

Antibacterial Thermoplastic Polyurethane Electrospun Fiber Mats Prepared by 3-Aminopropyltriethoxysilane-assisted Adsorption of Ag Nanoparticles*

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Abstract Antibacterial thermoplastic polyurethane (TPU) electrospun fiber mats were prepared by adsorption of Ag nanoparticles (Ag NPs) onto TPU/3-aminopropyltriethoxysilane (APS) co-electrospun fiber mats from silver sol. The use of APS can functionalize TPU fibers with amino groups, facilitating the adsorption of Ag NPs. The effects of pH of silver sol and APS content on Ag NP adsorption and antibacterial activity were investigated. Ag NP adsorption was evidenced by TEM, XPS and TGA. Significant Ag NP adsorption occurred at pH = 3–5. The main driving force for Ag NP adsorption is electrostatic interaction between —NH_3^+ of the fibers and —COO^- derived from the —COOH group capped on the surfaces of Ag NPs. The antibacterial activity of the Ag NP-decorated TPU/APS fiber mats was investigated using both gram-negative *Escherichia coli* and gram-positive *Bacillus subtilis*. The antibacterial rate increases with increasing APS content up to 5% where the antibacterial rates against both types of bacteria are over 99.9%.

Keywords PU; Electrospinning; Coupling agent; Immobilization; Bacteria

INTRODUCTION

Electrospinning is a simple and efficient method for producing ultrafine fibers. The electrospun fiber mats have shown many promising applications in the fields of filtration, catalysis, tissue engineering, wound dressings, sensors, *etc*^[1–11]. Thermoplastic polyurethanes (TPUs) are a class of widely used elastomers^[12, 13]. The electrospun TPU fiber mats are suitable for practical applications owing to their good mechanical properties and biocompatibility^[14–16]. In some of the applications such as antibacterial filtration and wound dressing, antibacterial activity is necessary. However, neat TPU electrospun mats do not have intrinsic antibacterial properties^[17] and hence, antibacterial function is usually introduced by incorporation of an antibacterial agent such as ionic liquid and AgCl nanoparticles into the electrospinning solution or after electrospinning^[18, 19]. For example, Xing *et al.*^[18] obtained multifunctional TPU electrospun mats by co-electrospinning TPU with a small amount of ionic liquid (IL), and the as-spun TPU/IL fiber mats have excellent antibacterial properties owing to the quaternary ammonium structure of the IL. Kang *et al.*^[19] reported that the TPU/AgCl composite fiber mat obtained by co-electrospinning TPU and AgCl nanoparticles can significantly improve the antibacterial properties of the TPU melt-blown nonwovens.

Silver has long been known as a safe, broad-spectrum and eco-friendly antibacterial agent with strong

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antibacterial activity^[20–22]. In recent years, silver nanoparticles (Ag NPs) have received considerable attention^[5, 6, 23]. They have been used for making polymer/Ag NP composite fiber mats applicable as antibacterial wound dressings, coatings for medical devices, textile fabrics, food-contact materials, sensors, etc^[23–26]. The main advantage of using Ag NPs is more efficient release of Ag⁺ than bulk silver because of their much larger specific surface area^[23, 26]. The incorporation of Ag NPs to polymer electrospun fibers is usually achieved by two strategies classified according to the stage they are incorporated: co-electrospinning and immobilization^[27]. In the former, a polymer and Ag NPs or a precursor such as AgNO₃ are co-electrospun to produce composite fibers; while in the latter, Ag NPs or a precursor are immobilized on the polymer fiber surfaces by adsorption or magnetron sputtering after electrospinning. When a precursor is used, a reduction usually follows to produce polymer/Ag NP composite fibers. Compared to the co-electrospinning method, the main advantage of the immobilization method is high efficiency of using the function of Ag NPs located solely on the fiber surfaces. However, magnetron sputtering needs special equipment, and for successful adsorption the electrospun fibers must have suitable functional groups such as carboxylate that has strong affinity for silver^[28].

It is well-known that amino group has strong affinity for noble metals such as gold and silver^[28–30]. This type of affinity has been used for adsorption of Au NPs or Ag NPs on support materials^[30–33]. For example, Rastogi *et al.*^[30] prepared silica nanoparticle-supported Ag NPs by the reduction of Ag⁺ in the presence of amino-functionalized SiO₂ nanoparticles. Mahanta and Valiyaveetil^[33] reported that both Au NPs and Ag NPs can be adsorbed on amino-functionalized polyvinyl alcohol electrospun fibers obtained by esterification with lysine.

Previously, our group reported a method to prepare amino-functionalized TPU electrospun fibers by co-electrospinning TPU with 3-aminopropyltriethoxysilane (APS), an aminosilane coupling agent known to easily undergo self-condensation upon exposure to air or heating^[31]. The amino-functionalized TPU electrospun fibers can easily adsorb Au NPs. In this work, Ag NPs are immobilized on amino-functionalized TPU electrospun fibers prepared similarly. The antibacterial activity of the Ag NP-decorated TPU/APS fiber mats are investigated using gram-negative *Escherichia coli* (*E. coli*) and gram-positive *Bacillus subtilis* (*B. sub.*). Another merit brought by APS is the improved hydrophilicity of the mat, facilitating the applications of Ag NP-decorated TPU mats in fields dealing with aqueous medium or moisture such as biomedical field and aqueous filtration. Compared with the existing methods for the preparation of antibacterial TPU fiber mats^[18, 19], the application situation of the current mats is different from that of TPU/IL fiber mats which are only effective under alkaline pH conditions because of their cationic surface activity^[34], and the existence of Ag NPs only on the fiber surfaces in the current case can facilitate more efficient use of the antibacterial function of Ag NPs than the homogeneous incorporation of AgCl nanoparticles in the case of TPU/AgCl co-electrospun fibers^[19]. Although the TPU fiber mats prepared by the existing methods have good antibacterial properties^[18, 19], the method reported in this work is complementary to the existing methods for preparing antibacterial TPU fiber mats.

EXPERIMENTAL

Materials

Silver nitrate (AgNO₃, AR), sodium chloride (NaCl, AR) and tetrahydrofuran (THF, AR) were purchased from Beijing Chemical Works. Sodium borohydride (NaBH₄, 98.0%–100%) was obtained from Fuchen Chemical Reagent Works, Tianjin. Trisodium citrate (Na₃C₆H₅O₇·2H₂O, AR) was purchased from Sinopharm Chemical Reagent Co., Ltd. *N,N*-dimethylformamide (DMF, AR) was obtained from Modern Oriental (Beijing) Science and Technology Development Co., Ltd. Thermoplastic polyurethane (TPU, polyester type, T1180PC, *M_w* = 107.8 kg/mol, *M_w*/*M_n* = 1.76) was obtained from Wanhua Chemical Group Co., Ltd., Yantai. 3-Aminopropyltriethoxysilane (APS) was purchased from Aladdin Chemistry Co., Ltd. Hydrochloric acid (HCl, 36%–38%) was obtained from Beijing Modern Oriental Fine Chemicals Co., Ltd. Agar was purchased from Biotopped. Tryptone and yeast extract were obtained from OXOID Ltd. All the aqueous solutions and sols were prepared with deionized water and all the chemicals were used as received.

Electrospinning

TPU/APS was dissolved in DMF/THF mixed solvent (volume ratio 2/3) at room temperature to prepare the electrospinning solutions. The mass ratios of APS to TPU were 0%, 1.5%, 3%, 4%, 5%, 8% and 13%. The total concentration of all the solutions are 23 wt%, and the solution was stirred for 18 h until the solute was dissolved completely. Then the solution was placed into a 10 mL syringe with a 12# blunt end needle. Electrospinning was conducted at ambient conditions, using electrospinning machine (HD-2335) bought from Beijing Ucalery Technology Development Co., Ltd. The applied voltage was 11 kV (+8.0 kV for the positive electrode and −3.0 kV for the negative electrode) with 15 cm receiving distance, and the feeding rate was 0.28 mL/h. The nanofibers were collected on a layer of aluminum foil attached to the negative electrode, and then post-treated at 60 °C for 24 h.

Synthesis and Adsorption of Ag NPs

The aqueous silver sol containing Ag NPs was prepared using the chemical reduction method reported in a previous work^[24]. NaBH₄ was used as the reducing agent, while Na₃C₆H₅O₇·2H₂O was used as the stabilizing agent in the process. The molar ratio of AgNO₃/NaBH₄/Na₃C₆H₅O₇·2H₂O was 1:5:1. The mean diameter of the Ag NPs was 11 nm, and the pH of the sol was 9.7.

The adsorption of Ag NPs onto the TPU/APS electrospun mats was achieved by filtration method^[24, 31]. 10 mL of silver sol adjusted freshly to pH = 3.0 or 4.0 or 5.0 by adding dropwise 1 mol/L HCl was filtered through the mat supported by an ordinary filter paper placed in a filtration funnel with diameter of 3 cm. The mat was then washed 5 times with deionized water and thoroughly dried.

Antibacterial Test

The antibacterial properties of the electrospun fiber mats were evaluated according to a method based on standard AATCC 100. For the evaluation of the antibacterial properties of Ag NP-adsorbed mats, mats with the same components and without Ag NPs were used as the control groups, and plate counting method was used. Bacteria used in this research were gram-negative *E. coli* and gram-positive *B. sub*.

The procedure of an antibacterial test is as follows. First, the mat was sterilized in 75% alcohol for 30 min and dried on the sterile console until the alcohol completely evaporated before being placed on a flat LB plate composed of 1 wt% NaCl, 1 wt% peptone, 0.5 wt% yeast extract, 2 wt% agar and deionized water. Next, 10 μL of pre-cultured bacterial suspension was inoculated on each electrospun fiber mat, and cultured in an incubator at 37 °C for a pre-determined time during which bacteria contacted with the Ag NPs adsorbed on the surface of the fiber mats and was killed. Then, the electrospun mat was immersed and oscillated in 1 mL of deionized water for 5 min in order to make sure all the bacteria on the mat were eluted. The concentration of the bacteria suspension after elution is equivalent to a dilution of 10² times compared with the original bacteria suspension. 0.1 mL of the suspension was taken out and stepwise diluted to dilutions of 10⁴, 10⁵, 10⁶, and 10⁷ times. 0.1 mL of each diluted suspension was placed on a LB plate, and then cultured for 18 h. After culture, we counted the number of colonies on each LB plate and calculated the concentration of the original bacteria suspension on the sample mat, which was denoted as A_1 (cfu/mL). The concentration of the original bacteria suspension on the control group was denoted as A_0 (cfu/mL). Finally, the antibacterial rate (P) was calculated by the following formula:

$$P = \frac{A_0 - A_1}{A_0} \times 100\%$$

Characterization

The morphology of the fiber mats was observed on a JSM-7401F field-emission scanning electron microscope (FESEM) operating at an accelerating voltage of 3 kV. The presence of Ag NPs on fiber surfaces was verified by transmission electron microscopy (TEM, JEM-2010). For TEM sample preparation, a few fibers were directly electrospun onto a Cu grid; a droplet of silver sol was deposited onto the fibers and allowed for 20 min; the fibers were washed thoroughly (10 times) with water and dried. X-ray photoelectron spectroscopy (XPS) data

were obtained from a Thermo Scientific ESCALab 250Xi multifunctional photoelectron spectroscopy analyzer with 200 W Al K α X-ray as the excitation source. The based vacuum was 3×10^{-8} Pa, and the take-off angle of the XPS was 45°. The silver content was quantitatively measured by thermogravimetric analysis (TGA, DTG-60) from room temperature to 550 °C with a heating rate of 20 K/min under nitrogen atmosphere using the principle that inorganic Ag NPs do not decompose at such conditions.

RESULTS AND DISCUSSION

Preparation and Characterization of Ag NP-decorated TPU/APS Fiber Mats

The preparation procedure for Ag NP-decorated TPU/APS electrospun fiber mats is shown in Fig. 1. First, TPU/APS were co-electrospun into fibers under ambient conditions using DMF/THF (2/3, *v/v*) as the solvent. Then, the self-condensation of APS was conducted in a ventilated oven at 60 °C for 24 h to convert APS into high molecular weight condensate and generate amino-functionalized TPU fibers^[31]. Finally, Ag NPs were adsorbed on amino-functionalized fibers by filtering a silver sol through the mat supported by an ordinary filter paper. The pH of the silver sol was adjusted to 3, 4, and 5 just before use to avoid sedimentation of Ag NPs.

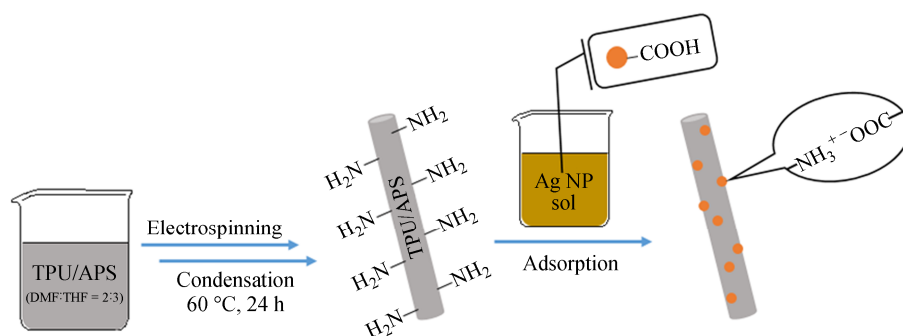


Fig. 1 Schematic representation for the preparation of Ag NP-decorated TPU/APS electrospun fiber mats

Figure 2 shows the FESEM images of Ag NP-decorated TPU/5% APS fibers prepared at three different pH values along with those of TPU/5% APS, neat TPU and Ag NP-decorated TPU fibers obtained at pH = 4 for comparison. The TPU/5% APS fibers are much thinner than the neat TPU fibers. The average fiber diameters of TPU/5% APS and neat TPU are $(1.17 \pm 0.02) \mu\text{m}$ and $(1.85 \pm 0.02) \mu\text{m}$, respectively. The incorporation of APS that has polar groups ($-\text{NH}_2$) significantly increases the solution conductivity, and thus decreases the fiber diameter^[31]. After the adsorption process, the morphology of both TPU and TPU/APS fiber mats has no obvious change in view of fiber diameter and mat porosity. It is hard to see Ag NPs even at high magnifications due to their small sizes. Therefore, TEM was used to observe the presence of Ag NPs on fiber surfaces. Although the preparation processes for TEM and FESEM samples are different, TEM samples can mimic to a great degree the fiber mats and provide useful information about the adsorption of Ag NPs on fiber surfaces. Figure 3 shows the TEM images of the same samples as described in Fig. 2. Both neat TPU and TPU/5% APS are smooth fibers. After the adsorption process, only few Ag NPs were found on neat TPU fibers treated at pH = 4 and many Ag NPs were observed on TPU/5% APS fibers in either monodisperse or slightly agglomerated states at all the three pH values. TEM observation is basically in agreement with the colors of the mats. After the adsorption process, neat TPU mat treated at pH = 4 is pale yellow-colored, while TPU/5% APS mats treated at pH = 3–5 are dark brown-colored. Both Ag NP-decorated TPU and Ag NP-decorated TPU/5% APS fiber mats prepared at pH = 4 were characterized by XPS and the surface atomic concentrations of Ag are 1.2% and 4.7%, respectively. The Ag contents determined by TGA are 0.3% and 5.4%, supporting the TEM observation and the color of the mats. Therefore, the amino-functionalized fiber mat has much better adsorption capacity than neat TPU fiber mat owing to the presence of amino groups.

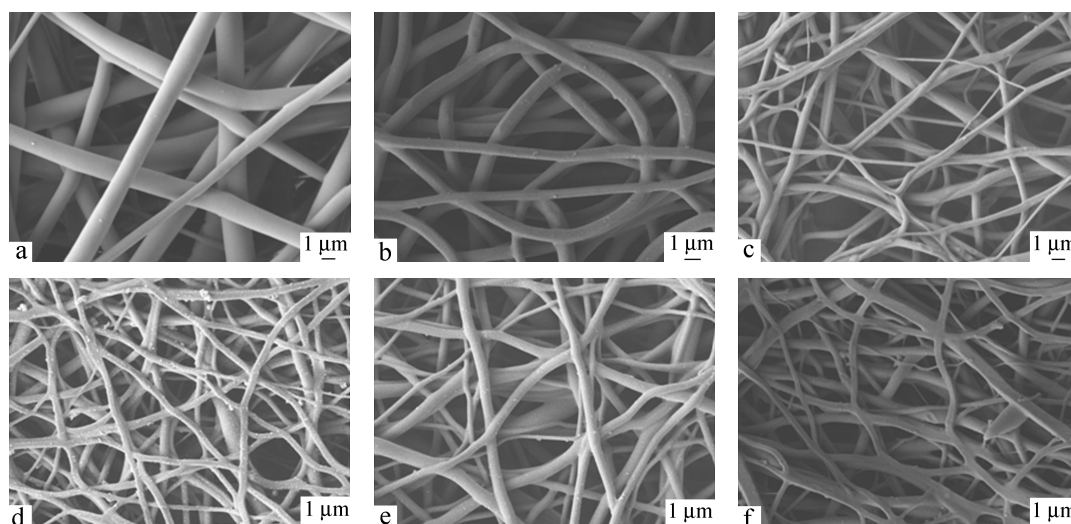


Fig. 2 FESEM images of TPU and TPU/5% APS electrospun fiber mats: (a) neat TPU, (b) Ag NP (pH 4)-decorated TPU, (c) TPU/5% APS, and (d–f) Ag NP-decorated TPU/5% APS at pH = 3, 4, 5, respectively

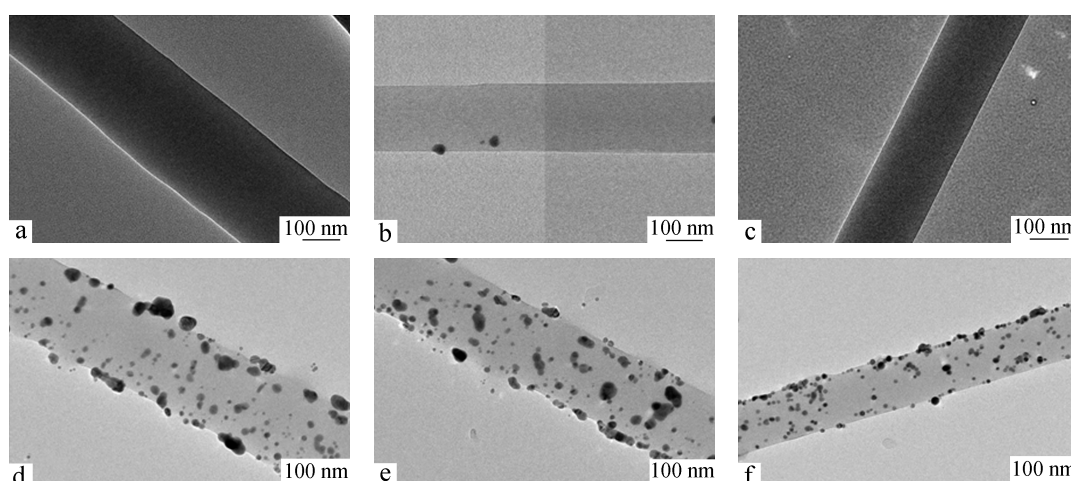


Fig. 3 TEM images of TPU and TPU/5% APS electrospun fiber mats: (a) neat TPU, (b) Ag NP (pH 4)-decorated TPU, (c) TPU/5% APS, and (d–f) Ag NP-decorated TPU/5% APS at pH = 3, 4, 5, respectively

The main driving force for the adsorption of Ag NPs on TPU/5% APS fiber mat is electrostatic interaction between —NH_3^+ of the fibers and —COO^- derived from the —COOH group of the stabilizing agent of Ag NPs by deprotonation in aqueous medium. Coordination between Ag NPs and the lone pair of electrons of amino groups may also occur^[35], but is thought of minor importance because with the as-prepared silver sol at pH of 9.7 only few Ag NPs were adsorbed on TPU/5% APS fiber mat. Neat TPU fiber mat hardly adsorbs Ag NPs at pH of 9.7 and does adsorb a small amount of Ag NPs at pH = 3–5 because of the hydrogen-bonding interaction between the urethane or ester groups of TPU and the carboxyl groups capped on Ag NPs, similar to the case of PCL fibers^[24].

Antibacterial Properties of Ag NP-decorated TPU/5% APS Fiber Mats

Figure 4 shows the time course of Ag NP-decorated TPU/APS and Ag NP-decorated TPU fiber mats obtained at pH = 4 against *E. coli* and *B. sub.* It is clear that the antibacterial rates of both mats increase rapidly within the first hour of incubation and reach a steady level after 2 h of incubation for *E. coli*. The time to the steady level is 5 h for *B. sub.* Similar trend was reported for Ag NP-decorated PCL/SEP fiber mats^[24].

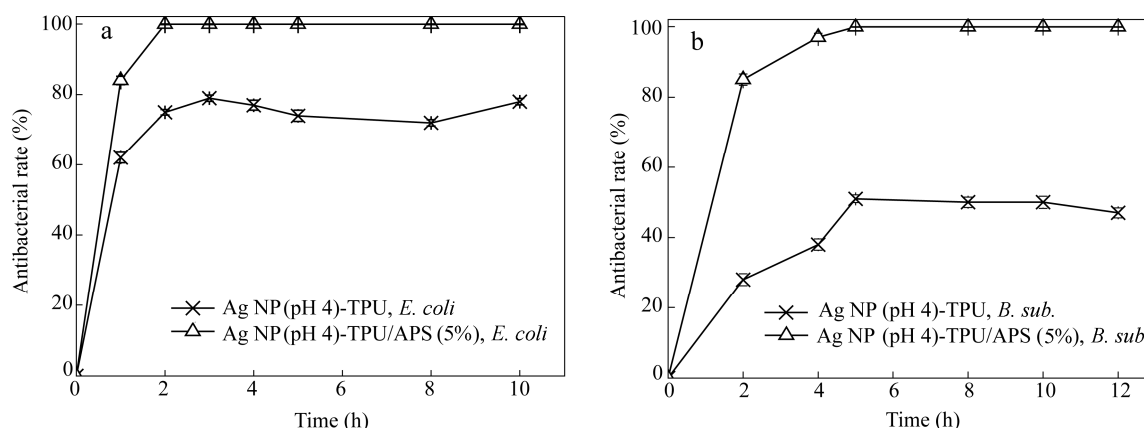


Fig. 4 Antibacterial rates of Ag NP-TPU and Ag NP-TPU/5% APS fiber mats at different contact times: (a) *E. coli* and (b) *B. sub.*

The antibacterial rates of Ag NP-decorated TPU/5% APS fiber mats prepared at pH = 3, 4 and 5 are summarized in Fig. 5 and compared with that of Ag NP-decorated TPU fiber mats. The time points are 2 h for *E. coli* and 5 h for *B. sub.* Excellent antibacterial activity was observed for all the three Ag NP-decorated TPU/5% APS fiber mats, much better than that of the Ag NP-decorated TPU fiber mats. For example, the antibacterial rates of the Ag NP-decorated TPU/5% APS fiber mats prepared at pH = 4 are over 99.9% against both *E. coli* and *B. sub.*, while those of the Ag NP-decorated TPU fiber mat prepared at pH = 4 are only $75\% \pm 0.8\%$ against *E. coli* and $51\% \pm 1.1\%$ against *B. sub.*, revealing that the functionalization by APS can indeed increase the antibacterial performance of TPU fiber mat.

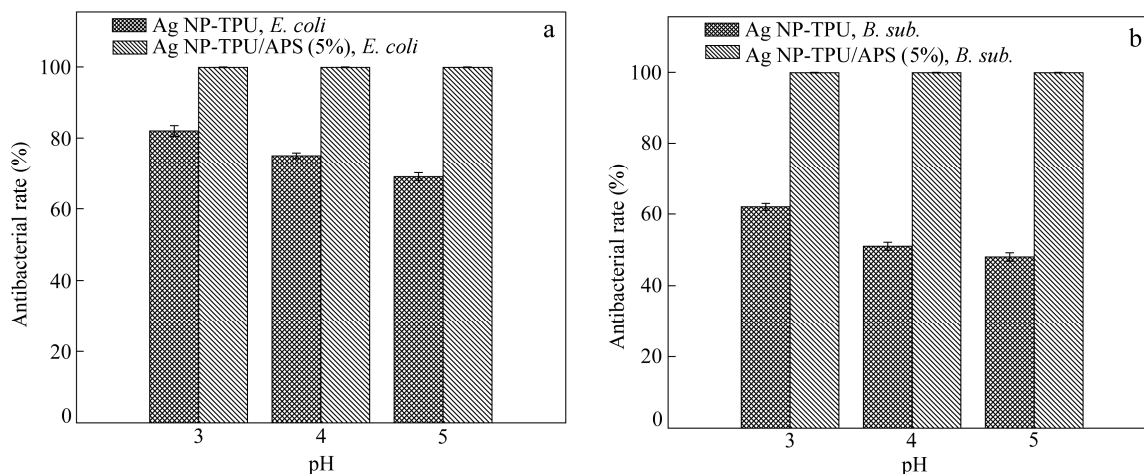


Fig. 5 Antibacterial rates of Ag NP-TPU and Ag NP-TPU/5% APS fiber mats: (a) *E. coli* and (b) *B. sub.*

Polymer nanocomposites containing Ag NPs usually show good antibacterial properties because of the excellent antibacterial activity of Ag NPs^[24, 36, 37]. Compared with Ag NP-decorated nylon 6 fiber mat^[36] and Ag NP-decorated PCL/SEP fiber mat^[24], the Ag NP-decorated TPU/APS fiber mats prepared in this work show similar performance against *E. coli*, and no colony of viable bacteria was found at a contact time of 2 h, although the Ag NP-adsorption mechanism is different.

Effect of the Amount of APS on Antibacterial Activity

Since the amino group is the key in increasing the antibacterial activity of TPU fiber mat, the effect of the

amount of APS on antibacterial properties of the TPU/APS fiber mats was investigated and the results are shown in Fig. 6. At low APS contents, the antibacterial rates against both *E. coli* and *B. sub.* increase with increasing APS content. When APS content is 5% or higher, the antibacterial rates against both *E. coli* and *B. sub.* are over 99.9%, indicating that incorporation of 5% APS to TPU is enough to get excellent antibacterial activity.

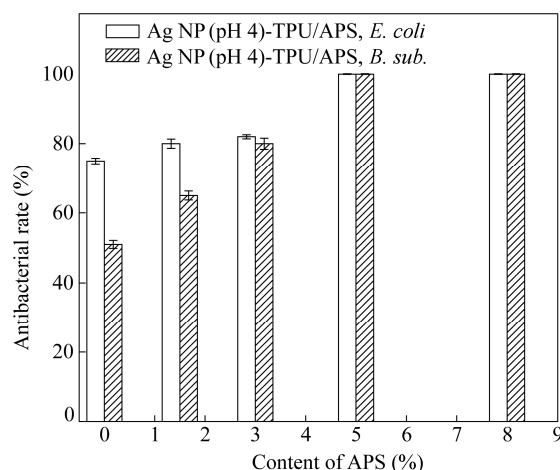


Fig. 6 Antibacterial rates of Ag NP (pH 4)-TPU/APS fiber mats with different contents of APS

CONCLUSIONS

Surface functionalization of TPU electrospun fiber mat with amino groups by co-electrospinning with a small amount of APS is a viable approach to increase the adsorption of Ag NPs and thus increases the antibacterial properties of TPU fibrous mat. The pH value of silver sol should be adjusted to 3–5 for better adsorption of Ag NPs using electrostatic interaction between $-\text{NH}_3^+$ of the fibers and $-\text{COO}^-$ derived from the $-\text{COOH}$ group capped on the surfaces of Ag NPs by deprotonation in aqueous medium. The antibacterial rates against both *E. coli* and *B. sub.* increase with increasing APS content up to 5% where the antibacterial rates against both *E. coli* and *B. sub.* are over 99.9%. Therefore, antibacterial thermoplastic polyurethane electrospun fiber mats that are potentially useful in a wide range of applications can be prepared by 3-aminopropyltriethoxysilane-assisted adsorption of Ag nanoparticles.

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