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Synthesis and Characterization of a Novel Fibrous Antibacterial Fiber with Organophorsphor Functional Groups

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ABSTRACT: A novel antibacterial fiber named ethyltriphenylphosphonium bromide-polyacrylonitrile fiber (ETPB-PANF) was synthesized by chemical modification of PANF reacted with ETPB. The PANF was first immersed in NaOH solution to get Na-PANF with —COONa groups. Na-PANF was then reacted with ETPB to get the final fiber. During the process of synthesis, this article investigated on the initial concentration of ETPB, the contact time, the reaction temperature, and the pH of the solution that may have effect on the properties of ETPB-PANF. ETPB-PANF was characterized by Fourier transform infrared spectroscopy, thermo gravimetric analyzer, scanning electron microscope, and X-ray photoelectron spectroscopy, and the releasing amount of ETPB from ETPB-PANF was examined by inductively coupled plasma atomic emission spectrometry. The antibacterial activity of ETPB-PANF was examined against *Escherichia coli* and *Staphylococci aureus* by improved shake flask method in sterile saline. The results showed that organophorsphor functional groups have been successfully grafted on PANF, and ETPB-PANF showed good antibacterial abilities for *E. coli* and *S. aureus*. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40935.

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INTRODUCTION

In 1935, Domagk discovered that benzalkonium chlorides were outstandingly effective for disinfection of skins, which thereby opened up a field of cationic surfactants as antibacterial agents.[1] To date, cationic nitrogen-containing compounds such as quaternary ammonium salts are generally extensive researched and used as antibacterial agents.[2–4] However, quaternary ammonium salts bear short duration, high medicinal dose, or long-term use which is easy to make bacteria resistant.[5] And in 1990, Graham pointed out that the emergence of a quaternary phosphonium salt was a new progress in the antibacterial field.

Quaternary phosphonium salts and quaternary ammonium salts have a similar structure, but phosphorus cation substitutes nitrogenous cation. The radius of phosphorus atom is larger than that of nitrogen atom, the same trend as ionic radius. The polarization increases as ionic radius increases, so that the positively charged around quaternary phosphonium salt increases, which can more easily adsorb negatively charged surface of microorganisms and kill them. Therefore, quaternary phosphonium salt is more stable than quaternary ammonium salt, and quaternary phosphonium salt does not react with general redox agent and acid or alkali, so its bactericidal activity will be better than that of quaternary ammonium salt. Kanazawa et al.[6] reported the polymeric phosphonium salt exhibited a higher activity by two orders of magnitude than the polymeric quaternary ammonium salt with the same structure except the cationic part.

As a new antibacterial agent, quaternary phosphonium salt has merits in efficient and broad-spectrum antimicrobial, low toxic, and excellent stability within the pH range 2–12, which has been extensively studied as active groups for preparing antibacterial materials,[7–11] but few studies were involved to prepare fibrous antibacterial fiber with organophousphor groups.

In this article, a new kind of fibrous material with organophosphor groups was synthesized by a simple, easy to operate, and even more environmentally friendly method. Polyacrylonitrile fiber (PANF) was selected as the parent fiber because it possesses good elasticity, shape retention, high strength, excellent heat resistance, and light resistance, and so forth. By virtue of the presence of nitrile groups along the framework of PANF, it

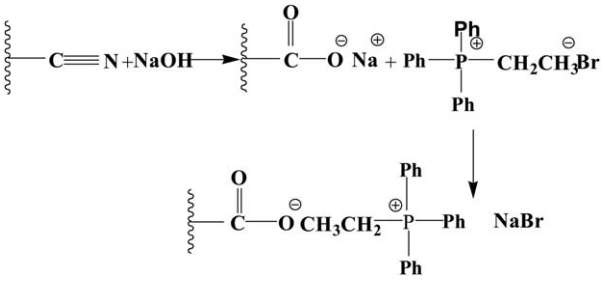

Figure 1. The reaction mechanism of ETPB-PANF.

offers multidirectional approaches to modify fiber for specific applications.[12] In this work, Na-PANF with —COONa groups was first prepared by immersing PANF into the NaOH solution. Then, Na-PANF was reacted with quaternary phosphonium salt to get the final fiber ethyltriphenylphosphonium bromide-PANF (ETPB-PANF) with organophousphor groups. ETPB-PANF was characterized by Fourier transform infrared spectroscopy (FTIR), thermo gravimetric analyzer (TGA), X-ray photoelectron spectroscopy (XPS), scanning electron microscope (SEM), and antibacterial activity against pathogenic *Escherichia coli* and *Staphylococci aureus*.

EXPERIMENTAL

Materials

PANF with length of 2–10 cm, linear density of 1–5 dtex, and content of acrylonitrile $\geq$ 90%, was purchased from Shanghai Petrochemical Co., China. PANF was immersed into NaOH solution to get Na-PANF with —COONa groups. The quaternary phosphonium salt, ethyltriphenylphosphonium bromide (ETPB) with purity 98% was purchased from Alfa Aesar China (Tianjin) Co. Peptone, beef extract, and sodium chloride were purchased from Beijing Aoboxing Biotechnology Co. Pathogenic *E. coli* and *S. aureus* were supplied by Henan Academy of Institute of Biology, Limited Liability Company, Zhengzhou, China.

Synthesis of ETPB-PANF

The reaction mechanism was shown in Figure 1. A certain amount of ETPB was dispersed in 100 mL deionized water, to which 0.5 g Na-PANF fiber was added. During different contact time, the whole system was shaken at 100 rpm in a temperature-constant shaker. After the reaction, ETPB-PANF was washed with deionized water until the washing liquor with 1% $AgNO_3$ solution was clear. The obtained fiber was dried at 40°C to constant weight. The weight gain was determined gravimetrically using the following relation:

$$\text{Weight gain} = \{(\text{mass of ETPB-PANF} - \text{mass of Na-PANF})/\text{mass of Na-PANF}\} \times 100$$

Characterization

FTIR spectra between 400 and 4000 cm^{-1} were collected on a Thermo Electron spectrometer (Nicolet 6700). Samples were prepared by attenuated total reflectance (ATR) method. XPS spectra for the Na-PANF and ETPB-PANF were analyzed by V G Scientific EACA Lab220i-XL XPS with a monochromated Al-Kα X-ray source. Both survey and high-resolution spectra were collected and calibrated to the binding energy of C1s at 284.6 eV. XPS peak 4.1 software was used to peak-fit the calibrated high-resolution spectra. SEM (SIGMA Carl Zeiss) was used to analyze the surface morphology of fibers before and after modification. The fibrous samples were fixed and sprayed by platinum for 90 s in 5-mA current. TG analyzer (Netzsch TG-20) was used for thermal stability determinations, and samples were heated from 0 to 800°C under N_2 flow at a scanning rate of 5°C/min.

Antibacterial Activity Assay

E. coli and *S. aureus* were the representative microorganism in this study. The media used were 10 g/L peptone, 5 g/L beef extract, and 5 g/L sodium chloride, at pH 7.2. After the bacteria were activated, 1 mL bacterial culture was centrifuged at 12,000 rpm for 2 min, and the thallus was cleaned with sterile saline, and then added to 20 mL sterile saline and dispersed uniformly. Twenty milligram ETPB-PANF was immersed into the bacterial suspension prepared by the procedure earlier. The mixed solution was incubated at 30°C with 180 rpm in a shaking incubator. After 24 h, 1 mL bacterial suspension was removed to make different dilutions of bacterial suspension (from $10^{-1}$to 10^{-6}). Finally, 100 μL bacterial suspension of 10^{-4}, 10^{-5}, and 10^{-6} were seeded on a sterile nutrient broth agar. The plates were put into an incubator at culture temperature 37°C for 24 h. The initial bacterial suspension (without fibers) was to be a negative control group. The number of living colonies was countered and three repeats were needed for each sample. The inhibition of cell growth can be quantified as follows:

$$\text{Antibacterial ratio (\%)} = (A - B)/A \times 100$$

where A and B are the number of the colonies detected from the negative control group (without fibers) and the testing group that fibers contacted with bacteria for some time, respectively.

Release of ETPB from ETPB-PANF Fiber

The concentration of phosphor was used to estimate the release of ETPB from ETPB-PANF. Twenty milligram ETPB-PANF was immersed into 20 mL distilled water and shaken at 30°C with 180 rpm in a shaker. After different time intervals, the concentration of phosphor from every sample was measured using inductive coupled plasma atomic emission spectrometer.

RESULTS AND DISCUSSION

Synthesis of ETPB-PANF

The effect of the concentration of NaOH, initial concentration of ETPB solution, contact time, pH of solution, and temperature on the weight gain of ETPB-PANF was discussed. Figure 2(a) showed the effect of the concentration of NaOH. When the concentration of NaOH was 0.1*M* or 0.05*M*, there was an ideal weight gain of more than 20%, as well as good fiber shape. When the concentration of NaOH was more than 0.3*M*, though there was a higher weight gain, the final fiber was destroyed into fragments, so the concentration of NaOH was 0.05*M* during the process that Na-PANF was prepared. Figure 2(b) showed the effect of pH. At pH $\leq$ 6.23, there was an increasing tendency in the weight gain and the maximum value of weight gain (19.44% $\pm$ 0.60%) could be examined at pH 6.23. But when pH > 6.23, there was a decreasing tendency, especially when pH > 8. So, pH 6.23 could be the suitable pH value during the synthesis of ETPB-PANF. Figure 2(c) showed the effect of initial

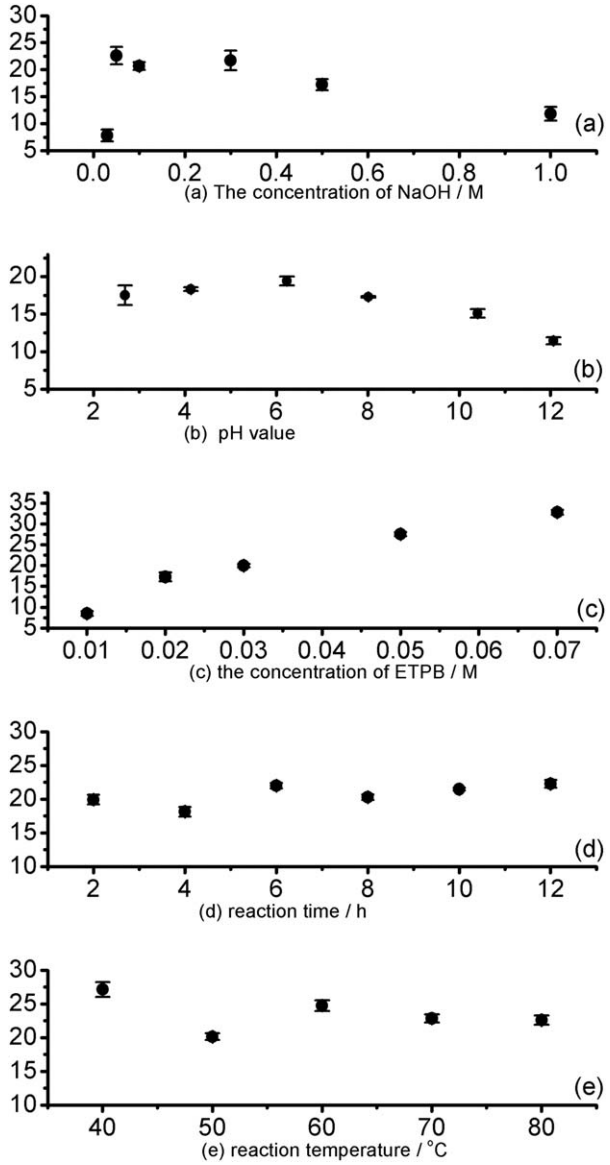

Figure 2. Parameters which have effect on the weight gain of ETPB-PANF [(a) the effect of the concentration of NaOH. The concentration of NaOH solution was from 0.03*M* to 1.0*M*, the concentration of ETPB was 0.03*M*, the reaction time was 8 h, pH was at 6.23, and the temperature was 50°C. (b) The effect of pH. At different pH values, 0.03*M* ETPB was mixed with 0.5 g Na-PANF in 100 mL distilled water at 50°C for 8 h in a shaker. (c) The effect of ETPB concentration. At different initial ETPB concentrations and pH = 6.23, ETPB was mixed with 0.5 g Na-PANF in 100 mL distilled water at 50°C for 8 h in a shaker. (d) The effect of reaction time. At different reaction time and pH = 6.23, 0.03*M* MTPB was mixed with 0.5 g Na-PANF in 100 mL distilled water at 50°C in a shaker. (e) The effect of reaction temperature. At different reaction temperature and pH = 6.23, 0.03*M* ETPB was mixed with 0.5 g Na-PANF in 100 mL distilled water for 8 h in a shaker).

concentrations of ETPB. The weight gain increased with the increasing concentration of ETPB. When the initial concentration of ETPB was from 0.01*M* to 0.07*M*, a sharp increase in weight gain (from 8.51% ± 0.60% to 32.78% ± 0.56%) could be observed. The proper concentration of ETPB was determined at

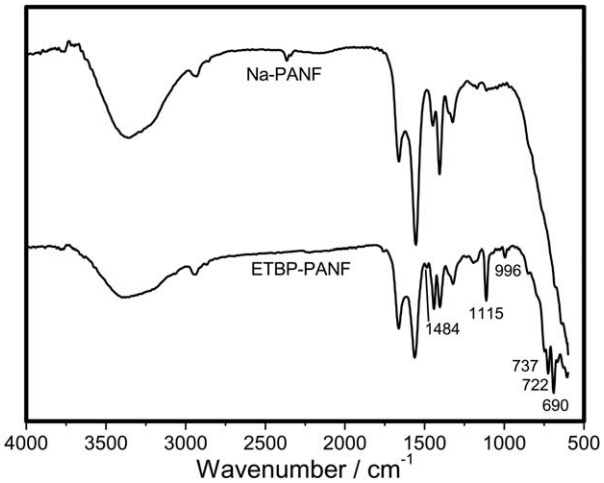

Figure 3. FTIR spectra of Na-PANF and ETPB-PANF.

0.03*M* to keep fiber in good shape and save cost. Figure 2(d) showed the effect of different contact time. When the concentration of ETPB was 0.03*M*, from 2 to 12 h, there was no sharp increase or decrease of weight gain, which could be concluded that the contact time did not have significant effect on the weight gain. Figure 2(e) showed the effect of different reaction temperature. The weight gain was more than 22% at the reaction temperature of 40, 60, 70, and 80°C, and there was a turning point when the temperature was at 50°C with the weight gain of about 20%. The figure showed that higher temperature did not help enhance the weight gain of the final fiber. So, the temperature of 40°C would be the favorable reaction temperature.

In conclusion, the best synthesis condition was in nearly neutral solution (pH 6.23), the concentration of ETPB was 0.03*M* with heated to 40°C in 2 h. That is just to coincide with the reaction mechanism. During this reaction, Na from Na-PANF was exchanged by organophosphur groups from ETPB, and the mechanism can be included to the mechanism of ion exchange. For the process of ion exchange, not higher temperature and neutral conditions will contribute to ideal experimental results. The initial concentration of ETPB during reaction could not be too high or too low, as the higher concentration may result in higher yield, but may lead to fragile fibers, whereas the lower concentration could bring lower yield with lower-density target groups on fibers.

Characterization

The FTIR spectra of Na-PANF and ETPB-PANF were shown in Figure 3. Compared with Na-PANF, some new peaks emerged. The peak at 1115 cm^{-1} was C-P (bending vibration absorption), 996 cm-1 was P-Ph (vibration absorption), and 737, 722, and 690 cm^{-1} were the characteristic peaks of =CH (bending vibration of the benzene ring). The peak at 1482 cm^{-1} appeared in the ETPB-PANF was very weak, but it did not exist in the Na-PANF, which was one important indicator of whether the benzene ring exist or not and represented the stretching vibration of the skeleton of benzene ring. Figure 3 suggested the presence of quaternary phosphonium groups of the ETPB-PANF.

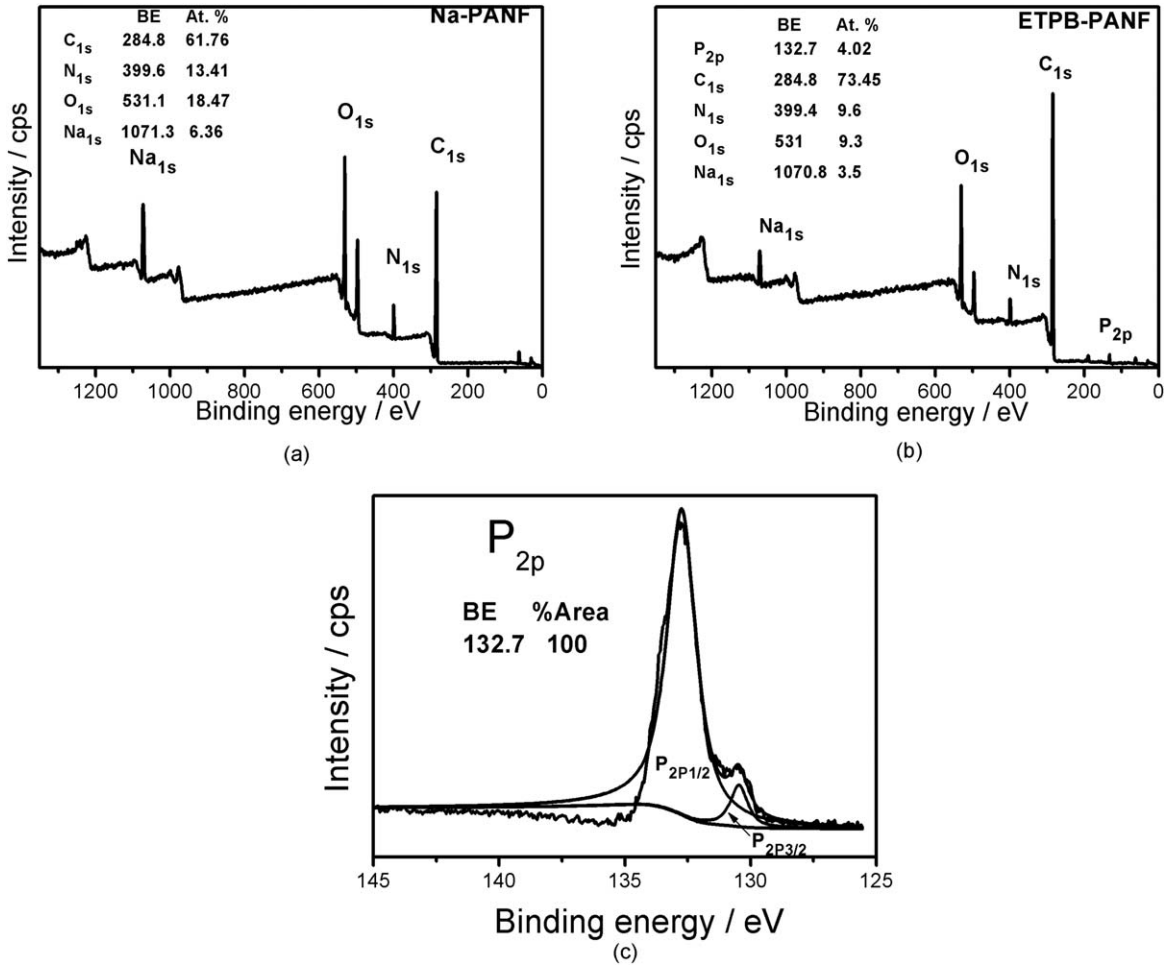

Figure 4. XPS spectra of (a) survey scan for Na-PANF and (b) survey scan for ETPB-PANF and (c) high-resolution scan of P_{2p} for ETPB-PANF.

XPS spectra of Na-PANF and ETPB-PANF were shown in Figure 4. Figure 4(a) showed the survey spectra of Na-PANF, the main elements C_{1s} (61.76 At %), N_{1s} (13.41 At %), O_{1s} (18.47 At %), and Na_{1s} (6.36 At %) could be examined. Figure 4(b) showed the survey spectra of ETPB-PANF, the main elements P_{2p} (4.02 At %), C_{1s} (73.45 At %), N_{1s} (9.6 At %), O_{1s} (9.3 At %), and Na_{1s} (3.5 At %) could be examined. A high-resolution spectra of P_{2p} in Figure 4(c) was fitted with a doublet peak of

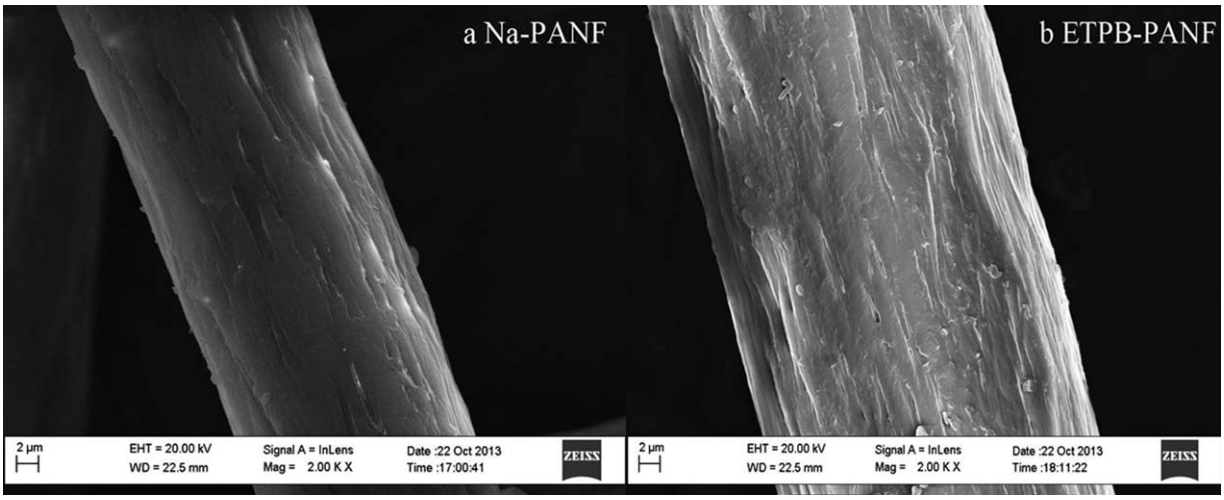

Figure 5. SEM images of Na-PANF and ETPB-PANF.

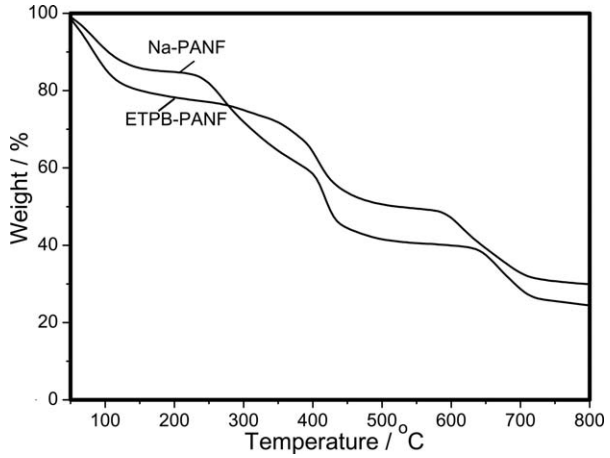

Figure 6. TGA curves of Na-PANF and ETPB-PANF.

$P_{2p1/2}$ situated at 132.7 eV and $P_{2p3/2}$ at 130.4 eV. And, it could be examined that if the atomic concentration of P (4.02 At %) was represented by the atomic concentration of Na (4.02 At % × 23/31 = 2.98 At %), the value was just nearly the same as the decreasing Na after reaction (6.36 At % − 3.5 At % = 2.86 At %), which suggests that the presence of organophosphorus group on ETPB-PANF was nearly 1 : 1 atomic exchanging with Na on Na-PANF during the process of synthesis.

SEM images of Na-PANF and ETPB-PANF were shown in Figure 5. The surface morphology of the fiber visibly changed from smooth to rough due to modifying between Na-PANF and ETPB-PANF. The ravines on the surface of ETPB-PANF were obviously much more than that on Na-PANF, indicating that the introduction of quaternary phosphonium group could help increase the fiber's surface area. From Figure 5, the ravines on the surface of ETPB-PANF were distributed evenly, and the overall structure of the fiber was kept fine after reaction.

The thermogravimetric curves of Na-PANF and ETPB-PANF were shown in Figure 6. A similar trend between Na-PANF and ETPB-PANF could be examined. The evaporation of free or absorbed water could be observed from 50 to 200°C. The weightlessness for Na-PANF and ETPB-PANF began at 382 and 242°C, respectively. At 400°C, the weightlessness rate of Na-PANF was up to 35.93%, while the weightlessness rate of ETPB-PANF was 41.55% due to the volatilization of phosphonium salts. Figure 6 showed that the introduction of phosphonium groups did not change the thermal stability of the parent fiber.

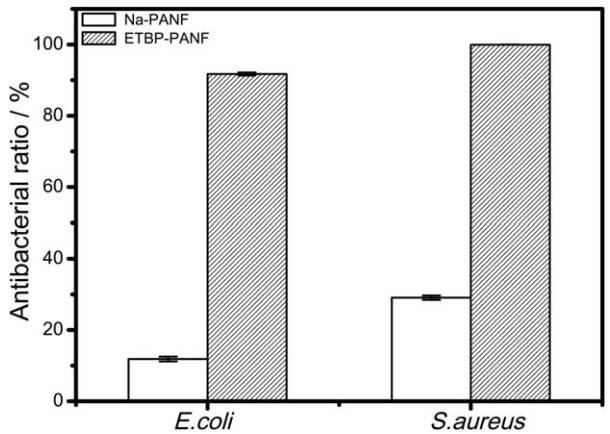

Figure 7. Antibacterial activity of ETBP-PANF against pathogens after disinfecting 24 h.

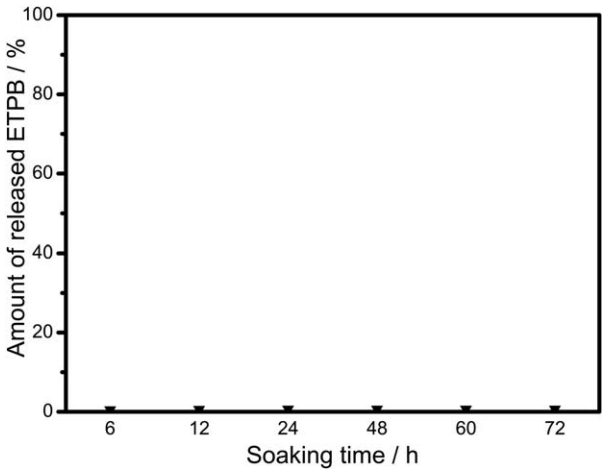

Figure 8. Released quantity of ETPB in distilled water at different soaking time.

Antibacterial Activities

In this work, the shaking method was applied to evaluate the antibacterial activity of ETPB-PANF. *E. coli* and *S. aureus* were the representative bacteria used. Figure 7 showed that the Na-PANF was not active against both of pathogens, whereas the ETPB-PANF showed great antiactivity. ETPB-PANF exhibited lower antiactivity against *E. coli* (91.74 ± 0.42%) than against *S. aureus* (99.96 ± 0.007%). The previous study showed that gram negative bacteria had protective structure in outer cell membrane, which was composed mainly of lipopolysaccharides and phospholipids, and the outer membrane took a significant role in protecting bacteria cells against foreign compounds such as antibacterial materials.[13] Thus, the lower sensitivity of MTPB-PANF toward *E. coli* appeared.

Release of ETPB from ETPB-PANF

Figure 8 showed the concentration of phosphor released from ETPB-PANF during different time intervals. After ETPB-PANF was continuously immersed in distilled water over 72 h, only 0.66% ETPB was released from ETPB-PANF, which demonstrated that the organophosphorus groups could stay stable on ETPB-PANF.

CONCLUSIONS

This work synthesized a novel antibacterial fiber in a simple, easy to operate, and environmentally friendly way. FTIR and XPS showed the presence of organophorsphur group on ETPB-PANF. SEM and TGA showed the fine microstructure and thermo stability of ETPB-PANF. The organophosphorus group could stay stable on ETPB-PANF. ETPB-PANF exhibited excellent antibacterial activities and higher activities against *S. aureus* than *E. coli* owing to different bacterial structure, and it could protest more than 99.9% of *S. aureus* and 91% of *E. coli*.

Therefore, this work suggested that the new fibrous material ETPB-PANF may be used in textiles, health care products, and hygienic applications.

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