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Carbohydrate Research 343 (2008) 230–237

Carbohydrate RESEARCH

Preparation of chitosan–nylon-6 blended membranes containing silver ions as antibacterial materials

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Received 23 July 2007; received in revised form 31 October 2007; accepted 5 November 2007

Abstract—Chitosan–nylon-6 blended membranes were prepared by combining solvent evaporation and a phase inversion technique, and then used to chelate silver ions. Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) were used to study the antibacterial properties of the membranes. Fourier-transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC) indicated hydrogen-bond interactions between chitosan and nylon-6. From the scanning electron microscopy (SEM) pictures, it was observed that with the increase of nylon-6 content, the blended membrane gradually became a material with porous morphology. After chelating silver ions, the tensile strength of the membranes increased. The antibacterial activity with the variation of chitosan content, the pH value and the concentration of the silver nitrate solution used to prepare Ag^+-loaded membranes were investigated systematically. The results indicated that the chitosan–nylon-6 blended membranes with Ag^+ were antibacterial to both Gram-positive bacteria and Gram-negative bacteria. The antibacterial activity improved with the increased chitosan content due to the larger amount of silver ions loaded. The antibacterial property of the chitosan–nylon-6 blended membranes could be primarily attributed to the content of chitosan and silver ions as well as the surface morphology of the membranes.

Keywords: Chitosan; Nylon-6; Silver ions; Antibacterial property

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1. Introduction

Chitosan is a natural nontoxic biopolymer derived by the deacetylation of chitin. Owing to its biocompatibility, biodegradability, antibacterial and antifungal activities, chitosan has become, in the past 20 years, a much sought-after material for a variety of applications including biomedical devices, in wound healing, controlled drug delivery,[1] food packing,[2] heavy metal chelating agents[3,4] and waste removal.[5,6] To extend the applications of chitosan, several approaches have been used for the modification of chitosan, such as graft polymerization[7] and blending with other natural or synthetic polymers.[8,9] In particular, modification of chitosan by means of blending with other polymers is a convenient and effective method for practical utilization.[10–13] However, little attention focuses on the effects of blending on the antibacterial properties of chitosan.[14]

The antibacterial activity of chitosan, arising from its polycationic nature, is well known for a variety of bacteria and fungi.[15] The interaction between the positively charged chitosan and the negatively charged microbial cell wall leads to the leakage of the intracellular constituents. The binding of chitosan with DNA and the inhibition of mRNA synthesis occurs via the penetration of chitosan into the nuclei of the microorganisms and interfering with the synthesis of mRNA and proteins.

However, the antibacterial activity of chitosan is variable and is affected by many factors such as molecular weight, pH value, and water solubility.[16,17] Also, chitosan shows antibacterial activity only in an acid medium,

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0008-6215/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2007.11.006

which is usually ascribed to the antibacterial mechanism and the poor solubility of chitosan at high pH value.[18,19] In addition, chitosan solution shows more effective antibacterial activity than chitosan membranes, which restricts its convenient usage in the field of packing and biomedical devices.

It is well known that chitosan has excellent metal-binding capacities, such as chelating silver ions.[20,21] Silver ions have powerful antibacterial activity and are widely used as some antibacterial agents and biomedical materials.[15] It is accepted that the amine groups in the chitosan molecules are mainly responsible for the uptake of metal cations by a chelation mechanism,[7,22] though hydroxyl groups (especially in the C-3 position) may also contribute to the sorption. In general, positive ions are bound to chitosan at high pH value, since the amine groups are unprotonated and the electron pair on the amine nitrogen is available for donation to silver ions. At low pH value, the anions are not bound to chitosan, since the amine group is protonated.[5]

Nylon is widely used in food packing due to its good gas-tightness, good stability and good wear resistance.[23] As we know, in the food industry, food quality and safety are major concerns since consumers prefer fresher and minimally processed products. To maintain food quality, the packing film carrying food additives such as antioxidants, antimicrobial, colorants, flavors, fortified nutrients and spices is one of the most effective methods available.[24]

The aim of this study is to extend the application of chitosan by blending it with nylon-6 and then endow the matrix with antibacterial properties by chelating with silver ions, which may then be used in food packing. Thus, in the present study, chitosan is blended with nylon-6 to prepare membranes by combining solvent evaporation and a phase inversion technique, and then silver ions are chelated to the membranes. The antibacterial properties of the membranes are then systematically investigated.

2. Experimental

2.1. Materials

Chitosan (M_w, 10,000; deacetylation degree of 90%) was purchased from Boao Technology Co. Ltd (Shanghai, China). Nylon-6 was donated by the State Key Laboratory of Polymer Materials Engineering (Sichuan University). All the other reagents were obtained from the Chemical Reagent Factory of Kelong, China, which were of analytical grade, and used without further purification. The evaluation with microorganisms was carried out in the Institute of Life Science of Sichuan University.

2.2. Preparation of membranes

Required amounts of chitosan and nylon-6 were dissolved in aq formic acid (90% HCO_2H). Then, the solution was spread on a glass plate for the solvent evaporation at room temperature for about 72 h. After peeling off the plates, the membranes were immersed into sodium hydroxide solution (2 M) to neutralize the residual acid. After washing thoroughly using copious amounts of distilled water, the membranes were dried at room temperature.

Chitosan–nylon-6 blended membranes containing Ag^+ were prepared by immersing the chitosan–nylon-6 blended membranes into $AgNO_3$ solutions of different concentrations (from 0.2 mmol/L to 1.4 mmol/L) and pH values (from pH 4 to 8), with stirring for 4 h, followed by washing thoroughly with copious amounts of distilled water. Finally, the membranes were dried at room temperature.

The samples were designated as CN10, CN30 and CN70, which indicated that the nylon-6 content was 10%, 30% and 70%, respectively, in the blended membranes before chelating with silver ions. The thickness of the membranes was about 250 μm, which was determined by a thickness tester.

2.3. Characterization of the membranes

To investigate the interaction between chitosan and nylon-6, an FTIR spectrum of the blended membrane was obtained using a Fourier-transform instrument (Nicolet-560, Nicolet Co. USA). For scanning electron microscopy (SEM) observation, the samples were dried at room temperature, and then attached to the sample supports and coated with a gold layer. The SEM images were recorded using an S-2500C microscope (Hitachi, Japan). Sensitive differential scanning calorimetry (DSC, EXSTAR 6000) was used to study the thermal stability of the chitosan–nylon-6 blended membranes. The samples were sealed into aluminum pans, and an empty pan was used as a reference. The temperature was raised from 40 °C to 300 °C at a heating rate of 10 °C/min.

Tensile testing was performed on each type of membrane as previously reported.[25] Briefly, the tensile strength of the membranes (dumbbell-shaped specimens, width 4 mm) were determined at room temperature with a universal tensile testing machine (AG-10TA) operated at a cross-head speed of 50 mm/min, pre-load of 1 kg, and grip-to-grip gap of 30 mm. For each type of membrane, five specimens were used.

2.4. Antibacterial activity tests

The antibacterial activity of the membranes was tested by an inhibition zone method.[26] In the method, two kinds of different bacteria including Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) were used.

For measurement of the antibacterial activity, all membranes were kept in distilled water for 1 h. Then, the membrane samples were punched to make disks (diameter = 8 mm), and the antibacterial activity was determined by a modified agar diffusion assay (disk test). The plates were examined for possible clear zones after incubation at 37 °C for 24 h. The presence of any clear zone that formed around the film disk on the plate medium was recorded as an indication of inhibition against the microbial species. Suspension cultures without any membranes were used as a control. The effect of the chitosan content in the blended membranes, the pH value and the concentration of the silver nitrate solution used for preparing the silver-ions-loaded membranes was investigated.

3. Results and discussion

3.1. Fourier transform infrared spectroscopy (FTIR) spectra

The FTIR spectra of the chitosan–nylon-6 blended membranes without silver ions are shown in Figure 1 to illustrate the interaction between the chitosan and nylon-6. Firstly, attention must be paid to the displacement of the amide I band of pure nylon-6, located at 1641.40 cm^{-1} toward smaller wavenumbers (lower energy) with an increase in chitosan content. Meanwhile, in the curve of CN30, a new peak appeared at about 1585.49 cm^{-1}, which may be attributed to the effect of hydrogen bonds formed between chitosan and nylon-6. According to the former literature,[26] the inter-hydrogen bonds formed between two different macromolecules are stronger than those formed between the molecules of the same polymer. Thereby, the inter-hydrogen bonds between chitosan and nylon were prone to formation.

To study the chelation between chitosan and silver ions, the spectra of the membranes containing silver ions were studied, as shown in Figure 2. It was revealed that with the addition of silver ions, the peak for the amide I band of chitosan shifted toward smaller wavenumbers. For pure chitosan and chitosan–Ag^+, the displacement number was about 33 cm^{-1} (from 1623.03 cm^{-1} to 1590.38 cm^{-1}), which indicated the strong interaction between chitosan and silver ions. Maybe the 'inductive effect' caused by the coordination interaction between NH_2 groups of the chitosan and silver ions led to the shift of the above peaks. Similarly, for the CN30 membranes, the shift in wavenumbers (from 1585.49 cm^{-1} to 1581.84 cm^{-1}) was also observed after chelating silver ions, but the shifted number was smaller than that for the pure chitosan. From the above data, it is suggested that the coordination interaction between chitosan and silver ions becomes stronger with an increase in chitosan content.

3.2. Surface morphology of the membranes

Figure 3 shows the scanning electron micrographs (SEM) of the prepared membranes. The pure chitosan membrane displayed a flat dense surface (Fig. 3a). With an increase in the amount of nylon-6 in the blended

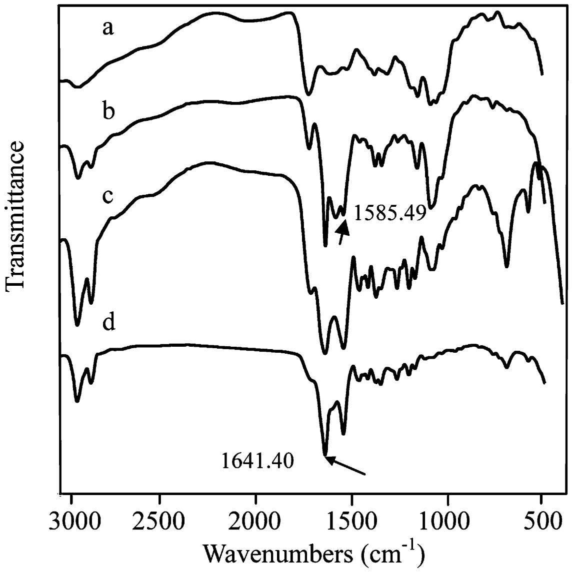

Figure 1. FTIR spectra of (a) pure chitosan membrane; (b) CN30 membrane; (c) CN70 membrane; and (d) pure nylon-6 membrane.

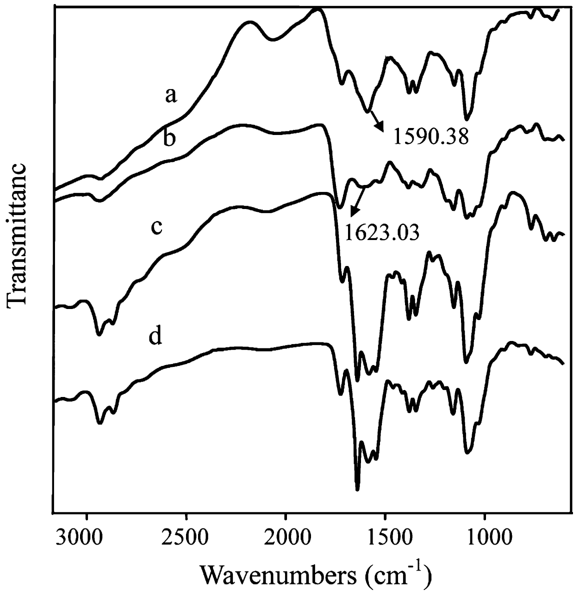

Figure 2. FTIR spectra of (a) chitosan–Ag^+ membrane; (b) chitosan membrane; (c) CN30–Ag^+ membrane; and (d) CN30 membrane.

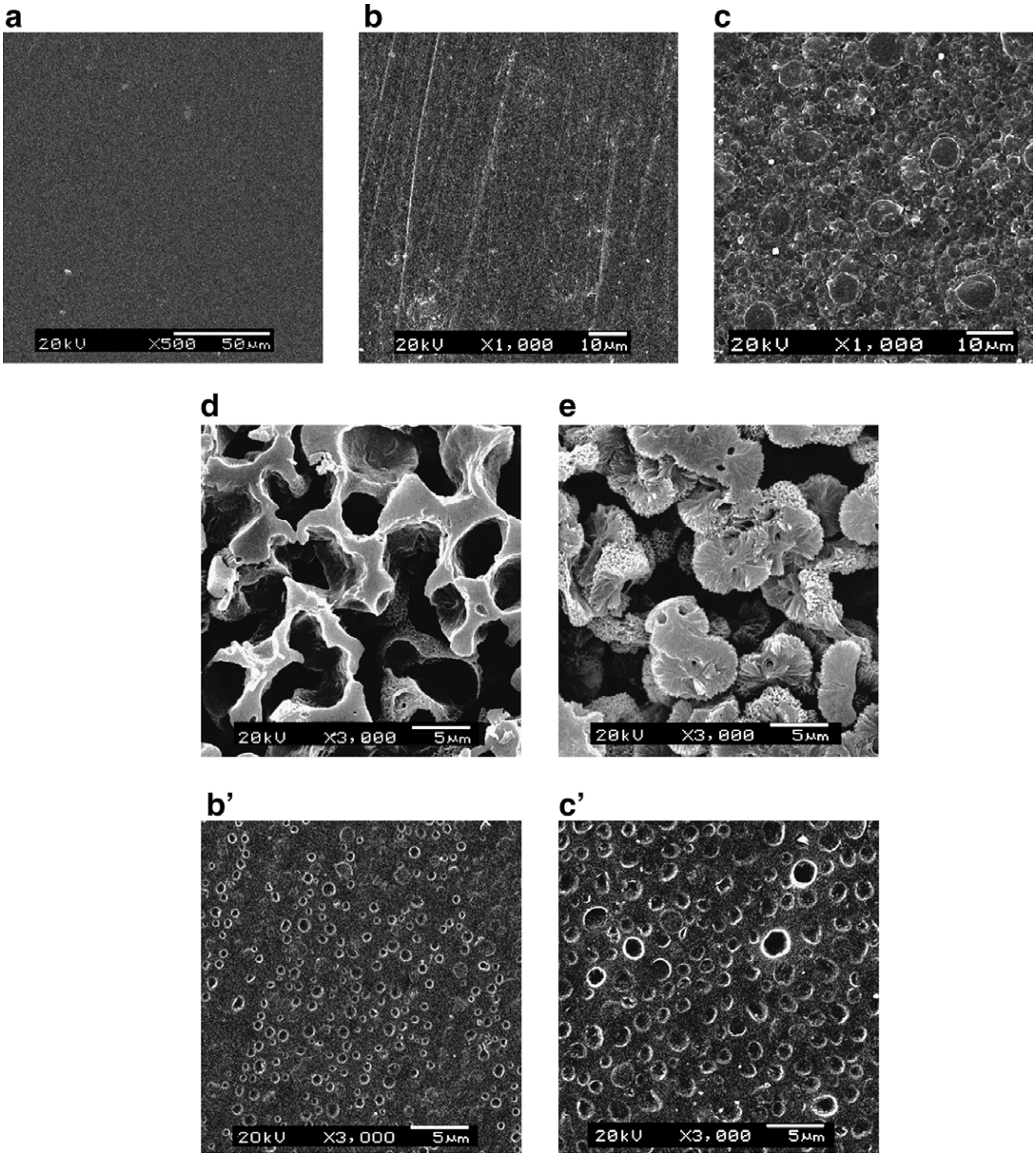

Figure 3. The scanning electron micrographs of the top surface of the prepared membranes. (a) Pure chitosan; (b) CN10; (c) CN30; (d) CN70; (e) pure nylon-6; (b′) CN10–Ag^+; and (c′) CN30–Ag^+.

membranes, the smooth surface gradually disappeared. The flat dense surface of the chitosan membrane became progressively undulating. This result is in accord with the previous report.[26] For the blended membrane CN30, a scattered round plaque-like structure was observed as shown in Figure 3c, which was possibly due to the immiscibility of the two polymers.[27] When the chitosan ratio increased to 70%, as the membrane CN70, a porous structure was observed, as shown in Figure 3d. For the pure nylon-6 membrane, it showed a particular morphology (Fig. 3e), which might be called a 'snowflake' structure. These were caused by the phase inversion when the membranes were immersed in sodium hydroxide after the solvent evaporation, since the solvent was not vaporized completely for 72 h. When silver ions were incorporated into the membranes, lipid droplet-like dents were observed on the membrane surfaces. Generally, the membrane structure could be modulated by the variation of the chitosan ratios in the blended polymers.

3.3. Thermal analysis of the membranes

To study the thermal properties of the chitosan–nylon-6 blended membranes, the differential scanning calorimetry (DSC) curve (first scan) of the prepared membranes is shown in Figure 4. Glass transition temperatures (T_g) were not observed both for the pure chitosan and nylon-6. A broad endotherm was observed around 60 °C for the chitosan–Ag^+ membrane, which is

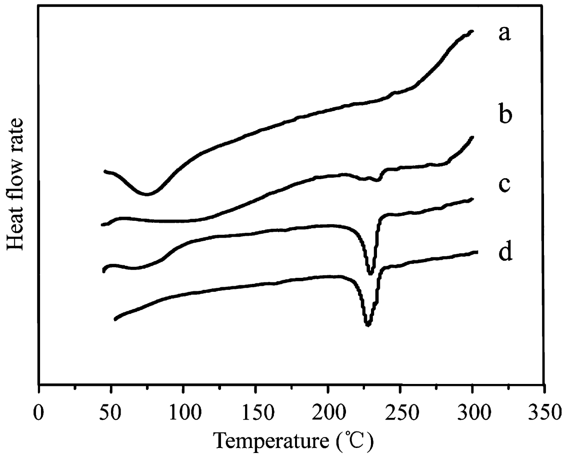

Figure 4. The DSC curves (first scan) of the blended membranes. (a) Chitosan–Ag^+; (b) CN30–Ag^+; (c) CN70–Ag^+; and (d) nylon-6.

an endothermic peak that exists for most polysaccharides at low moisture contents.[28] In the nylon-6 curve, a melting endothermic peak was observed at 225 °C, which was associated with the crystalline polymer fraction. The characteristic peak of chitosan at about 60 °C became smaller with increasing nylon-6 content, and the melting peak of nylon at 225 °C became smaller with decreasing the nylon-6 content, but no obvious change in the melting temperature was observed. This indicated that the nylon crystallization decreased after blending with chitosan, and there was interaction between chitosan and nylon-6. From the FTIR and DSC analysis, it is suggested that inter-hydrogen bonds exist between chitosan and nylon.

3.4. Tensile strength of the membranes

Figure 5 shows the tensile strength of the prepared membranes. As shown in the figure, it is clearly observed that all the membranes containing silver ions displayed an increased tensile strength over that of the corresponding membranes without silver ions, which may be attributed to the strong interaction between chitosan and silver ions. As illustrated in the FTIR analysis (Fig. 2), the coordination bonds formed between NH_2 groups of chitosan and silver ions made the inter-molecule interaction more intense, and thus improved the tensile strength.

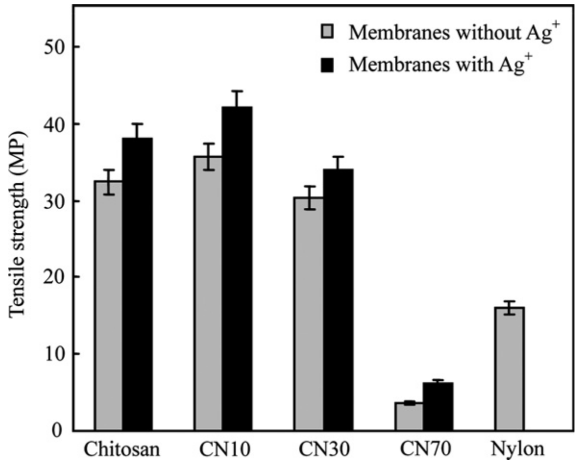

Figure 5. Tensile strength of the blended membranes. Data are expressed as the means ± SD, $n = 5$.

When the nylon content is lower than 30% in the blended membranes, the difference of the tensile strength is not distinct; however, the CN10–Ag^+ membrane showed increased tensile strength, with the values of 42.08 MPa. Besides the coordination interaction between chitosan and silver ions, there were inter-hydrogen bonds between chitosan and nylon, which made the molecules tight binding, so the tensile strength increased. With more nylon-6 incorporated, the strength decreased, which could be due to the formation of the porous structure induced by the preparation process, as shown in SEM photos (Fig. 3d and e). Similarly, the tensile strength obtained for pure nylon-6 was lower than the theoretical value (40–60 MPa), which could also be attributed to the snow-like structure formed in the preparation process. As a result, the proper hydrogen bond formed between chitosan and nylon can improve the mechanical strength of the blended membranes, and the dense and uniform structures are beneficial in maintaining higher mechanical strengths.

3.5. Antibacterial activity of the membranes

3.5.1. Effect of the chitosan content. Figure 6 shows the photographs of the antibacterial test. It is clearly observed that the membranes loaded with silver ions showed antibacterial activity against both *S. aureus* and *E. coli*. The effect of chitosan content in the Ag^+-loaded membranes on the antibacterial activity against *S. aureus* and *E. coli* as determined by the disk method is shown in Figure 7. As shown in the figure, the inhibition zone diameter became larger with more chitosan incorporated, and when the chitosan content was more than 70%, the differences of the antibacterial activity were not obvious. However, the nylon-6 membrane did not exhibit antibacterial activity, even after immersing in silver nitrate solution, since there was no interaction between nylon-6 and Ag^+. In the test, pure chitosan membrane without silver ions showed small inhibition zone, which could be due to the decreased amount of the positive charge of chitosan after being partly neutralized with sodium hydroxide; thus the interaction with the negative cell wall of the bacteria became weaker. It is well known that chitosan has antibacterial activity, and the antibacterial activity of chitosan membrane is usually lower than that for chitosan solution. After incorporating silver ions, the antibacterial activity of

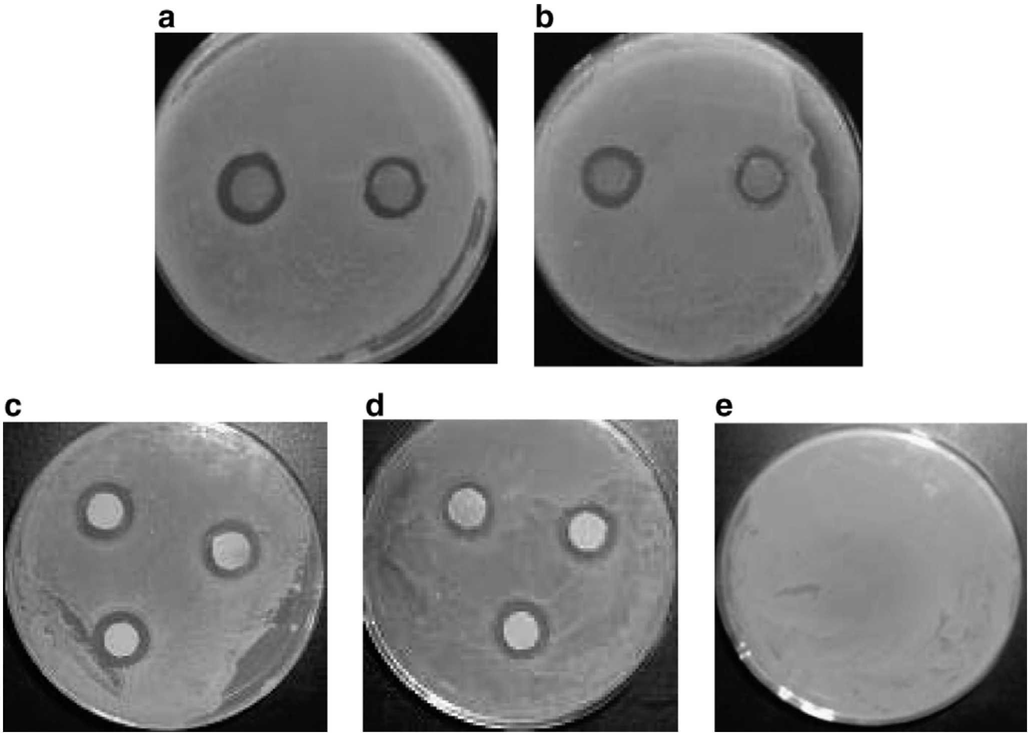

Figure 6. Photographs of the antibacterial test results of the blended membranes with different chitosan content against *E. coli* (a, c) and *S. aureus* (b, d). (a) Chitosan–Ag^+ membrane against *E. coli*; (b) chitosan–Ag^+ membrane against *S. aureus*; (c) CN30–Ag^+ membrane against *E. coli*; (d) CN30–Ag^+ membrane against *S. aureus*; and (e) agar plate incorporated bacteria without any membrane as control.

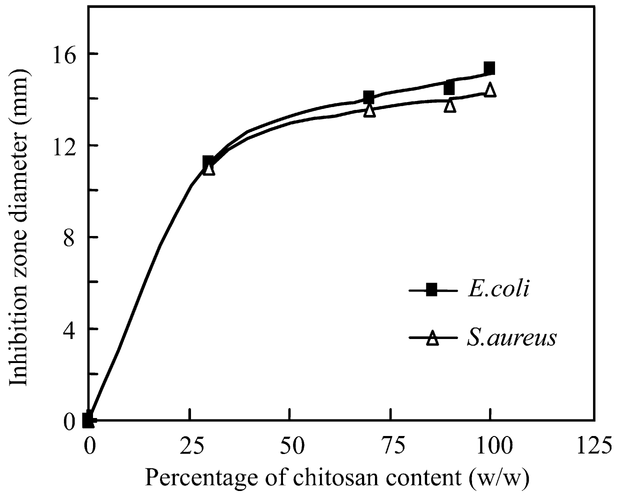

Figure 7. Effect of chitosan content in the chitosan–nylon-6 blended membranes on the antibacterial activity against both *E. coli* and *S. aureus*. (The pH value and concentration of the silver nitrate solution used to treat the membranes were 6 and 0.5 mmol/L, respectively.) Duplicate experiments gave similar results.

chitosan greatly increased, and the inhibition zone diameter increased to about two times larger (data not shown). As we know, silver ions have effective antibacterial activity. The increased antibacterial activity of the Ag^+-incorporated membranes was caused by the Ag^+. It is reported that Ag^+ adheres to the negative charged cell wall, changing the cell wall permeability. This action, coupled with protein denaturation, induces cell lysis and death.[29] The antibacterial activity of Ag^+ is also related to its ability to modify the DNA replication mechanisms as well as to cause abnormalities in the size, cytoplasmic contents, cell membranes, and outer cell layers of sensitive cells.[30–32]

For further understanding of the effect of silver ions on antibacterial activity, the amount of Ag^+ in the blended membranes was investigated as shown in Figure 8. It was clearly observed that with the increase of nylon-6 content in the membranes, the amount of loaded silver ions decreased. Combined with Figure 7, it was easy to conclude that the silver ions had a great effect on the antibacterial activity, which further proved the point of the above discussion. In addition, the morphology of the chitosan–nylon-6 blended membranes also affected the antibacterial activity. With an increase in the nylon-6 content of the membranes, the flat dense surface of the chitosan membrane became progressively undulating, which might be conducive to bacterial adhesion and colonization. This was in accord with the findings of the previous research: the rougher surfaces may be more conducive to cellular and bacterial colonization.[14,33,34] In a word, after incorporating silver ions, the antibacterial activity of the chitosan–nylon-6 blended membrane was improved greatly. In addition, the antibacterial activity is affected by the content of

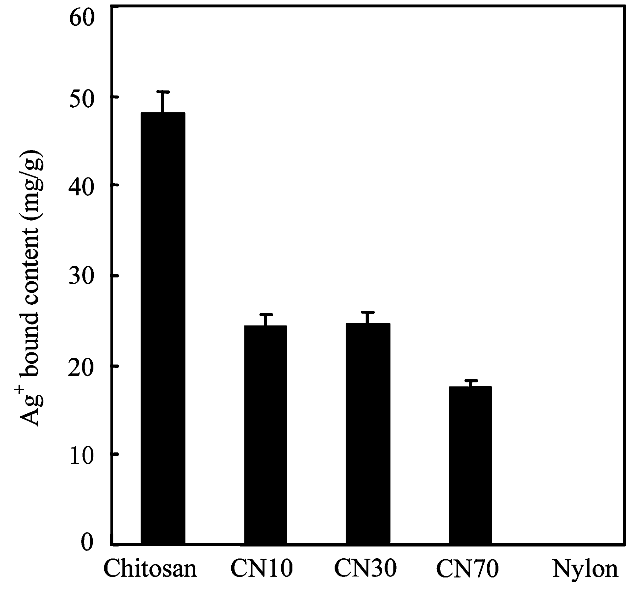

Figure 8. The amount of the silver ions bound to the chitosan–nylon-6 blended membranes after immersing into silver nitrate solution (pH 6 and a concentration of 0.5 mmol/L). Data are expressed as the means ± SD, $n = 3$.

chitosan and the morphology of the chitosan–nylon-6 blended membranes.

3.5.2. Effect of the pH value of the silver nitrate solution.

Since silver ions had a great effect on the antibacterial activity, we examined the effect of the silver nitrate solution used to treat the CN30 membrane on the antibacterial property. Figure 9 shows the antibacterial activity of the CN30–Ag$^+$ membrane as a function of the pH value of the silver nitrate solution. As shown in the figure, the membrane exhibited the best antibacterial activity at pH 6. Chitosan is a weak polybase with a pK_a of 6.5,[28] which may be more suitable for the unprotonated amine nitrogen to donate the electron pair to silver ions. Thus, larger amounts of the silver ions were incorporated to the membrane, and the antibacterial activity improved.

3.5.3. Effect of the concentration of the silver nitrate solution.

Figure 10 shows the effect of the concentration of the silver nitrate solution used to prepare the Ag$^+$-loaded membranes on the antibacterial activity. As shown in the figure, when the concentration of the silver nitrate solution increased from 0.2 to 0.5 mmol/L, the antibacterial activity increased. However, there seemed no significant difference when the concentration increased over 0.5 mmol/L.

As shown in Figure 8, when the concentration of the silver nitrate solution was 0.5 mmol/L, the Ag$^+$-bound capacity in the pure chitosan membrane was 48.2 mg per gram of chitosan, which is in agreement with the previous report.[24] But for the CN30 membrane, the Ag$^+$-bound capacity is lower than that of the pure chitosan membrane, with a value of 26.6 mg per gram of chitosan. This is probably attributed to the hydrogen bonds formed between NH_2 groups of chitosan and nylon, which served to decrease the number of NH_2 groups in chitosan used for coordination with silver ions. The data indicate that the Ag$^+$-incorporated in the CN30 membrane may have reached saturation, and no obvious difference of the antibacterial activity was observed when the concentration of the silver nitrate solution was increased to over 0.5 mmol/L.

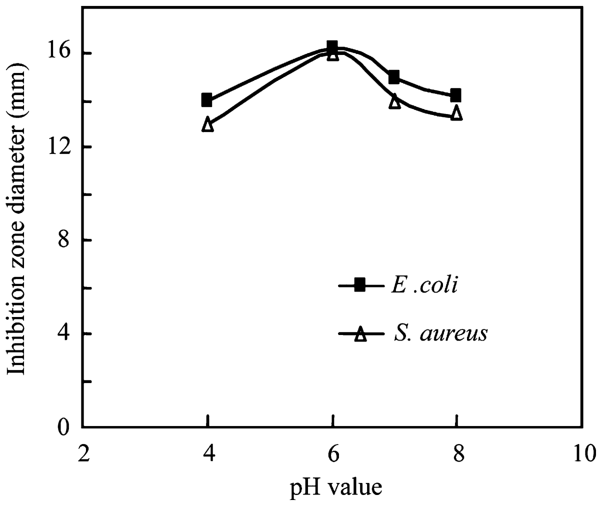

Figure 9. Antibacterial activity of the CN30–Ag$^+$ membrane with the variation of pH values of the silver nitrate solution (concentration of 0.5 mmol/L) used to treat the membranes. Duplicate experiments gave similar results.

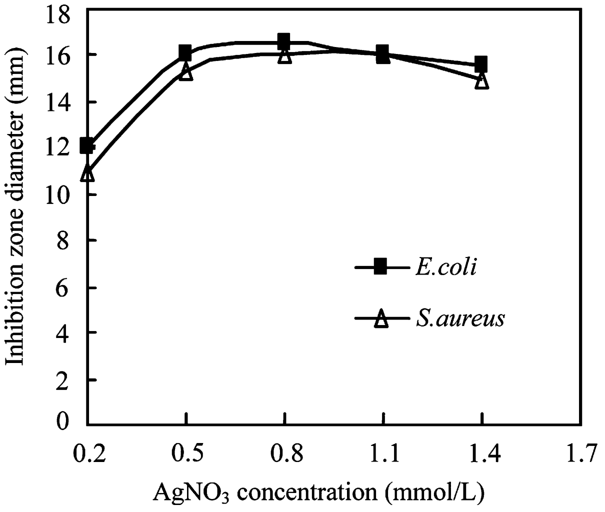

Figure 10. Antibacterial activity of the CN30–Ag$^+$ membrane with the variation of concentration of the silver nitrate solution (pH value of 6) used to treat the membranes. Duplicate experiments gave similar results.

In conclusion, the content of chitosan and silver ions incorporated in the blended membranes has a great effect on the antibacterial activity. Thus, the pH value and the concentration of the silver nitrate solution, which can affect the chelated content of silver ions in the blended membranes, affects the antibacterial activity.

4. Conclusions

In summary, all the chitosan–nylon-6 blended membranes containing silver ions were antibacterial to both Gram-negative bacteria (*E. coil*) and Gram-positive bacteria (*S. aureus*), and there was no significant difference between the two types. With increased nylon-6 content, the antibacterial property of the blended membranes decreased, which may be attributed to the decreased chitosan and Ag^+ content in the blended membranes. Besides, the surface morphology of the blended membranes may also affect the antibacterial activity of the blended membranes. From the mechanical analysis, it is clear that the tensile strength of the membranes increased after incorporating Ag^+. In this study, a simple way was proposed to extend the application of chitosan by blending with nylon-6 and endow the material with antibacterial properties that render it useful for food packing. Of course, by blending chitosan with other synthetic polymers, new food packing materials with antibacterial activity may be obtained.

Acknowledgments

This work was financially sponsored by the National Natural Science Foundation of China (Nos. 50403028, 50673064).

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