

Structural Changes and Their Effect on Mechanical Properties of Silk Fibroin/Chitosan Blends

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ABSTRACT: Silk fibroin/chitosan blend films were prepared by the solvent casting method. Miscibility between silk fibroin and chitosan was examined by dynamic mechanical thermal analysis. Structural changes of silk fibroin by the addition of chitosan were investigated by IR spectroscopy. The conformational transition of silk fibroin from random coil form to β -sheet structure induced by blending with chitosan resulted in the increase of crystallinity and density of the blend films. The blend film containing 30 wt % chitosan exhibited a maximum increase in crystallinity and density. It was found that the tensile strength and initial tensile modulus of blend films were greatly enhanced with increasing the chitosan content and showed a maximum value at the composition of 30 wt % chitosan. © 1999 John Wiley & Sons, Inc. *J Appl Polym Sci* 74: 2571–2575, 1999

Key words: silk fibroin; chitosan; blend, structural change; mechanical property

INTRODUCTION

Although many synthetic polymers have been used as biomaterials, natural polymers with the inherent compatibility to the living body have been noted as excellent biomaterials.^{1,2} Silk fibroin is a linear polypeptide that is composed of 17 amino acids, and its main components are nonpolar ones such as alanine and glycine, and so forth.^{3,4} It has been reported that silk fibroin has two kinds of crystalline modifications, silk I and silk II, as well as the random coil form, depending on the sample preparation conditions.⁵ Magoshi et al.⁶ have extensively studied the structure of silk fibroin from *Bombyx mori* and its structural changes, particularly the conformational transition from random coil form to β -sheet form induced by treatments such as heating, stretching, and/or immersion in polar solvents. This transition makes silk fibroin attractive as a biomaterial

because silk fibroin with a β -sheet structure is resistant to water and has good mechanical properties. There have been some reports on silk fibroin as an enzyme immobilization matrix that can be used as a good biosensor.⁷ It has been also reported that silk fibroin film has good dissolved oxygen permeability in the wet state, similar to that of human skin, which suggests the promising application of silk fibroin as a wound dressing artificial skin.⁸ However, the silk fibroin film is too brittle to be used by itself. The poor mechanical properties of silk fibroin could be improved by blending it with other natural or synthetic polymers.^{9–12}

Chitosan is the second most abundant biomass and has a repeating structure of 1,4-linked 2-amino-2-deoxy- β -D-glucan. It has useful functional groups such as hydroxyl and amino groups that can be modified to various derivatives.^{13,14} Chitosan is also a biomaterial well known for its biocompatible and biodegradable properties.^{15,16} Chitosan can be easily shaped into fibers and films, which are commercially useful in sutures, hollow fibers, membranes, medical gauze, and

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wound dressings. For these reasons, chitosan was chosen and blended in this work to improve the mechanical properties of silk fibroin films. We investigated the miscibility and structural changes of silk fibroin/chitosan blends as a function of blend ratios. Finally, the effect of structural changes on mechanical properties of silk fibroin/chitosan blends was also studied.

EXPERIMENTAL

Materials

Degummed silk was completely dissolved in 9.3M lithium bromide solution at 40°C.¹⁷ The solution was dialyzed using cellulose tubular membranes (Sigma Co., 250–257 μm) in distilled water for 3 days at room temperature. After centrifugation at 15,000 rpm for 15 min to remove the impurities, the supernatant was collected and lyophilized. Chitosan ($M_v = 5.0 \times 10^5$, degree of deacetylation = 82%) was purchased from Sigma and purified by reprecipitation using 1N sodium hydroxide solution.¹⁸ The precipitates were sufficiently washed with distilled water, methanol, and ether, followed by drying in a vacuum at 50°C. The solutions of silk fibroin and chitosan in formic acid with different mixing ratios [90/10, 70/30, and 50/50 silk fibroin/chitosan (w/w)] were cast onto polystyrene plates and dried at room temperature.

Characterization of Blend Films

The IR spectra of the blend films were obtained using an FTIR spectrometer (Midac Co.) with a resolution of 2 cm^{-1} and a repetition of 100 scans. It was essential to ensure that films were sufficiently thin to be within an absorption range where the Beer–Lambert law is obeyed. The dynamic mechanical properties were measured by a Rheometric Scientific DMTA MK III at 3 Hz under a nitrogen atmosphere. The temperature range was from 30 to 270°C, and the heating rate was 2°C/min. Changes in the crystalline state were monitored by a wide-angle X-ray diffractometer (MAC MXP18A-HF) with Ni-filtered $\text{CuK}\alpha$ radiation at 12 kV and 50 mA. The density of the membrane was measured by a density gradient column prepared from mixture systems of carbon tetrachloride/heptane at 25°C. Mechanical tests were performed by an Instron 4201. The gauge

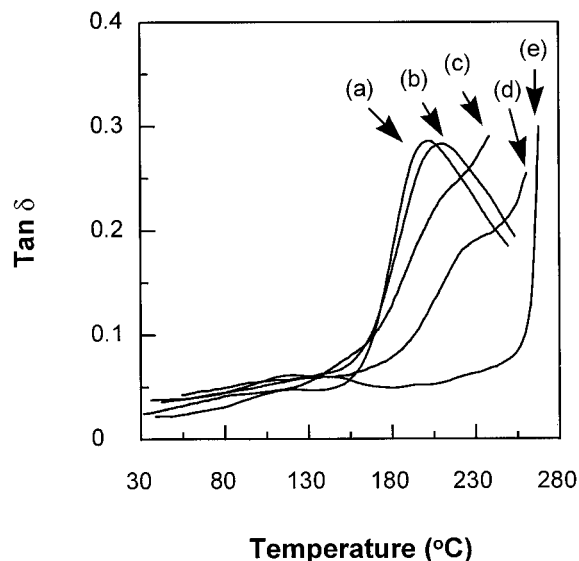


Figure 1 Temperature dependence of the loss tangent ($\tan \delta$) of silk fibroin/chitosan blends and pure polymer films measured by DMTA at 3 Hz. Silk fibroin/chitosan (w/w) = (a) 100/0, (b) 90/10, (c) 70/30, (d) 50/50, and (e) 0/100.

length was 20 mm, and the crosshead speed was 10 mm/min.

RESULTS AND DISCUSSION

Miscibility of Silk Fibroin/Chitosan Blends

The most general criterion for miscibility in a polymer blend is the existence of a single glass transition, which means the interaction between two pure polymers at the molecular level. It is well known that dynamic mechanical thermal analysis (DMTA) is very sensitive to the glass transition of a polymer system. Figure 1 shows the temperature dependence of the $\tan \delta$ values of silk fibroin/chitosan blends and pure polymers. It is generally accepted that the temperature at the maximum value in $\tan \delta$ corresponds to the glass–rubber transition. Accordingly, the $\tan \delta$ peak at 201°C of silk fibroin, which is related to the molecular motion in the amorphous region, is designated as the α relaxation reflecting the glass transition temperature of silk fibroin. The glass transition temperature of chitosan, however, seems to exist above 250°C, in spite of significant thermal decomposition similar to cellulose.^{19,20} The α -relaxation peak of silk fibroin obviously shifts to the higher temperature region with the addition of

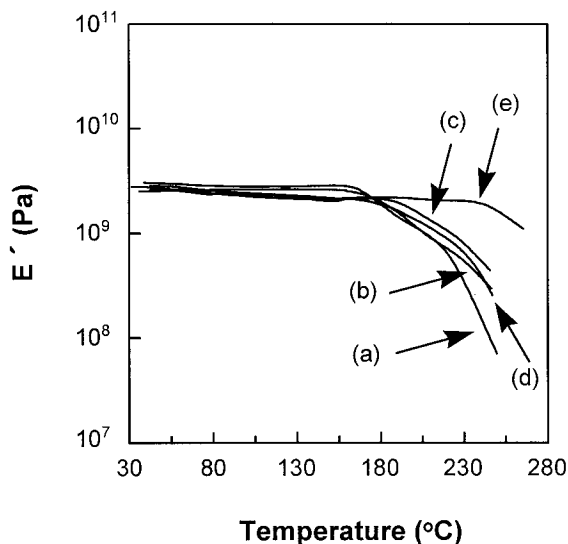


Figure 2 Temperature dependence of the dynamic storage modulus (E') of silk fibroin/chitosan blends and pure polymer films measured by DMTA at 3 Hz. Silk fibroin/chitosan (w/w) = (a) 100/0, (b) 90/10, (c) 70/30, (d) 50/50, and (e) 0/100.

chitosan, presumably toward the glass transition temperature region of chitosan. Figure 2 shows the temperature dependence of the dynamic storage modulus (E') of silk fibroin/chitosan blends. It was reported that an immiscible blend showed a step character corresponding to the glass transition temperatures of individual components, but a miscible blend showed no step character in the sharp drop region.²¹ A monotonous change in the first plateau region, as well as no step character in the sharp drop region, was observed for silk fibroin/chitosan blends. These results led to the conclusion that silk fibroin/chitosan blends exhibit considerable miscibility at the molecular level. No trace of phase separation for silk fibroin/chitosan blends was observed by scanning electron microscopy (data not shown).

Structural Change of Silk Fibroin/Chitosan Blends

The structural change of blend films was studied by IR spectroscopy. The absorption bands at 1630 (amide I), 1530 (amide II), 1265 (amide III), and 700 cm^{-1} (amide V) were assigned to the β -sheet structure of silk fibroin (spectra not shown). The absorption bands at 1660 (amide I), 1540 (amide II), 1275 (amide III), and 650 cm^{-1} (amide V) were assigned to the random coil form.¹¹ The characteristic absorption bands of chitosan were observed at 1659 (amide I), 1562 (amide II), and

1317 cm^{-1} (amide III). A small but distinct change of the amide I band was observed in the silk fibroin/chitosan blends, and it reached maximum with the 70/30 blend ratio (Fig. 3). If there is no interaction between silk fibroin and chitosan, the amide I band of the blends may be located between those of each polymer. Therefore, such a distinct shift of the amide I band by blending suggests the specific intermolecular interaction between silk fibroin and chitosan resulting from the conformational change of silk fibroin from random coil form to β -sheet structure.³

Figure 4 shows X-ray diffraction patterns of silk fibroin/chitosan blends. The crystalline peak of silk fibroin or chitosan seems to be diffused by mixing with each other. For the 70/30 blend a characteristic diffraction pattern was observed at $2\theta = 20.5^\circ$, corresponding to the crystalline spacing of 4.39 \AA attributed to the β -form structure of silk fibroin.²² It may be assumed that unoriented small crystals are created by specific interactions between silk fibroin and chitosan, such as hydrogen bonding. Therefore, we may conclude that crystallization of silk fibroin can be synergistically promoted by mixing of 30 wt % chitosan with structural changes from random coil form to β -sheet form.

The change of crystallinity of silk fibroin/chitosan blends was also investigated by density measurements. The densities of silk fibroin and chitosan films were 1.348 and 1.434 g/mL , respectively. The expected density of a simple mixture is calculated using the following equation:

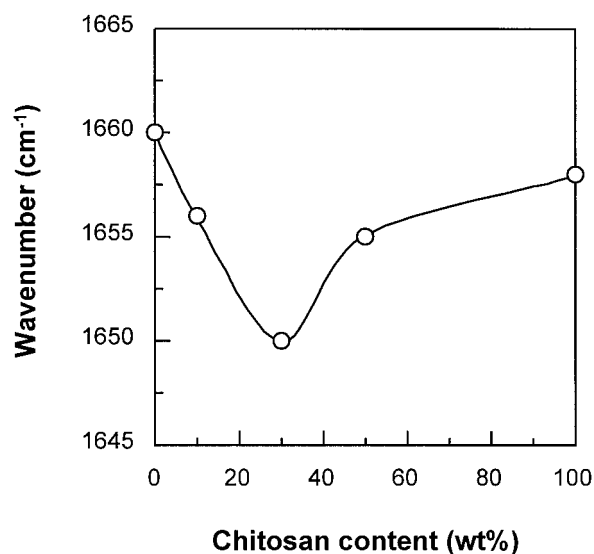


Figure 3 Changes in the amide I band of silk fibroin/chitosan blend films.

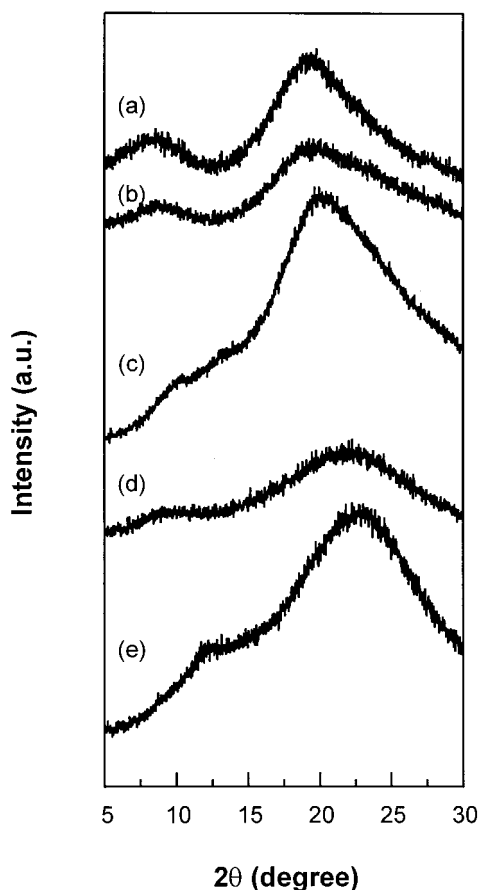


Figure 4 X-ray diffraction diagrams of silk fibroin/chitosan blends and pure polymer films. Silk fibroin/chitosan (w/w) = (a) 100/0, (b) 90/10, (c) 70/30, (d) 50/50, and (e) 0/100.

$$1/\rho = w_1/\rho_1 + w_2/\rho_2 \quad (1)$$

where ρ , ρ_1 , and ρ_2 are the densities of the mixture, component 1, and component 2, respectively; and w_1 and w_2 are the weight fractions of the components. Because we obtained higher density values for the blend films than those of the simple mixtures, differences between the observed and the expected values from eq. (1) are plotted in Figure 5. Particularly, silk fibroin/chitosan blend containing 30 wt % chitosan shows a maximum enhancement in density. Such a density change is in good agreement with earlier results by X-ray diffraction.

Mechanical Properties of Silk Fibroin/Chitosan Blends

Figure 6 shows the tensile strength of the blend films as a function of chitosan content. The tensile

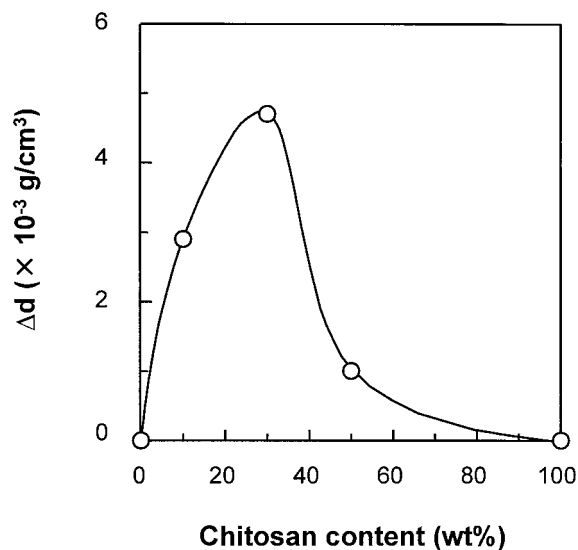


Figure 5 Density difference (Δd) between the observed and the calculated values under the assumption of a simple mixture of silk fibroin and chitosan.

strength of silk fibroin and chitosan are 29 and 50 MPa, respectively. The tensile strength of the blend films shows a higher value than that of a pure polymer and reaches a maximum value at the 70/30 blend ratio. A maximum value of the tensile strength with the 70/30 blend is attributed to structural changes of silk fibroin from random coil form to β -sheet crystalline form, resulting in the increase of modulus and toughness. Figure 7 shows the initial tensile modulus of the blend

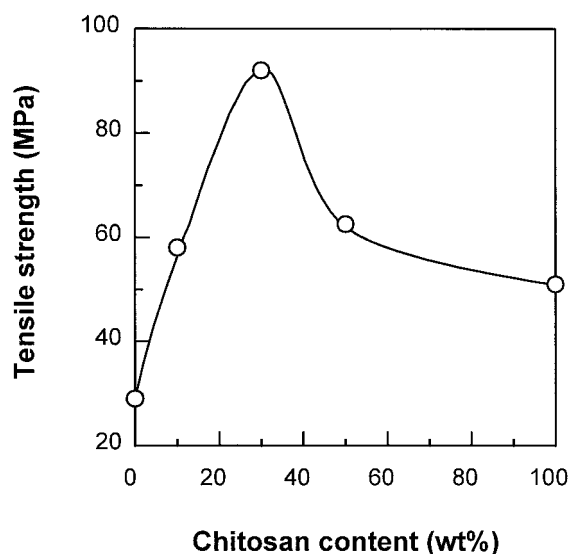


Figure 6 Tensile strength of silk fibroin/chitosan blend films as a function of chitosan content.

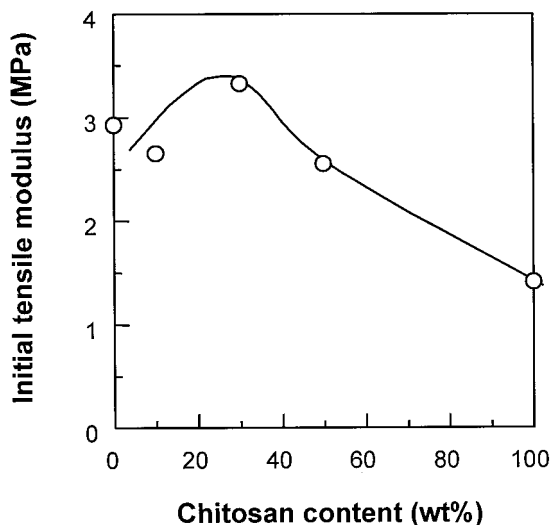


Figure 7 Initial tensile modulus of silk fibroin/chitosan blend films as a function of chitosan content.

films. The silk fibroin film exhibits a large initial modulus value because of its brittleness. The maximum increase of the 70/30 blend is also ascribable to the increase in crystallinity as shown in Figures 4 and 5.

CONCLUSIONS

Silk fibroin/chitosan blends were miscible in the range of 10–50 wt % chitosan content. The α transition of silk fibroin measured with DMTA shifted to a higher temperature with the addition of chitosan. Conformational changes of silk fibroin from random coil form to β -sheet form were observed by IR spectroscopy. This conformational transition by blending with chitosan resulted in an increase of crystallinity and an enhancement of the mechanical properties of silk fibroin. This blend film may have versatile and potential applicability to biomedical uses.

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