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Preparation and Characterization of a Novel Antibacterial Fiber Modified by Quaternary Phosphonium Salt on the Surface of Polyacrylonitrile Fiber

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Abstract: A novel antibacterial fiber named MTPB-PANF was synthesized by chemical modification of polyacrylonitrile fiber (PANF). The PANF was firstly reacted with alkali solution to get Na-PANF with -COONa functional groups. Na-PANF was then reacted with different concentration of methyltriphenyl phosphonium bromide (MTPB) into flasks, and the whole system was immersed into a to and fro vibrator. During the synthesis process, this paper investigated on the initial concentration of MTPB, the contact time, the reaction temperature and the pH of the solution that may have effect on the properties of the final fiber. The properties of MTPB-PANF were discussed by fourier transform infrared spectroscopy (FT-IR), thermo gravimetric analyzer (TGA), scanning electron microscope (SEM) and the stability of organophosphorus groups on MTPB-PANF examined by inductively coupled plasma atomic emission spectrometry (ICP-AES). The antibacterial activity of MTPB-PANF was examined against pathogenic *Escherichia coli* and *Staphylococci aureus* by improved shake flask method in sterile saline and was evaluated by the viable cell counting method. The obtained results showed an excellent antibacterial activity of MTPB-PANF. And the antibacterial mechanism was discussed by the concentration of K^+ released from cells after bacterial testing.

Keywords: Polyacrylonitrile fiber, Chemical modification, Quaternary phosphonium salt, Characterization, Antibacterial activity

Introduction

With the growing of public health awareness of disease transmissions and cross-infection caused by the microorganisms, several groups have shown keen interest in developing antibacterial fibers and fabrics for various applications, such as clothing for hospital staff and patients, hospital beddings, sports clothing, armbands, underwear, ladies tights, shoe linings, sleeping bags, toys for children, water purification systems, etc [1].

The antibacterial moieties in polymers include pyridine [2], imidazole [3], N-halamines [4], metal ions such as silver ions, copper ions, zinc ions [5-7], various types of quaternary ammonium [8-14] and phosphonium salts [15-19], Nevertheless, the applications of inorganic antibacterial materials are limited because an accumulation of heavy metals will result in serious environmental problems and may be harmful to humans in the case of high metal concentration [20]. And Joanna Feder-Kubis and Krzysztof Tomczuk [21] studied antibacterial activities of ammonium derivatives, alkylimidazolium derivatives, alkoxymethylimidazolium derivatives, and pyridinium derivatives against several types of bacteria, and the results showed ammonium derivatives demonstrated the best antibacterial activities. Moreover, some studies showed quaternary phosphoniums have better antibacterial properties than quaternary ammoniums [15,22,23]. Quaternary phosphonium salt is a new generation of efficient, broad-spectrum, low toxicity antiseptic. Compared with quaternary ammonium salts, though they both have a similar structure, phosphorus atomic radius 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, it does not react with general redox agent and acid or alkali and its bactericidal activity is better than quaternary ammonium salt. Kanazawa [23] reported the polymeric phosphonium salt exhibited a higher activity by 2 orders of magnitude than the polymeric quaternary ammonium salt with the same structure except the cationic part. With a number of advantages of low foam, strong capability of sludge stripping and excellent stability within the pH range 2-12, it has been extensively studied as active groups for preparing antibacterial materials [24-28].

This paper was the first time to focus on a novel antibacterial material of fibrous material PANF reacted with quaternary phosphonium salts in a simple, easy-control synthesis route with mild reaction conditions. Fibrous material PANF was used as the parent fiber, and then quaternary phosphonium salt was grafted on the PANF. The whole synthesis mechanism was two steps: the PANF was firstly reacted with alkali solution to get Na-PANF with -COONa functional groups. Na-PANF was reacted with different concentration of MTPB into flasks, and the whole system was immersed into a to and

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fro vibrator. The process of synthesis avoided the harsh reaction conditions such as radiation, high pressure, and high temperature. And the advantage is that the obtained material MTPB-PANF was fibers, which will have more resourceful models in practice, e.g. fibers can be in filament or staple or woven in thread and cloths, otherwise, the most importance is fibers have much wider specific surface than that of resins and granular materials, which will result in a more wonderful antibacterial activity.

Here FTIR, TGA, TEM, antibacterial tests against *Escherichia coli* (*E.coli*) and *Staphylococci aureus* (*S.aureus*) were applied to measure the properties of MTPB-PANF.

Experimental

Materials

PANF was sodiumized in laboratory to get Na-PANF with -COONa groups, and the cation exchange capacity were 5.0 mmol/g. The quaternary phosphonium salt, methyltriphenyl-phosphonium bromide (MTPB) with purity 98 % was purchased from Alfa Aesar China (Tianjin) Co., Ltd. Peptone, beef extract and sodium chloride were purchased from Beijing Aoboxing Biotechnology Co., Ltd. Pathogenic *E.coli* and *S.aureus* were supplied by Henan Academy of Institute of Biology, Limited Liability Company, Zhengzhou, China.

Synthesis of MTPB-PANF

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

Weight gain = {(Weight of fiber after modification − Initial weight of fiber) / Initial weight of fiber} ×100

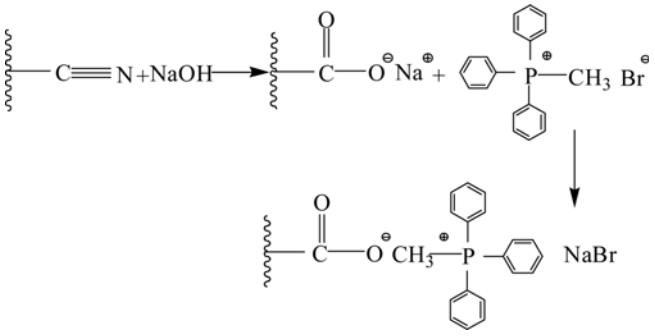

Figure 1. The reaction mechanism of MTPB-PANF.

Characterization

Fourier transformed infrared spectrometer (FTIR NICOLET 200) spectra between 400 and 4000 cm^{-1} were collected on a Thermo Electron spectrometer. Samples were prepared in the pellet of KBr. A SIGMA CARL ZEISS scanning electron microscopy (SEM) was applied to observe the fiber's surface morphology before and after reaction. Samples were sprayed by platinum for 90 seconds in 5 mA current. A thermogravietry analyzer (NetzschTG-20) was used for thermal stability determinations, and samples were heated from 0 °C to 800 °C under N_2 flow at a scanning rate of 5 °C/min. A Labthink XLW (L) PC auto-tensile tester was used for testing the stretching resistance of fibers. Samples were dried at about 45 °C in the air oven and 60 fiber filaments were tested for average calculation.

Antimicrobial Activity Assay

In this study, *E.coli* and *S.aureus* were as the representative of Gram negative and Gram positive bacteria respectively. The media used were 10 g/*l* peptone, 5 g/*l* beef extract and 5 g/*l* sodium chloride, at pH 7.2. After activated bacteria, 1 m*l* bacterial culture was centrifuged at 12000 rpm in two minutes, and thallus was cleaned with sterile saline. Then added to 20 m*l* sterile saline and dispersed uniformly. 20 mg MTPB-PANF was immersed into the bacterial suspension prepared by the procedure above. The mixed solution was incubated at 30 °C with 180 rpm in a shaking incubator. After 24 h, 1 m*l* bacterial suspension was removed, to make different dilutions of bacterial suspension (from 10^{-1} to 10^{-6}). Finally, 100 u*l* bacterial suspension of 10^{-4}, 10^{-5} and 10^{-6} were seeded on an 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 Antibacterial ratio can be quantified as follows:

$$\text{Antibacterial ratio } (\%) = (A - B) / A \times 100 \quad (1)$$

Where A and B are the number of the colonies detected from the negative control group and treated samples after anti-bacteria, respectively.

Stability of Organophosphorus Groups on MTPB-PANF Fiber

The concentration of phosphor was used to estimate the stability of organophosphorus groups on MTPB-PANF Fiber. 20 mg MTPB-PANF was immersed into 20 m*l* 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 (ICP-AES). The Released ratio was calculated from the proportion of the mass of released phosphor and MTPB-PANF.

Results and Discussion

Synthesis of MTPB-PANF Fiber

In the process of reaction, the effect of initial concentration of MTPB solution, contact time, pH of solution and temperature on weight gain of the obtained MTPB-PANF were discussed. Figure 2(a) showed the effect of pH. At pH $\leqslant$6.46, there was an increasing tendency in the weight gain and the maximum value of weight gain (17.89 %) could be examined at pH 6.46. But when pH>6.46, there was a decreasing tendency, especially when pH>10. So pH 6.46 could be the suitable pH value during the synthesis of ETPB-PANF. Figure 2(b) showed that at the same conditions except different initial concentration of MTPB, the weight gain increased with increasing concentration. From 0.01 M to 0.02 M, a sharp increase in weight gain (3.95 % to 11.98 % correspondingly) could be observed, while there was a slight increase when the concentration was from 0.03 M to 0.15 M. And the highest was up to 33 % at C_0=0.15 M. However, 0.03 M was selected as the satisfactory initial concentration of MTPB for the following experiment, in order to avoid higher cost, though the weight gain of final fiber was higher at C_0=0.15 M. Figure 2(c) showed the effect of different contact time on the weight gain. When C_0=0.03 M, from 2 to 12 h, there was not obvious 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(d) showed the effect of different reaction temperature. When the temperature was 40, 60, or 70 °C, the weight gain was all less than 20 %. But there was a turning point when heated to 50 °C, and the weight gain was 24.75 %.

In conclusion, the best synthesis condition was in nearly neutral solution, C_0 of MTPB was 0.03 M with heated to 50 °C in 2 hours. That's just to coincide with the reaction mechanism. During this reaction, Na^+ from PANF-COONa was exchanged by organophosphur groups from MTPB, 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 experiment. The initial concentration of MTPB 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, while the lower concentration could bring lower yield with lower density target groups on fibers.

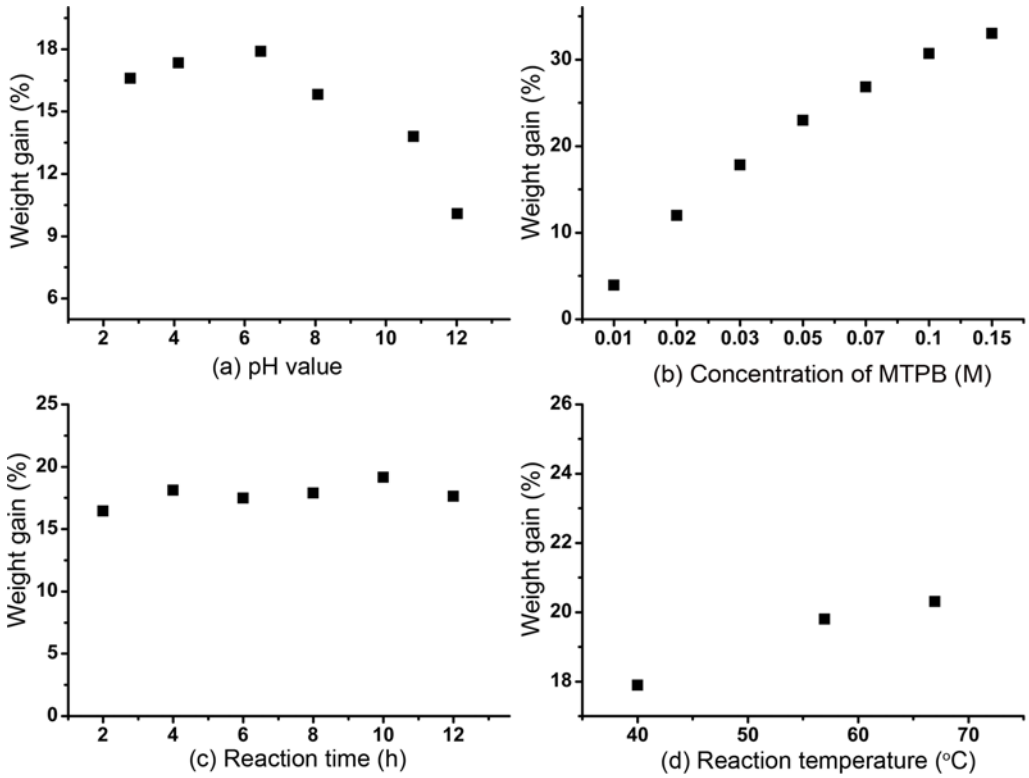

Figure 2. Parameters which have effect on the weight gain of final fibers. (a) The effect of pH value. At different pH values, 0.03 M MTPB was mixed with 0.5 g Na-PANF in 100 m*l* distilled water at 50 °C for 8 h in a shaker. (b) The effect of MTPB concentration. In different initial MTPB concentrations and pH=6.46, MTPB was mixed with 0.5 g Na-PANF in 100 m*l* distilled water at 50 °C for 8 h in a shaker. (c) The effect of reaction time. At different reaction time and pH=6.46, 0.03 M MTPB was mixed with 0.5 g Na-PANF in 100 m*l* distilled water at 50 °C in a shaker. (d) At different reaction temperature and pH=6.46, 0.03 M MTPB was mixed with 0.5 g Na-PANF in 100 m*l* distilled water for 8 h in a shaker.

Properties of MTPB-PANF Fiber

The FTIR spectra of Na-PANF and MTPB-PANF were shown in Figure 3. From Figure 3, the peak at 1662 cm^{-1}, 1557 cm^{-1} was the C=C stretching vibration, and 1443 cm^{-1}, 1406 cm^{-1} was the C-H deformation vibration of -CH_3. The peak at 1116 cm^{-1} was C-P bending vibration absorption peak, peak 901 cm^{-1} was was vibration absorption peak of P-Ph, and 749 cm^{-1}, 719 cm^{-1} and 690 cm^{-1} were the characteristic peaks of =CH bending vibration of the benzene ring. 510 cm^{-1} was the absorption peak of the C-H deformation vibration of benzene ring. That suggested that MTPB had been grafted to Na-PANF and the organophorsphur groups kept stable on the final fiber.

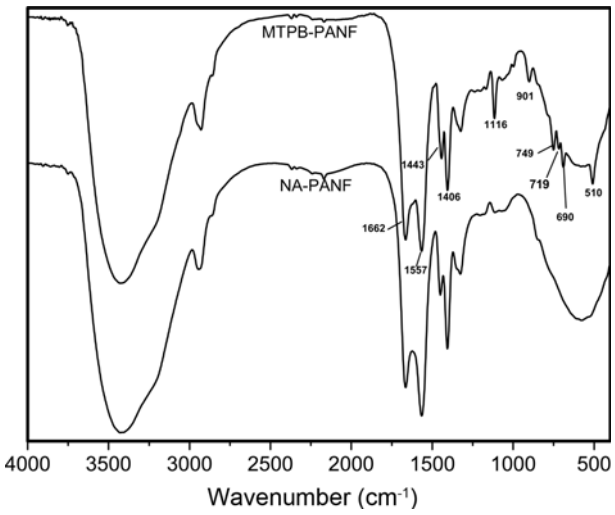

Figure 3. FTIR spectra of Na-PANF and MTPB-PANF.

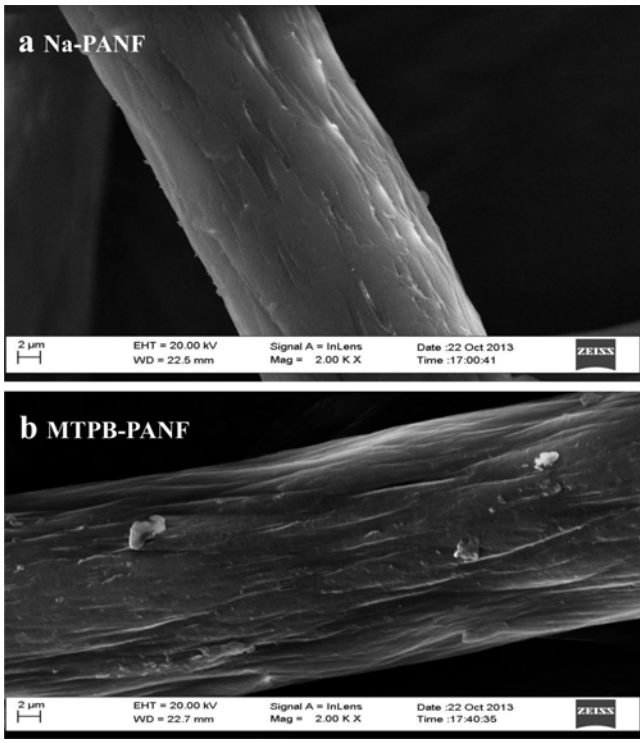

Figure 4. SEM images of Na-PANF and MTPB-PANF.

SEM images of Na-PANF and MTPB-PANF were shown in Figure 4. The surface morphology of the Na-PANF visibly changed from smooth to rough due to modifying. The ravines of MTPB-PANF surface increased significantly, and some places had significantly protrusions, indicating that modification caused the surface of fiber rough, which could reduce the mechanical properties of the fiber, so suitable reaction conditions should be considered for better final fibers with high density of target functional groups and satisfactory fibrous configuration, but which could also help to increase the fiber's surface area and promote the process of modification.

The stretching resistance of fibers before and after modification was tested to value the mechanic strength. The average stretching resistance of Na-PANF was about 0.055N but that of MTPB-PANF was 0.043 N. The ratio of reduction was about 21 % compared with the average stretching resistance of the Na-PANF fibers.

TG analysis (Figure 5) showed a similar trend between Na-PANF and MTPB-PANF. Between 50 ^{o}C and 200 ^{o}C was the evaporation of free or absorbed water. At 239.5 ^{o}C, MTPB-PANF began to lose weight while the Na-PANF bagan to lose weight at 382 ^{o}C. When to 403 ^{o}C, the weight loss rate of MTPB-PAN was 41.4 %, as the sharp volatilization of phosphonium salts emerged at this stage. So the introduction of phosphonium salt did not significantly change the thermal stability of the PANF fiber, the fiber at range from room temperature to 220 ^{o}C could still maintain the original fiber's physical and chemical properties.

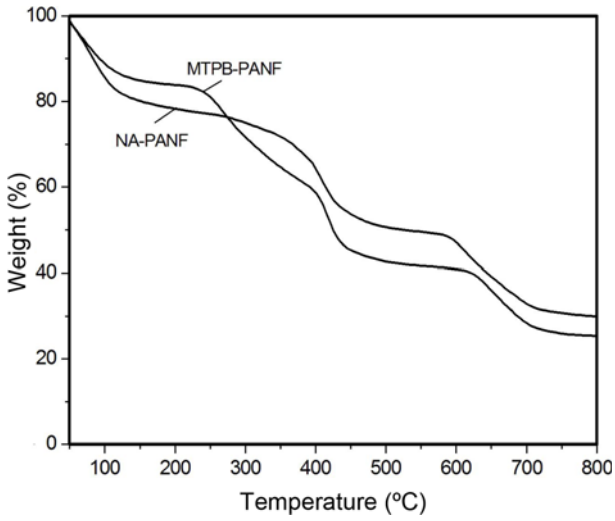

Figure 5. TGA curves of Na-PANF and MTPB-PANF.

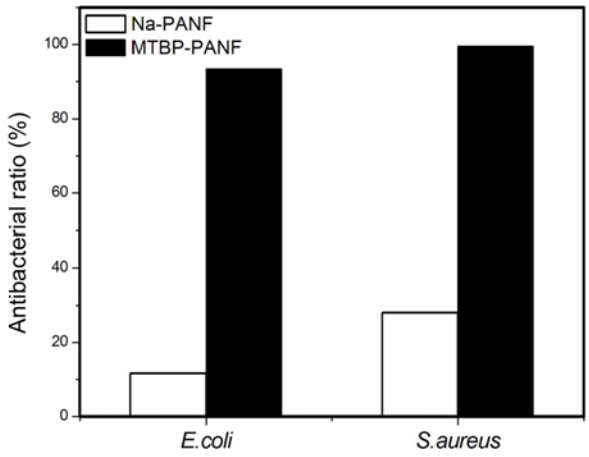

Figure 6. Antibacterial activity of MTBP-PANF against pathogens after disinfecting 24 hours.

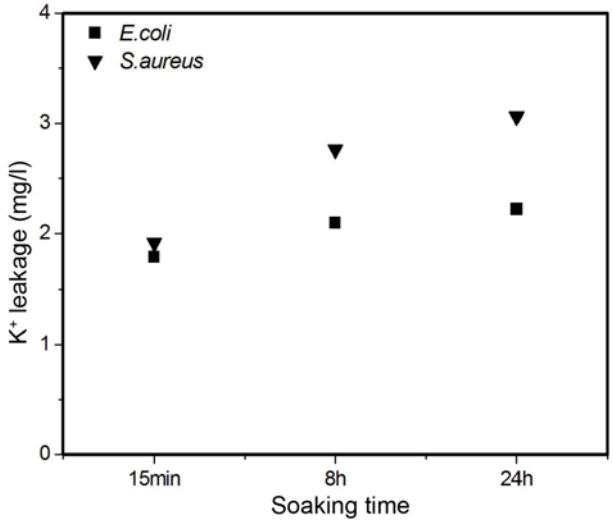

Figure 7. Leakage of K^+ from *E.coli* and *S.aureus* after 24 h interaction with MTPB-PANF.

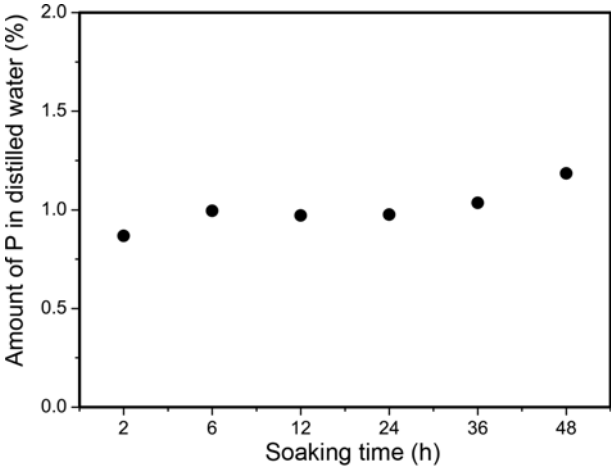

Figure 8. Released ratio of MTPB in distilled water at different soaking time.

Antimicrobial Activities

In this work, improved shake flask method was applied to evaluate the antibacterial activity of MTPB-PANF. Pathogenic *E.coli* and *S.aureus* were the representative bacteria employed. After disinfecting 24 h, Figure 6 showed that the Na-PANF was not active against both of the pathogens while the MTPB-PANF showed great anti-activity. However, MTPB-PANF exhibited lower anti-activity against *E.coli* than against *S.aureus*. Because Gram negative bacteria have the protective structure of their outer cell membrane, which is composed mainly of lipopolysaccharides and phospholipids, and the outer membrane takes a significant role in protecting bacteria cells against foreign compounds such as antibacterial materials [29]. Thus, the lower sensitivity of MTPB-PANF towards *E.coli* appeared.

K^+ exists universally in bacteria and plays a part in the regulation of polysome content and protein synthesis [30]. So whether K^+ could be detected or not could be as a symbol of whether cell death or living. Figure 7 showed the concentration of K^+ released from cells after the antibacterial test.After different incubation time, the bacteria suspension centrifuged and supernatants were collected for measuring K^+ with ICP-AES. We can see there's an increase of K^+ concentration with the increasing incubation time. Within 15 min, 1.913 mg/*l* and 1.789 mg/*l* of K^+ were released from *E.coli* and *S.aureus* cells after antibacterial testing. After 24 h, the concentration of K^+ in suspension containing *E.coli* and *S.aureus* were highly up to 2.220 mg/*l* and 3.065 mg/*l*. We also see before 8 hours, there exited a sharply increased curve, which suggested that the cell membranes of most bacteria were probably damaged by MTPB-PAN. However, after 8 hours, the flat curve showed the decreasing number of surviving bacteria. In comparison of the leakage of K^+ concentration from *E.coli* and *S.aureus*, it could be seen that the concentration of K^+ from *S.aureus* was significantly higher than that from *E.coli*, which suggested that the changes of *S.aureus* membranous permeability and destruction of the cell membrane were more serious than that of *E.coli*. So, we speculated that the MTPB-PANF fibers could mainly damage the cell membranes of bacteria, further lead to the release of cell inclusion such as K^+ penetration, and eventually kill the pathogens.

Stability of Organophosphorus Groups on MTPB-PANF Fiber

Phorsphor was measured by ICP-AES when MTPB-PANF was immersed in distilled water after 2, 6, 12, 24, 36 and 48 h. Figure 8 showed from 2 h to 48 h, 0.86-1.18 % of P could be measured in water solution which demonstrated that organophosphorus groups could stay stable on MTPB-PANF in water.

Conclusion

A novel fibrous antibacterial material MTPB-PANF has been prepared in a simple, mild and easily-control way. The organophorsphur functional groups have been successfully grafted onto the parent fibers. The modification conditions had little damage in the surface of the final fiber. And the organophorsphur functional groups existed stable with only 1.18 % phorsphor released from MTPB-PANF after immersed in distilled water for 48 hours.

MTPB-PANF exhibited excellent antibacterial activities and higher activities against *S.aureus* than against *E.coli* owing to different bacterial structure. More than 93 % *E.coli* and 99 % *S.aureus* could be killed by MTPB-PANF, and the concentration of K^+ released from cells after the antibacterial test further showed that the bacteria was not prohibited reproducing, but killed by MTPB-PANF.

This work may supply a new method with the fibrous antibacterial material to kill the hazardous microorganism in flexible staple fibers, thread, cloth, or other applicable forms.

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