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Novel starch/chitosan blending membrane: Antibacterial, permeable and mechanical properties

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ABSTRACT

Antibacterial membranes were prepared from a mixture of hydrolyzed starch and chitosan. Glycerin was incorporated in the membranes to as plasticizer agent. The effects of component ratio on the mechanical and permeable properties of the prepared membranes were investigated. The elongation-at-break and water vapor transmission rate of starch/chitosan blending membranes were largely improved compared with each single component due to the interaction formed between the hydroxyl groups of starch and the amino ones of chitosan, which was confirmed by FT-IR characterizations. With the help of optical microscope, the influence of component ratio on the morphologies of starch/chitosan membranes was systematically investigated. It comes to a conclusion that extreme low or high starch content will cause an asymmetric membrane surface. To prove the antibacterial activity of obtained membranes, *Escherichia coli* (*E. coli*) was chosen as the target bacteria *via* optical density method. The resulted starch/chitosan membranes exhibited an outstanding antibacterial activity against *E. coli*.

1. Introduction

In recent years, degradable membranes have attracted widespread interest due to the increasing problem of environmental pollutants caused by using synthetic polymers based on petroleum chemistry. Degradation of conventional plastic requires an extraordinarily long time, which may be confronted with secondary pollution and much high cost. So, as a way to solve the above problems, environmental friendly biodegradable membranes have been developed recently.

The usual materials adopted for preparing environmentally friendly polymer membranes are generally classified into two categories: degradable synthetic polymers [polycaprolacton, polylactide, PVA, polyesteramide, etc. Fadnis, Illiger, Rao, & Demappa, 2008; John & Thomas, 2008; Mawad et al., 2008; Vidéki, Klébert, & Pukánszky, 2005)] and degradable natural ones [chitin, chitosan, pectin, starch, cellulose, etc. (Chen, Wu, Long, & Liu, 2006; Chwojnowski, Dudzińska, Wojciechowska, & Łukowska, 2008; Ma & Hanna, 1999; Nagahama et al., 2008)]. For abundant existing in nature and being cheap, renewable natural polymer shows a wider application prospect in the food, packaging and medical industries rather than its synthetic counterpart.

Among various degradable membrane materials, chitosan has attracted considerable attention for its unique properties, the most important one of which is abundant commercial supplies and antibacterial properties. Chitosan is the N-deacetylation resultant of chitin which is the second most naturally occurring biopolymer after cellulose (Guibal, 2005). Since the amine group NH_2 can be protonated to NH_3^+, which may endow chitosan with a favorable gel and membrane forming properties, it has been extensively examined in the medical industry for its potential in the development of artificial skin. Although chitosan membranes are highly impermeable to oxygen, they have relatively poor water vapor barrier characteristics due to its excellent hydrophilicity, which is not favorable for the usage as artificial skins (Willför, Sundberg, Tenkanen, & Holmbom, 2008). Srinivasa had reported that adding plasticizers had negative effects on barrier properties in spite of positive effects on mechanical properties (Srinivasa, Ramesh, & Tharanathan, 2007). Many efforts have been done to improve water barrier capability of chitosan by means of weakening its hydrophilic property, among which blending chitosan with some hydrophobic materials had attached most interest.

Starch, containing about 30% amylase, 70% amylopectin and less than 1% lipids and proteins from plants, is a fine additive for forming membrane owing to its insoluble property. In the present work, chitosan was blended with starch to form heterogeneous membrane. The mechanical properties and water vapor transmission rate were detected as a function of the interaction between chitosan

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and starch. In addition, the antibacterial properties imply the potential application of the resultant membrane in medical field.

2. Experimental

2.1. Materials

All the chemicals were of reagent grade and were purified before used. Solutions were prepared with deionized water (electrical resistivity ⩾ 18.2 MΩ cm/25 °C). Maize starch ($(C_6H_{10}O_5)_n$ ⩾ 99%) with a polymerization degree of 2000–3000 was used for the membrane forming process. Chitosan (deacetylation degree ⩾92%) used was purchased from Huoshuo Fine Chemical Co. Ltd., Shanghai, China. Glycerin (purchased from Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) was diluted into 25% solution to be used as the plasticizer.

2.2. Gelatinization of starch powder

Starch powder with different mass and 50.0 mL distilled water were put into a 250 mL three-necked flask equipped with a stirrer, thermometer and nitrogen pipe. The slurry was mechanically stirred and gelatinized at 90 °C for 30 min under a nitrogen atmosphere, which was then cooled to room temperature, forming a homogeneous solution.

2.3. Preparation of starch/chitosan membrane

Chitosan powder was first dissolved in 1% acetate solution under continuous stir and added to the obtained starch solution with several drops of glycerin. The mixed solution was continuously stirred for several hours, which was then poured into a Teflon-coated dish to evaporate water in an oven at 50 °C overnight. After conditioned at 50% RH and 25 °C for 48 h, the obtained membrane was cut into 10×20 mm and ø15 mm pieces for elongation-at-break and water vapor transmission rate measurements, respectively.

2.4. Mechanical properties

Mechanical property of elongation-at-break was measured with a constant force applied to two ends of 10 × 20 mm piece of starch/chitosan membrane, following the guidelines of ASTM standard Method D 882-91 (ASTM, 1995a). Elongation-at-break was calculated as the ratio of the final length at the point of sample rupture to the initial length of a specimen, which was repeated five times for each type of membrane.

2.5. Water vapor transmission rate

Water vapor transmission rate (g/m^2 h) was measured by means of a modification of ASTM Method E 96-95 (ASTM, 1995b). Membrane specimens (ø 15 mm) were placed to cover glass cups containing a certain amount of distilled water. The cups were placed in an environmental chamber where the value of relative humidity (HR) was confirmed to be 50% with the help of hygrometer, at 25 °C for 10 h, so the water vapor transmission process would been induced by the difference of relative humidity, as a driving force, between the outside (50%) and inside (100%) of water-included glass cup. The weights of the cups were recorded every hour to calculate water vapor transmission rate and an average value was obtained.

2.6. Water absorption

Water absorption of starch/chitosan membranes was tested as described: membranes dried naturally at room temperature were immersed completely in deionized water for absorbing process. After sufficient swelling overnight, the swollen membranes were taken out from the water and the excess water on the surface was wiped off with filter paper until a constant weight was obtained. The water absorbing quantity (noted as WAQ) of starch/chitosan membranes was calculated as $WAQ = (W_w - W_d)/W_d$, where W_w and W_d are the weights of wet and dry membranes, respectively.

2.7. Antimicrobial activity of membranes

Antimicrobial activity test on membranes was carried out using agar disk diffusion method according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (NCLC, 2003). The inhibition zone assay on culture medium was used for determination of the antimicrobial ability of obtained membranes against *Escherichia coli* O157:H7. The starch/chitosan membranes were cut into ø 5 mm disks and placed on agar-covered plates, where *E. coli* had been previously cultured. Then the plates were incubated at 37 °C for 24 h. Digital images were taken to vividly show how starch/chitosan membranes inhibit the growth of *E. coli.*

2.8. Fourier-transform infrared characterization

Fourier-transform infrared (FT-IR) spectra of membranes with different starch/chitosan ratios were obtained using an IR spectrometer (FT-IR, Tensor 27, Bruker Inc.) in attenuate total reflection (ATR) mode. To examine the interactions between chitosan and starch, 64 consecutive scans at 1 cm^{-1} resolution were averaged.

2.9. Morphology observation

The morphologies of starch/chitosan membranes were examined by optical microscope (Nikon E600POL, Japan) using a transmission mode. The tested samples were prepared by cutting prepared film into 2 × 2 cm pieces. Prior to observation, samples were clamped by two glass slides to avoid the crinkling of membranes.

3. Results and discussion

3.1. Elongation-at-break (E)

The *E* values of chitosan/starch blend membranes with the different starch masses are shown in Fig. 1, while the mass of chitosan

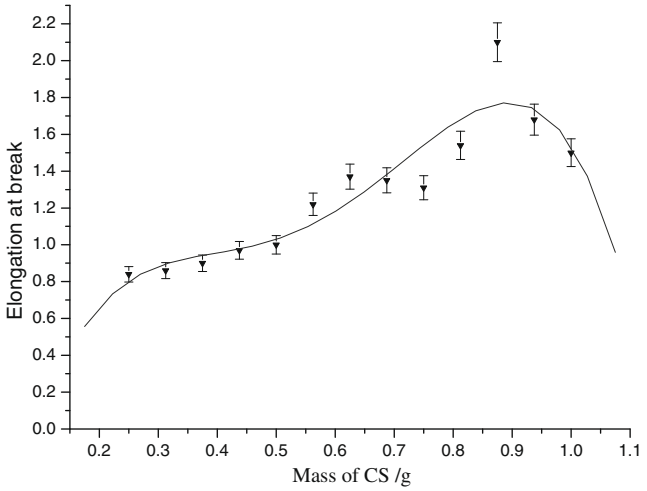

Fig. 1. Elongation-at-break (*E*) values of chitosan/starch blend membranes with the different starch masses.

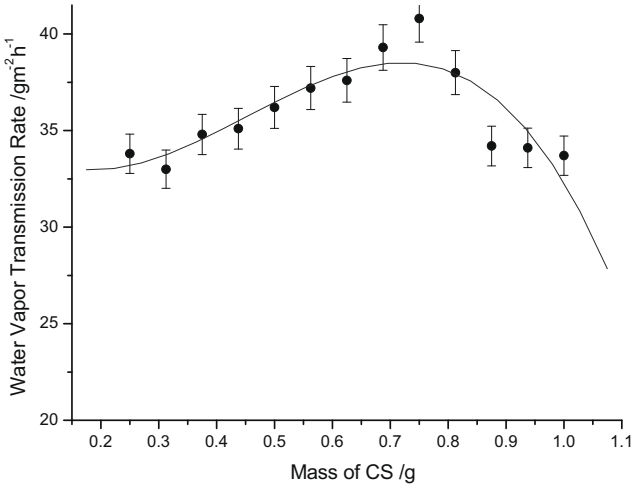

Fig. 2. Water vapor transmission rate of starch/chitosan blend membrane with the different starch masses.

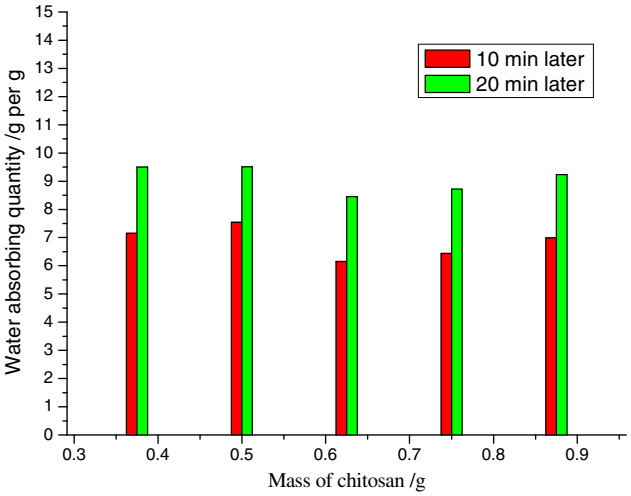

Fig. 3. Water absorbing quantity (WAQ) of starch/chitosan membranes immersed in water for different time.

retained 0.5 g. As observed, the *E* values of membranes were affected by the starch contents. This phenomenon has also been reported by Hanna and the co-workers (Xu, Kim, Hanna, & Nag, 2005). With the mass of starch increasing, the E value of obtained membrane increased in the initial stage until reaching the maximum at starch mass of 0.875 g, after which the curve started bending downwards. The variation in Fig. 1 could be explained that: with the addition of starch, the E value of blend membrane increases owing to the formation of hydrogen bonds between NH_3^+ of the protonated chitosan and OH^- of the starch. However, when the addition of starch was too much, the flexibility of obtained membrane was lowered and the E value also decreased for the brittle nature of CS membrane (Kim, Na, & Park, 2003). Thus, its comparatively difficult to form homogeneous starch/chitosan membrane with higher content of CS.

3.2. Water vapor transmission rate

Water vapor permeability of starch/chitosan blend membrane was determined by means of water vapor transmission rate (WVTR), and the result was shown in Fig. 2. The WVTR behaved in a similar fashion as that of the Elongation-at-break, i.e. with the increasing of starch, the WVTR value of obtained membrane increased at the beginning until reached the maximum at starch mass of 0.75 g with 0.5 g chitosan, following which the curve started to bend downwards. This observation should be caused by the forming of numerous microcracks due to the incompatibility of the two components, which enhanced the moisture penetration

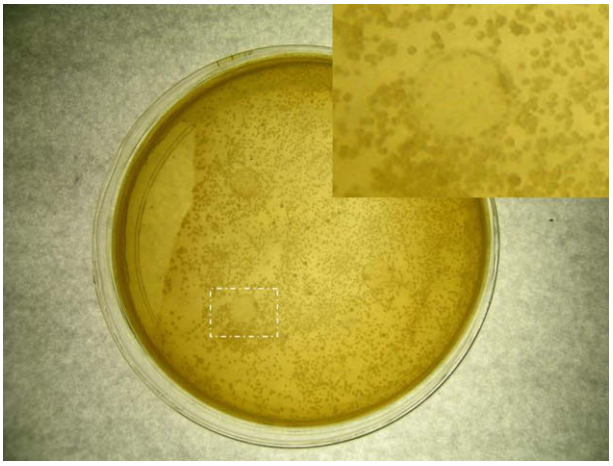

Fig. 4. Picture of antimicrobial activity test on membranes: the area braced by white dotted line was magnified as an inset.

through the as-obtained membranes and consequently increased the WVTR value. As low WVTR could enlarge the applications of this blend membrane, especially in a highly humid environment, low starch content will be considered to incorporate into the chitosan matrix to form interactions between the two components,

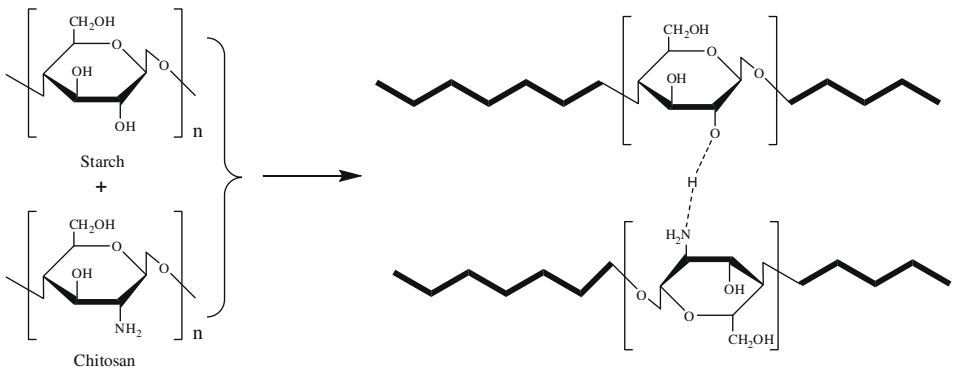

Fig. 5. Scheme of formation of starch/chitosan membranes.

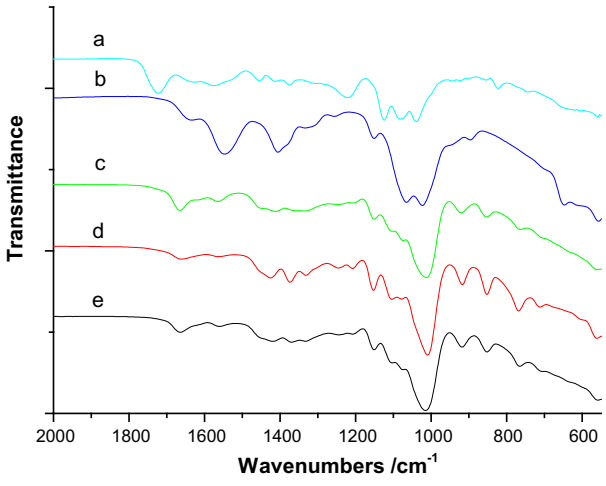

Fig. 6. FT-IR curves of starch powder (a), chitosan powder (b) and starch/chitosan membranes with starch-to-chitosan ratios of 7:4 (c), 5:4 (d) and 3:4 (e).

which may prevent water molecules from diffusing through the membranes.

3.3. Water absorption

Water sensitivity is another important property for practical applications of starch/chitosan membranes. To analyze the hydrophobic/hydrophilic characters of the obtained membranes, the properties of water absorption were tested and the results were given in Fig. 3. The value of WAQ of membranes showed minor variation irrespective of the component ratios. With the prolonging of swelling time, WAQ enlarged to get the equilibrium. By comparing WAQ values of membranes with different starch contents, it comes to the conclusion that the contents of starch did not obviously affect the water absorption, although the presence of starch enhanced the hydrophobicity of blend membranes as a result of the hydrophobic chains of starch.

3.4. Antimicrobial activity

Inhibitory activity of starch/chitosan membranes was measured based on the comparison between growth conditions of *E. coli* at areas far from and near the circular membrane disk. If all the *E. coli* around the plate grows in the same scale, its assumed that there is no inhibition. The result was presented in Fig. 4, in which the area around the membrane disk was magnified for a clearer visualization. After growing for 10 h in the culture medium, abundant *E. coli* had covered the bottom of culture disk except for where starch/chitosan membrane disks existed, as displayed in the inset in Fig. 4. Thus, it can be concluded that the as-prepared starch/chitosan membranes had an outstanding antibacterial activity, for the absence of *E. coli* (dark points) on the surface of membrane disks against abundant *E. coli* around. The conclusion above agrees well with the finding of Zhai et al. (Zhai, Zhao, Yoshii, & Kume, 2004). As Zhai tested the antimicrobial activity by measured the turbidity of the medium, here we gave the appearance of inhibition zone as substitute for more direct viewing.

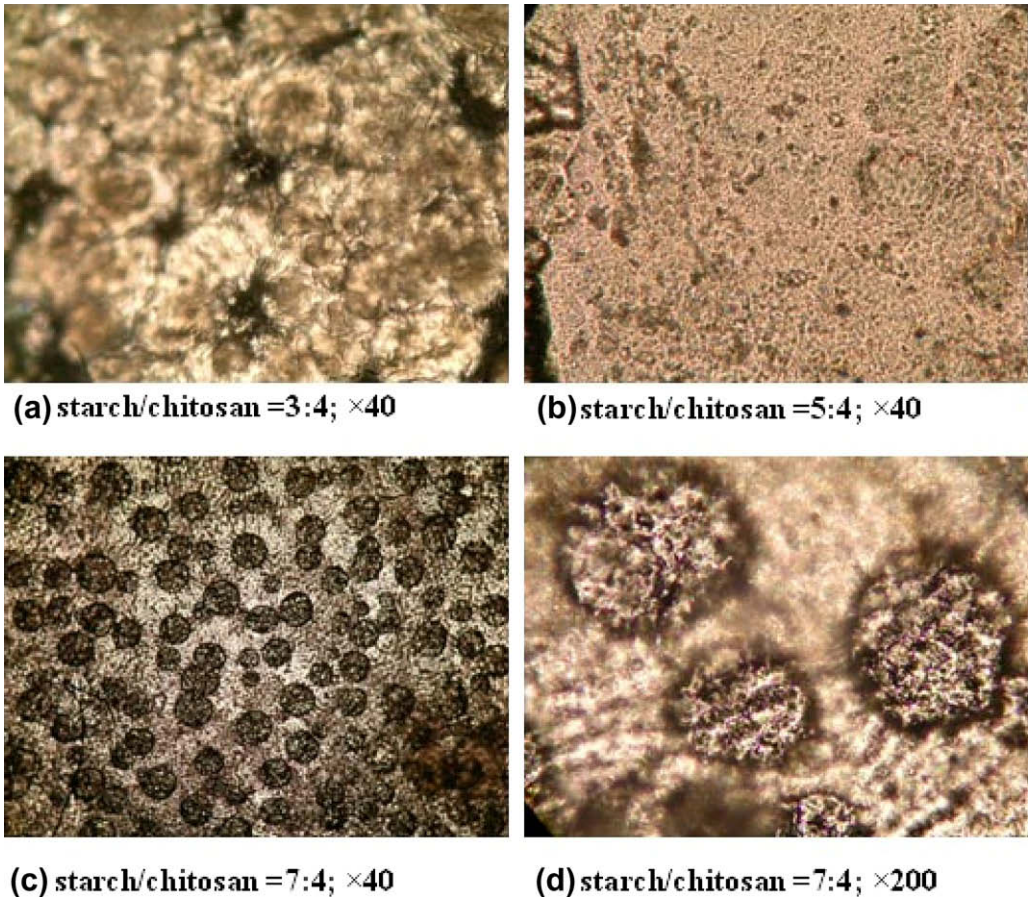

Fig. 7. Optical microscope pictures of starch/chitosan. Starch-to-chitosan ratio = 3:4 (a), 5:4 (b), 7:4 (c).

3.5. Fourier-transform infrared (FT-IR)

The main chain elements of starch and chitosan are similar to each other except for the amino group existing only in chitosan at **C**-2 position as same as its counterpart –OH in starch (the chemical structures of chitosan and starch are displayed in Fig. 5). Hence, the only difference between FT-IR spectra of pure chitosan and starch come down to peaks at 1655 and 1578 cm^{-1}, assigned to C=O stretching (amide I) and NH bending (amide II), respectively (Nunthanid, Puttipipatkhachorn, Yamamoto, & Peck, 2001). To investigate the interaction between two components, FT-IR spectra of chitosan/starch membranes with different ratios are shown in Fig. 6. Moreover, the FT-IR spectra of pure starch and chitosan were shown in the same figure for comparison. To avoid the disturbance of absorbed water, the samples were placed at 50 °C in vacuum overnight to make sure that the IR spectra were taken on completely dried samples.

With the analysis of peaks in FT-IR spectra, it can be confirmed that there were some physical or chemical interactions between mixed substances (Griffiths, 1983). In a typical spectrum of chitosan/starch, the amino peak at 1665 cm^{-1} (amide I) and 1564 cm^{-1} (amide II) shifted to a higher wavenumber, which indicated that some interactions had occurred between the hydroxyl groups of starch and the amino ones of chitosan (Meenakshi et al., 2002).

3.6. Morphology observation of starch/chitosan membrane

To explore the relationship between the morphology and components ratio, samples with starch-to-chitosan mass ratio of 3:4, 5:4 and 7:4 were tested by optical microscope, and the digital images were displayed in Fig. 7. Using a transmission mode, the phase separation of prepared blend membranes can be easily observed.

At lower starch content, a spot of starch will not greatly mix with chitosan to form an asymmetric membrane as a result of the phase separation. There were many microcracks on the surface of starch/chitosan membrane caused by the crinkle of starch link in the surrounding of abundant chitosan (see Fig. 7a), which will decrease the mechanical and permeable properties. With too much starch added, chitosan links will crinkle to form a flat cake shape and the whole membrane was like the configuration of bilayer lipid membrane (see Fig. 7c). Furthermore, chitosan cake unit was magnified and displayed in Fig. 7d, in which the interface between two components could be obviously made out. Only with the appropriate ratio can a symmetrical membrane formed (see Fig. 7b), which was also reflected in the mechanical and permeable properties discussed above. The chain structures of starch and chitosan are similar except the groups at **C**-2 positions demonstrated in Fig. 5, so the two can form a uniform mixture theoretically. But the membranes can present different morphologies due to different composing as described above.

4. Conclusion

The results obtained demonstrate that starch/chitosan blending membranes of appropriate component ratio exhibit outstanding mechanical and permeable properties. The origin of the enhancement phenomenon had been examined by FT-IR to be the interaction between the hydroxyl groups of starch and the amino groups of chitosan. Recur to the antimicrobial activity test, obtained membranes were confirmed to be propitious to applications in package and medical industries.

Acknowledgments

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