

Research Article

Synthesis and Antibacterial Testing of Silver/Poly (Ether Amide) Composite Nanofibers with Ultralow Silver Content

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Received 1 April 2014; Revised 17 July 2014; Accepted 17 July 2014; Published 11 August 2014

Academic Editor: Tianxi Liu

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Antimicrobial materials have attracted much attention all over the world. Herein, a new kind of antimicrobial material, poly (ether amide) (Pebax[®]) nanofibers containing Ag nanoparticles, was prepared by electrospinning method. The Ag/Pebax[®] composites were characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), differential scanning calorimetry (DSC), and thermogravimetric analyzer (TGA) measurements. The antimicrobial properties of Ag/Pebax[®] composites against *Escherichia coli* (*E. coli*; ATCC25922 and avirulent) and *Staphylococcus aureus* (*S. aureus*; ATCC6538 and avirulent) were evaluated by membrane adhering method. It was found that the Ag content played an important part in the antimicrobial ability of Ag/Pebax[®] composites. When the mass ratio of AgNO₃ to Pebax[®] in the precursor was 0.15%, the inhibition rate can reach >99.9% and antimicrobial activity against *E. coli* and *S. aureus* was 5.8 and 5.6, respectively, exceeding the antimicrobial testing standards JIS Z 2801. The above results indicated that the Ag/Pebax[®] composite was a promising antimicrobial material that can be used in many applications.

1. Introduction

Nowadays, human beings are facing a variety of infectious diseases, not only conventional sicknesses (e.g., *malaria*) but also emerging viral influenza (such as SARS and *avian flu*), all of which were spread by microorganism. Therefore, in the past few years, antimicrobial agents have been paid more and more attention, in which silver and its compounds were proved to possess potent antimicrobial efficiency against microorganism [1, 2]. It is generally believed that the bacterial cell will be killed upon the contact with silver, because the positively charged Ag cations strongly bind to electron donor groups containing sulphur, oxygen, or nitrogen in the cell membrane and enzymes, resulting in structural and functional changes in the cell and finally death of the microorganism [3–7]. Moreover, multiplication of bacteria may be prevented owing to the interaction between the Ag⁺

and DNA structure [4, 5]. In addition, because of immediate death of microorganisms upon contact with Ag⁺, there are minimal chances for development of bacterial resistance [8]. In comparison with Ag ions, Ag nanoparticles are superior in heat resistance, chemical stability, release rate of Ag⁺, and service time, bringing about more opportunities for antimicrobial applications [9].

On the basis of the aforementioned advantages, combining Ag nanoparticles with polymers to form Ag/polymer composites has attracted much interest of many researchers, because the Ag/polymer composite can be applied in diverse fields, such as medical use, daily necessities, and clothing. Recently, Ag/polymers composites with different polymer matrixes, for example, cellulose acetate [10], polyurethane [8], poly (ethylene oxide) [11], poly (vinyl alcohol) [1], and poly (lactic-co-glycolic acid) [12], have been fabricated by electrospinning. The electrospinning is a popular technique

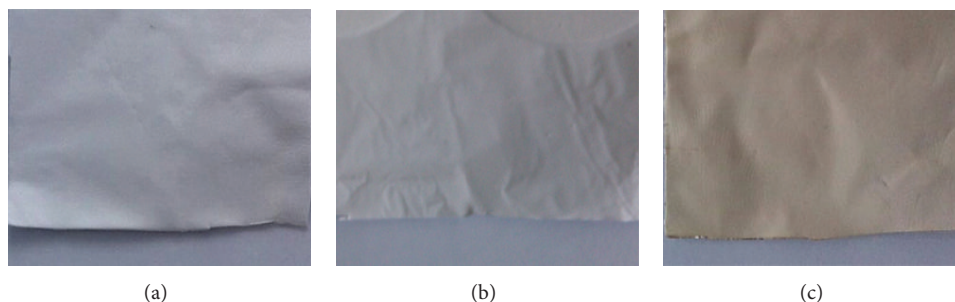


FIGURE 1: Optical photographs of an electrospun nanofiber web: (a) Pebax, (b) AgNO₃/Pebax-3, and (c) Ag/Pebax-3.

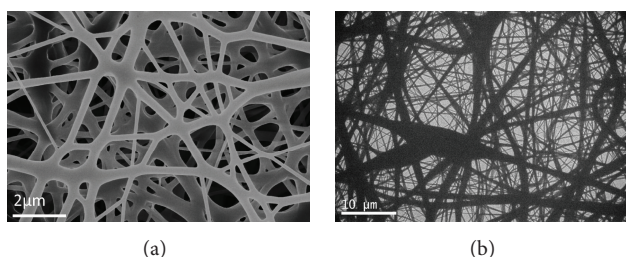


FIGURE 2: (a) SEM and (b) TEM images of Pebax nanofibers.

to prepare high-performance polymer products due to its cost effectiveness, versatility, and simplicity. Briefly, electrospinning is a process by which a polymer solution is ejected under high electric fields to produce polymeric fibers [13]. The fibers are collected as a nonwoven and porous web with a high surface to volume ratio, small pore sizes, and high porosity [1, 8, 14], which is suitable for biomedical use, disinfection, water purifying systems, and so on.

Pebax is a kind of copolymer, and its structure unit consists of dodecyl amide and ether components, which offers a perfect combination of mechanical strength, breathability, water barrier property, gas barrier property, flexibility, chemical resistance, no skin allergy, and ease of processing. So it can be used to make urinary catheter, arterial catheter, PTCA (percutaneous transluminal coronary angioplasty) catheter device, infusion bags, and so forth [15–18]. Furthermore, Pebax is a high-performance thermoplastic polyamide elastomer with excellent durability and workability, which can be widely used as high-performance sports materials [19–21]. If the antibacterial ability can be rendered to the Pebax by embedding Ag nanoparticles, the performance of Pebax will be dramatically improved with certainty. However, to the best of our knowledge, the preparation of Ag/Pebax composite has not been reported so far.

Herein, we employed electrospinning technique and ultraviolet treatment to prepare Ag/Pebax composites without any other reducing agent. The size-uniform Ag nanoparticles were well dispersed in the Pebax matrix. The antimicrobial properties of this Ag/Pebax composite against *Escherichia coli* and *Staphylococcus aureus* were studied via membrane adhering method. Study results showed that Ag/Pebax

presented excellent antimicrobial properties at an ultralow AgNO₃ content in the precursor (0.15‰), which was much lower than that of conventional antimicrobial Ag/polymer composites reported in the literature [1, 8–10, 12], making Ag/Pebax composite a promising antimicrobial material with low-cost and excellent antimicrobial properties, which could be widely used in many fields in the near future.

2. Experimental Section

2.1. Preparation of Ag/Pebax Composites. First, Pebax 2533 (ARKEMA Firm) was added into isopropanol (Shanghai Lingfeng, >99%) and the mixture was magnetically stirred for 5 h at 60°C to obtain Pebax solution with a concentration of 66.7 mg mL⁻¹. Meanwhile, 6.7 mg mL⁻¹ AgNO₃ (Shanghai Lingfeng, 99.99%) in isopropanol solution was prepared in dark. Then a given amount of AgNO₃ solution was added into the Pebax solution and the mass ratio of AgNO₃ to Pebax was controlled from 0.05‰ to 0.25‰. After sonication, the homogeneous solution was fed into a positively charged spinneret with a diameter of 0.6 mm attached to an electrospinning apparatus [22]. The voltage used for electrospinning was 13 kV and the collection distance was 10 cm. The solution was spun under its own gravity without flow control equipment and the product obtained (AgNO₃/Pebax) was dried freely in air. Finally, the as-prepared samples were treated under UV light (254 nm, 14 W) for 6 h to obtain Ag/Pebax composites, which was denoted as Ag/Pebax-1, Ag/Pebax-2, Ag/Pebax-3, Ag/Pebax-4, and Ag/Pebax-5 for the sample with the mass ratio of AgNO₃ to Pebax at 0.05‰, 0.10‰, 0.15‰, 0.20‰, and 0.25‰, respectively.

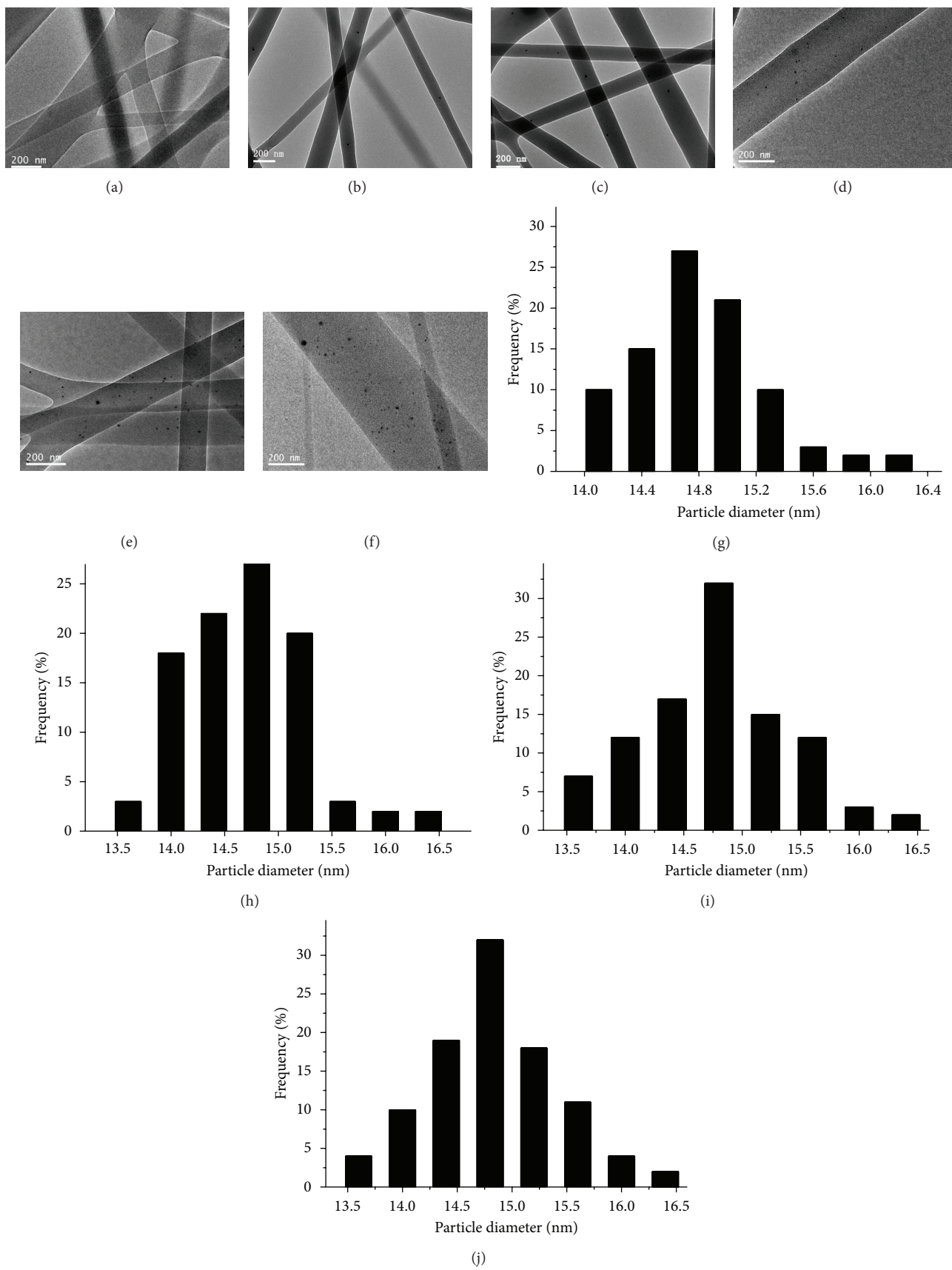


FIGURE 3: Continued.

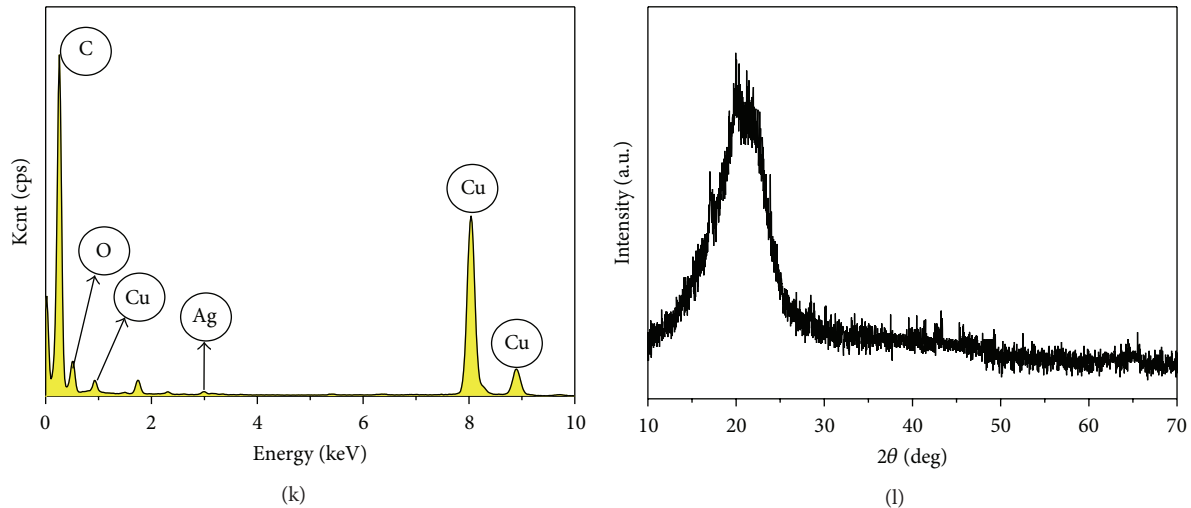


FIGURE 3: ((a)–(f)) TEM images of (a) Pebax, (b) Ag/Pebax-1, (c) Ag/Pebax-2, (d) Ag/Pebax-3, (e) Ag/Pebax-4, and (f) Ag/Pebax-5. ((g)–(j)) The particle size distribution of Ag nanoparticles of (g) Ag/Pebax-2, (h) Ag/Pebax-3, (i) Ag/Pebax-4, and (j) Ag/Pebax-5. (k) The EDS spectrum of Ag/Pebax-5. (l) The XRD patterns of Ag/Pebax-5.

2.2. Characterizations. The morphology of the resulting electrospun nonwoven fiber mat was evaluated using scanning electron microscopy (SEM, Zeiss, SUPRA 55, Germany). The transmission electron microscopy (TEM) image was obtained on a JEOL JEM-2100 microscope operating at 200 kV equipped with energy dispersive spectrometer (EDS). The crystalline structure of the sample was identified by X-ray diffraction (XRD, Rigaku D/max 2500PC) using Cu $K\alpha$ radiation. A simultaneous differential scanning calorimeter (PerkinElmer, DSC 8500, USA) was operated in the temperature range from -40°C to 80°C with a heating rate of $10^{\circ}\text{C min}^{-1}$ under a nitrogen atmosphere. The thermal degradation behaviors of Ag/Pebax nanofibers were studied on a thermogravimetric analyzer (PerkinElmer, Pyris 1 TGA, USA) from 30°C to 800°C at a heating rate of $10^{\circ}\text{C min}^{-1}$ under nitrogen at a flow rate of 20 mL min^{-1} . The chemical state of the sample was examined by X-ray photoelectron spectroscopy (XPS) on a VG Scientific ESCALABMKLL spectrometer using Al $K\alpha$ X-ray source (10 mA, 15 kV).

2.3. Bacterial Culture and Test for Antibacterial Activity. The bacteria used for the test were *Escherichia coli* (*E. coli*: ATCC25922) and *Staphylococcus aureus* (*S. aureus*: ATCC6538). Any water used was distilled and all reagents were of a grade appropriate for microbiological purposes. The stock culture was stored at 5°C to 10°C on an appropriate medium and the bacteria were transferred from the stock culture to a slant culture medium and incubated at $(35 \pm 1)^{\circ}\text{C}$ for 24 h. Then bacteria were transferred onto another slant culture medium and incubated for another 20 h. After that, the bacteria were transferred into 1/500 NB (nutrient broth) prepared in advance, obtaining a bacterial concentration between 2.5×10^5 and 10×10^5 cells mL^{-1} , which was used as the test inoculum. Then 0.4 mL of the test inoculum was pipetted onto the test surface and covered by a piece of square Ag/Pebax film with side length of 40 mm and a thickness of

$\sim 1\text{ mm}$. The test specimen was incubated at $(35 \pm 1)^{\circ}\text{C}$ with a relative humidity of 90% for 24 h. After incubation, 20 mL of neutralizer was added to wash the specimen and then the viable bacteria count was determined by pour-plate culture method. For the testing, all petri dishes should be inverted and incubated at $(35 \pm 1)^{\circ}\text{C}$ for 40 h, and then the number of colonies in petri dishes was counted [22].

The number of viable bacteria was calculated by

$$N = C \times D \times V, \quad (1)$$

where N is the number of viable bacteria recovered per test specimen; C is the average plate count for the duplicate plates; D is the dilution factor for the plate counted; and V is the volume in mL of neutralizer added to the specimen.

The antibacterial activity was calculated by

$$R = U_t - A_t, \quad (2)$$

where R is the antibacterial activity; U_t is the average of the common logarithm of the number of viable bacteria recovered from the untreated test specimens after 24 h; and A_t is the average of the common logarithm of the number of viable bacteria recovered from the treated specimens after 24 h [23, 24].

3. Results and Discussion

The electrospun Pebax was tough and flexible, and it is white in color (Figure 1(a)). The SEM and TEM images (Figure 2) indicated that the electrospun Pebax was mesh on a microlevel formed by the connection between Pebax nanofibers, proving that the electrospinning technique can be applied successfully on Pebax.

In order to confer antibacterial ability to Pebax, AgNO_3 was electrospun with Pebax simultaneously and the as-prepared samples were treated with UV light to decompose AgNO_3 to metallic Ag. As shown in Figure 1(c),

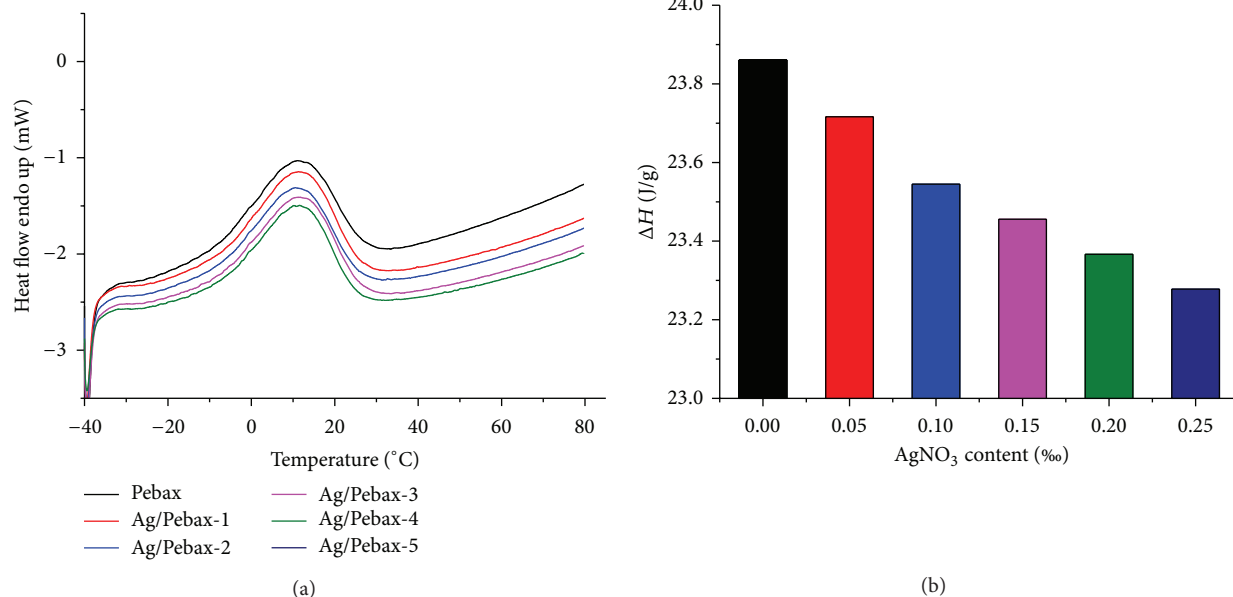


FIGURE 4: (a) DSC diagrams and (b) the heat of fusion for pure Pebax nanofibers and Ag/Pebax composites with different AgNO₃ contents.

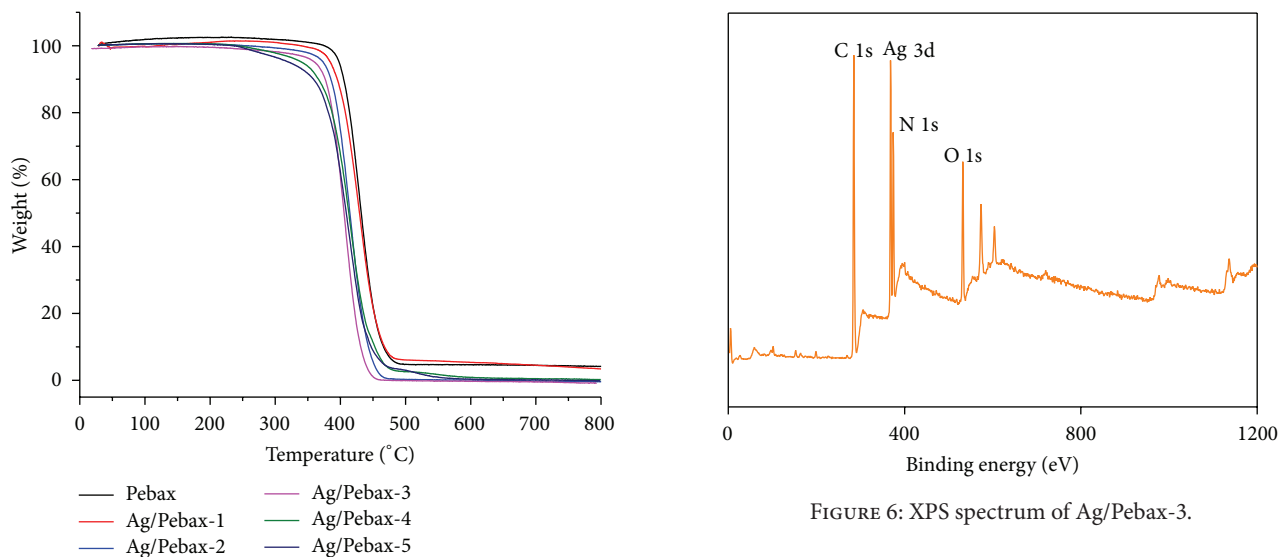


FIGURE 6: XPS spectrum of Ag/Pebax-3.

FIGURE 5: TGA data of pure Pebax nanofibers and Ag/Pebax composite with different AgNO₃ contents.

the Ag/Pebax composite was pale yellow in color which was obviously different from that of pure Pebax (Figure 1(a)) and AgNO₃/Pebax composite (Figure 1(b)). The color change should come from the presence of metallic Ag in Ag/Pebax composite, so we further studied the composite by TEM. As shown in Figures 3(b)–3(f), the metallic Ag was in near spherical morphology and Ag nanoparticles were dispersed homogeneously within the fiber matrix. Although the number density of Ag nanoparticles in the Pebax matrix was increased with the AgNO₃ content, no aggregation was observed. In addition, the Ag particle diameter distribution

of Ag/Pebax-2 to Ag/Pebax-5 is shown in Figures 3(g) to 3(j), respectively, which was obtained by measuring about 200 Ag nanoparticles for each sample. Both of the particle diameters of the two samples ranged between 13.5 nm and 16.5 nm, with average particle size at 14.8, 14.9, 14.7, and 14.8 nm for Ag/Pebax-2 to Ag/Pebax-5, respectively, on the assumption of Gaussian distribution. Since the density of Ag particle in Ag/Pebax-1 was very low, its average particle size was calculated to be 14.8 nm by using the arithmetic mean value of all Ag particles. The above results indicated that the mean particle size of Ag nanoparticles nearly did not change with the increase of Ag content, which may be due to the fact that the Ag content was very low and AgNO₃ was completely dissolved in the solution; thus the AgNO₃ crystals after electrospinning were distributed

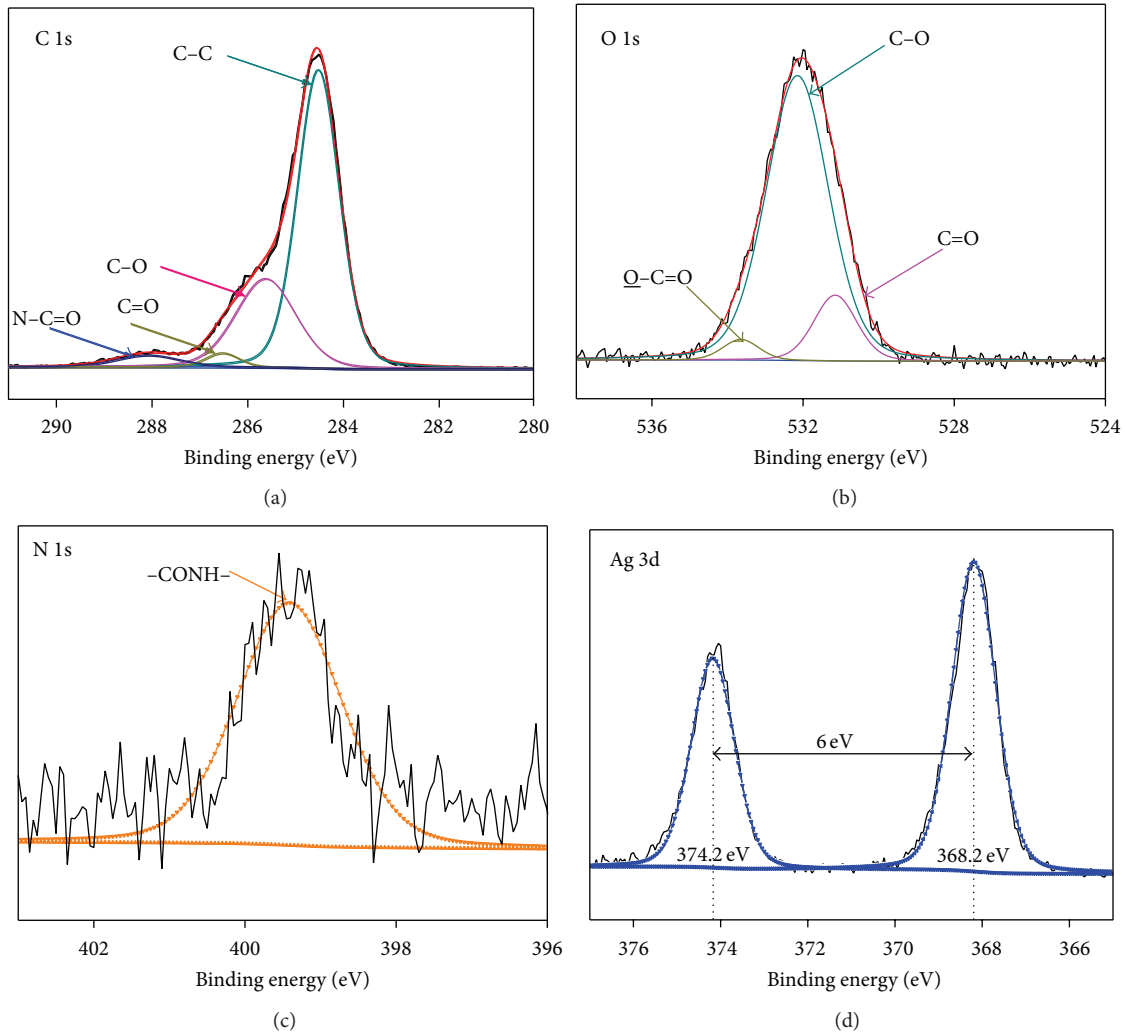


FIGURE 7: XPS deconvolution spectrum of (a) C 1s, (b) O 1s, (c) N 1s, and (d) Ag 3d core levels of Ag/Pebax-3.

uniformly in the Pebax matrix, resulting in the fact that the Ag nanoparticles reduced were also monodisperse and the mean particle size only fluctuated slightly at different Ag loadings. Additionally, the EDS spectrum of Ag/Pebax-5 in Figure 3(k) confirmed the ultralow content of Ag content in the composite. The crystalline information of Ag/Pebax-5 was analyzed by XRD. As shown in Figure 3(l), the diffraction peak at 20° was ascribed to pure Pebax 2533 [25]. According to JCPDS 04-0783 (Ag), the crystalline Ag should be revealed by diffraction peaks at 2θ values of 38.12° , 44.28° , and 64.43° . However, no distinctive diffraction of silver was observed in the Ag/Pebax composite even for Ag/Pebax-5 with the highest concentration of AgNO_3 (0.25%) (Figure 3(l)). It was probably because of the fact that the concentration of Ag included in the composite was below the detection limit of the XRD method [26].

To investigate the effect of silver on the crystallinity of Pebax, we performed DSC measurements in a N_2 atmosphere. Formula for calculating the degree of crystallization (X_c) was $X_c = \Delta H / \Delta H_0$, where ΔH is the heat of fusion of the sample in this experiment and ΔH_0 is the heat of fusion

of pure Pebax with 100% crystallinity which is a constant here. Figure 4(a) shows the DSC thermograms for Pebax and the Ag/Pebax composite between -40°C and 80°C . It was found that the ΔH of Ag/Pebax composites was lower than that of pure Pebax, indicating that the X_c of Pebax polymer was reduced in the presence of Ag nanoparticles. Furthermore, the reduction in crystallinity was enhanced with the increase of the AgNO_3 content (Figure 4(b)). In addition, the glass transition temperature (T_g) for Pebax, Ag/Pebax-1, Ag/Pebax-2, Ag/Pebax-3, Ag/Pebax-4, Ag/Pebax-5, and Ag/Pebax-6 is -6.80°C , -7.34°C , -7.52°C , -7.95°C , -8.13°C , -8.25°C , and -8.36°C , respectively, indicating that the T_g of Pebax polymer is gradually reduced with the increase of the AgNO_3 content. Generally, the regular structure of ether segments is in favor of the crystallization [27]. In this work, with the increase of Ag content, the regularity of the internal structure of Pebax was reduced, whereas the flexibility of the polymer chain was improved [28], which may result in the decrease of the X_c and T_g .

The thermal degradation behaviors of Ag/Pebax nanofibers were studied with a TGA under nitrogen atmosphere.

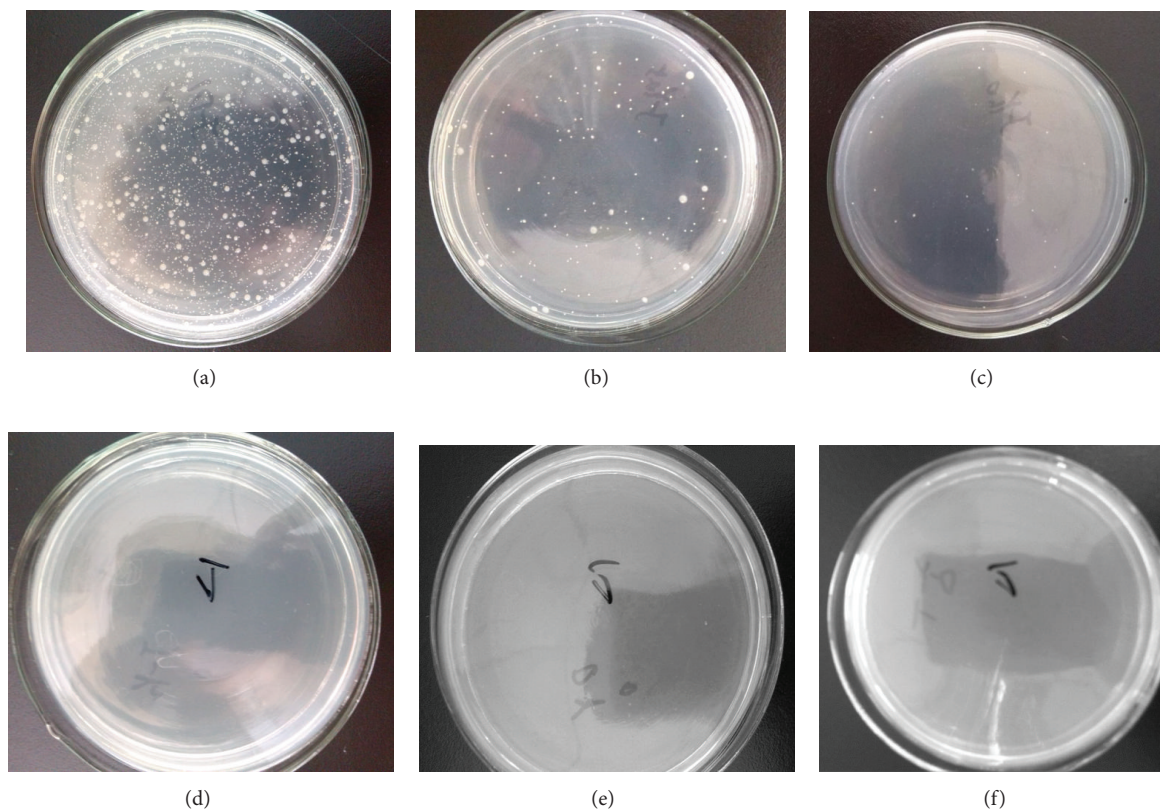


FIGURE 8: Comparison of the antibacterial ability of (a) Pebax, (b) Ag/Pebax-1, (c) Ag/Pebax-2, (d) Ag/Pebax-3, (e) Ag/Pebax-4, and (f) Ag/Pebax-5 against *E. coli*.

With the increase of Ag content, the onset decomposition temperature of the Ag/Pebax composite gradually decreased. As shown in Figure 5, the onset decomposition temperature of Pebax, Ag/Pebax-1, Ag/Pebax-2, Ag/Pebax-3, Ag/Pebax-4, and Ag/Pebax-5 is 350.14°C, 330.62°C, 310.77°C, 291.30°C, 273.10°C, and 250.21°C, respectively. Because the Ag nanoparticles dispersed uniformly in the nanofibers and their particle size nearly did not change at different Ag contents, the increase of Ag content in nanofibers will increase the number of Ag nanoparticles, which can weaken the tightness of the Pebax molecule arrangement and expand the volume of noncrystalline region in the nanofiber, resulting in the decrease of thermal stability of the whole composite, that is, a drop of the onset decomposing temperature. Additionally, Ag nanoparticles may also play a catalytic role in the decomposition during DSC measurement.

The chemical state and near surface composition of the Ag/Pebax composite were studied by XPS. With a survey range of 0–1200 eV, four elements can be detected: carbon, silver, nitrogen, and oxygen (Figure 6). Figure 7 shows the high-resolution XPS spectra of Ag/Pebax-3. From the analysis of C 1s spectrum (Figure 7(a)), we can see carbon atoms in the C–C bond, C–O bond, C=O bond, and N–C=O at 284.5 eV, 285.6 eV, 286.5 eV, and 288.1 eV, respectively. The peak at 532.1 eV in the O 1s spectrum (Figure 7(b)) is attributed to the oxygen in the C–O bond and the peaks at

531.1 eV and 533.7 eV are assigned to the oxygen of C=O and O–C=O bonds, respectively [29]. The N 1s spectrum presents a peak at 399.4 eV which comes from the nitrogen in the –CONH– group. In the XPS spectrum of Ag 3d (Figure 7(d)), two peaks were observed at 368.2 and 374.2 eV, which can be ascribed to Ag (3d_{5/2}) and Ag (3d_{3/2}) peaks, respectively. Moreover, the splitting of the 3d doublet of Ag was 6.0 eV, which indicates that these Ag/Pebax composites only contain metallic Ag (Ag⁰) [30–32], indicating the complete reduction of AgNO₃ by UV.

The potential use of Ag/Pebax composite as antibacterial material was assessed by observing their antibacterial activity against two kinds of common bacteria, *E. coli* and *S. aureus*. The electrospun Pebax without Ag was evaluated in a control experiment. The bacteria were incubated in a growth medium containing various nanofibers and the antibacterial properties of samples were tested by membrane adhering method, following the antibacterial testing standard (JISZ 2801:2010). The changes in the content of Ag had a significant effect on the antibacterial properties of Ag/Pebax composite. The number density of bacterial colony for pure Pebax was high (Figure 8(a)), and both of the antibacterial rate (1) and antimicrobial activity (2) were nearly zero (Figure 9); that is, there was no antibacterial activity observed for Ag-free pure Pebax. In contrast, the samples added with small amount of AgNO₃ in the Pebax precursor solution showed

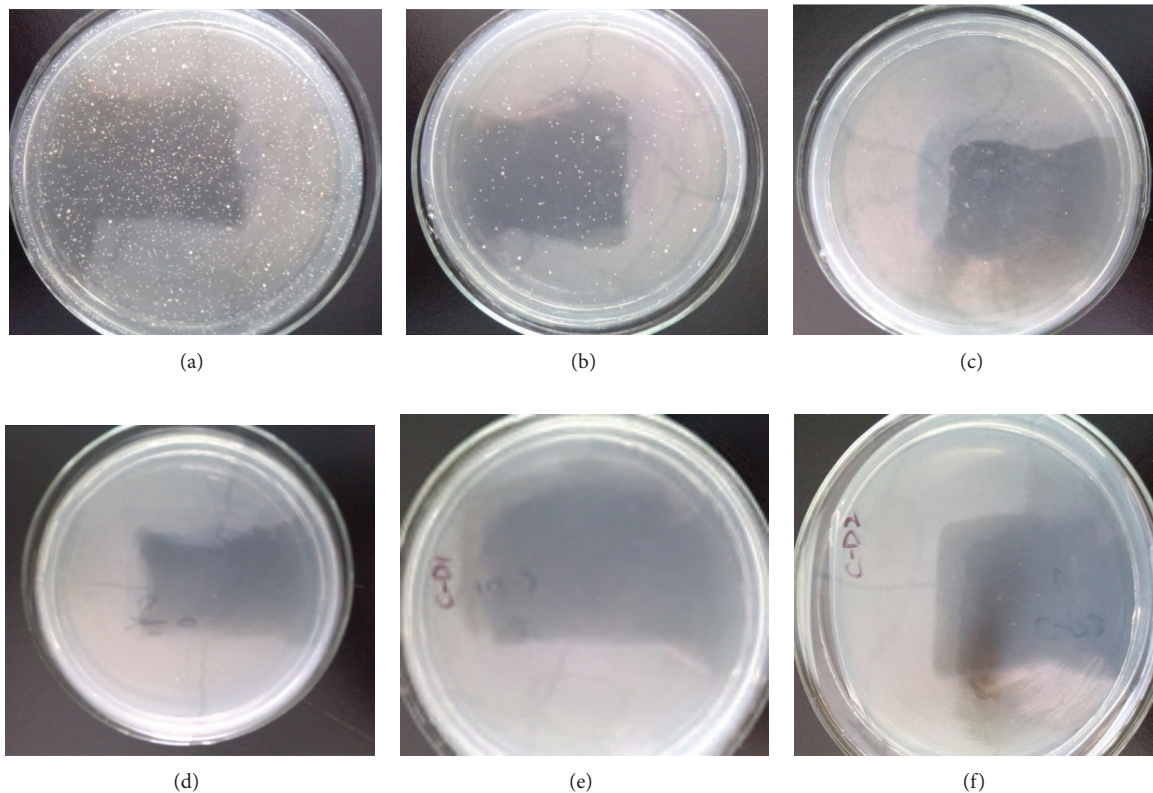


FIGURE 9: Comparison of the antibacterial ability of (a) Pebax, (b) Ag/Pebax-1, (c) Ag/Pebax-2, (d) Ag/Pebax-3, (e) Ag/Pebax-5, and (f) Ag/Pebax-6 against *S. aureus*.

an inhibitory effect on *E. coli* and *S. aureus*. Although the content of AgNO_3 was only 0.05‰ (Figures 8(b) and 9(b)), the antibacterial rate against *E. coli* and *S. aureus* can reach 88.2% and 76.1%, respectively. Furthermore, the antibacterial properties of Ag/Pebax composite were enhanced gradually with the increase of AgNO_3 concentration (Figures 8(b)–8(d), 9(b)–9(d) and 10). When the AgNO_3 content reached 0.15‰, no bacterial colony can be observed (Figures 8(d) and 9(d)) and the inhibition rates all larger than 99.9%, and the value of antimicrobial activity against *E. coli* and *S. aureus* was 5.8 and 5.6, respectively (Figure 10). Further increasing the content of Ag failed to enhance the antibacterial properties of Ag/Pebax any more (Figures 8(e)–8(f), 9(e)–9(f) and 10). Because the Pebax did not show antibacterial ability, Ag nanoparticles were responsible for the antibacterial ability of Ag/Pebax composites. It was reported that the antimicrobial property of material containing silver is mainly determined by the total release rate of silver ions [32]. In this work, owing to the stable particle size, the increase of Ag content will lead to the enlargement of the number and the total surface area of Ag nanoparticles; thus the release rate of silver ions was accelerated, giving rise to the improvement of antibacterial ability of Ag/Pebax composites (Figure 10). According to the antimicrobial testing standard JISZ 2801:2010, the sample whose antimicrobial activity is greater than 2 ($R > 2$) passes the antibacterial test of the strain ($R > 2$ means the antibacterial efficiency can reach 99% or more). According

to the experimental results, it can be concluded that the Ag/Pebax composite with AgNO_3 content larger than 0.15‰ presented excellent antibacterial properties, and this Ag content was much lower than that of Ag/polymer composites reported in the literature (usually larger than 1 wt.%) [1, 7, 8, 11, 33–35], making Ag/Pebax a promising antibacterial material with potential application in a number of fields, such as biomedical materials, sports apparatus, and laminating films.

4. Conclusions

This study has described a simple route to prepare well-dispersed and size-uniform Ag nanoparticles in Pebax nanofibers via an electrospinning process. Ag nanoparticles with an average diameter of ca. 15 nm were dispersed homogeneously in Pebax nanofibers. The antimicrobial activities of Ag/Pebax composite against *E. coli* and *S. aureus* were evaluated by membrane adhering method. Study results indicated that the Ag/Pebax composites presented outstanding antimicrobial properties at ultralow Ag content; for example, the inhibition rate can reach >99.9% and antimicrobial activity against *E. coli* and *S. aureus* was 5.8 and 5.6 at the critical value of AgNO_3 content (0.15‰), respectively, which suggested that the Ag/Pebax composite can be used as a promising antimicrobial material.

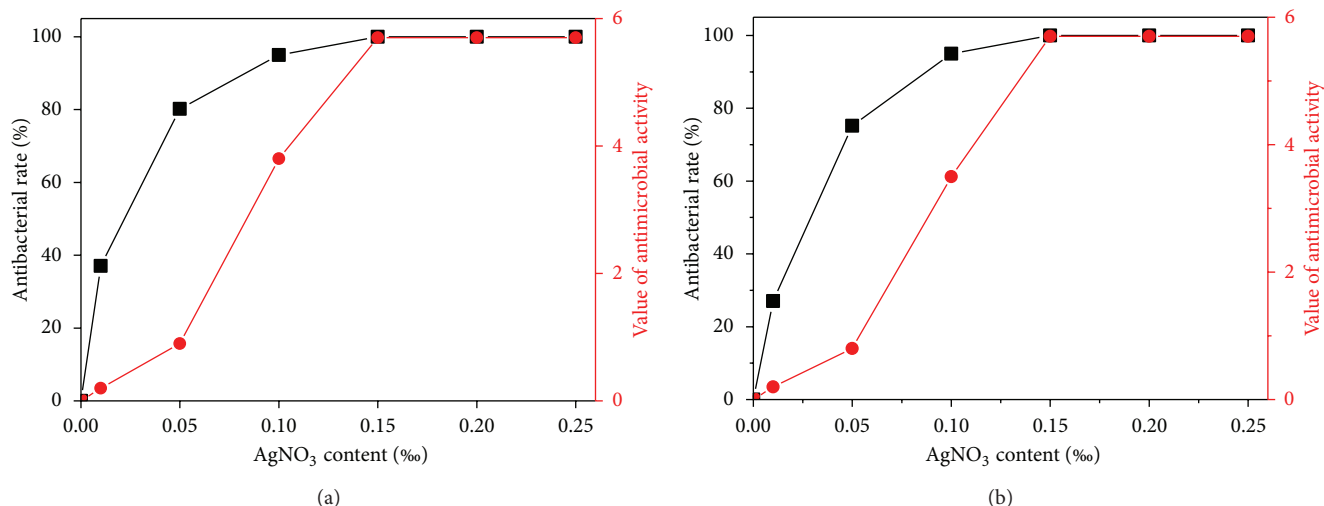


FIGURE 10: The antibacterial rate and antimicrobial activity of Ag/Pebax against *E. coli* and *S. aureus* as a function of AgNO₃ content in the precursor.

Conflict of Interests

The authors declare that they have no financial and personal relationships with other people or organizations that can inappropriately influence their work; there is no professional or other personal interest of any nature or kind in any product, service, and/or company that could be construed as influencing the position presented in, or the review of, the paper entitled.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China under Grant (no. 21373034); the Specially Hired Professorship-Funding of Jiangsu province under Grant (no. SCZ1211400001); the start-up funds from Changzhou University Jiangsu province; Jiangsu Key Laboratory of Advanced Catalytic Material and Technology; and Key Laboratory of Fine Petrochemical Engineering and PAPD of Jiangsu Higher Education Institutions. This trademark belongs to Arkema France and is related to polyether block amides.

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