Antibacterial Activities of Acrylic Acid-Grafted Polypropylene Fabric and Its Metallic Salt

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ABSTRACT: Acrylic acid (AAc) was grafted onto polypropylene (PP) fabric by a preirradiation method using a ^{60}Co gamma ray. The effects of the absorbed dose, the reaction temperature, reaction time, storage time, as well as the addition effect of ferrous ion and sulphuric acid on the degree of grafting, were determined. It was shown that the synergistic effect of a strong acid with ferrous sulfate can increase the grafting yield. Antibacterial activities on metallic complexes of acrylic acid-grafted polypropylene (AAc-*g*-PP) fabric were evaluated by a viable cell counting method. An Ag complexed fabric had strong biocidal effect for all bacteria. Fe, Cu, and Zn complexed fabrics had different antibacterial activities depending on each bacterium. However, AAc-*g*-PP fabric and Ni-complexed fabric did not have bactericidal effect. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci 69: 2213–2220, 1998

Key words: acrylic acid; polypropylene fabric; ferrous sulfate; grafting

INTRODUCTION

Various kinds of plastics are usually sterilized by means of either dry/wet heat, ethylene oxide, or ionizing radiation. However, these polymers can be contaminated or infected by microorganisms such as bacteria if they are exposed to the atmosphere. One method of solving the problem is to develop polymer materials that have bactericidal activity themselves. An organic or inorganic biocide is usually added to the polymers during processing of the material, such as mastication of rubber or mixing of polymer compound.[1–2]

Another method is to endow a biocidal function to the polymer after processing. The grafting method can be used for this purpose. The grafting process needs free radicals or peroxides, and the production of these initiation species is possible by UV,[3–4] plasma, radiation,[5–6] and chemicals. Radiation-induced grafting is one of the most effective methods to modify polymers because of its rapid and uniform creation of active radical sites on the existing polymer matrix. The methods of achieving a grafting reaction using radiation can be divided into simultaneous irradiation and preirradiation.

In this study, a preirradiation grafting method was used to give a biocidal function to PP fabric that is suitable for air filter. The preirradiation grafting technique is favorable from the viewpoint of the formation of a less homopolymer. When organic polymers are subjected to ionizing radiation, the trapped radical or macromolecular peroxide and hydroperoxides, capable of initiating graft copolymerization reaction, are generally formed.[7–8] Unfortunately, homopolymerization is usually accompanied as an undesirable side reaction.

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Additives are currently used in a wide range of radiation polymerization processes. In some cases, they accelerate the rate of reaction; for example, in grafting, they increase grafting yield and reduce homopolymerization.[9–10] It is well known that the addition of certain metal salts to the reaction mixture can suppress the formation of the homopolymer, thus leaving the monomers free to take part in the grafting reaction and facilitating the isolation of the resulting copolymers.[11–12] Recently, it has been reported that the grafting reactivity of radiation-peroxidized polyethylene film can also be kept at room temperature for more than 3 months.[13]

In this experiment, the grafting of AAc onto PP fabric was performed by the preirradiation method. The effects of storage time, absorbed dose, AAc concentration, reaction temperature, and reaction time on the degree of grafting were determined. The effects of ferrous sulfate and sulphuric acid on the grafting yield were evaluated.

After AAc was grafted to PP fabric, various metals were introduced to AAc-*g*-PP fabric to evaluate their antibacterial activities. Antibacterial activities of modified fabrics were examined by viable cell counting method against *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa.*

EXPERIMENTAL

Materials

Commercial PP fabric (140 g/m^2, Chonbang Industries Co. Ltd.) and acrylic acid (Junsei Chemical Co., LTD.) were used without further treatment. Other chemicals were reagent grade.

Irradiation

γ-ray irradiation from ^{60}Co was carried out at an exposure rate of 5.9 kGy/h in the circumstance of air.

Grafting Procedure

The grafting experiments were performed in a glass ampoule having a cock, with the distilled water and additives being added first, followed by the monomer. The irradiated PP fabric was immersed in the monomer solution, purged by bubbling nitrogen. The grafting reaction was carried out by placing the ampoules in water bath set at the proper temperature. After the grafting reaction, the grafted samples were taken out of the monomer solution in glass ampoules and washed with methanol and distilled water to remove the remaining homopolymer.

The degree of grafting was determined as follows:

$$\text{Degree of grafting } (\%) = \frac{W_g - W_0}{W_0} \times 100$$

where W_0 and W_g are the weights of the PP fabric samples before and after grafting, respectively.

Metallic Complex of AAc-*g*-PP Fabric

AAc-*g*-PP fabric (6.94 mM COOH/g) was immersed into $2 \times 10^{-2}M$ metallic solution containing $AgNO_2$, $FeSO_4 \cdot 7H_2O$, $(CH_3COO)_2Zn \cdot 2H_2O$, $(CH_3COO)_2Ni \cdot 4H_2O$, $CuSO_4 \cdot 5H_2O$, $CoCl_2 \cdot 6H_2O$, respectively, for 3 h at room temperature. The content of metals adsorbed by AAc-*g*-PP was evaluated by ICP (PQ3, Fisons).

Antibacterial Assessment

Bactericidal activity was evaluated from examining the killing rate by the viable cell counting technique against *E. coli, S. aureus,* and *P. aeruginosa.* One loopful of the bacteria was inoculated in 150 mL of nutrient broth (peptone 5.0 g/L, beef extract 3.0 g/L, pH 6.8) at 36°C for 24 h, and 1 mL from former bacteria solution was cultured again in nutrient broth at 36°C for 20 h in a test tube shaker at 100 rpm. At this stage, the culture of *E. coli* involves approximately 10^9 cells/mL; that of *S. aureus* and *P. aeruginosa* involves 10^8 cells/mL. AAc-*g*-PP fabric, and its metallic complexes were cut into the weight of 0.145 g. These cut fabrics were contacted with 5 mL solution having 10^9 cells/mL for *E. coli* and 10^8 cells/mL for *S. aureus* and *P. aeruginosa* to assess their bactericidal activities. At a specified time, 1 mL of same culture was added to 9 mL of distilled water, and several decimal dilution was repeated. From this diluted solution, the surviving bacteria were counted by the spread plate method. After inoculation, the plates were kept at 36°C, and the colonies were counted after 12 h.

Scanning Electron Microscopy

Bacteria adherent on the metallic complexed polypropylene fabric surfaces are fixed with 2.5% glu-

taraldehyde in PBS for 10 min at room temperature. The bacteria fixed on the surfaces were dehydrated in an ethanol-grade series (50, 60, 70, 80, 90, and 100%) for 10 min each after washing with PBS and allowed to dry on a clean hood at room temperature. The bacteria fixed on the surfaces were examined by a scanning electron microscope (SEM, JSM-840A, JEOL Co., Japan) with a tilt angle of 45° after gold deposition in vacuum.

RESULTS AND DISCUSSION

If crystalline polymers are subjected to radiation, a number of polymer radicals remain immobilized in the rigid polymer matrix and may be survived for a considerable length of time. Since the radiation-induced radicals and peroxides can be kept for a long time, depending on the storage condition, it is possible to utilize them for initiating grafting reactions. The key factor to influence the trapping of radicals is the physical state of the irradiated polymer, such as the degree of crystallinity and main chain mobility and storage temperature after irradiation of polymer. The degree of crystallinity of polypropylene used in this experiment was found to be 62%, based on 147 J/mol of polypropylene having 100% crystallinity.

When a PP fabric is irradiated in air, the free radicals and peroxides capable of initiating the grafting reaction are formed and kept long, depending on the storage condition. However, the yield of free radicals and peroxy radicals are different, depending on the presence of oxygen when polypropylene is irradiated. The schematic mechanism of PP fabric during irradiation is as follows.[14]

$$P^{\bullet} + O_2 \rightarrow PO_2^{\bullet} \quad (3)$$

$$PO_2^{\bullet} + PH \rightarrow POOH + P^{\bullet} \quad (4)$$

$$PO_2^{\bullet} + P^{\bullet} \rightarrow POOP \quad (5)$$

$$PO_2^{\bullet} + PO_2^{\bullet} \rightarrow POOP + O_2 \quad (6)$$

$$P^{\bullet} + P^{\bullet} \rightarrow P - P \quad (7)$$

In these schemes, P represents the polypropylene and $P^{\bullet}$ represents radicals produced by irradiation. When the diperoxides and hydroperoxides are heated, the dissociation of those are possible as follows.

$$POOP \rightarrow PO^{\bullet} + {}^{\bullet}OP \quad (8)$$

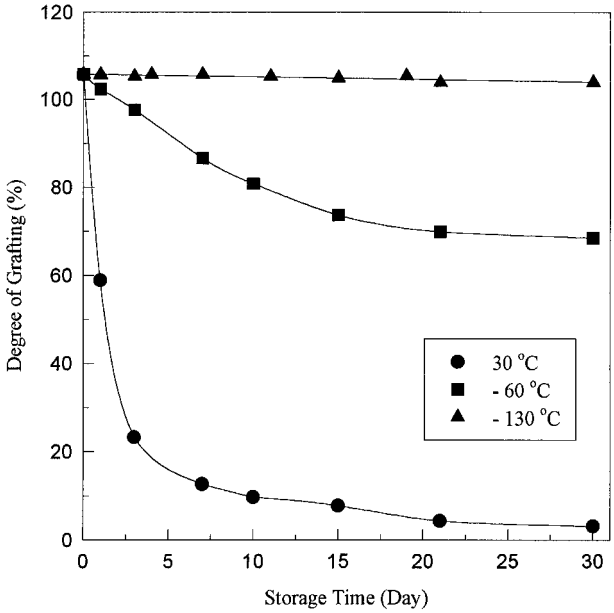

Figure 1 Effect of storage condition on the grafting of acrylic acid onto 5 kGy irradiated PP fabric at 30°C in $2 \times 10^{-1} M$ H_2SO_4 and $1 \times 10^{-3} M$ $FeSO_4 \cdot 7H_2O$ solution. Monomer concentration 30% (v/v). Reaction time is 1 h.

$$POOH \rightarrow PO^{\bullet} + {}^{\bullet}OH \quad (9)$$

If a monomer is allowed to diffuse into trapped radicals or oxide radicals formed from dissociation of peroxides, grafting takes place during the diffusion process.

The polypropylene fabric was stored at various storage condition for a certain period of time after irradiation. Figure 1 shows the effect of storage temperature and time on the grafting of acrylic acid onto polypropylene fabric preirradiated to a total dose of 5 kGy. The grafting yield of the irradiated polypropylene fabric stored at room temperature was found to decrease rapidly with storage time. On the other hand, the grafting yield at storage temperature −130°C remained nearly constant up to 30 days. In the case of storage temperature −60°C, the grafting yield decreased slightly with increasing storage time. A constant grafting yield even after 30 days' storage at −130°C can be attributable to the stoppage in termination of free radicals [eqs. (5), (6), and (7)], which comes from the high crystallinity of polypropylene and the restriction of chain segmental motion at such a low temperature. The termination rate of various active sites increases with increasing storage temperature of irradiated poly-

mer. The reaction temperature is one of the most important factors to control in the grafting process. As shown in Figure 2, the grafting reaction at 70°C was possible even for samples kept at room temperature for 30 days. This means that the reactive sites in the backbone polymer were generated by the decomposition of diperoxides [eq. (8)] or hydroperoxides [eq. (9)] at high temperature and lead to the grafting reaction. That is, the grafting reaction at this temperature was mostly due to the decomposition of peroxides. Most of the grafting reaction at a particularly low reaction temperature, such as 30°C, can be attributable to the trapped radicals.

The peroxides, which are produced by gamma irradiation, are stable at room temperature, while the trapped radicals may decay and be partly transferred to the stable species, such as POOP or POOH.[15]

By heating the irradiated PP fabric samples in AAc aqueous solution, the grafting of AAc to PP fabric initiated by peroxyradicals proceeds. However, undesirable homopolymerization initiated by the •OH radical formed in the thermodecomposition reaction takes place simultaneously. It leads to not only the lower grafting yield but also an enhancement of homopolymerization, and the homopolymers anchored on the PP fabric are difficult to remove. To overcome homopolymerization, O'Neill reported a method using a reducing agent to decompose the peroxy species by converting •OH to inactive ions. For this purpose, a ferrous ion has been commonly used. The reaction of Fe^{2+} on the POOH is as follows.[16]

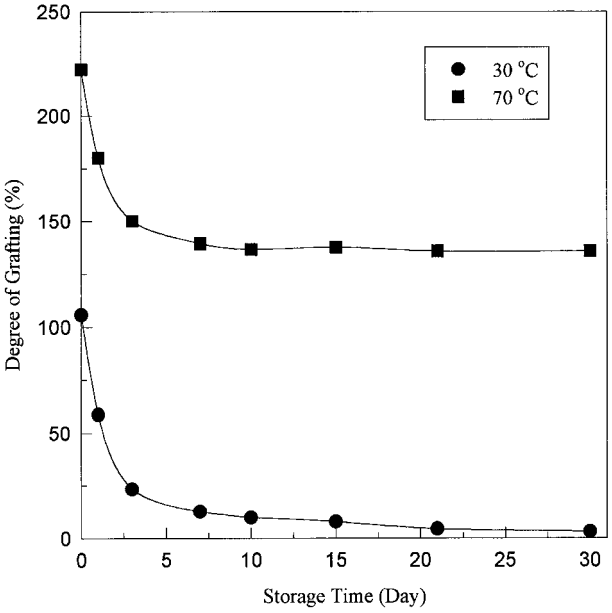

Figure 2 Effect of reaction temperature on the grafting of acrylic acid onto 5 kGy irradiated PP fabric in 2 $\times$ $10^{-1}M$ H_2SO_4 and 1 $\times$ $10^{-3}M$ $FeSO_4 \cdot 7H_2O$ solution. Storage conditions: room temperature; monomer concentration, 30% (v/v); reaction time, 1 h.

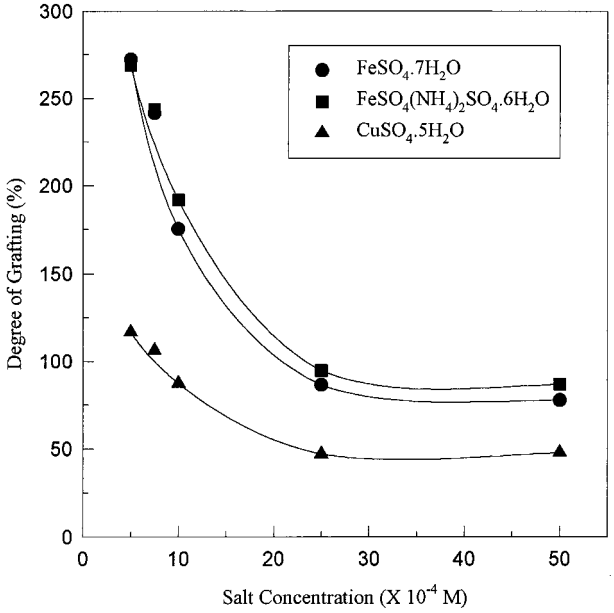

Figure 3 Effect of salt concentration on the grafting of acrylic acid onto 5 kGy irradiated PP fabric at 30°C in 2 $\times$ $10^{-1}M$ H_2SO_4 solution. Monomer concentration is 30% (v/v); reaction time is 2 h.

$$POOH + Fe^{2+} \rightarrow PO^{\bullet} + Fe^{3+} + OH^{-} \quad (10)$$

Figure 3 shows the effect of metallic salt concentration on the grafting yield in the presence of sulphuric acid. The grafting reaction was severely limited without the metallic salt because of the homopolymer formation during the grafting reaction. The grafting yield decreased with increasing the concentration of ferrous sulfate. Metallic salt plays a important role in decomposing the hydroperoxides by a redox reaction, as shown in [eq. (10)]. In addition, the deactivation process of the grafting chain radical growth is as follows.

$$PO^{\bullet} + Fe^{2+} \rightarrow PO^{-} + Fe^{3+} \quad (11)$$

This reaction leads to reduce the grafting reaction. The grafting yield was much higher in the case of ferrous sulfate and Mohr's salt than cupric sulfate because Fe^{2+} can be transferred to Fe^{3+} by oxidation to dissociate hydroxides. However, it should be noted it is impossible to oxidize Cu^{2+}.

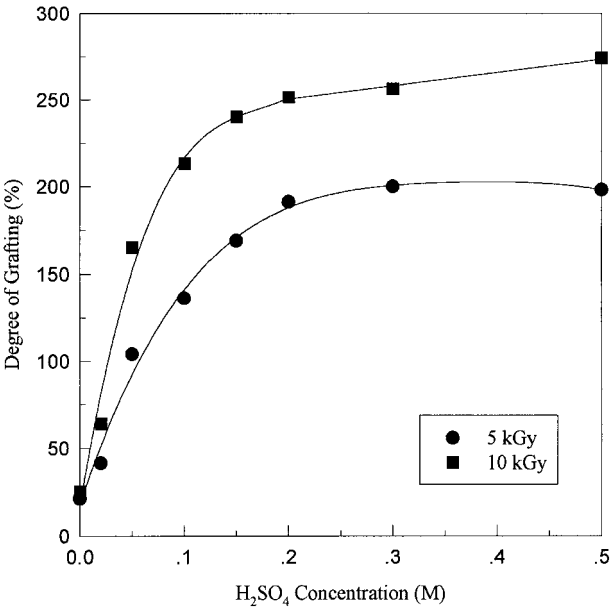

Figure 4 Effect of H_2SO_4 concentration and dose on the grafting of acrylic acid onto preirradiated PP fabric at 30°C in $1 \times 10^{-3}M$ $FeSO_4 \cdot 7H_2O$ solution. Monomer concentration is 30% (v/v); reaction time is 2 h.

The degree of grafting was low in the absence of sulphuric acid; however, it increased with the addition of sulfuric acid (Fig. 4). This means that the addition of sulphuric acid and the appropriate concentration of Fe^{2+} can greatly enhance the grafting. Sulphuric acid accelerates the decomposition of hydroperoxides in the presence of Fe^{2+} to form radicals, which can initiate the grafting reaction. The OH^- accumulated in the reaction medium can be consumed by H^+, which originates from the dissociation of H_2SO_4. Therefore, eq. (10) can progress easily forward in the presence of acid. In the previous work, we reported the results of the inclusion of a cationic salts in the monomer solution on the photosensitized simultaneous grafting of styrene in the presence of acid. The enhancement in the grafting reaction by addition of acid was explained by partition mechanism. In this grafting reaction, it is assumed that acid plays a role in enhancing the redox reaction and partition effect.

Figure 5 shows the effect of acid type on the grafting yield. The addition of sulphuric acid led to the highest grafting yield. Acetic acid had almost no effect on the grafting reaction. The results in Figure 6 illustrated the influence of the variation of methanol–H_2O composition on the grafting of AAc at 30% monomer concentration. These results clearly indicated that the increase in methanol content was accompanied by a significant decrease in grafting yield. This may be explained by the following mechanisms.

$$P^{\bullet} + M(\text{monomer}) \rightarrow PM^{\bullet} \qquad (12)$$

$$P^{\bullet} + CH_3OH \rightarrow PH + {}^{\bullet}CH_2OH \qquad (13)$$

When reaction (12) proceeds, the grafting reaction occurs; whereas with reaction (13), the backbone polymer radical abstracts H atoms from the methanol, leading to ${}^{\bullet}CH_2OH$ radicals, which produces homopolymer than graft. Therefore, the grafting yield is decreased by the increase methanol content in the solution.

AAc-*g*-PP fabric (6.94 mM COOH/g) was reacted with $2 \times 10^{-2}M$ of metallic solution containing $AgNO_2$, $FeSO_4 \cdot 7H_2O$, $(CH_3COO)_2Zn \cdot 2H_2O$, $(CH_3COO)_2Ni \cdot 4H_2O$, $CuSO_4 \cdot 5H_2O$, $CoCl_2 \cdot 6H_2O$ for 3 h at room temperature. The contents of metals introduced in AAc-*g*-PP fabric (6.94 mM COOH/g) are shown in Table I.

Figure 7 shows log plots of viable cell number of *E. coli* after contacting with a AAc-*g*-PP fabric and its metal complexed PP fabrics. About 10^9 cells/mL of bacteria contacted with each treated fabric of 0.145 g. An Ag complexed fabric had strong biocidal effect in killing all bacteria within

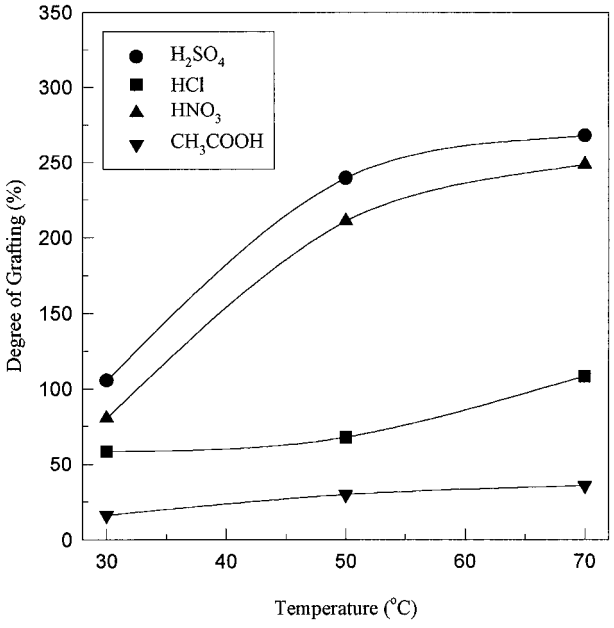

Figure 5 Effect of acid type on the grafting of acrylic acid onto 5 kGy irradiated PP fabric in $2 \times 10^{-1}M$ acid and $1 \times 10^{-3}M$ $FeSO_4 \cdot 7H_2O$ solution. Monomer concentration is 30% (v/v); reaction time is 1 h.

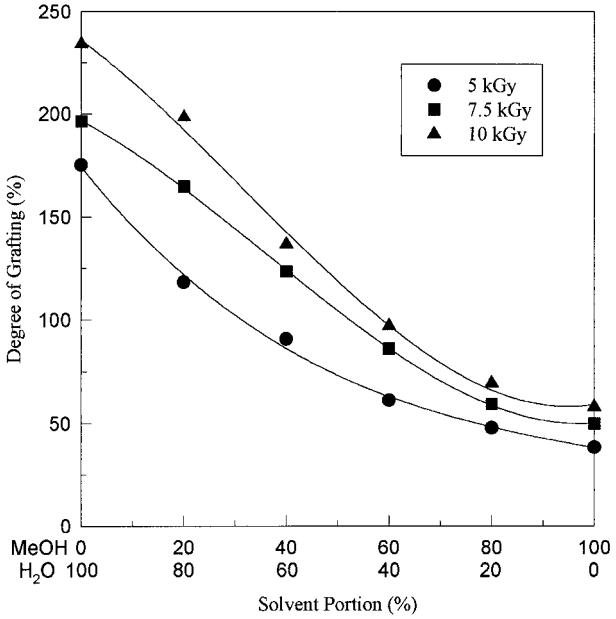

Figure 6 Effect of solvent portion on the grafting of acrylic acid onto 5 kGy irradiated PP fabric at 30°C in $2 \times 10^{-1}M$ H_2SO_4 and $1 \times 10^{-3}M$ $FeSO_4 \cdot 7H_2O$ solution. Monomer concentration is 30% (v/v); reaction time is 1 h.

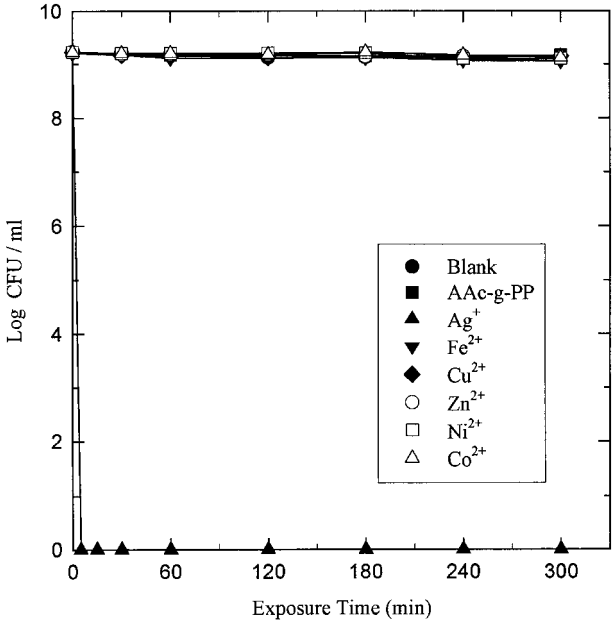

Figure 7 Changes in log plot of viable cell number of *E. coli* with the exposure time for the metal complexed AAc-*g*-PP.

5 min. The other metal complexed fabrics and AAc-*g*-PP fabric did not have bactericidal effect.

S. aureus was applied to the same samples as in Figure 7 (Fig. 8). An Ag complexed fabric had strong biocidal effect in killing all bacteria within 5 min, Cu complexed fabric had intensive biocidal effect in killing all bacteria within 30 min, and Zn complexed fabric was capable of killing *S. aureus* within 180 min. The other metal complexed fabrics did not have any bactericidal effect against *S. aureus,* showing different pattern from those of *E. coli.*

P. aeruginosa was applied to the same sample (Fig. 9). Ag complexed fabric had strong biocidal effect to kill all bacteria within 30 min. On the other hand, Fe complexed fabric was capable of killing *P. aeruginosa* within 180 min, with Cu and Co complexed fabrics having little biocidal effect.

From the antibacterial activity of metallic salts of AAc-*g*-PP fabric, it was found that Ag complexed fabric had strong biocidal effect for all bacteria. Fe, Cu, and Zn complexed fabrics had different antibacterial activity depending on each bac-

Table I The Content of Metals Introduced in AAC-*g*-PP Fabric (6.94 m*M* COOH/g)

Metal	Ag	Fe	Cu	Zn	Ni	Co
Content (mmol/g)	0.10	0.28	0.37	1.96	1.75	0.08

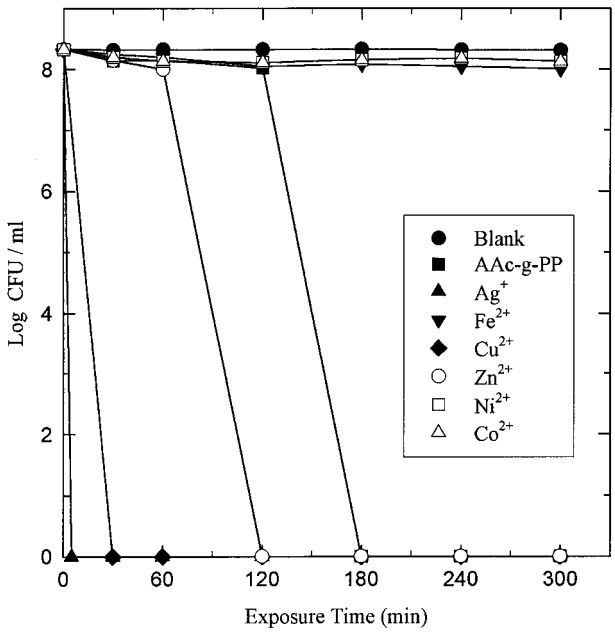

Figure 8 Changes in the log plot of viable cell number of *S. aureus* with the exposure time for the metal complexed AAc-*g*-PP.

terium. On the other hand, AAc-*g*-PP fabric and Ni complexed fabric did not have bactericidal effect at all.

The mode of action of the biocides of metallic complexes of acrylic acid-grafted polypropylene can be interpreted on the basis of each elementary process described as follows. The target site of the cationic biocides is the cytoplasmic membranes of bacteria, and the following elementary processes have been proposed for their mode of action: (1) adsorption onto the bacterial cell surface; (2) diffusion through the cell wall; (3) binding to the cytoplasmic membrane; (4) disruption of the cytoplasmic membrane; (5) release of the cytoplasmic constituents, such as K^+ ions, DNA, and RNA; and (6) death of the cell. It is well known that the bacterial cell surfaces are negatively charged. Then, adsorption onto the negatively charged cell surface (process 1) is expected to be enhanced with increasing charge density of the cationic biocides.

It is known that there is much difference in the structure of cell walls between gram-positive and gram-negative bacteria. The gram-positive bacterium, such as *S. aureus,* has a simple cell wall structure in which, outside the cytoplasmic membrane, there is only a rigid peptidoglycan layer. The peptidoglycan layer, though relatively thick, is composed of networks with plenty of pores, which allow foreign molecules to come into the cell without difficulty. Thus, *S. aureus* killed faster than gram-negative bacteria, such as *E. coli* or *P. aeruginosa,* though nonmotile. On the other hand, gram-negative bacteria, such as *E. coli* and *P. aeruginosa,* have very complicated cell walls. There is another membrane outside the peptidoglycan layer, which is called the outer membrane, and has a structure similar to that of the cytoplasmic membrane. Also, *E. coli* is motile by peritrichous flagella or nonmotile, where *P. aeruginosa* is motile by one or several polar flagella; that is, they are rarely nonmotile.

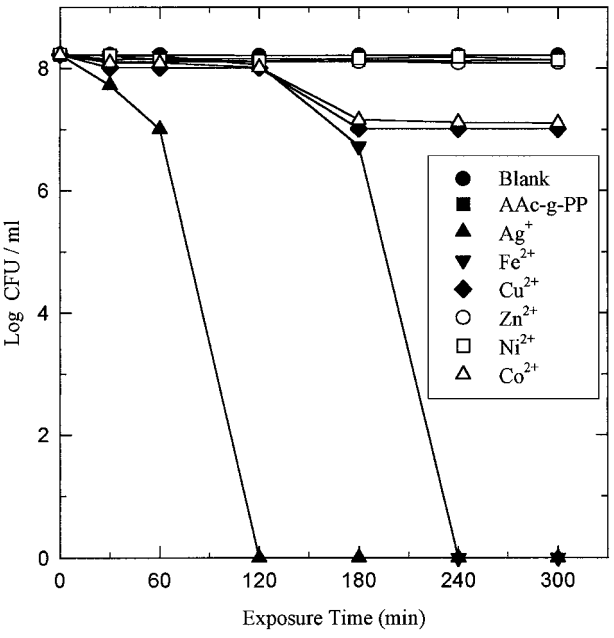

Figure 9 Changes in log plot of viable cell number of *P. aeruginosa* with the exposure time for the metal complexed AAc-*g*-PP.

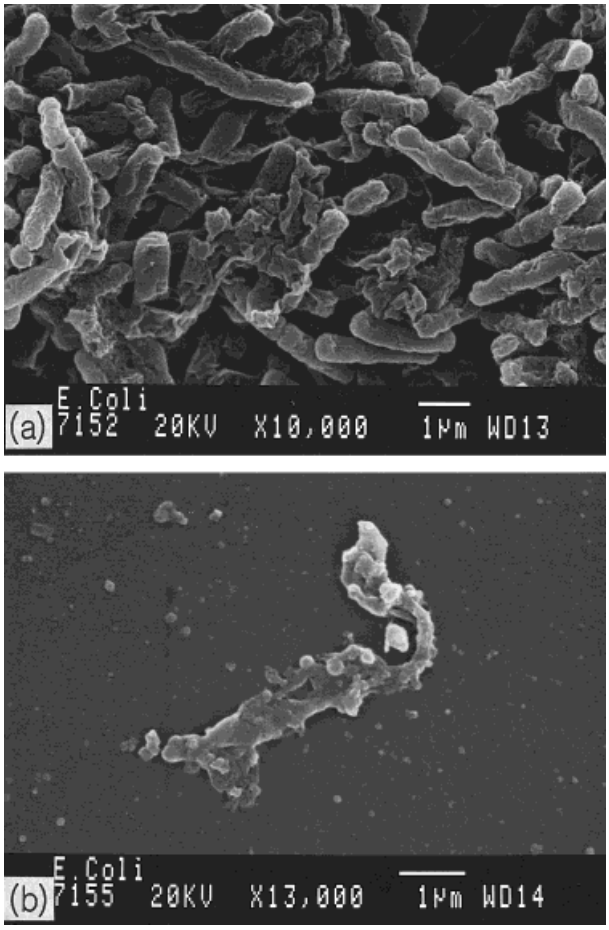

Figure 10 SEM photographs of Ag complexed polypropylene fabric surfaces on which *E. coli* was inoculated.

Figure 10 shows the scanning electron micrographs of Ag complexed polypropylene fabric surfaces which *E. coli* was inoculated on and incubated at 37°C for 30 min. As shown in Fig. 10(a) and (b), the bacteria are shrunken and deformed.

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