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Antibacterial and anti-mildew behavior of chitosan/nano-TiO_2 composite emulsion

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Abstract−A novel chitosan/nano-TiO_2 composite emulsion (CTCE) was prepared by inverse suspension technology, and its multi-function properties were studied. As can be seen from the results, the gauze treated with chitosan/nano-TiO_2 composite emulsion showed excellent antibacterial activities against *Escherichia coli*, *Aspergillus niger* and *Candida albicans* that the bactericidal ratios could reach 99.96%, 100% and 78.3% after 24 h, respectively. At the same time, the chitosan/nano-TiO_2 composite emulsion could really kill the bacteria, not just inhibit its growth, and the bactericidal ratio for *E. coli* could reach 70.3% after 1 h. The bactericidal ratio for *E. coli* reached 98.8% at 27 °C compared with 58.7% at 18 °C after 8 h due to the activity of bacteria being weakened at low temperature. Furthermore, the antibacterial ability of antibacterial gauze was hardly influenced with the increase of storage time, which could be reused for up to 8 times without loss of antibacterial ability.

Key words: Chitosan, Nano-TiO_2, Antibacterial, Anti-mildew, Textile

INTRODUCTION

Natural fibers or synthetic fibers may be destroyed by erosion of bacteria or mildew, such as discoloration, breakage and so on. Acetate fibers with irregular section may be easily accreted by microorganisms. Many microorganisms, involving unhealthy ones, exist in people's daily lives. Not only the fabric but also human health may be harmed by microorganisms. The microbial metabolite will produce odor and other intermediate products, which are irritating to the skin. Contamination by microorganisms such as mildew may result in the damage and discoloration of fabrics. In addition, fabrics may be damaged if the finishing agent is consumed by some microorganisms and cotton or latex is consumed by mildew [1]. Therefore, finding good antibacterial and anti-mildew agents is an urgent problem faced by the textile industry. Traditional antibacterial agents are organic agents, strictly speaking, which belong to pesticides. Especially, the security issue must be considered first. Antibacterial agent PCP (pentachlorophenol) which was used widely in the early period is toxic, carcinogenic and teratogenic compound [2]. Some organic and inorganic metal compounds which were used as textile finishing agents or fiber modifiers all contained a variety of heavy metal ions. These heavy metal ions are absorbed and deposited in the liver, bones, kidneys, heart and brain of humans. A heavy accumulation of heavy metal ions can lead to serious harm. The harm to children is more serious for the higher absorption capacity for heavy metal ions [3]. Researchers have turned to looking for substitutes of organic antibacterial agents-natural antibacterial agents and inorganic antibacterial agents, because of the limitations of traditional organic antibacterial agents, such as poor durability, temperature variability, easy decomposability in UV irradiation and biological resistance [4].

Chitosan, as one kind of inartificial antibacterial agents, is nontoxic, biocompatible and biodegradable. Besides, the antibacterial activity of chitosan is particularly of interest and has been observed against various bacteria [5]. It was reported [6,7] that chitosan could be used as anti-crease finishing agent for cotton fabric [8]; anti-shrink, antibacterial agent, or dyeing finishing agent for wool fabric [9], and comfort finishing agent for polyester fabric [10]. In recent years, chitosan has been applied widely in biomedical membrane [11], food packaging materials [12], and other fields [13]. Based on its excellent properties and understanding of the antibacterial mechanism, chitosan is the optimal antimicrobial agent. However, the stability of chitosan should be improved.

Nanometer titanium dioxide (TiO_2), as an antibacterial material, belongs to the non-dissolution-type inorganic antibacterial agent. Nano-TiO_2 is nontoxic, flavorless and non-simulative. It has antibacterial activity, and UV shielding property [14]. Its antibacterial mechanism was studied by several researchers [15]. It has been widely used in cosmetics, environmental photocatalysis [16], antimicrobial coating [17-19], and so on. It has also been approved as a food additive by national departments [3]. In the textile field, TiO_2 is mostly used for shielding UV [20], and the antibacterial ability of nano-TiO_2 is mainly depending on the UV irradiation, which limited its application. Chitosan/gelatin/TiO_2 antibacterial composite membrane has been reported [21]. However, it has not been studied whether chitosan and nano-TiO_2 compounded to be used in textiles.

In this work, a novel chitosan/nano-TiO_2 composite emulsion was prepared with chitosan and nano-TiO_2 by inverse suspension technology. The antibacterial abilities of gauze treated with chitosan/nano-TiO_2 composite emulsion were studied. The results showed that the antibacterial gauze had excellent antibacterial ability under invisible light. Meanwhile, the bactericidal rate and durability of the treated gauze have been improved.

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MATERIALS AND METHODS

1. Materials

Chitosan was obtained from shrimp shells with 90% degree of deacetylation. Molecular weight measurement using a solution viscosity method suggested that the Mv of chitosan was approximately 100,000. A nanometer TiO_2 range from 50 nm to 80 nm in diameter was obtained from the University of Science and Technology Beijing. Medical gauze was obtained from a drugstore in Beijing. Epichlorohydrin and acetic acid were of analytical grade and obtained from the Chemical Reagent Company of Beijing. Beef peptone, beef extract powder, yeast extract powder and agar were of biochemical grade. *E. coli*, ATCC 25922; *A. niger*, ATCC 16404; *C. albican*, ATCC 10231 were obtained from the American Type Culture Collection. They were cultured with LB medium, Czapek's agar and wort agar, respectively. All the culture media were sterilized with normal autoclaving before being used. Slide and Petri dishes with 60 mm diameter were used. Deionized water was used in all experiments.

2. Preparation

2-1. Preparation of the Chitosan/nano-TiO_2 Composite Emulsion (CTCE)

0.1 g chitosan was dissolved in 2.5% acetic acid solution, epichlorohydrin was added into the above emulsion with stirring for 10 min at room temperature. 0.05 g TiO_2 was then added into above emulsion under stirring for 2 h. TiO_2 powder was homogeneously dispersed by ultrasonic vibrator for 15-20 min.

2-2. Preparation of the Antibacterial Gauze

Each gauze sample (5×5 cm) was soaked by chitosan emulsion, nano-TiO_2 emulsion and chitosan/nano-TiO_2 composite emulsion for 1 h, respectively. After natural drying, different treated gauze samples were obtained.

3. Test of Antibacterial Activity

3-1. Shake-flask Method [3]

Treated gauze or untreated gauze was dipped into a flask containing 100 mL normal saline (0.9% saline) with a cell concentration of $2.5\text{-}4.0\times10^3$/mL for *E. coli* or *C. albican* ($0.5\text{-}1.0\times10^3$/mL for *A. niger*). The flask was then shaken at 170 rpm/min on a rotary shaker at 27 °C. The antibacterial efficacy was determined by spreading 0.2 mL appropriate dilution of strain suspension on agar plate which was obtained after photocatalysis in the dark and incubated for 48 h. Afterwards, the number of colonies on the plate was counted. Antimicrobial efficacy was determined based on duplicated test results. The bactericidal ratio was calculated as follows:

$$\text{Bactericidal ratio}=(N_0-N_1)/N_0\times100\% \qquad (1)$$

Where, N_0 and N_1 are the number of colonies of the strain suspension at initial and at irradiation time, respectively.

3-2. Antibacterial Test According to AATCC-100

0.2 mL normal saline containing *E. coli* was spread on the surface of gauze samples. Culture medium was added. After being incubated for 24 h, the number of colonies on the gauze sample was counted.

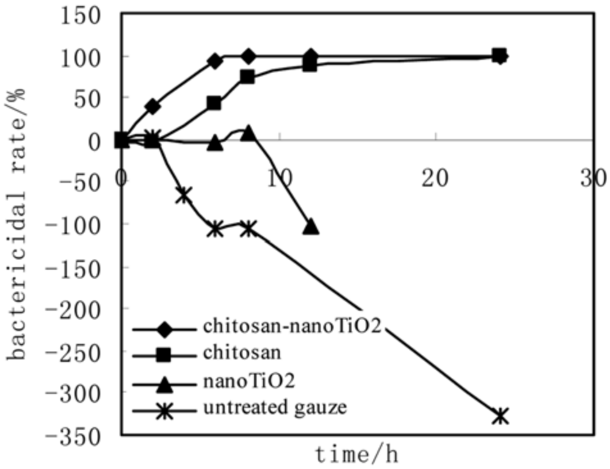
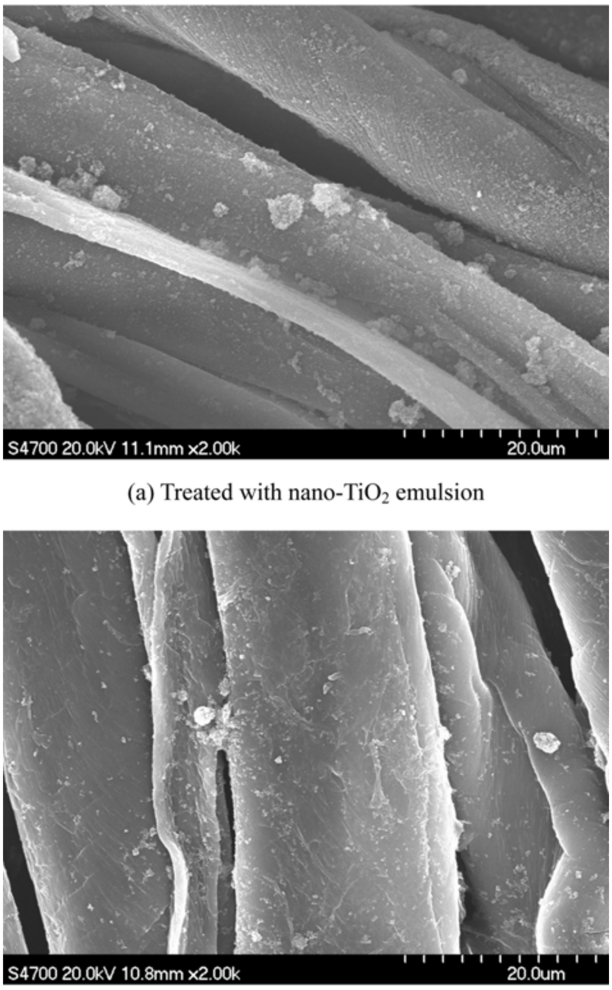


Fig. 1. Comparison of CTCE with other antibacterial agents.



(a) Treated with nano-TiO_2 emulsion

(b) Treated with chitosan/nano-TiO_2 composite emulsion

Fig. 2. SEM images of (a) before and (b) after adding the chitosan to treated the gauze sample.

RESULTS AND DISCUSSION

1. Antibacterial Activities of CTCE

1-1. Comparison with Other Antibacterial Agents

Different gauzes were treated with chitosan/nano-TiO_2 composite emulsion, chitosan and nano-TiO_2, respectively. The comparisons of their antibacterial activities are shown in Fig. 1. The number of colonies of *E. coli* in the blank control group was doubled after 10 h, and increased by 3-4 times 24 h later. No obvious antibacterial activity of the gauze treated with nano-TiO_2 emulsion was observed. The reasons were probably that the electronic hole induced by photocatalysis was filled with ions in aqueous solution [6]. Another reason could be that the nano-TiO_2 was easily reunited in aqueous solution, and nano-TiO_2 powder sticking on the gauze sample was easily shed. These reasons may cause an unfavorable outcome. The gauze treated with chitosan emulsion had better antibacterial activity. But compared with the chitosan/nano-TiO_2 composite emulsion, the bactericidal ratios of the chitosan emulsion apparently declined. As can be seen from Fig. 1, the bactericidal ratio of the chitosan/nano-TiO_2 composite emulsion was very quick and reached 92.3% after 6 h. The SEM images of gauzes treated with nano-TiO_2 and chitosan/nano-TiO_2 composite emulsion are shown in Fig. 2. The surface of the fiber is rough and the nano-TiO_2 distinctly reunited in picture (a). On the contrary, in picture (b) the surface of the fiber is smooth and the nano-TiO_2 well dispersed. It indicates that the presence of chitosan increased the dispersion of the nano-TiO_2 powder in solution. At the same time, the functional group (-NH_2) on the chitosan could prevent to reunite the electronic hole with ions on the nano-TiO_2 surface. But this needs to be further researched.

1-2. Antibacterial Stability of CTCE

The antibacterial stability of chitosan/nano-TiO_2 composite emulsion was studied. The results indicated that the composite emulsion had excellent antibacterial activity: the bactericidal ratio could reach 100% after 24 h and be unchanged in the inspection time of 84 h. This demonstrated that the chitosan/nano-TiO_2 composite emulsion could really kill the bacteria, not just inhibit its growth (see Fig. 3).

1-3. Influence of Temperature on the Antibacterial Efficiency

As shown in Fig. 4, the bactericidal ratio reached 70.3% at 27 ºC after 1 h, and 98.8% after 8 h; however the bactericidal ratio only reached 13.2% at 18 ºC after 1 h, and 98.2% 24 h later. The bactericidal ratio at 27 ºC was obviously faster than at 18 ºC. Generally speaking, the appropriate growing temperature for bacteria is about 30-40 ºC. The activity of bacteria and the adaptability to the environment would be changed with the temperature changing. The activity of bacteria was weakened at low temperature, and was not sensitive to the change of environment, which is why the bactericidal function at 27 ºC presented better than at 18 ºC.

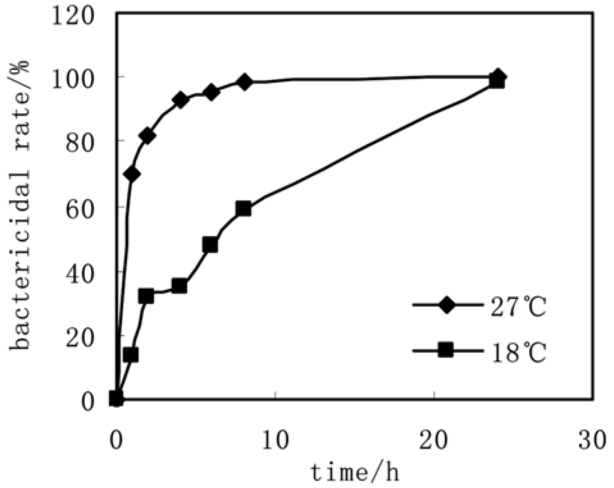


Fig. 4. The influence of temperature on the antibacterial efficiency.
(*E. coli* 170 rpm/min)

1-4. Influence of Washing Time

The practicability and stability of the chitosan/nano-TiO_2 composite emulsion were directly influenced by the combinative ability between composite emulsions with the textile fiber. After the antibacterial gauzes were used, they were immersed into deionized water and kept shaking at 170 rpm/min on a rotary shaker at 27 ºC for 1 h, 2 h, 4 h, and 6 h, respectively. Afterwards, the gauzes were taken out and contaminated with *E. coli*; the antibacterial efficacy was checked again by shake-flask method. The results indicated that the antibacterial capabilities were not markedly influenced by the washing time (see Fig. 5). The bactericidal ratio reached more than 90% after 4 h. Therefore, the antibacterial gauze has a better stability and could be washed a long time without losing its antibacterial ability.

2. The Batches for Reusing

2-1. Influence of Contaminated Batches

The antibacterial gauze had excellent antibacterial activity for *E.*

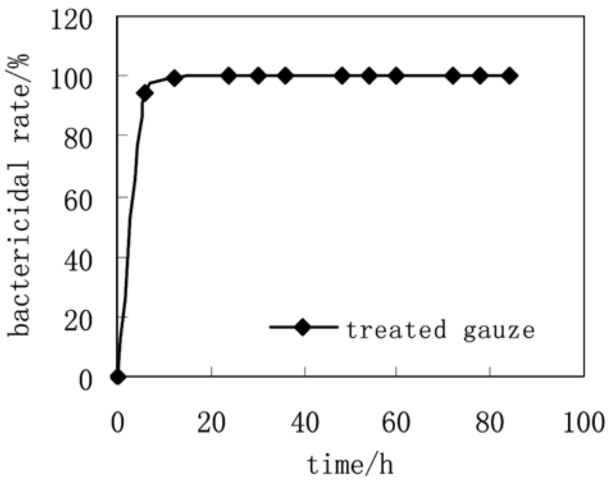


Fig. 3. Antibacterial stability of CTCE.
(*E. coli* 27 ºC, 170 rpm/min)

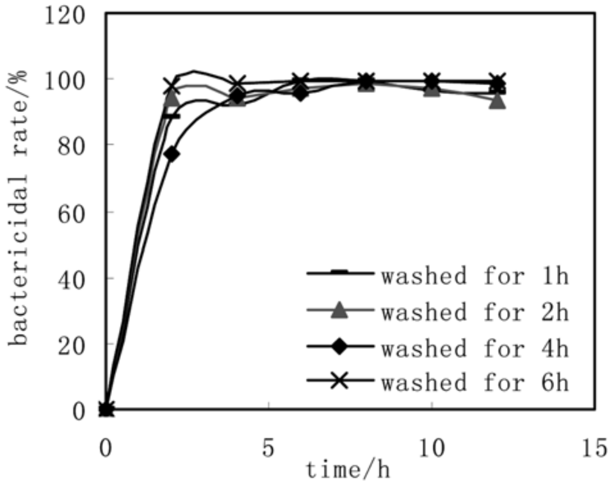


Fig. 5. Influence of washing time on the antibacterial activities.
(*E. coli* 27 ºC, 170 rpm/min)

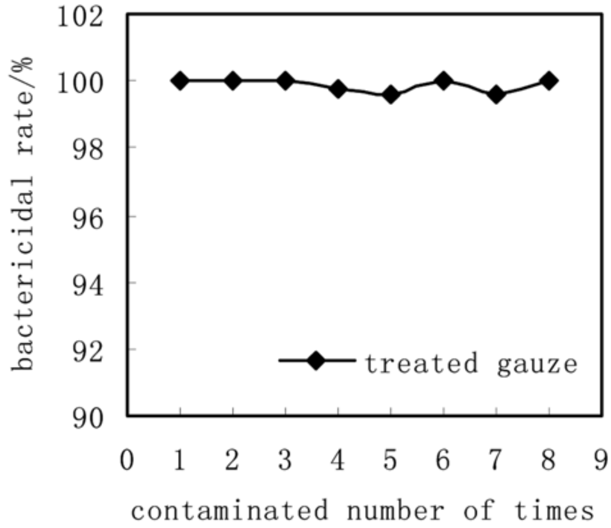


Fig. 6. Influence of contaminated batches on the antibacterial activities.

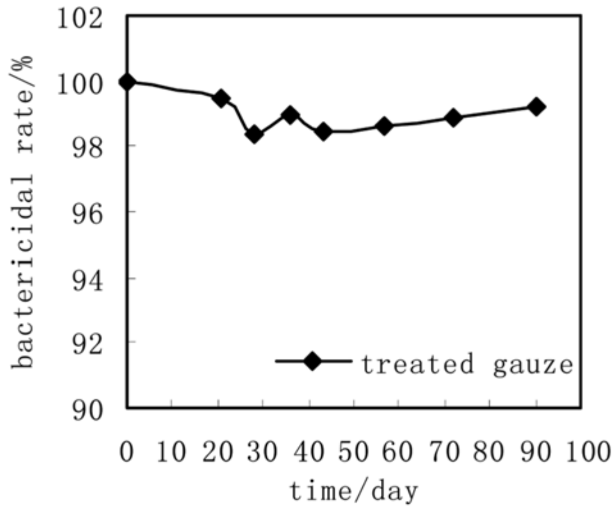


Fig. 7. Influence of storage time on the antibacterial activities.

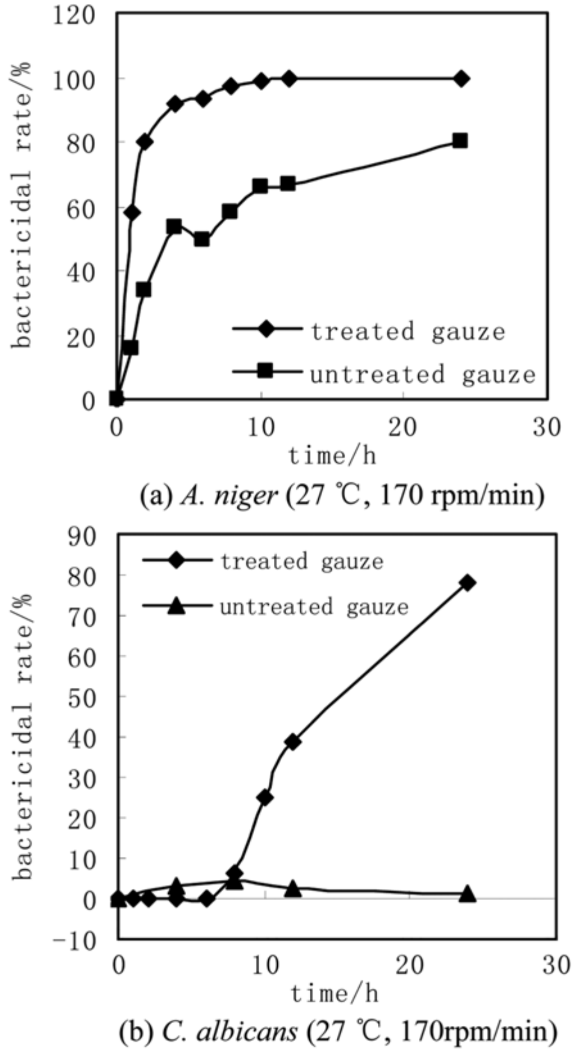


Fig. 8. Antibacterial effect on other bacteria.

coli, and could be reused for up to 8 times according to AATCC-100 without loss of performance. As can be seen from Fig. 6, the bactericidal ratios for *E. coli* could still reach more than 99.5% when the gauze had been contaminated after eight times.

2-2. Stability of the CTCE on Storage

The stability of an antibacterial product is very important. Hereby, the antibacterial activity for *E. coli* of the chitosan/nano-TiO_2 composite emulsion was tested according to AATCC-100. The period of inspection was 90 days. As seen from Fig. 7, the antibacterial activity was not markedly influenced by the storage time. All of the bactericidal ratios could reach 98.33% in the whole period of inspection. It indicated that gauze treated with the chitosan/nano-TiO_2 composite emulsion was stable under natural conditions, so the treated gauze could be stored for a long time.

3. Antibacterial Activities for Other Bacteria

The antibacterial activities against *A. niger* and *C. albicans* are also shown in Fig. 8. The bactericidal ratios for *A. niger* could reach more than 90% after 4 h. However, the number of colonies in the blank control group also declined with the increase of time. The potential reason was that the optimum pH value for the growth of mold is about 5 to 6. But the pH value of normal saline in the blank control group is about 7, so it may be harmful to the growth of *A. niger*, and some parts of the cells may get autolysis or death with the time increased, eventually causing a reduction of the number of *A. niger*. And for *C. albicans*, the bactericidal ratio almost reached 80% after 24 h. The number of colonies in the blank control group was almost constant in 24 h, because the normal saline is a lack of nutrition for the growth of *C. albicans,* the cell division and cell death should be kept in balance under the isotonic condition. It is known that *C. albicans* belongs to gram-positive bacteria, which has a thicker cell wall structure than that of the gram-negative bacteria. Hereby, killing the *C. albicans* is more difficult than the *E. coli*. Specifically, ($-NH_3^+$) on the chitosan molecules adsorbed on the surface of bacterial cells could cause asymmetric distribution to the negative charge on the cell wall and cell membrane of the gram-positive bacteria. As a result, the synthesis of the cell wall was destructed, and then the bacteria were finally killed. Compared to gram-negative bacteria, $-NH_3^+$ directly penetrated into the gram-negative bacteria cells (*E. coli* and *A. niger*) and complexed with anion material in cells. Consequently, the normal physiological activities of cells were disrupted and the bacteria were finally killed [2].

CONCLUSIONS

In this paper, a chitosan/nano-TiO_2 composite emulsion has been designed as a novel antibacterial coating for textiles. It was prepared via inverse suspension technology and its multi-function properties were studied. The gauze treated with chitosan/nano-TiO_2 composite emulsion showed broad-spectrum and stabilized antibacterial properties against *E. coli, A. niger* and *C. albicans*. And the antibacterial activity was hardly influenced with the increase of contamination time or storage time. Using the excellent functions of the gauze to explore green textiles would be a new development.

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