Physical Properties of Silk Fibroin/Chitosan Blend Films

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ABSTRACT: Silk fibroin/chitosan blend films were examined through IR spectroscopy to determine the conformational changes of silk fibroin. The effects of the fibroin/chitosan blend ratios (chitosan content) on the physical and mechanical properties were investigated to discover the feasibility of using these films as biomedical materials such as artificial skin and wound dressing. The mechanical properties of the blend films containing 10–40% chitosan were found to be excellent. The tensile strength, breaking elongation, and Young's modulus were affected by the chitosan contents of the blend films, which were also related to the density and degree of swelling. The coefficient of water vapor permeability of the blend films increased linearly with the chitosan content, and the values of 1000–2000 g m^{-2} day^{-1} were comparable to those of commercial wound dressings. Silk fibroin/chitosan blend films had good oxygen and water vapor permeabilities, making them useful as biomaterials. In particular, the blend film containing 40–50% chitosan showed very high oxygen permeability. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 80: 928–934, 2001

Key words: silk fibroin; chitosan; blend film; mechanical properties; permeability

INTRODUCTION

Natural polymers as biotechnological or biomedical resources have been investigated because of their unique properties including nontoxicity, degradability, and biological compatibility. Silk fibroin, one of the typical natural protein polymers, has been studied as a source of textile fiber and biomaterials. However, silk fibroin films in the dry state, being very brittle, are not suitable for use by themselves. The physical properties of silk fibroin film could be enhanced by mixing it with other synthetic or natural polymers, such as poly(vinyl alcohol) (PVA),[1] sodium alginate,[2] and so forth.

Chitosan is a deacetylated product of chitin treated with alkali. While chitin is insoluble in water and many commercial solvents, chitosan is readily soluble in various acidic solvents including formic and acetic acids and others. Chitosan film has been investigated as a biomedical material because of its good biocompatibility and mechanical and physical properties.[3]

Liang and Hirabayashi[4] reported on the good mechanical properties of the fibroin/chitosan blend. The silk fibroin in silk fibroin/chitosan blend membranes showed a β-sheet conformation while pure silk fibroin showed a random coil conformation.[4–6] The pervaporation properties of the blend membranes exhibited pH and ion sensitivities of a chitosan/silk fibroin interpenetrating polymer network.[7] Although several researchers investigated the conformations and mechanical properties of the fibroin/chitosan blend films, some important physical characteristics of the blend films such as the density, morphology, and water vapor and oxygen permeabilities are not yet reported. The physical, as well as the mechanical, properties of the films should satisfy the

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basic requirements for biomedical applications such as artificial skin and wound dressings.

In this study, silk fibroin/chitosan blend films prepared in varying blend ratios were examined to discover their effects on the physical characteristics. The properties of silk fibroin/chitosan blend films including the density, degree of swelling, wet strength, elongation, and water vapor and oxygen permeabilities were measured.

EXPERIMENTAL

Materials

Raw silk produced by *Bombyx mori* silkworms was degummed twice with a marseillus soap [0.5% on the weight of the fiber (owf)] and sodium carbonate [0.3% owf] solution at 100°C for 1 h and washed thoroughly with distilled water. Degummed silk was dissolved in a mixed solvent of $CaCl_2$, H_2O, and ethanol with a 1 : 8 : 2 molar ratio. The solution was filtered and cast at 40°C to obtain a regenerated fibroin film after dialysis against distilled water for 4 days.

The chitosan was purchased by Aldrich Company. The degree of deacetylation was 84%, and the molecular weight was 200,000.

The silk fibroin/chitosan blend films were prepared by casting the mixture of 5% (w/v) silk fibroin and 5% (w/v) chitosan dissolved in formic acid solution onto a polystyrene plate and allowing the solvent to evaporate at room temperature. The thickness of the blend films was controlled at about 50 μm. All the samples in this study were treated with methanol prior to measurements.

Measurements

Fourier transform IR (FTIR) spectra of the fibroin/chitosan films were obtained with a Midac M series spectrometer. The morphology of the fractured surface of the blend films was observed by a scanning electron microscope (JSM-5410LV, Jeol) after gold coating.

The density of the blend film was measured using a Mattler density kit. First the weights of the sample in air (W_a) and in water (W_w) were measured, and the density of the sample was compensated with the density of water (ρ_w) at the measured temperature.

$$\text{density} = W_a/(W_a - W_w) \times \rho_w$$

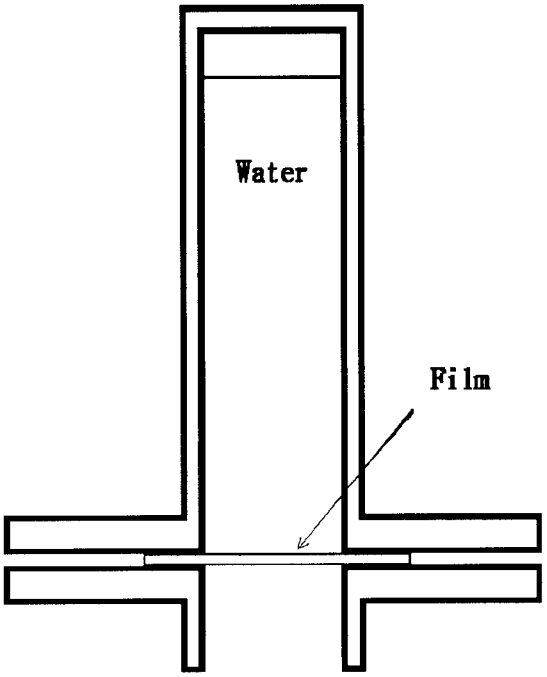

Figure 1 A schematic diagram of the apparatus for the water vapor permeability measurement.

The swelling ratio of the blend films was calculated with the following equation:

$$\text{swelling ratio (\%)} = (W_s - W_d)/W_d$$

where W_s is the weight of the swollen samples and W_d is the weight of the dry samples. The weight of the completely dried sample was measured directly and that of the sample swollen in distilled water at 37°C for 72 h was measured after centrifuging to remove the excess water from the surface of the swollen sample.

The tensile strength and percent of elongation were measured after the sample was soaked in water for over 24 h using a Minimat (Rheometric Scientific). The experimental conditions were 200-N initial load cell, 5 mm/min tensile speed, and 5 × 40 × 0.05 mm sample dimensions.

Water vapor permeability was measured in the incubator with a constant temperature of 36°C to avoid possible variations attributable to ambient conditions. Figure 1 shows the schematic diagram of the apparatus for measuring the water vapor permeability. The weight of the sample was measured as a function of time, and the coefficient of water vapor permeability (g cm cm^{-2} s^{-1}) was calculated by dividing the weight changes by the cross-sectional area.

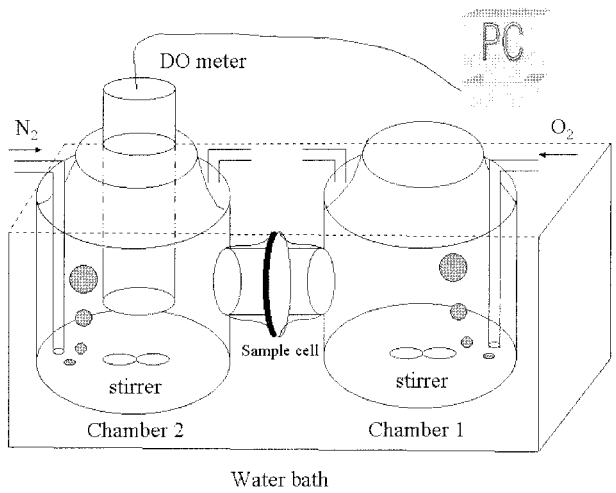

Figure 2 A schematic diagram of the apparatus for the oxygen permeability measurement.

Dissolved oxygen permeating through the samples was measured using an oxygen permeability apparatus (Fig. 2). First the sample was placed in between the two oxygen chambers, and N_2 gas was purged through the chambers. Chamber 1 was then saturated with O_2 gas, and the amount of dissolved oxygen, which permeated through the sample cell from chamber 1 to chamber 2, was measured using an oxygen meter (Oxi 538, WTW). The permeability coefficient P [cm(STP) cm cm^{-2} s^{-1} $cmHg^{-1}$] was calculated from the following equation:

$$\text{oxygen permeability coefficient} = (VB)/(AH) \times 10^{10}$$

where V is the volume of oxygen per unit time (cm^3/s), B is the thickness of the samples (cm), A is the surface area of the samples (cm^2), and H is the pressure of the transmission (cmHg).

RESULTS AND DISCUSSION

FTIR Spectra

FTIR spectra of fibroin/chitosan blend films with different compositions are shown in Figure 3. The silk fibroin film treated with methanol showed absorption bands at 1625 (amide I), 1527 (amide II), and 1260 cm^{-1} (amide III), which were attributed to the β-sheet conformation of silk fibroin. On the other hand, the chitosan film showed absorption bands at 1154 and 900 cm^{-1}, which were attributed to the saccharide structure, and at 1598 and 1651 cm^{-1}, which were attributed to the amino group of chitosan and the amide group of chitin, respectively.[8,9] The blend films exhibited the characteristic absorption bands of both fibroin and chitosan with only intensity differences due to varying compositions of the two materials.

IR spectra, representing typical absorption bands sensitive to the molecular conformation of silk fibroin, were used by many researchers to determine the conformation of silk fibroin. The absorption bands at 1660 (amide I), 1540 (amide II), and 1235 cm^{-1} (amide III), which were assigned to the random coil structure of silk fibroin, shift to 1630, 1530, and 1265 cm^{-1}, in accordance with the structural changes into β-sheet conformation.[10–13]

Nakamura et al.[14] reported that IR spectra of fibroin and a fibroin/chitosan blend showed the characteristic peaks of a random coil structure of fibroin. On the other hand, other researchers[4–6] reported that the conformational transition of silk fibroin to a β-sheet structure was induced by chitosan. It is uncertain if the chitosan components affect the conformation of silk fibroin in the fibroin/chitosan blend. In our study, because the fibroin/chitosan blend films were treated with methanol and the conformation of silk fibroin changed to the β-sheet structure, IR spectra of the blend films showed all the characteristic bands of chitosan and the β-sheet structure of silk fibroin.

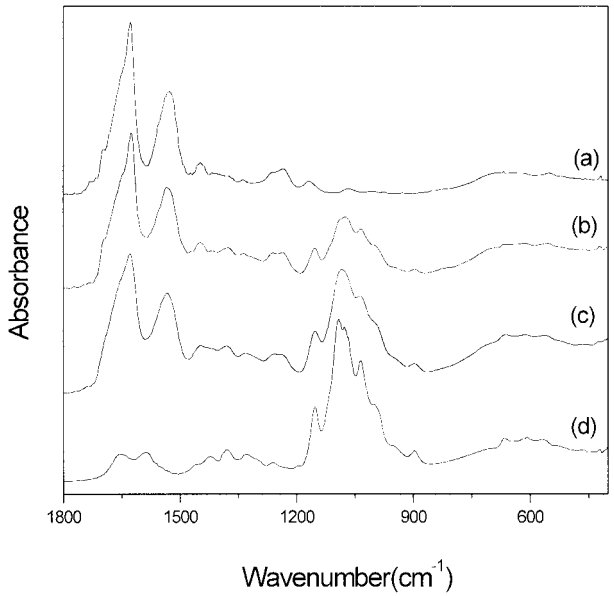

Figure 3 FTIR spectra of silk fibroin/chitosan blend films with blend ratios of (a) 100/0, (b) 60/40, (c) 40/60, and (d) 0/100.

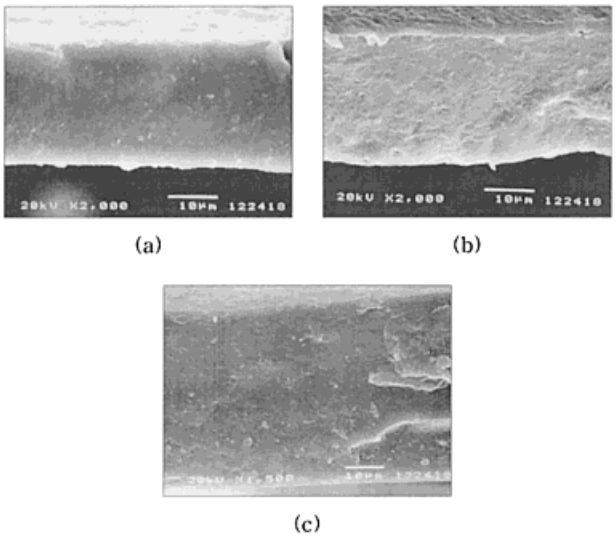

Figure 4 SEM photographs of silk fibroin/chitosan blend films with blend ratios of (a) 70/30, (b) 50/50, and (c) 30/70.

Surface Characteristics

The surface morphology of the silk fibroin/chitosan blend films was observed with scanning electron microscopy to verify the compatibility of the mixtures of fibroin and chitosan. Figure 4 shows the fractured surface of the blend film with a smooth surface structure, regardless of the blend ratios.

The compatibility and phase separation behavior were studied for the fibroin blends such as fibroin/PVA,[1,15–17] fibroin/polyacrylamide,[18] and fibroin/cellulose[19] among others. These fibroin blend films were classified according to the occurrence of the microscopic phase separation. The silk fibroin/chitosan blend films prepared in this study did not show microscopic phase separation morphology as other fibroin/polysaccharide blends such as fibroin/cellulose did, which was probably due to the interaction between fibroin and chitosan molecules. Several researchers[4–6,14] reported that intermolecular hydrogen bonds between fibroin and chitosan might be formed in the fibroin/chitosan blends.

Density

The density of the silk fibroin/chitosan blend film was measured and plotted as a function of composition (Fig. 5). The density value of the pure fibroin film was 1.40 g/cm^3, slightly higher than those reported for degummed silk fiber and fibroin film,[19–21] which are due to the differences in the measurement technique and solvent system used to prepare the blend dope solution. The density of the blend films, showed a increase linear general, with chitosan content. In particular, the density of the blend films containing 10–20% chitosan content was somewhat lower than that of the pure fibroin film.

The density of the favorable polymer blends was generally higher than that of the mixture rule while the lower density was caused by unfavorable polymer blends attributable to the void induced by the steric hindrance of the two polymers.[22] From these results a small amount of chitosan (10–20%) was found to disturb the interaction between fibroin molecules in blend films, thus giving a lower density value.

Swelling

The degree of swelling of fibroin/chitosan blend films was measured in terms of the equilibrium swelling ratio (Fig. 6). Considering the possible applications of these biomaterials in artificial skin and wound dressings, the degree of swelling is one of the important factors in determining the usefulness of the biomaterials. The swelling ratios of silk fibroin and chitosan film were 40 and 120%, respectively. Chitosan film had an excellent swelling power compared with silk fibroin, and the degree of swelling of the fibroin blend films could be enhanced by the addition of a chitosan component. In particular, fibroin/chitosan blend films containing 10–20% chitosan showed much higher swelling ratio values of 150–210% due to the disordered nature of the structure (Figs. 5, 6). When the chitosan content in the

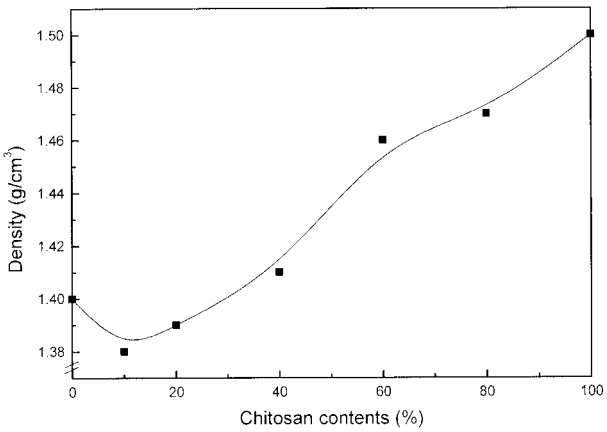

Figure 5 The density of silk fibroin/chitosan blend films.

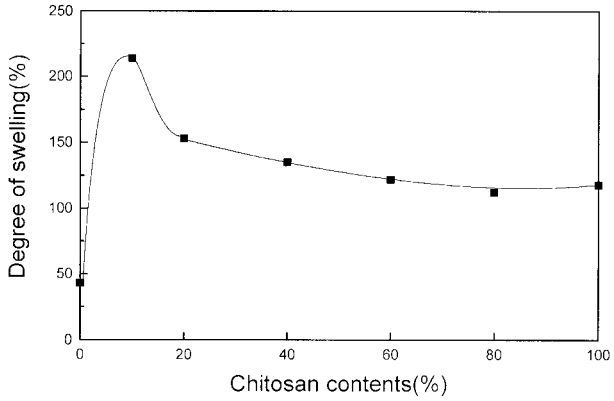

Figure 6 The degree of swelling of silk fibroin/chitosan blend films.

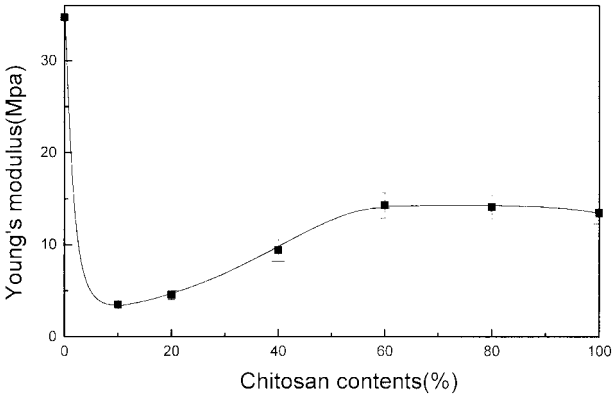

Figure 8 Young's modulus of silk fibroin/chitosan blend films measured under wet conditions.

blend film was higher than 40%, however, the degree of swelling was not much different than that of the pure chitosan film.

Mechanical Properties

Mechanical properties are of primary importance for determining the performance of materials expected to undergo various types of stresses during use. Figure 7 shows the tensile strength and strain of fibroin/chitosan blend films with varying chitosan contents. The stress–strain curves were obtained in the wet state, which is more important in practical applications of biomaterials than the dry state.

The tensile strength of silk fibroin film was 4.5 MPa, while that of chitosan film was 30 MPa. Compared with the other results,[18,19] the strength of silk fibroin film was observed to be weaker because of the sample condition undergoing the tensile test in the wet state, as well as the solvent system used for dissolution and film preparation. In the fibroin/chitosan blend films, the tensile strength increased with the increase in the chitosan content. At present the strength of blend films appears to be sufficient for practical applications. However, a high content of the chitosan component could result in a much stronger film if necessary.

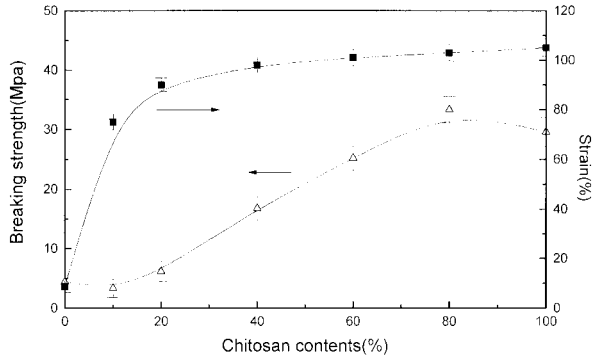

Figure 7 The breaking strength and strain of silk fibroin/chitosan blend films measured under wet conditions.

The elongation at break was 10 and 100%, respectively, for silk fibroin and chitosan film. The silk fibroin film is very brittle in dry conditions, but the elongation increases as the film absorbs water in the wet state. The chitosan film has excellent breaking elongation, as well as tensile strength. Interestingly, the breaking elongation of the blend films did not increase linearly with the chitosan content. The blend film containing even only a 20% chitosan component showed as high as 90% breaking elongation, close to the value of pure chitosan film.

Figure 8 shows the Young's modulus of fibroin/chitosan blend films calculated using the stress–strain curve. Silk fibroin film, which has an extremely high Young's modulus, is very stiff. The addition of the chitosan component to silk fibroin (containing about 10–40% chitosan) is effective for the improvement of the flexibility of blend films. The Young's modulus of the blend films showed trends similar to the tensile strength and breaking elongation for the effect of chitosan content on the mechanical behavior of the film (Figs. 7, 8).

The mechanical properties of the fibroin/chitosan blend films are quite important from a practical point of view. According to our results, con-

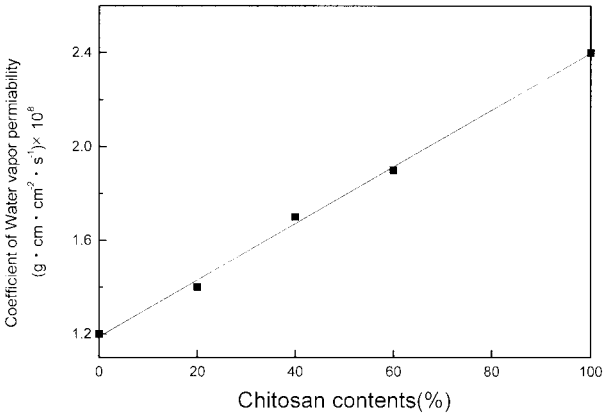

Figure 9 The coefficients of water vapor permeability for silk fibroin/chitosan blend films.

trolling the chitosan content in blend films can be useful for improving the mechanical properties. Silk fibroin film, which has poor mechanical properties, can be used in a wide range of biomaterial applications when blended with chitosan. The mechanical properties were significantly affected by the chitosan content of the blend films and were related to the characteristics of the physical and morphological structures.

Water Vapor Permeability

Figure 9 shows the coefficients of the water vapor permeability for silk fibroin/chitosan blend films. The coefficient of the chitosan film (2.4×10^{-8} g cm cm^{-2} s^{-1}) is much higher than that of the fibroin film (1.2×10^{-8} g cm cm^{-2} s^{-1}). As the chitosan content increased, the vapor permeability coefficient of the blend films linearly was increased because of the high permeability power of the chitosan component.

In order to apply the fibroin/chitosan blend films in wound dressings or artificial skin, the water vapor permeability must be carefully controlled. A large amount of water flux of moisture will result in dehydration of the wound surface and disruption of the wound–graft interface, whereas a small amount will cause the accumulation of exudative fluid beneath the graft and lift the film from the wound bed.[23] The water vapor permeating rate for normal skin is 204 ± 12 g m^{-2} day^{-1} while that for injured skin can range from 279 ± 26 g m^{-2} day^{-1} for a first degree burn to 5138 ± 202 g m^{-2} day^{-1} for a granulating wound with a surface temperature of 35°C.[24] That of commercial dressings at a static state was reported to be 90–2890 g m^{-2} day^{-1}.[25] Yannas et al.[26] suggested the optimum rate of water flux through the graft to be approximately 1200 g m^{-2} day^{-1}. The water vapor permeation rates of the fibroin and chitosan film were 1037 and 2074 g m^{-2} day^{-1}, respectively, on the basis of the film thickness of 100 μm. Therefore, the blend films are expected to control the excess water vapor evaporated from the wound skin.

Oxygen Permeability

Good oxygen permeability in biomedical applications as artificial skin or cornea inhibits the growth of bacteria and accelerates wound healing and skin regeneration. A typical oxygen permeability curve for the fibroin/chitosan blend film is shown in Figure 10. The oxygen permeation rate of the sample swollen in water for 24 h was determined from the slope of the oxygen permeability curve. The oxygen permeability coefficients of silk fibroin/chitosan blend films are listed in Table I.

The oxygen permeability coefficient of the chitosan film was higher than that of the silk fibroin film. The coefficient markedly increased as the chitosan content increased up to 50%. However, the permeability coefficients hardly changed for the blend films containing higher than 60% chitosan content. The oxygen permeability of silk fibroin film can be improved by blending the film with chitosan. In particular, the 50/50 silk fibroin/chitosan blend film showed excellent oxygen permeability and could be used as a biomaterial requiring high oxygen permeability.

While the chitosan film showed a better oxygen permeability than the fibroin film, in the silk fibroin/chitosan blend films, the oxygen permeabil-

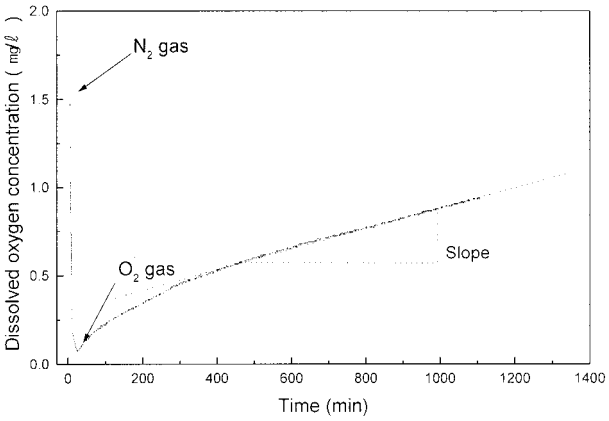

Figure 10 The typical dissolved oxygen permeability curve for fibroin/chitosan blend films.

Table I Calculated Oxygen Permeability Coefficients of Silk Fibroin/Chitosan Blend Films

Chitosan Content in Silk Fibroin/ Chitosan Blend Films (%)	Coefficient of Oxygen Permeability [cm^3(STP) cm/(cm^2 s cmHg) $\times 10^{10}$]
0	0.249
20	0.446
30	0.462
40	0.524
50	0.579
60	0.430
70	0.438
80	0.423
100	0.395

ity was excellent, regardless of the content of each component. However, the permeability of the blend films did not always increase linearly with the increase in chitosan content. The structure, morphology, and properties of the blend films can affect the oxygen permeability. Although direct evidence to explain this result was not found in this study, the degree of swelling, as well as the density, seemed to be related to the oxygen permeability of the blend films.

CONCLUSIONS

The silk fibroin/chitosan blend films were prepared, and physical and mechanical properties were examined with varying chitosan contents (blend ratios). Microscopic phase separation did not occur in the blend films, regardless of the blend ratios. The density, degree of swelling, and mechanical properties were strongly affected by the chitosan content in the blend films. The mechanical properties can be markedly improved by blending silk fibroin with 10–40% chitosan component. The coefficient of water vapor permeability of the blend films was comparable to that of commercial wound dressings. In particular, the blend film containing 40–50% chitosan content showed very high oxygen permeability. Therefore, this silk fibroin/chitosan blend film can be used as a wound dressing and artificial skin because of its good mechanical properties and good water vapor and oxygen permeabilities.

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