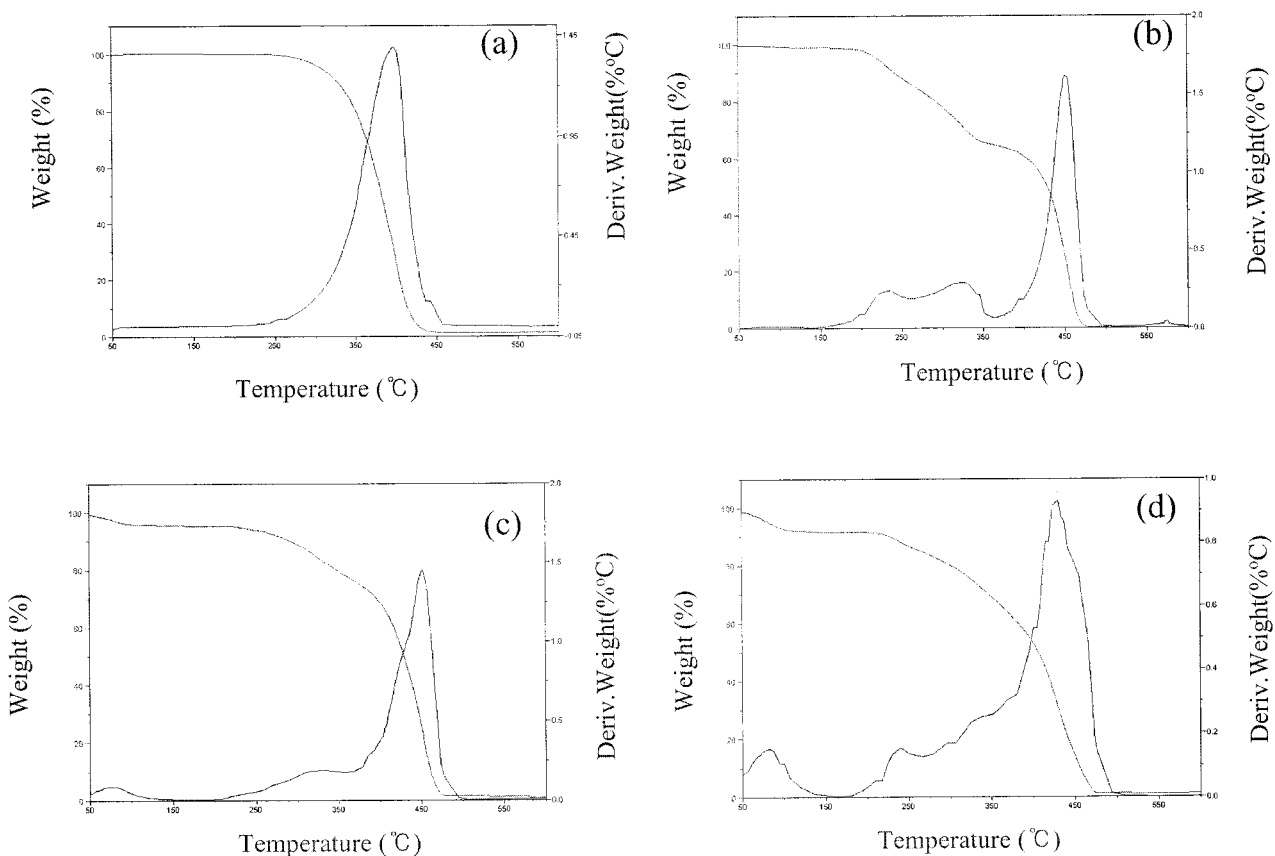


Figure 6 TGA curves of (a) original PP film and (b) 55% grafted PP film, and (c) the TETA group of 1.12 mmol/g and (d) the TETA group of 1.95 mmol/g modified PP film with the same grafting yield.

the TETA group onto the GMA-grafted polymer chains.

Figure 6 shows TGA curves of (a) the original PP film, (b) the 55% GMA-grafted PP film and (c) the TETA of 0.95 mmol/g, and (d) the TETA group of the 1.95 mmol/g-modified PP film with the same degree of grafting. A considerable change in the thermal behavior of the PP film occurred upon grafting. In Figure 6(b), the first weight loss around 230°C was interpreted as the deesterification of poly-GMA on the surface, while the second weight loss around 350°C, as the decarboxylation of that. In Figure 6(c,d), the weight loss was observed at three different temperature regions. The first weight loss around 100°C is due to the loss of moisture on the surface with the amine group, suggesting the existence of the hydrophilic group on the surface of the PP film.

Adsorption of Urokinase by the Aminated PP Films

Figure 7 shows the UV absorption spectra of an urokinase solution with various TETA group con-

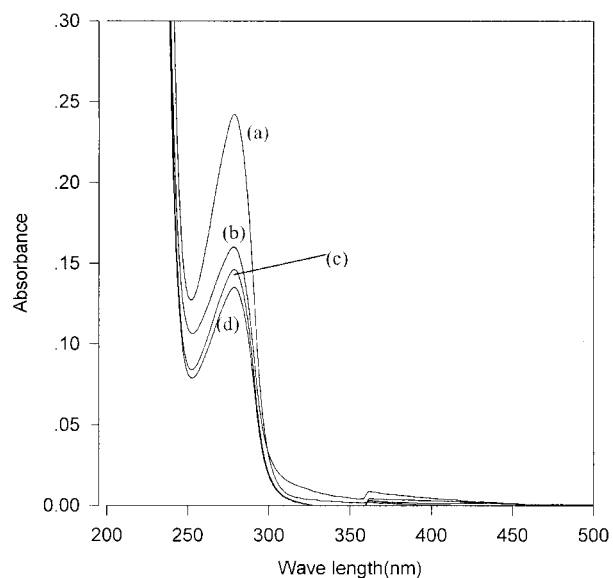


Figure 7 UV spectra of urokinase solution with various TETA group contents after the adsorption by the TETA group: (a) starting UK solution and (b) 1.12, (c) 1.18, and (d) 1.95 mmol/g. Adsorption conditions: 37.3°C; pH 7.4; 24 h.

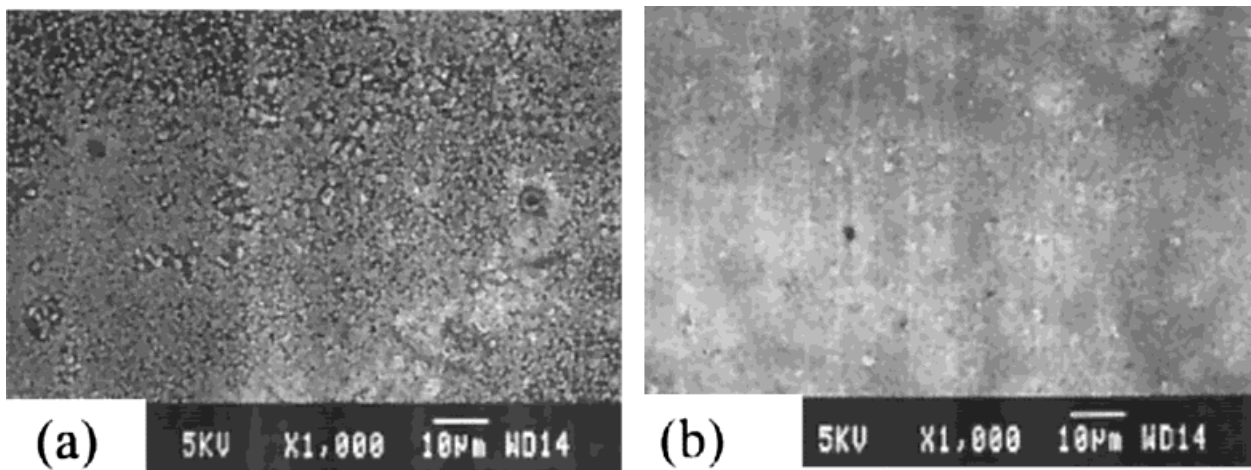


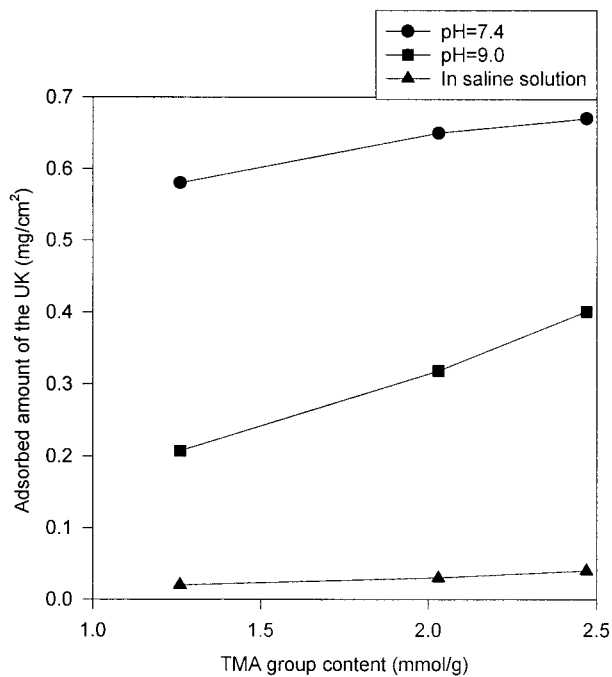
Figure 8 Surface morphology of the PP film with the TETA group of (a) 0.95 mmol/g and (b) 1.95 mmol/g.

tents after the adsorption by the PP film with the TETA group. The concentration of urokinase was 1.14 mg/mL in the PBS solution (at pH 7.4 and 37.3°C). The UV absorbance of the urokinase solution decreased with increasing TETA group content at 278.6 nm. However, in this study, change of the activity of the urokinase adsorbed was not determined.

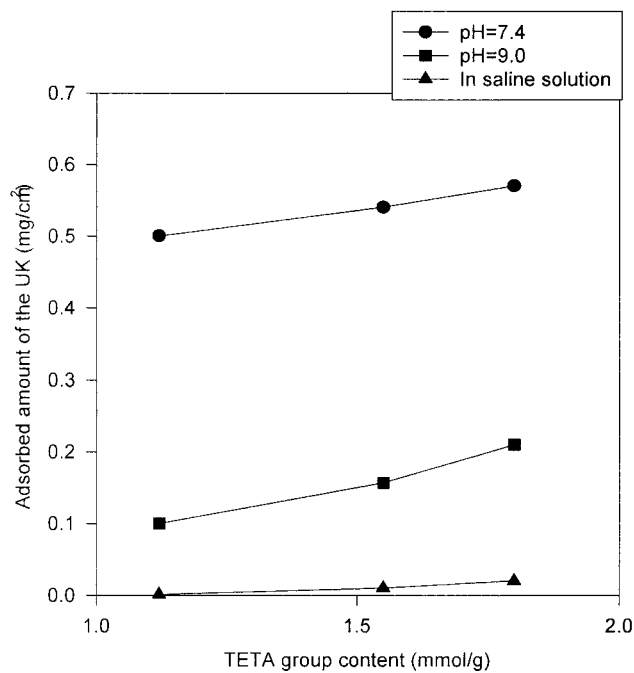
Figure 8 shows SEM photographs of (a) the

0.95 mmol/g and (b) the 1.95 mmol/g TETA-modified PP film. Both PP films modified with the TETA group showed a high adsorption, which suggested that the TETA-group-introduced PP film is a good adsorbent. As the TETA content increased, the adsorption of urokinase increased.

Figure 9 shows the adsorption of urokinase for the PP films with different (a) TMA and (b) TETA contents and pH values. Furthermore, the ad-



(a)



(b)

Figure 9 Relationship between the urokinase uptake and the amount of (a) TMA and (b) TETA groups as a function of pH value.

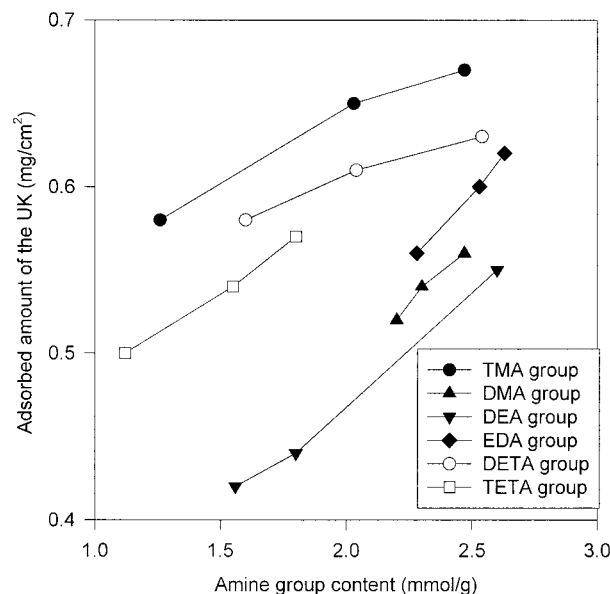


Figure 10 Relationship between the urokinase uptake and the amount of amine groups. Adsorption conditions: pH 7.4; 37.3°C; 24 h.

sorption characteristics of urokinase in a saline solution was examined. In pH 7.4, the adsorbed amount of urokinase was 0.54, 0.62, and 0.68 mg for TMA contents of 1.26, 2.03, and 2.47 mmol/g, respectively. In pH 9, the adsorption amount of urokinase was 0.20, 0.30, and 0.39 mg for a TMA content of 1.26, 2.03, and 2.47 mmol/g, respectively. On the other hand, the urokinase decomposed in pH 4.0 at 37.3°C for 24 h. In pH 7.4, the adsorbed amount of urokinase was 0.50, 0.53, and 0.58 mg for a TETA content of 1.12, 1.55, and 1.80 mmol/g, respectively. In the saline solution, the adsorbed amount of urokinase was very small. These results suggested that the adsorption amounts of urokinase by the PP film with the TMA and TETA groups at pH 7.4 was higher than that at pH 9.

Figure 10 shows the adsorption of urokinase for the PP films for various amine groups in the PBS solution (pH 7.4 at 37.3°C). In the range of amine group content from 1.0 to 2.7 mmol/g, the adsorption of urokinase by the PP films modified with six kinds of amines was in the following order: TMA > DETA > TETA > EDA > DMA > DEA. On the other hand, the introduction of the TEA and HAD groups to poly-GMA was lower than that of other amine groups such as the TMA, DMA, DEA, EDA, DETA, and TETA groups.

CONCLUSIONS

PP films with various amine groups were prepared by radiation-induced grafting of GMA onto

PP films and subsequent amination of poly-GMA graft chains. The adsorption characteristics of urokinase for the PP films with various amine groups were examined. From the results, conclusions were drawn as follows:

1. The adsorption of urokinase by PP film with various amine groups increased by increasing the amine group content.
2. At pH 7.4 and 9.0, the adsorption amount of urokinase by PP film with TMA and TETA groups at pH 7.4 was greater than that at pH 9.0.
3. In the range of amine group content from 1.0 to 2.7 mmol/g, the adsorption amount of urokinase by PP film modified with various amine groups was in the order of TMA > TEA > TETA > EDA > DMA > DEA.
4. The data presented here may be used for further developments in practical application of the TMA and TETA groups-modified adsorbent for recovery of urokinase in human urine.

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