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Carbohydrate Polymers 57 (2004) 435–440

Carbohydrate Polymers

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Preparation and characterization on mechanical and antibacterial properties of chitsoan/cellulose blends

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Received 13 February 2004; revised 3 May 2004; accepted 28 May 2004

Available online 2 July 2004

Abstract

Polysaccharides-based membranes of chitosan and cellulose blends were prepared using trifluoroacetic acid as a co-solvent. Morphology and mechanical property of prepared membranes were studied by Instron and dynamic mechanical thermal analysis. The mechanical and dynamic mechanical thermal properties of the cellulose/chitosan blends appear to be dominated by cellulose, suggests that cellulose/chitosan blends were not well miscible. It is believed that the intermolecular hydrogen bonding of cellulose is supposed to be break down to form cellulose–chitosan hydrogen bonding; however, the intra-molecular and intra-strand hydrogen bonds hold the network flat. The reduced water vapor transpiration rate through the chitosan/cellulose membranes indicates that the membranes used as a wound dressing may prevent wound from excessive dehydration. The chitosan/cellulose blend membranes demonstrate effective antimicrobial capability against *Escherichia coli* and *Staphylococcus aureus*, as examined by the antimicrobial test. These results indicate that the chitosan/cellulose blend membranes may be suitable to be used as a wound dressing with antibacterial properties.

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Keywords: Chitosan; Cellulose; Mechanical; Antibacterial

1. Introduction

Chitosan [Poly(1,4-β-D-glucopyranosamine)] is the *N*-deacetylated polysaccharide from chitin which possesses valuable properties for biomedical applications (Molinaro, Leroux, Damas, & Adam, 2002; Park et al., 2001; Shu & Matthew, 2000). It is a biopolymer that has the same β-(1 → 4)-D-glucopyranose units backbone as cellulose, expect for the 2-hydroxy is replaced by an acetamide group. Chitosan has been well known as being able to accelerate the healing of wound in human (Cho, Cho, Chung, & Ko, 1999; Ueno et al., 1999). It has also been documented that chitosan confers considerable antibacterial activity against a broad spectrum of bacteria (Hirano & Nagano, 1989; Muzzarelli et al., 1990). Owing to the advantages, some of the applications of chitosan have included wound dressings, gauzes and medical sutures (Kaessmann & Hark, 1997; Kim et al., 1999; Sagar, Hamlyn, & Wales, 1990).

The modification of chitosan by means of blending with other polymers may be a convenient and effective method to improve physical properties for practical utilization. Investigation of blends of chitosan with synthetic and naturally occurring macromolecules has attract much interesting in the recently years. It has been reported that the hydrophilic property of chitosan could be modified via blending with PEG and PVA (Kweon & Kang, 1999; Zhang, Li, Gong, Zhao, & Zhang, 2002). Chitosan was also blended with several polymers such as polyamides, poly(acrylic acid), gelatin, silk fibroin and cellulose to enhance mechanical properties (Arvanitoyannis, Nakayama, & Aiba, 1998; Isogai & Atalla, 1992; Lee et al., 1999; Park, Lee, Ha, & Park, 1999; Ratto, Chen, & Blumstein, 1996).

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doi:10.1016/j.carbpol.2004.05.013

Cellulose is a linear polymer of β-(1-4)-D-glucopyranose units. It forms crystals (cellulose I_a) where intra-molecular and intra-strand hydrogen bonds hold the network flat allowing the more hydrophobic ribbon faces to stack (Isogai & Atalla, 1992). This tendency to form crystals utilizing extensive intra- and intermolecular hydrogen bonding makes it completely insoluble in normal aqueous solutions. Cellulose has many uses as emulsifier, stabilizer, dispersing agent, thickener, and gelling agent, especial for the preparation of textile. The similarity of cellulose and chitosan in primary structures suggests that there may be sufficiently similar to facilitate the formation of homogeneous composite films. However, it is known that a deficiency of chitosan is its poor tensile strength after wetting in water. Blending cellulose with chitosan is expected to be a useful method to improve the mechanical properties of chitosan. Several studies reported that there are in the presence of specific interactions between cellulose and chitosan molecules based on the analysis of Raman and ^{13}C NMR spectroscopy, and X-ray diffractometry (Hasegawa, Isogai, Onabe, & Usuda, 1992a; Hasegawa, Isogai, Onabe, Usuda, & Atalla, 1992b; Isogai & Atalla, 1992; Hasegawa, Isogai, Kuga, & Onabe, 1994).

In the present study, blends of chitosan and cellulose have been prepared by casting films from trifluoroacetic acid (TFA). Morphology and mechanical properties are investigated for various blend compositions of chitosan/cellulose composites, using Instrun, and dynamic mechanical analysis (DMA). Water vapor permeation and antibacterial test were studied to examine its possibility of such a chitosan/cellulose blends to be used as a biomaterial for wound dressing.

2. Experimental

2.1. Materials

Chitosan was purchased from Alderich Chemical Co. (Switzerland). Cellulose was a gift from Chian Man-mode Fiber Corporation (Taiwan). Trifluoroacetic acid was a commerical product from Lancaster, Chemical Co. All other reagents and solvents used were of reagent grade.

2.2. Chitosan/cellulose blends processing

Cellulose and chitosan were dissolved in aqueous acetic acid (2.0 wt%) and tetrafluoroacetic acid, respectively, for the preparation of cellulose and chitosan solution. The cellulose and chitosan solution were blended together in different ratios. Transparent films were obtained by casting these solutions to produce blends with final compositions 3/1, 1/1 and 1/3 (w/w). The films prepared in this manner were soaked in 1N NaOH at room temperature for 1 day to remove the acids. The films were separated from the plate during the immersion procedure. They were then washed with water, and dried at room temperature.

2.3. Water vapor transpiration rate

Water vapor transpiration rate (WVTR) were determined according to the ASTM method E96-90, Procedure D. An evaporimeter was constructed in a closed glass chamber to prevent variations owing to ambient conditions. It consists of a glass chamber with a cover, isothermal bath at 35 °C, a digital hygrometer with a continuous % relative humidity (RH), temperature and dew point display, and a reservoir of a saturated magnesium chloride solution. A permeability cup (cylinder, 3 cm in diameter and 5 cm in height) made from high-density polyethylene (HDPE) was placed in the closed glass chamber. The permeability cup was filled with 20 g of deionized distilled water and the test membrane fixed onto its opening. Evaporation of water through the test membrane was monitored by measurement of loss of weight of the cup. An open cup was used as the control. The RH in the evaporimeter after equilibration was approximately 40%.

2.4. Mechanical and dynamic mechanical thermal analysis

In the mechanical property measurement, test samples were cut from each studied group using a razor blade. Stress–strain curves of the test samples were determined by an Instron (Intron Mini 44, USA) at a constant speed of 50 mm/min. The strain-at-fracture was taken as the percent strain at the point of fracture, while the ultimate-tensile-strength was taken as the force at which fracture occurred divided by the initial cross-sectional area.

Dynamic mechanical thermal analysis (DMTA) was performed with a DMA-2980 (TA Instrument). Dynamic mechanical properties were measured from -30 °C up to 225 °C while heating the sample at 5 °C/min. Film stripes of about $40 \times 4 \times 0.1$ mm were tested at a frequency of 5 Hz.

2.5. Antibacterial test

Testing of antibacterial activity of the chitosan membranes was performed according to the method described by Grzybowski, Antos, and Trafny (1996). The samples used for the antibacterial assay were sterilized at 121 °C by an autoclave over a period of 30 min. In the antibacterial assay, 16-mm-diameter test samples cut from the sterilized chitosan, cellulose, and the chitosan/cellulose blend membranes were placed on the bottoms of the wells in a 24-well plate (the diameter of each well is about 16 mm). Subsequently, 50 μl of bacterial broth culture was seeded onto the chitosan, cellulose, and the chitosan/cellulose blend membranes (10^6 CFU/ml), respectively. The bacterial broth cultured in the well without containing any chitosan membrane was used as control. The bacteria used for the test were *Escherichia Coli* and *Staphylococcus aureus*.

Subsequently, the membranes were put in the moisture incubator and incubated at 37 °C. After 1, 2, or 4 h of incubation, each chitosan membrane was placed into test tubes containing 1 ml PBS and sonicated for 75 s in an ultrasonic washer (64 kHz). Subsequently, 50 μl of the incubated medium taken out from each tube was seeded on agar plates containing nutrient broth and incubated at 37 °C for 24 h. Finally, the units of colony formation in each agar plate were calculated to examine the antibacterial ability of each chitosan membrane.

3. Results and discussion

3.1. Mechanical and dynamic mechanical thermal analysis

Fig. 1 shows the tensile strength of the blend films as a function of chitosan content. The tensile strength of cellulose and chitosan are 63 and 34N, respectively. The tensile strength of the blend films reaches at about 50N without regarding to the cellulose/chitosan blend ratio (3/1, 1/1 and 1/3), suggests that tensile strength of the blend films is cellulose dominated. It has been reported that the presence of chitosan limits the crystalline of cellulose (Hasegawa et al., 1994). However, in the present study, it is interesting to find that the tensile strengths almost approach to the strength value of original cellulose.

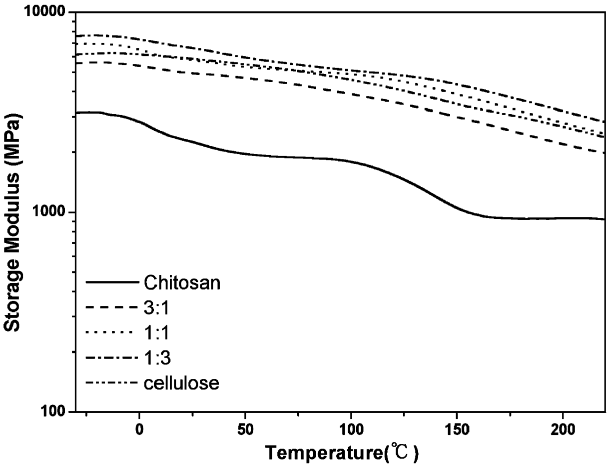

Fig. 2. Storage modulus (E') of cellulose, chitosan and the chitosan/cellulose blend films.

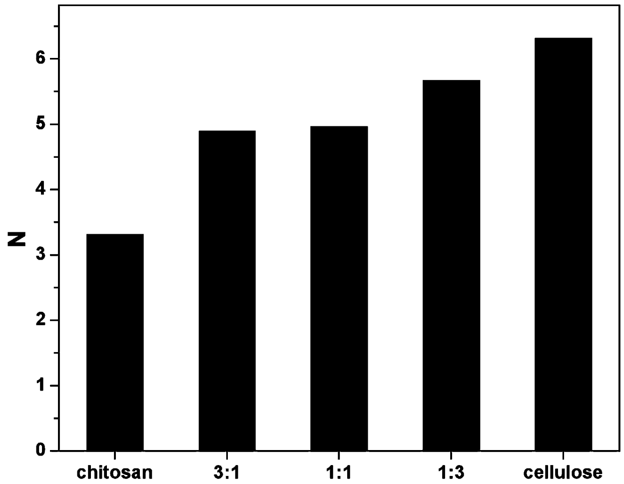

Fig. 1. Tensile strength of cellulose, chitosan and the chitosan/cellulose blend films.

Figs. 2 and 3 show temperature dependence of the storage (E') modulus and loss (E'') modulus of cellulose/chitosan blends and pure polymers. The E' and E'' vs. temperature curve shows the main relaxation characteristic of cellulose, chitosan and cellulose/chitosan blends. The curve of storage (E') modulus of chitosan shows a peak at 0–30 °C, which is not seen in the curves of cellulose and the chitosan/cellulose blends. The peak is attributed to the hydration of side groups (–CH_2OH) on chitosan. The E' values indicate that chitosan has a lower storage modulus as compared to the cellulose and the chitosan/cellulose blends. Blending with cellulose improves mechanical properties of chitosan; however, the E' values for three types of chitosan/cellulose blends (chitosan/cellulose blend ratios are 3/1, 1/1 and 1/3) almost approach to the E' value of original cellulose.

Fig. 4 shows the temperature dependence of the tan δ values of cellulose/chitosan blends and pure polymers. It is generally accepted that the temperature at the maximum value in tan δ corresponds to the glass–rubber transition. Accordingly, the tan δ peak at 190 °C of cellulose, which is related to the molecular motion in the amorphous region, is designated as the α relaxation reflecting the glass transition temperature of cellulose. Chitosan demonstrate two tan δ peaks at around 15–22 °C and 153 °C. The tan δ peak at 15–22 °C is characterized as the β relaxation attributed to the hydration of side groups (–CH_2OH) on chitosan. Another tan δ peak at 153 °C is designed as the α relaxation reflecting the glass transition temperature of chitosan.

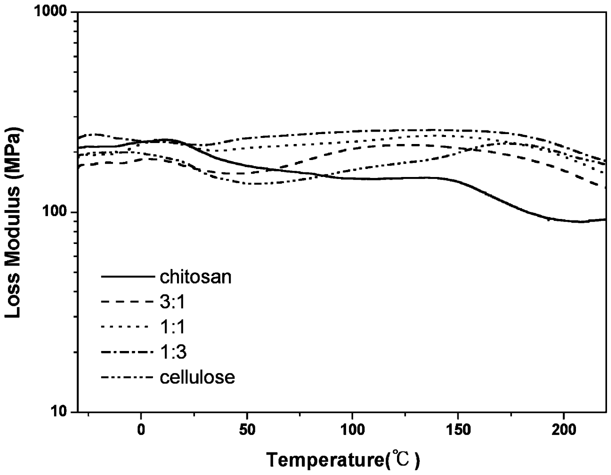

Fig. 3. Loss modulus (E'') of cellulose, chitosan and the chitosan/cellulose blend films.

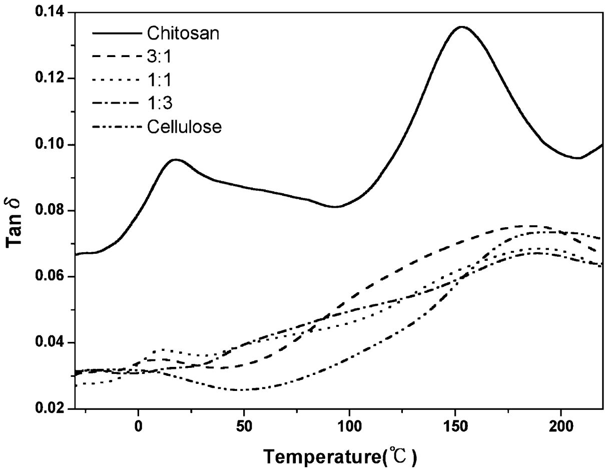

Fig. 4. Temperature dependence of the tan δ values of cellulose, chitosan and the chitosan/cellulose blend films.

The existence of a single and sharp peak of glass transition in a polymer blend can be considered as the most general criterion for miscibility, which means the interaction between two pure polymers at the molecular level. The α relaxation peak of cellulose becomes board with the addition of different contents of chitosan; however, the shift of α relaxation peak of cellulose was not obvious. The broadened glass transition region suggesting heterogeneity, possibly due to the presence of, respectively, cellulose-rich and chitosan-rich regions. Nevertheless, it was found that β relaxation at 15–22 °C of chitosan was disappeared after blending with cellulose. This can be attributed to the interaction of side groups ($-CH_2OH$) on chitosan with $-OH$ groups on cellulose, which displaces the hydrogen bonding to water.

These results lead to the conclusion that chitosan/cellulose blends exhibit considerable immiscibility. The mechanical and dynamic mechanical thermal properties of the chitosan/cellulose blends appear to be dominated by cellulose. This is not surprising in view of the crystal forms of cellulose. The intermolecular hydrogen bonding of cellulose is supposed to be break down to form cellulose–chitosan hydrogen bonding; however, the intra-molecular and intra-strand hydrogen bonds hold the network flat. It is suggested that cellulose holds its mechanical properties in the chitosan/cellulose blends because of the retention of its intra-molecular and intra-strand hydrogen bonding.

3.2. Water vapor transpiration rate

An ideal dressing would control the evaporative water loss from a wound at an optimal rate. The rate for normal skin is 204 g/m^2 per day, while that for injured skin can range from 279 g/m^2 per day for a first degree of burn wound to 5138 g/m^2 per day for a granulating wound. The water vapor permeability of a wound dressing should prevent both excessive dehydration as well as buildup of exudate. It was recommended that a rate of 2500 g/m^2 per day, which being in the mid-range of loss rates from injures skin, would provide an adequate level of moisture without risking wound dehydration. Chitosan has been reported to be blend with several polymers to prepare membrane for the separation of water-ethanol mixtures (Ghazali, Nawawi, & Huang, 1997; Shieh & Huang, 1998). The water vapour transpiration rates of various chitosan/cellulose membranes are summarized in Table 1. The chitosan films has relative higher water vapour transpiration rate (1063 g/m^2 per day) than the cellulose films (880 g/m^2 per day), while the water vapour transpiration rates for all compositions of chitosan/cellulose blend membranes are almost approached to the transpiration rates of original cellulose (816–864 g/m^2 per day). It is believed that the presence of chitosan in blend membranes will increase the water vapour transpiration due to stronger affinity of chitosan to water molecules, and due to the larger volume of $-NH_2$ side groups (19 cm^3/mol) on chitosan than that of the $-OH$ side groups (13 cm^3/mol) on cellulose (Shieh & Huang, 1998). The water vapour

Table 1
The water vapour transpiration rates of various chitosan/cellulose membranes

Condition	Water vapour transpiration rates (g/m^2 per day)	Relative humidity (RH%)	Temperature (°C)
Molecular weight = 560,000 open (no membrane)	1927.824		
Chitosan:cellulose = ratio			
Chitosan:cellulose = 1:0	1063.392	41–42%	36.5–37
Chitosan:cellulose = 3:1	838.080		
Chitosan:cellulose = 1:1	903.408		
Chitosan:cellulose = 1:3	852.240		
Chitosan:cellulose = 0:1	880.656		

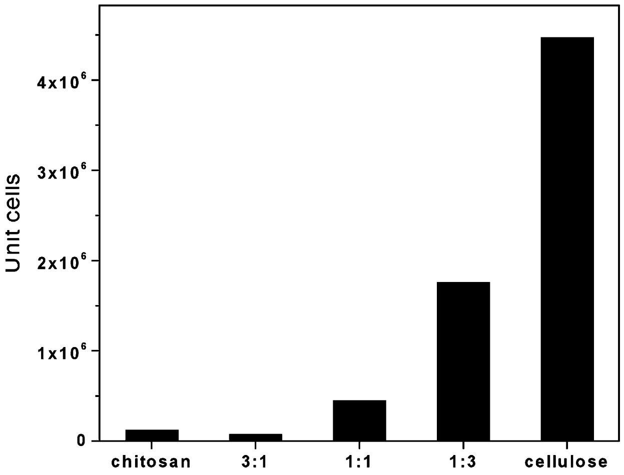

Fig. 5. Bacteriostatic effect of cellulose, chitosan and the chitosan/cellulose blends on the growth of *Escherichia coli*.

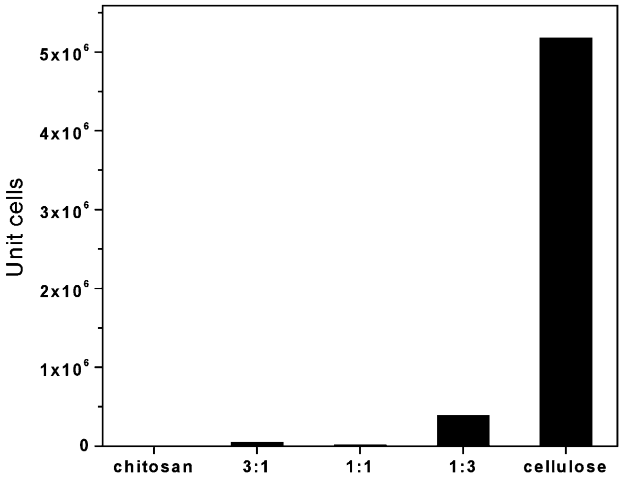

Fig. 6. Bacteriostatic effect of cellulose, chitosan and the chitosan/cellulose blends on the growth of *Staphylococcus aureus*.

transpiration rates observed in this study indicated that chitosan and cellulose are immiscible to a great extent in the blend membranes. Cellulose holds its intra-molecular and intra-strand hydrogen bonding to form compact structure in the blend membranes, which dominates the water vapor transpiration.

3.3. Antibacterial ability

In this study *E. coli* and *S. aureus* were used as the test bacteria to examine the antibacterial properties of chitosan, cellulose and chitosan/cellulose blend membranes. The numbers of colony of these bacteria formed on the test membranes are shown in Figs. 5 and 6. It was noted that the numbers of colony of all test bacteria formed on the chitosan/cellulose blend membranes were decreased with the increase of chitosan concentration. The chitosan/cellulose blend membranes demonstrate more effective antimicrobial ability against *E. coli* than that of *S. aureus*, as indicated by the lower colony unit. These results indicate that the chitosan/cellulose blend membranes may be suitable to be used as a wound dressing with antibacterial properties.

4. Conclusion

In this work, the blend membranes were successfully prepared from chitosan and cellulose using trifluoroacetic acid as a solvent. It is interesting to find that the mechanical and dynamic mechanical thermal properties of the cellulose/chitosan blends are almost dominated by cellulose, suggests that cellulose/chitosan blends were considerably immiscible. It is believed that the intermolecular hydrogen bonding of cellulose is break down to form cellulose–chitosan hydrogen bonding; however, the intra-molecular and intra-strand hydrogen bonds hold the network flat. The studies of water vapor transpiration through the chitosan/cellulose membranes, and the antimicrobial capability against *E. coli* and *S. aureus* indicates that the membranes used as a wound dressing may prevent wound from excessive dehydration and infection. These results indicate that the chitosan/cellulose blend membranes may be suitable to be used as a wound dressing with antibacterial properties.

Acknowledgements

The authors wish to thank the National Science Council of Taiwan, ROC for the financial support of this project (NSC 88-2216-E-012-001).

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