Preparation and Properties of Silver-Containing Nylon 6 Nanofibers Formed by Electrospinning

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ABSTRACT: Nylon 6 nanofibers containing silver nanoparticles (nylon 6/silver) were successfully prepared by electrospinning. The structure and properties of the electrospun fibers were studied with the aid of scanning electron microscopy, transmission electron microscopy, energy-dispersive spectroscopy, and X-ray diffraction. The structural analysis indicated that the fibers electrospun at maximum conditions were straight and that silver nanoparticles were distributed in the fibers. Finally, the antibacterial activities of the nylon 6/silver nanofiber mats were investigated in a broth dilution test against *Staphylococcus aureus* (Gram-positive) and *Klebsiella pneumoniae* (Gram-negative) bacteria. It was revealed that nylon 6/silver possessed excellent antibacterial properties and an inhibitory effect on the growth of *S. aureus* and *K. pneumoniae*. On the contrary, nylon 6 fibers without silver nanoparticles did not show any such antibacterial activity. Therefore, electrospun nylon 6/silver nanocomposites could be used in water filters, wound dressings, or antiadhesion membranes. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 112: 2320–2326, 2009

Key words: nanocomposites; nylon; electrospinning

INTRODUCTION

In the last few years, there has been increasing interest in the subject of textile materials having antibacterial properties because of various environmental pollutants.[1] The prevention of odor and the discoloration of textiles are significant reasons to use antimicrobials. For these reasons, consumers are showing increasing interest in antimicrobial products.[2] It is known that silver and its compounds exhibit a strong toxicity to a wide range of microorganisms.[3] Some forms of silver have been demonstrated to be effective against burns, severe chronic osteomyelitis, urinary tract infections, and central venous catheter infections.[4] Spadaro et al.[3] found that silver ion had a biocidal effect on as many as 16 different kinds of bacteria, including *Escherichia coli* and *Staphylococcus aureus*. Feng et al.[4] investigated the mechanism of the inhibition of silver ions on Gram-negative *E. coli* and Gram-positive *S. aureus* and showed that the interaction of Ag^+ with thiol groups played an essential role in bacterial inactivation. In addition, silver nanoparticles are small enough to pass through outer cell membranes and enter the cell's inner mechanism.

Nanofiber technology forms an important object for recent material research studies. An elegant method of nanofiber production is now well known as electrospinning, which was introduced in the early 1930s.[5,6] Recently, much attention has been paid to the electrospinning process as a unique technique because it can produce polymer nanofibers with a diameter ranging from several micrometers down to 10s of nanometers, depending on the polymer and processing conditions.[7] Most of the silver-releasing products are in the form of a sheet, sponge, or fiber, particularly a nanofiber. So far, silver-containing nanofibers, for example, poly(L-lactide),[8] activated carbon,[9] polypropylene,[10] nylon 6,6,[11] cellulose acetate,[12] and poly(ethylene terephthalate)/chitosan,[13] have been electrospun successfully, and their antibacterial activity has been observed. However, to date, no report has yet been published showing the antibacterial activity of nylon 6/Ag nanofibers produced by electrospinning.

In this study, silver nanoparticles were incorporated into nylon 6 nanofibers by electrospinning, and the antibacterial activity of the electrospun fibers was evaluated against *S. aureus* (Gram positive) and *Klebsiella pneumoniae* (Gram negative) with a modified

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broth dilution test. The surface properties of the nanofibers and the influence of silver on the textural properties were also studied by scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), a conductivity meter, and energy-dispersive spectroscopy (EDS).

EXPERIMENTAL

Materials

The nylon 6 used in this study, with an average molecular weight of about 23,000 g/mol, was supplied by KP Chemical Co. (Korea). Formic acid (HCOOH > 85%) was purchased from Duksan Pure Chemical Co., Ltd. (Korea). The silver nanoparticle suspension (the mean diameter of the particles was approximately 8 nm with a standard deviation of ±2.4 nm), with a density of 6.54 g/cc, was kindly donated by Hunion Co. (Korea). The bacteria, *S. aureus* and *K. pneumoniae*, were obtained from the Korean Cell Line Bank and the Korean Collection for Type Cultures, respectively. All other chemicals used in this study were analytical grade and were used without further purification.

Preparation of the polymer solution

Nylon 6 was dissolved in formic acid at various concentrations (15–30 wt %), and the solutions were stirred overnight at room temperature to ensure complete dissolution. To remove insoluble fraction or impurities, the solutions were filtered with a nonwoven fabric. Then, certain amounts of the silver nanoparticle suspension (0–2000 ppm) were mixed with the nylon 6/formic acid solutions and stirred by a shaking incubator for 24 h in an ice-water bath to get the nylon 6/silver solutions. The prepared solutions was then subjected to the electrospinning experiments.

Electrospinning

The electrospinning experiments were performed at room temperature, and the apparatus for electrospinning was assembled on the basis of a study carried out by Lee et al.[14] The polymer solution was placed into a 10-mL glass syringe fitted with a needle and with an inner diameter of 0.4 mm. A clamp connected to a high-voltage power supply, which could supply a positive voltage from 0 to 50 kV, was attached to the needle. A piece of aluminum foil was placed at a distance of 10 cm from the needle tip. The polymer jets generated from the needle by high voltage flew to the collector and formed the nanofiber mesh. The polymer solutions were electrospun with a fixed mass flow rate of 0.1–0.3 mL/h and a voltage between 8 and 22 kV. Finally, the electrospun samples were dried overnight at 40°C to remove moisture.

Surface characterization

The viscosity and conductivity of the nylon 6 blend solutions were measured by a rotational viscometer (LVDV II+, Brookfield Co.) and an electric conductivity meter (model 125 A+, Thermo Scientific), respectively. Morphologies of the electrospun nanofiber were observed with a field emission scanning electron microscope (FE-SEM S-4300, Hitachi, Japan) with an accelerating voltage of 20 kV, and the obtained images were analyzed by image analyzing software (ImagePro). An aluminum sheet with a nanofiber mat was cut and mounted on aluminum specimen stubs with double-sided adhesive tape, and the samples were sputter-coated with gold. For each sample, the average value and its standard deviation were calculated from 50 measured diameters. The morphologies of the nylon 6/silver nanofibers were observed through TEM (H-7600, Hitachi). The silver nanoparticle distribution on the surface and cross section of the nylon 6/silver fibers were investigated by EDS. The fibers were analyzed by XRD (Rigaku X-ray diffractometer), which was measured on a RINT2000 wide-angle goniometer with Cu Kα radiation at 40 kV and 100 mA. With this procedure, the angles of diffraction (2θ's) of all the samples were measured from 25 to 80° in the equatorial direction.

Antibacterial assessment

The antimicrobial activity of the nylon nanofibers with and without silver nanoparticles was tested against Gram-positive *S. aureus* and Gram-negative *K. pneumoniae* by the nonwoven fabric attachment method.[15] Briefly, sterilized Luria–Bertani (LB) broth was measured (8 mL) into sterile tubes. The silver-containing nylon 6 nanofibers were introduced into the LB broth solutions, which contained about 1.5×10^5 colony-forming units of *S. aureus* and *E. coli*, respectively. The mixtures were cultured at 37°C in a shaking incubator for 18 h. Nylon 6 nanofibers were also tested as a control. A 100-μL portion of each of these cell solutions was seeded onto LB agar with a surface spread plate technique. The plates were incubated at 37°C for 24 h. Then, the number of bacterial colonies (colony-forming units) was counted. The counts were used to calculate the surviving number of bacteria.[16] The antibacterial efficacy (ABE; %) of the specimens was calculated according to the following equation:

$$\text{ABE } (\%) = (V_c - V_t)/V_c \times 100$$

where V_c and V_t are the number of viable bacterial colonies of the control (nylon 6 nanofiber) and the test specimens (nylon 6/silver nanofiber), respectively.

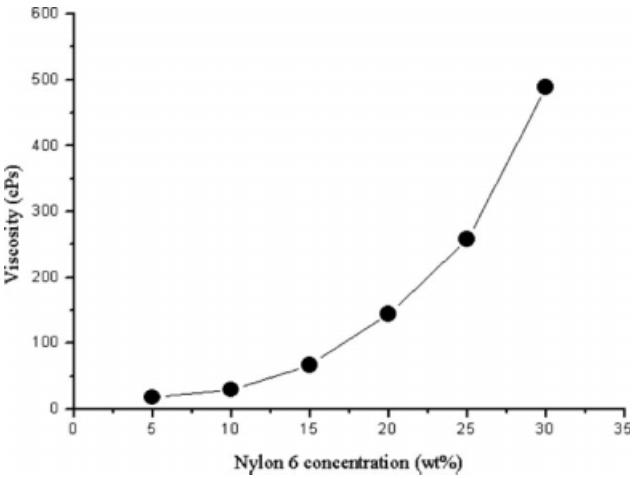

Figure 1 Relationship between the nylon 6 concentration in formic acid and the solution viscosity.

RESULTS AND DISCUSSION

Surface and morphology of the nylon 6/silver nanofiber mat

The effects of the solution viscosity on the electrospinnability were examined, and the resulting morphologies of the electrospun deposition were observed to distinguish the spinnabilities of the following three classes: (1) the coexistence of beads and fibers, (2) homogeneous fibers (very few or no beads), and (3) the failure of the spinning jet. Figure 1 represents the relationship between the nylon 6 concentration in formic acid by weight percentage (from 15 to 30 wt %) and the solution viscosities in centipoises. The viscosities of nylon 6 exponentially increased with the concentration. In Figure 1, three kinds of areas are indicated according to the resulting morphologies of the electrospun depositions: area A (ca. 5–60 cP), fibers and coexisting beads; area B (ca. 60–500 cP), deposition of a homogeneous fiber network; and area C (>500 cP), failure of the spinning jet because of a high viscosity. The SEM micrographs of the nanofibers electrospun from nylon 6 solutions with different concentrations or viscosities ranging from 15 to 30 wt % are shown in Figure 2. At the concentration below 15 wt % (area A), beads or beaded fibers were generated by electrospinning. The solution viscosities rapidly increased from 15 wt % nylon 6 (Fig. 1) because of the onset of chain entanglement.[17] So long, uniform and continuous nanofibers were obtained at concentrations above 15 wt % (area B), and the best fibers were found at a concentration of 25 wt %, as shown in Figure 2(c). Therefore, we concluded that extensive chain entanglements were necessary to produce continuous fibers by electrospinning. The solution concentration of 25 wt % was chosen to fabricate nonwoven randomly arranged nylon 6 fibers with nanometer-scale diameters.

Figure 3(A) shows SEM images of the nylon 6 nanofibers electrospun from 25 wt % nylon 6 solutions with different amounts of silver nanoparticles

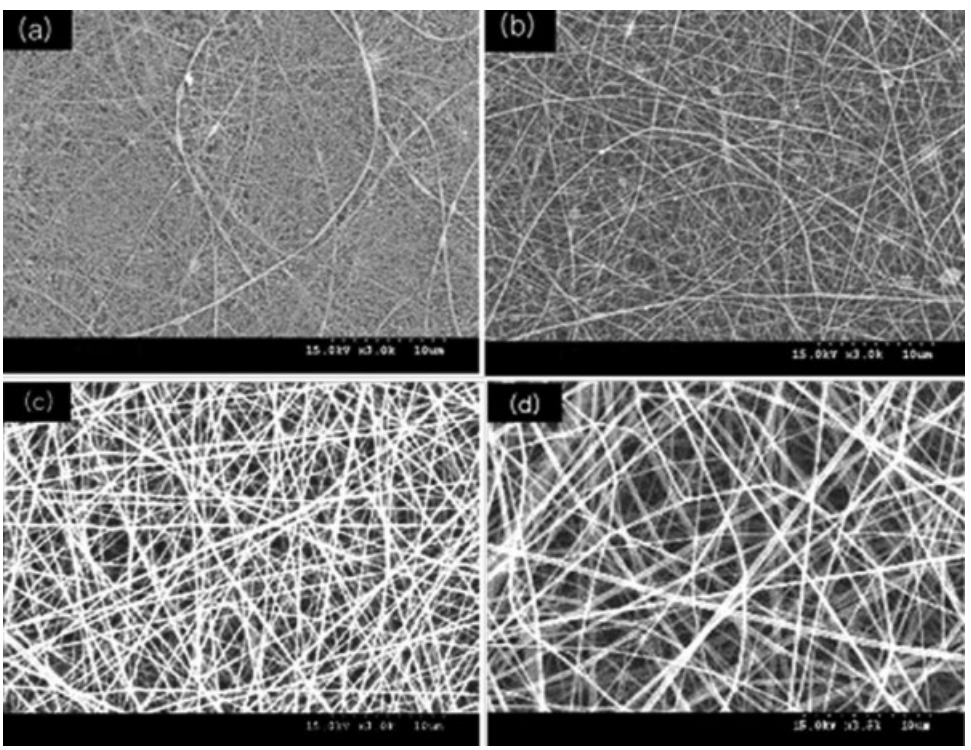

Figure 2 SEM images of electrospun nylon 6 nanofibers with polymer solutions of different concentrations: (a) 15, (b) 20, (c) 25, and (d) 30 wt %.

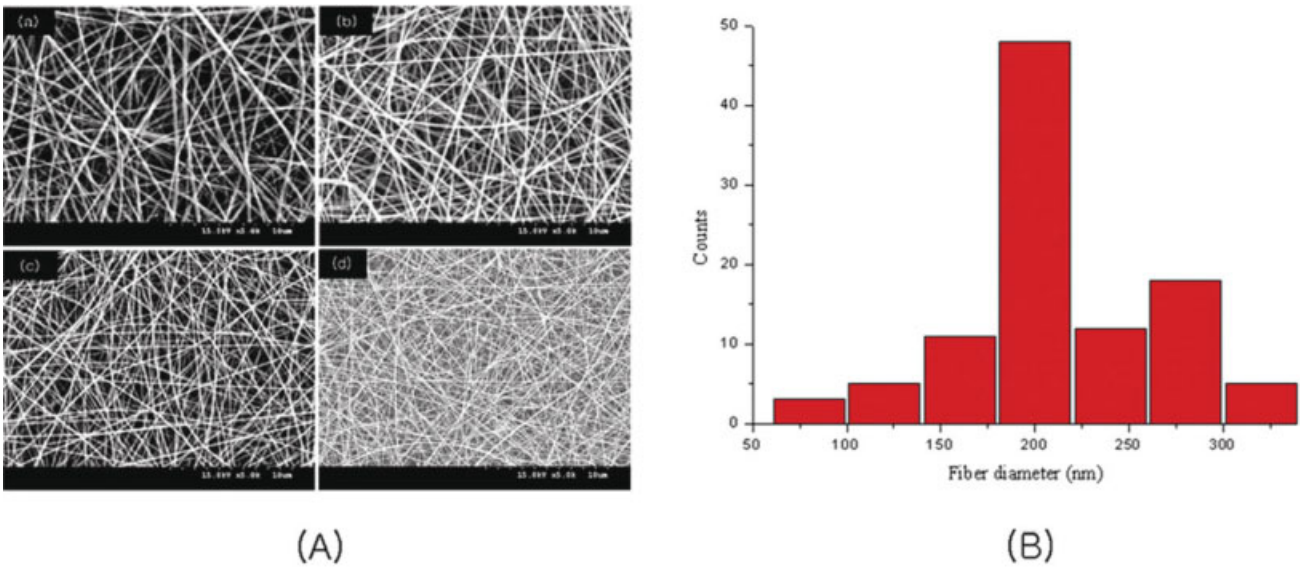

Figure 3 (A) Effect of the silver content on the diameter of electrospun nylon 6 nanofibers: (a) 0, (b) 300, (c) 1000, and (d) 2000 ppm. (B) Diameter distribution of nylon 6/silver (1000 ppm) nanofibers electrospun from a 25 wt % nylon 6 solution. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

(0–2000 ppm). The electrospun fibers with 1000 ppm silver nanoparticles possessed relatively homogeneous, longer, and stronger fibers [Fig. 3(c)] than the fibers with 0, 300, and 2000 ppm silver nanoparticles [Fig. 3(a,b,d)]. The diameter of the electrospun nylon 6 fibers decreased with increasing silver content. This was because the conductivity of the nylon 6/formic acid solution was proportional to the content of silver, as shown in Figure 4. The distribution of fiber diameter, which was electrospun with 25 wt % nylon 6 solution, is shown in Figure 3(B). Under this solution concentration, the average diameter of the electrospun fibers was around 180 nm (from image analysis), and their diameters ranged from 150 to 250 nm. Figure 4 shows the changes in the electric conductivity of the nylon 6/silver blend solutions according to the amount of silver nanoparticles added. The conductivities of the nylon 6/silver blend solutions linearly increased with increasing amount of silver nanoparticles. The addition of silver enhanced the charge density of the nylon 6 solutions, and thus, stronger elongation forces were imposed on the ejected jets under the electrical field, which resulted in substantially straighter and finer nylon 6 fibers.[18,19]

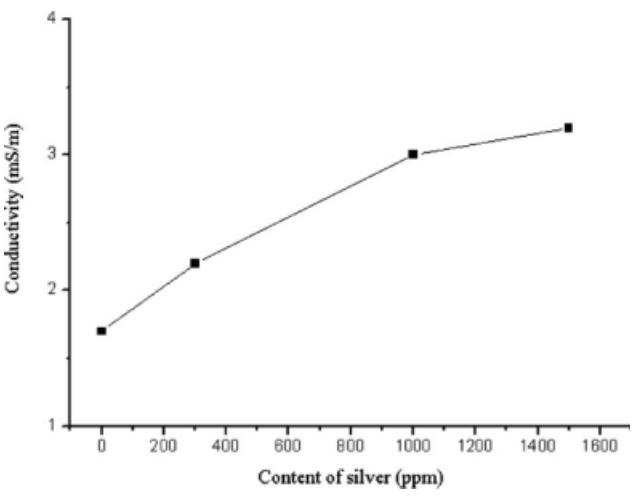

Figure 4 Conductivity of nylon 6 solutions as a function of the silver content.

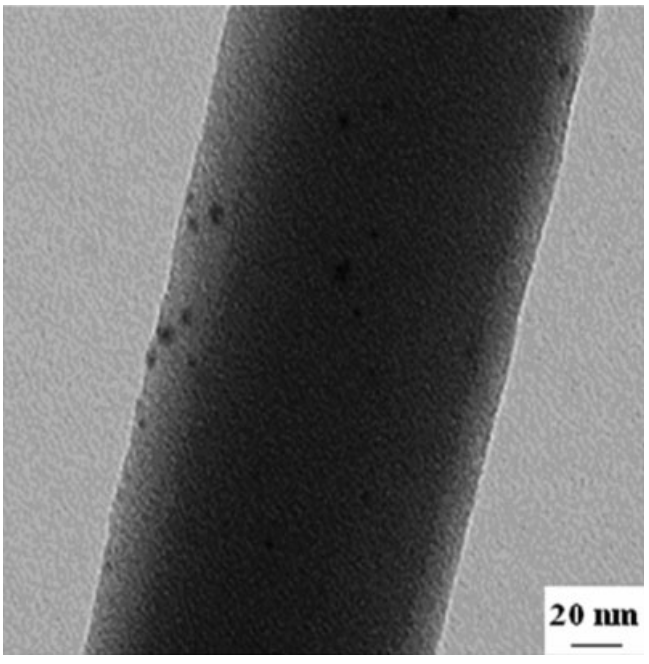

Figure 5 TEM image of a nylon 6 nanofiber containing 1000 ppm silver.

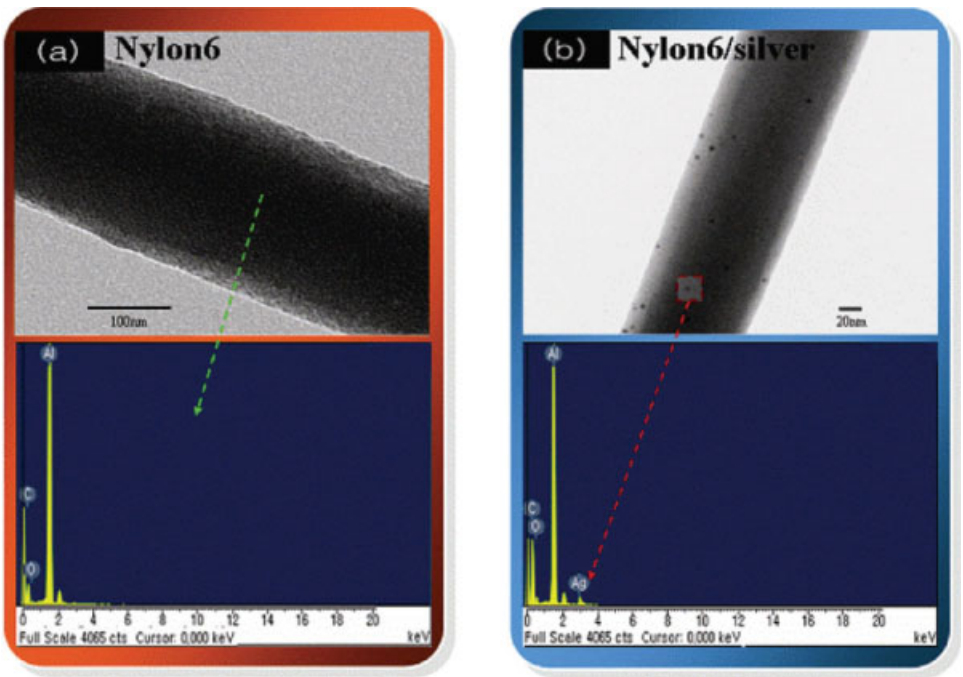

Figure 6 EDS of (a) nylon 6 and (b) nylon 6/silver nanofibers. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 5 shows the TEM image of the nylon 6 nanofibers containing 1000 ppm silver. As shown, the silver nanoparticles were distributed in the nylon 6/silver fibers, and only a few particles seemed to aggregate to some extent. The distributed silver particles were spherical, and the average size of a single particle was about 5–10 nm. The silver nanoparticles embedded in the nylon 6 fibers were also confirmed by EDS [Fig. 6(b)].

The XRD patterns of the nylon 6/silver nanofibers are shown in Figure 7. Four crystalline peaks were observed around 2θ values of 37.7, 44.0, 64.5, and 77.2°. Their intensities were markedly enhanced with increasing silver content in the spinning solution. They were attributed to silver crystals because they corresponded to the diffraction planes of (111), (200), (220), and (311) of silver, respectively.[20]

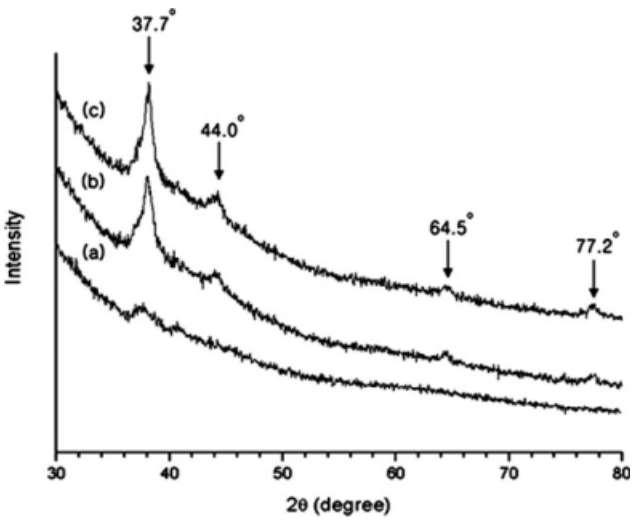

Figure 7 XRD patterns of nylon 6/silver nanofibers: (a) nylon 6, (b) nylon 6/silver (1000 ppm), and (c) nylon 6/silver (2000 ppm).

Antibacterial activity

Growth inhibition rate test

The antibacterial capacities of the nylon 6/silver composite fibers against Gram-positive *S. aureus* and Gram-negative *K. pneumoniae* were explored by a

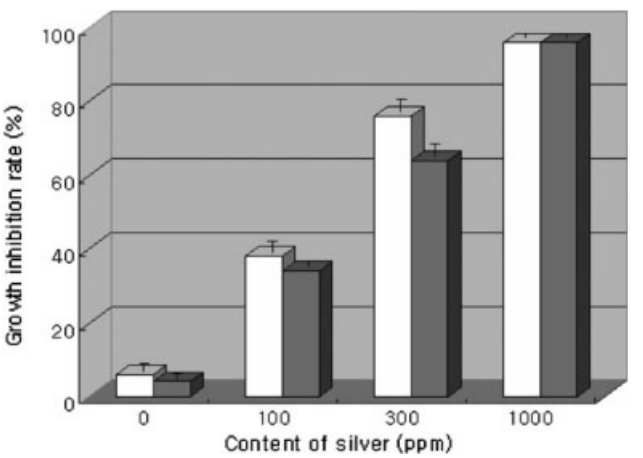

Figure 8 Growth inhibition rates of (□) *S. aureus* and (■) *K. pneumoniae* on silver-containing nylon 6 nanofiber mats.

viable cell counting method. Figure 8 shows the results of the antibacterial tests for the nylon 6 nanofiber mat containing 0–1000 ppm silver nanoparticles against *S. aureus* and *K. pneumoniae*, respectively. The results demonstrated that the growth inhibition rate of the bacteria gradually increased with increasing silver content, and it reached a maximum value of 99.9% for both types of bacteria when the silver content was 1000 ppm. These results indicate that the silver nanoparticles were responsible for the antibacterial activity of the composite fibers and that this activity was quite strong. In the literature,[21] only ionic silver has shown antibacterial activity; thus, silver-loading nylon 6 fibers was of the elution type. The antibacterial activity of this fiber was attributed to the trace amounts of silver ion released from the fiber, which attached to the negatively charged bacteria and prohibited the growth of bacteria.

Inhibition zone test

Mats of nylon 6 fiber containing 2000 ppm silver nanoparticles and pure electrospun nylon 6 fibers as a control were placed on a lawn of organisms in an agar plate and incubated for 24 h at 37°C. The inoculants used were both Gram positive (*S. aureus*) and Gram negative (*K. pneumoniae*). After 24 h of incubation, antibacterial responses were observed in both test cases. The bactericidal activity displayed a fairly good zone of inhibition, or the region in which the bacterial species refused to propagate around the nylon 6/silver fiber mat, but there was no such growth inhibition zone around the nylon 6 fibers, as shown in Figure 9. Interestingly, the gram positive bacteria (*S. aureus*) were the most affected. Because all of the incubations were performed on the same media (blood agar), this difference may have been due to dissimilarities in the cell wall materials of the two types of bacteria. Gram-positive cell walls are principally composed (90%) of peptidoglycan, a macromolecule composed of amino acids and sugar, which has the ability to form more than 20 layers in the cell wall. In addition, bacillus species have the ability to form endospores, which are highly resistant to a wide range of biocidal agents. Gram-negative bacteria, however, are low in peptidoglycan content but high in lipid content.[22]

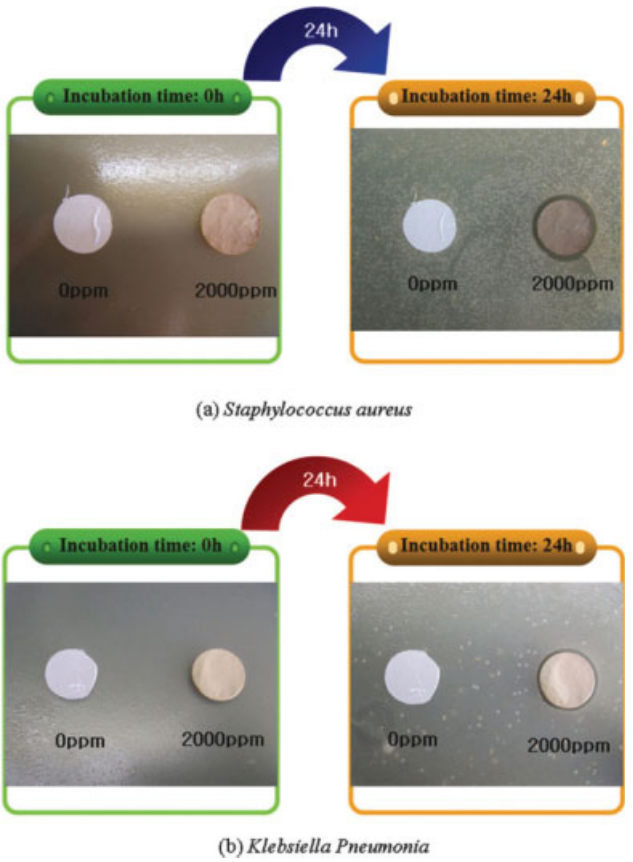

Figure 9 Effect of silver nanoparticles on the formation of zone inhibition: (a) *S. aureus* and (b) *K. pneumoniae*. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

CONCLUSIONS

Nylon 6 nanofibers with a diameter in the range 150–250 nm containing silver nanoparticles were successfully prepared via an electrospinning technique and were characterized by SEM, TEM, XRD, and EDS. The results demonstrated that the fibers electrospun at maximum conditions were straight and that silver nanoparticles were distributed throughout the fibers. Finally, the results of antibacterial tests showed that the nanofibers containing silver had strong antibacterial activities against *S. aureus* (Gram-positive) and *K. pneumoniae* (Gram-negative) bacteria. The ABE in 24 h was as high as 99% for both tests when the fibers contained 1000 ppm silver and created a clear inhibition zone around the fibers when the fibers contained 2000 ppm silver. On the contrary, nylon 6 nanofibers without silver nanoparticles did not show any antibacterial activity. Therefore, the obtained nylon 6/silver nanofiber mats may find practical applications in areas such as water filters, wound dressings, and antiadhesion membranes.

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