

Wettability and Antibacterial Assessment of Chitosan Containing Radiation-Induced Graft Nonwoven Fabric of Polypropylene-g-Acrylic Acid

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ABSTRACT: The grafting of acrylic acid (AA) onto polypropylene (PP) nonwoven fabric was induced by γ -ray irradiation without degassing to obtain PP-g-AA-modified nonwoven fabric. Chitosan was immobilized onto the PP-g-AA-modified nonwoven fabric with 1-ethyl-3-(3-dimethylamino propyl) carbodiimide to get the chitosan-containing PP-g-AA-modified nonwoven fabric. The PP-g-AA-modified nonwoven fabrics were characterized by IR spectroscopy. In addition to the absorption peaks associated with pure PP nonwoven fabric, absorption peaks at 1700 cm^{-1} (C=O) and at about 2950 cm^{-1} for the COOH group were also found for the PP-g-AA-modified nonwoven fabric. The chitosan-containing PP-g-AA nonwoven fabric obtained had an absorption peak at about $1475\text{--}1580\text{ cm}^{-1}$ (CO—NH), in addition to those of the PP-g-AA nonwoven fabric. The effects of acrylic acid grafting content and chitosan on the

wettability and antibacterial assessment were investigated. The wicking time of water on the PP-g-AA-modified nonwoven fabric decreased with increasing acrylic acid grafting content and chitosan, whereas the water content on the PP-g-AA-modified nonwoven fabric increased. Because the concentration of *Pseudomonas aeruginosa* on the PP-g-AA-modified nonwoven fabric decreased with increasing acrylic acid grafting content and chitosan, it means that the antibacterial activity of the PP nonwoven fabric was enhanced by the modification of γ -ray radiation-induced grafting of acrylic acid and the immobilization of chitosan onto the PP-g-AA-modified nonwoven fabric. © 2003 Wiley Periodicals, Inc. *J Appl Polym Sci* 90: 1331–1336, 2003

Key words: graft copolymers; wettability; nonwoven fabrics; antibacterial activity; irradiation

INTRODUCTION

Grafting technology has long been known in polymer chemistry. Ikada et al.^{1–8} reported on a series of studies of the surface modification of polymers for medical applications by graft polymerization methods. In our previous studies,^{9–15} the grafting of various vinyl monomers onto a membrane of thermoplastic rubber by the γ -ray irradiation method was reported. Because graft polymerization is a time-consuming and expensive oxygen degassing process in industrial applications, Uchida et al.^{16,17} reported on a method for surface graft polymerization of ionic monomer onto poly(ethylene terephthalate) by ultraviolet irradiation without degassing. In our studies, we reported on a method for surface graft polymerization of vinyl monomers onto styrene–butadiene–styrene triblock

copolymer membrane by ultraviolet photografting without degassing.^{18–22}

Because nonwoven fabric is characteristically porous with larger surface area, dust free, is easily processable, and the surface mechanism readily modifiable, it can readily be conditioned to serve as an excellent dressing material. Chen et al.²³ recently reported a method for preparation of easily removable temporary wound dressing materials by radiation grafting of *N*-isopropylacrylamide onto nonwoven fabric.

Poly(acrylic acid) is a kind of hydrogel that exhibits the ability to swell in water and retain a significant fraction of water within its structure without dissolving. Hydrogels have physical properties similar to those of human tissues and possess excellent tissue compatibility. They may interact less strongly with immobilized species than more hydrophobic materials. Thus, molecules and cells immobilized on or within hydrogels may be more likely to retain their biological activity for longer periods of time. The main disadvantage of hydrogels is their poor mechanical properties after swelling. To eliminate this disadvantage, a hydrogel was grafted onto some base polymers with good mechanical properties. In our previous

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study, the preparation of poly(acrylic acid)-modified polyurethane by UV radiation without degassing was reported.²⁴ The cytotoxicity test and both cell adhesion and proliferation assays in cell culture were conducted to evaluate the biocompatibility of the membrane and an animal primary test was also done.

Chitosan [poly- β (1-4)-D-glucosamine], a cationic polysaccharide, is obtained by alkaline deacetylation of chitin, the principal exoskeletal component in crustaceans. Because the combination of properties of chitosan are desirable, such as water-binding capacity, fat-binding capacity, bioactivity, biodegradability, nontoxicity, biocompatibility, and antifungal activity, chitosan and its modified analogs have found many applications in medicine, cosmetics, agriculture, biochemical separation systems, biomaterials, and drug controlled-release systems.²⁵⁻³²

Radiation, such as γ -ray, provides a sterilization effect and, because no initiator is required for copolymerization, no environmental pollution and biological toxic substances are generated. In this study, the preparation of poly(acrylic acid)-modified poly(propylene) (PP) by γ -ray-induced radiation without degassing is reported. The effects of dose rate, irradiation time, and concentration of acrylic acid on the solution on the degree of grafting were studied. Chitosan was immobilized onto the PP-g-AA-modified nonwoven fabric. The wicking time and water content on the PP-g-AA-modified nonwoven fabric were evaluated. Antibacterial assessment of the PP-g-AA-modified nonwoven fabric was evaluated.

EXPERIMENTAL

Materials

The polypropylene (PP) nonwoven fabric used was from Formosa Inc. (Taiwan). PP was washed with distilled water by ultrasonication, and then dried under reduced pressure. Chitosan was from Aldrich (Milwaukee, WI). Acrylic acid, 1-ethyl-3(3-dimethylamino propyl) carbodiimide, ethanol, and acetic acid were of reagent grade and were used directly.

Preparation of PP-g-AA nonwoven fabric

PP was immersed into acrylic acid (AA) solution with ethanol as solvent and irradiated with ⁶⁰Co γ -ray (29,000 Ci) to achieve graft copolymerization reaction of acrylic acid onto the PP nonwoven fabric surface. After copolymerization, the PP-g-AA nonwoven fabric was washed by ultrasonication to remove the homopolymer, followed by drying in a vacuum oven. The degree of grafting (in wt %) was calculated by the following equation:

$$\text{Grafting (\%)} = \frac{W_f - W_i}{W_i} \times 100$$

where W_f and W_i are the final and initial weights of the PP nonwoven fabric after and before radiation-induced copolymerization, respectively.

Preparation of chitosan-containing PP-g-AA nonwoven fabric

1-Ethyl-3-(3-dimethylamino propyl) carbodiimide (EDAC) served as an intermediate to ligate chitosan to PP-g-AA nonwoven fabric. Initially, water-soluble EDAC was linked to PP-g-AA in MES buffer containing 20 mM of MgCl₂ at pH 5.0 to react at 4°C for 24 h. The PP-g-AA was then immersed into chitosan solution with acetic acid as solvent (2 g/0.1M \times 100 mL) to react for 24 h to get the chitosan-containing PP-g-AA nonwoven fabric. After the reaction, the chitosan-containing PP-g-AA nonwoven fabric was washed by ultrasonication, followed by drying in a vacuum oven. The degree of immobilization (in wt %) was calculated by the following equation:

$$\text{Degree of immobilization (\%)} = \frac{W_k - W_f}{W_f} \times 100$$

where W_k and W_f are the final and initial weights of the PP-g-AA nonwoven fabric after and before reaction with chitosan, respectively.

Measurement of water content and wicking time of water on the modified PP nonwoven fabric

The modified PP nonwoven fabric was cut into 8 \times 8-cm² pieces and dried in a vacuum oven for 24 h to determine the dry weight (W_d). Water content was measured by immersing the modified PP nonwoven fabric in distilled water. The wet weight with different immersion times (W_t) was determined by wiping off the surface water with a piece of filter paper. The absorbed water content was then calculated by

$$\text{Water content (W\%)} = \frac{W_t - W_d}{W_t}$$

The measurement of the wicking time on the modified PP nonwoven fabric is the same as that in the study of Chen et al.²³

Antibacterial assessment

Bactericidal activity was evaluated by examining the killing rate by the viable cell counting technique against *Pseudomonas aeruginosa*. One loop-full of the bacterial was inoculated in 150 mL of nutrient broth at 37°C for 18 h in a test tube. At this stage, the culture of *Pseudomonas aeruginosa* involves approximately 10⁹ cells/mL. PP-g-AA-modified nonwoven fabrics were cut into pieces weighing 0.05 g. These cut fabrics were

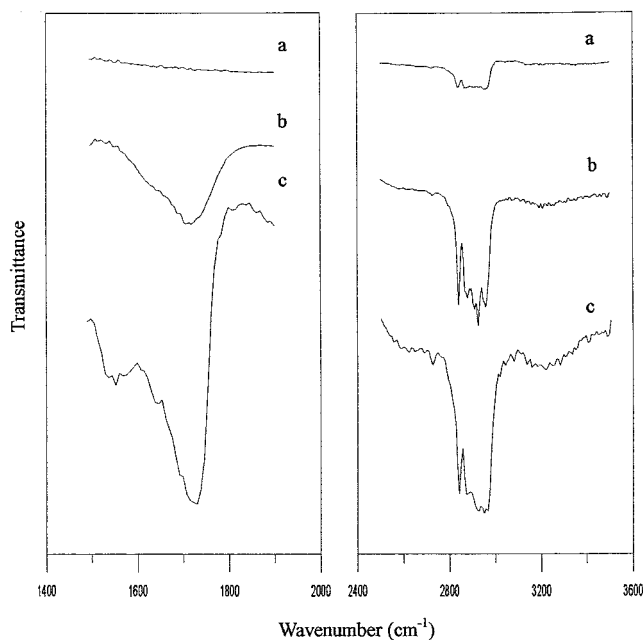


Figure 1 FTIR spectra of (a) polypropylene nonwoven fabric, (b) PP-g-AA nonwoven fabric, and (c) chitosan-containing PP-g-AA nonwoven fabric.

put into contact with 2 mL solution having 10^9 cells/mL for *Pseudomonas aeruginosa* to assess their bactericidal activities. After 18 h, 1 mL of the same culture was added to 9 mL of distilled water, and several decimal solutions were repeated. From this diluted solution, the surviving bacteria were counted by the spread-plate method. After inoculation, the plates were kept at 37°C , and the colonies were counted after 18 h.

RESULTS AND DISCUSSION

Identification of modified PP nonwoven fabric

Figure 1(a)–(c) shows the absorption spectra of pure PP nonwoven fabric, PP-g-AA nonwoven fabric, and chitosan-containing PP-g-AA nonwoven fabric, respectively. In addition to the absorption peaks associated with pure PP nonwoven fabric, the PP-g-AA nonwoven fabric also had absorption peaks at 1700 cm^{-1} (C=O) and at about 2950 cm^{-1} for the COOH group. Because the unreacted acrylic acid and its homopolymer were removed by ultrasonication, the presence of these absorption bands confirms that the spectra of Figure 1(b) were those of PP-g-AA nonwoven fabric. By comparing Figure 1(b) and 1(c), the chitosan-containing PP-g-AA nonwoven fabric obtained has an absorption peak at about $1475\text{--}1580\text{ cm}^{-1}$ (CO—NH), in addition to those of the PP-g-AA nonwoven fabric. Thus the chitosan has been bonded onto the PP-g-AA nonwoven fabric.

Study of grafting behavior

The effect of the distance between the radiation source and the sample is shown in Figure 2. There was no significant effect of radiation on the degree of grafting when the distance between the radiation source and the sample was longer than 25 cm. When the distance between the radiation source and the sample decreased, the degree of grafting increased. Because the dose rate of the radiation source on the sample is inversely proportional to the square of the distance between the radiation source and the sample, the decrease of the distance resulted in a significant increase of the dose rate. When the distance between the radiation source and the sample was within 10 cm, the appearance of sample roughening was found and degradation of the sample occurred. To evaluate the effect of dose rate on the degree of grafting of the sample, the results of samples irradiated with different dose rates for 6 h are also shown in Figure 2. The degree of grafting increases with total dose for the radiation system. A quadratic dependency of the degree of grafting (G) on the total dose (E) is given by the following formula:

$$G = -0.0828825E^2 + 1.88562E - 0.625001$$

When $G = 0$, the value of total dose (E) is about 23 kGy. This means that there is an induction time for the grafting to take place because of the existence of oxygen in the monomer mixture. Because this grafting

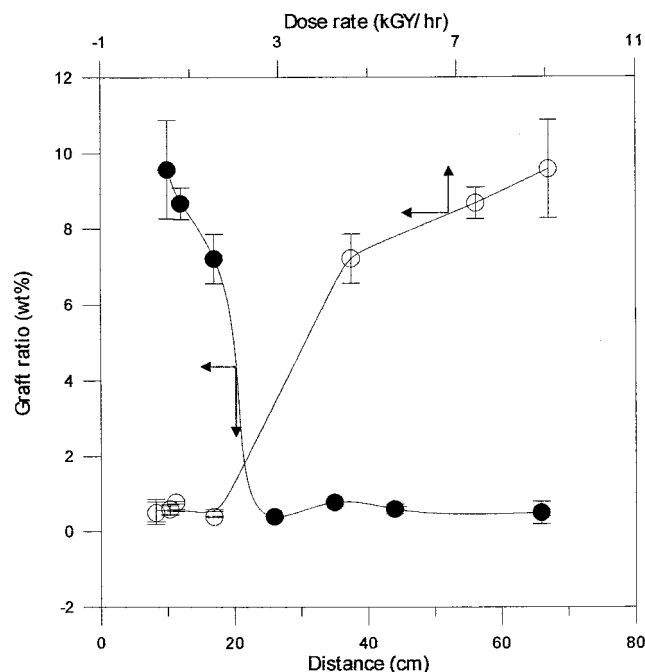


Figure 2 Effect of irradiated distance (cm) (●) and dose rate (kGy/h) (○) on the graft ratio. Radiation time, 6 h; concentration of acrylic acid, 30% (v/v).

polymerization takes place without degassing, oxygen present in the monomer acts as an inhibitor of radical polymerization.

As shown in Figure 3 when samples were irradiated with a constant dose rate, 4.63 kGy/h, the degree of grafting of the samples increases with irradiation time, but seems to level off at about 6 h. As reported in our previous study,²¹ after the active sites on the modified material had reacted to a certain extent, no additional graft copolymerization occurred and only homopolymers of acrylic acid were produced.

The relationship between the AA concentration of various AA-ethanol solutions and the degree of grafting is shown in Figure 3. The degree of grafting increased with acrylic acid concentration.

A quadratic dependency of the degree of grafting (G) on the monomer concentration (C) is given by the following formula:

$$G = 0.00785C^2 - 0.525C - 0.00200001$$

Although the grafting degree of acrylic acid on the PP-g-AA nonwoven fabric was different within this study, no significant difference in surface chitosan content on the chitosan-containing PP-g-AA-modified nonwoven fabric was observed. The surface chitosan content of all chitosan-containing PP-g-AA-modified nonwoven fabrics was about 18.6 ± 1.1 .

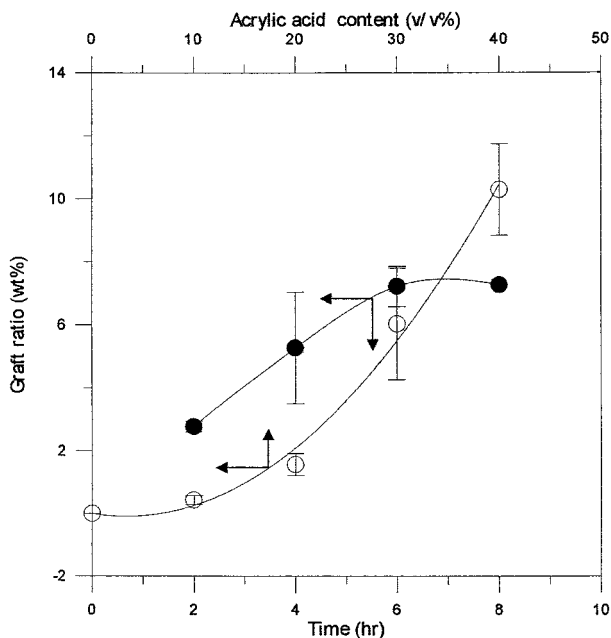


Figure 3 (a) Effect of irradiation time on the graft ratio (●). Concentration of acrylic acid, 30% (v/v); dose rate, 4.63 kGy/h. (b) Effect of acrylic acid concentration on the graft ratio (○). Dose rate, 7.3 kGy/h with irradiated time 6 h. Time (h) versus graft ratio (wt %) in 30% acrylic acid; dose rate, 4.63 kGy/h. Acrylic acid content (○) versus graft ratio (wt %); dose rate, 7.3 kGy/h, irradiated time 6 h.

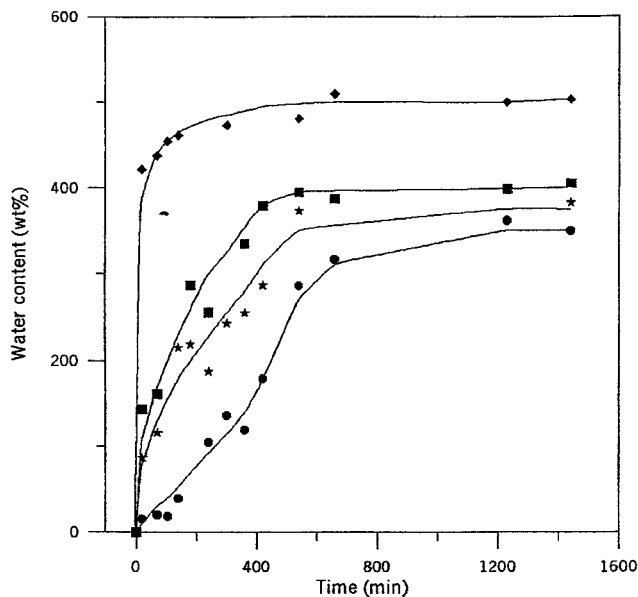


Figure 4 Water content of various PP-g-AA nonwoven fabrics: (●) PP nonwoven fabric; (★) PP-g-AA10; (■) PP-g-AA20; and (◆) PP-g-AA40.

Wettability and antibacterial assessment

Figure 4 and Figure 5 show the relationship between the water absorption and the soaking time for the PP-g-AA-modified nonwoven fabric and chitosan-containing PP-g-AA-modified nonwoven fabric, respectively. The following results were found: (1) The water content increased with the soaking time and then leveled off. (2) The water absorptions, both for the PP-g-AA nonwoven fabric and chitosan-contain-

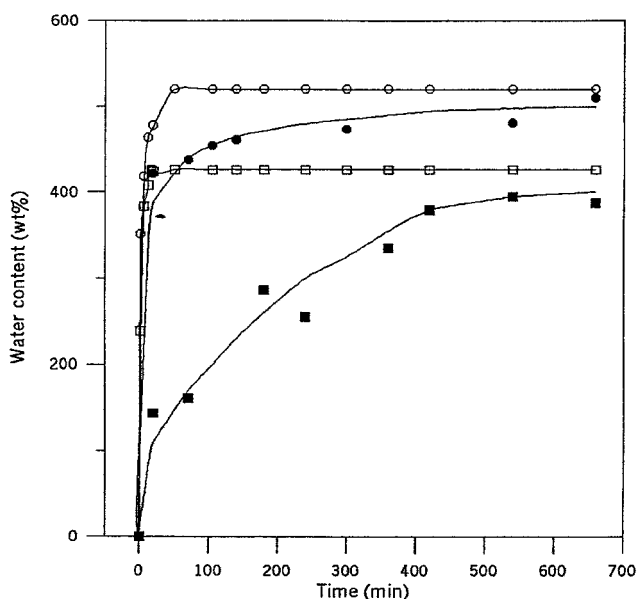


Figure 5 Water content of various modified PP-g-AA nonwoven fabrics: (■) PP-g-AA20, (□) PP-g-AA20-chi, (●) PP-g-AA40, and (○) PP-g-AA40-chi.

ing PP-g-AA nonwoven fabric, also increased with the degree of grafting of AA onto PP nonwoven fabric. (3) By comparing the results of Figure 4 and Figure 5, the soaking time to reach the equilibrium state is less for the chitosan-containing PP-g-AA nonwoven fabric than for the PP-g-AA nonwoven fabric. These observations may be explained as follows.

Polypropylene nonwoven fabric is hydrophobic, whereas polyacrylic acid and chitosan are hydrophilic. After the irradiation, acrylic acid was grafted onto the surface of PP nonwoven fabric and the surface property on the PP nonwoven fabric was changed from hydrophobic to hydrophilic. Because chitosan is a cationic polysaccharide with water-binding capacity, the wettability on PP-g-AA-modified nonwoven fabric was enhanced after the immobilization of chitosan onto the PP-g-AA-modified nonwoven fabric.

The preceding results also can be confirmed from the relative wicking times shown in Table I. The wicking time decreases with the increasing grafting degree of acrylic acid. After the treatment of chitosan on the PP-g-AA-modified nonwoven fabric, the wicking time decreased further.

Table II shows the viable cell number of *Pseudomonas aeruginosa* after coming into contact with PP-g-AA-modified nonwoven fabric. From the antibacterial activity of PP-g-AA-modified nonwoven fabric, it was found that the viable cell number decreased with increasing acrylic acid grafting on the PP nonwoven fabric. After the treatment with chitosan on the PP-g-AA-modified nonwoven fabric, the viable cell number decreased further.

A number of investigations were conducted to provide polymeric materials with surface antibacterial activity by incorporating antibacterial agents covalently onto the surface of the polymeric materials.³³⁻³⁷ The ability to capture bacterial cells onto their

TABLE I
Graft Ratio, Degree of Immobilization, and Wicking Time of Various Samples

Sample	Graft ratio ^a (wt %)	Degree of immobilization ^b	Wicking time (min)
PP ^c	0		159
PP-g-AA10 ^d	0.5		146
PP-g-AA20 ^e	1.6		140
PP-g-AA30 ^f	6.0		137
PP-g-AA40 ^g	10.3		136
PP-g-AA20-chi		19.7	136
PP-g-AA30-chi		17	130
PP-g-AA40-chi		19.4	106

^a Graft ratio of acrylic acid onto PP nonwoven fabric.

^b Degree of immobilization of chitosan onto PP-g-AA20, PP-g-AA30, and PP-g-AA40 nonwoven fabric, respectively.

^c Polypropylene nonwoven fabric.

^{d-g} The samples were prepared by soaking PP nonwoven fabric in 10, 20, 30, and 40 (v/v %) acrylic acid solution, respectively.

TABLE II
Antibacterial Assessment of Various Samples

Sample	<i>Pseudomonas aeruginosa</i> (cells/mL) $\times 10^{-7}$
PP nonwoven	3360
PP-g-AA10	20
PP-g-AA20	14.5
PP-g-AA30	6.1
PP-g-AA40	1.2
PP-g-AA20-chi	1.0
PP-g-AA30-chi	0.022
PP-g-AA40-chi	0.015

surface is strongly strain dependent.³⁸ The adsorption of bacterial cells by the immobilized biocides seemed to be affected by hydrophobicity/hydrophilicity of the immobilized system and the bacterial cells.

In this study, with the increasing grafting degree of acrylic acid, the hydrophilicity of the modified nonwoven fabric increased and enhanced the antibacterial activity. Because chitosan, [poly- β (1-4)-D-glucosamine], is a cationic polysaccharide and the bacterial cell surfaces are negatively charged, increasing charge density on the chitosan-containing PP-g-AA nonwoven fabric will enhance the adsorption of the negatively charged cell surface to the nonwoven fabric, resulting in the decrease of the viable cell number.

CONCLUSIONS

In this study, chitosan-containing PP-g-AA-modified nonwoven fabric was prepared by a γ -ray irradiation-induced method with acrylic acid grafted onto polypropylene nonwoven fabric. Chitosan was immobilized onto the PP-g-AA-modified nonwoven fabric with 1-ethyl-3-(3-dimethylamino propyl) carbodiimide (EDAC).

The wettability and antibacterial assessment of the modified PP nonwoven fabric were enhanced with the grafting ratio of acrylic acid and the immobilization of chitosan on the PP-g-AA-modified nonwoven fabric. Because the improvement of wettability and antibacterial assessment of the PP nonwoven fabric has now been achieved, the properties and application of the chitosan-containing PP nonwoven fabric for biomaterials will be evaluated in the future.

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