Structure and Physical Properties of Silk Fibroin/Polyacrylamide Blend Films

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ABSTRACT: This article deals with the characterization of blend films obtained by mixing silk fibroin (SF) and polyacrylamide (PAAm). The DSC curves of SF/PAAm blend films showed overlapping of the main thermal transitions characteristic of the individual polymers. The exothermic peak at 218°C, assigned to the β-sheet crystallization of silk fibroin, slightly shifted to a lower temperature by blending. The weight-retention properties (TG) of the blend films were intermediate between those of the two constituents. The TMA response was indicative of a higher thermal stability of the blend films, even at low PAAm content ($\leq 25\%$), the final breaking occurring at about 300°C (100°C higher than pure SF film). The peak of dynamic loss modulus of silk fibroin at 193°C gradually shifted to lower temperature in the blend films, suggesting an enhancement of the molecular motion of the fibroin chains induced by the presence of PAAm. Changes in the NH stretching region of silk fibroin were detected by FTIR analysis of blend films. These are attributable to disturbance of the hydrogen bond pattern of silk fibroin and formation of new hydrogen bonds with PAAm. The values of strength and elongation at break of blend films slightly improved at 20–25% PAAm content. A sea–island structure was observed by examining the air surface of the blend films by scanning electron microscopy. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 71: 1563–1571, 1999

Key words: silk fibroin; polyacrylamide; blend films; physical properties; thermal stability

INTRODUCTION

The silk spun by the domestic silkworm *Bombyx mori* is a widely studied biopolymer, whose chemical, physical, and mechanical properties have been reviewed by various authors.[1–4] Besides its use as textile fiber, silk is considered an interesting starting material for nontextile applications, i.e. for developing new materials and devices for biotechnological and biomedical utilization. As it is well known, silk fibroin is available not only in form of fiber, but can also be prepared in form of gel, powder, compact, and porous membranes after dissolution with suitable solvents. Surgical sutures,[5] biosensors developed by the enzyme immobilization technique,[6,7] membranes with selective permeability,[8,9] biocompatible devices with controlled drug release,[10] wound-repairing, and bone-binding functions[11] are some of the applications already developed or suggested for silk.

Amorphous, water-soluble silk fibroin films prepared by casting an aqueous solution at room temperature can be made insoluble by means of simple

physicochemical treatments,[8,12] which are effective in inducing random coil→β-sheet conformational transition.[12,13] However, these films suffer from poor tensile properties, because they are very brittle and almost unsuitable for practical use in the dry state.[8,14,15] The inferior tensile properties of silk films can be improved by blending with other natural or synthetic polymers. Blends of silk with chitosan,[16] sodium polyglutamate,[17] sodium alginate,[18] cellulose,[19] tussah (*Antherea pernyi*) silk fibroin,[20] and polyvinyl alcohol (PVA)[15,21] have been reported. Water absorption, mechanical properties, and thermal stability of silk fibroin films were improved by blending with sodium alginate.[18] The addition of cellulose to silk fibroin permitted preparation of films with excellent elastic behavior.[19] Silk/PVA blend films showed increased permeability to neutral salts.[15] Recently, Sun et al.[22] reported the properties of blend fibers obtained by mixing silk with an acrylic polymer (PAC). The addition of up to 30% silk resulted in an increase of moisture absorption, while the mechanical properties slightly decreased compared to pure PAC fibers. The blend fibers showed a skin–core structure, with silk fibroin mainly located in the skin. The specific interactions (hydrogen bonding) in silk fibroin/poly(acrylonitrile-*co*-methyl acrylate) blends have recently been investigated by means of FTIR spectroscopy.[23]

The polymer blending technique can be considered a useful tool for the preparation of silk fibroin films with improved physical properties and performance.[15–21] In most cases it has been shown that formation of specific intermolecular interactions through hydrogen bonds between silk and the added polymer is responsible for the observed mixing behavior and properties of the blends.[18,19,22,23] The selection and use of polymers potentially able to form hydrogen bonds when mixed with silk fibroin, as well as the study of the properties of the resulting blend films, may be of great interest in view of developing new silk-based materials for biomedical and biotechnological applications. The present study focuses on the preparation and characterization of a new kind of blend between silk fibroin (SF) and polyacrylamide (PAAm). PAAm appeared a suitable candidate for blending with silk because it is a water-soluble polymer with several primary amide groups on its chain backbobe. Moreover, PAAm is a polymer of biomedical and pharmaceutical interest widely studied as hydrogel for blood-compatible applications.[24] SF/PAAm blends were prepared by casting the mixed polymer aqueous solutions at room temperature. Physical proper-

ties and morphology of the blend films were studied by means of various thermoanalytical techniques (DSC, TG, TMA, and DMA), tensile measurements, and scanning electron microscopy. A deeper insight into the molecular interactions, particularly hydrogen bonds, between SF and PAAm in the blends was achieved by means of FTIR measurements. We think that the results reported in this study may contribute basic information for developing silk-based blend systems with enhanced functional performance.

EXPERIMENTAL

Materials

The aqueous solution of silk fibroin (SF) was obtained by gently dispersing into water the content of the posterior silk gland excised from full grown larvae of *Bombyx mori* silkworm. The solution concentration was about 0.3% w/v. The polyacrylamide (PAAm) solution was prepared by diluting with water the commercial 10% w/v solution purchased from Wako Pure Chemicals, Lot No. LTJ2704.

Pure and blend films with different blending ratios were prepared by casting the respective aqueous solutions on a polyethylene plate at 20°C and 65% relative humidity (RH) for 48 h. The films were then subjected to further drying under vacuum for 2 days. Films of about 20-30 μm thickness were obtained. These were allowed to set in standard conditions (20°C and 65% RH) before measurements.

Measurements

Heat-flow differential scanning calorimetry (DSC) measurements were performed with a Rigaku Denki Co., Ltd. instrument (DSC-10A) at a heating rate of 10°C/min. The DSC range and sample weight were 2.5 mcal/s and ca. 2 mg, respectively. The open aluminium cell was swept with N_2 gas during the analyses.

Thermogravimetric (TG) analyses were run in a flowing nitrogen atmosphere on a Rigaku Denki Co., Ltd. thermogravimetric Thermoflex system, using 3 mg of specimen. The temperature was raised to 400°C at a heating rate of 10°C/min.

Dynamic mechanical measurements (DMA) were carried out with a Toyoseiki Rheolograph Solid-S. The temperature range studied was from −50 to 260°C. Film specimens of 3-mm width and

15-mm length were heated at 2°C/min. The temperature dependence of storage (E') and loss (E'') moduli was measured at a frequency of 10 Hz, with an initial tension applied to samples of 30 gf.

Thermomechanical analyses (TMA) were performed with a CN-8361 instrument (Rigaku Denki Co., Ltd.) at a heating rate of 10°C/min, under N_2 to provide the inert atmosphere. Initial force applied and TMA full scale were 1.5 g and ±1000 μm, respectively.

Stress–strain measurements were performed at 20°C and 65% relative humidity with a Toyo Baldwin Co. tensile testing machine, mod. Tensilon UTM-II, at a gauge length of 20 mm and strain rate of 8 mm/min. Each value is the average of 20 measurements.

The infrared spectra were obtained directly on the films with a Perkin-Elmer FTIR 1725 spectrophotometer.

The surface morphology was examined by using a scanning electron microscope mod. Stereoscan 440 (LEO) after gold coating.

Figure 1 DSC curves of silk fibroin (a) and blend films. SF/PAAm composition: (b) 85/15; (c) 75/25; (d) 60/40; and (e) 40/60.

RESULTS AND DISCUSSION

DSC Curves

The thermal bahavior of SF/PAAm blend films was investigated by means of DSC measurements (Fig. 1). The pure SF film [Fig. 1(a)] showed the characteristic pattern of amorphous silk fibroin, as obtained by casting from a dilute aqueous solution at room temperature.[25] An endothermic shift at about 175°C marked the transition from the glassy to the rubbery state (T_g).[26] It was followed by an exothermic peak at 218°C and a prominent endothermic peak at 280°C, attributed to the heat-induced β-sheet crystallization and to the thermal decomposition of silk fibroin with unoriented β-sheet structure, respectively.[26]

The sequence of DSC events typical of silk fibroin also characterized the thermal behavior of blend films containing ≤25% PAAm [Fig. 1(b) and (c)]. However, some changes were observed, such as a slight broadening of the decomposition endotherm in the lower temperature range, and a downwards shift of the crystallization peak (from 218 to 212°C). With increasing the amount of PAAm in the blend [Fig. 1(d) and (e)], a broad endotherm appeared at about 221°C. It is worth noting that the DSC curve of pure PAAm (curve not shown) was characterized by an intense endothermic peak at about 220°C, which can be

attributed to various thermally induced transitions, such as melting of PAAm chains and beginning of thermal degradation (see TMA and TG results). Accordingly, its intensity increased as a function of the PAAm content in the blend. The tendency of the endotherm at 280°C to broaden and shift to lower temperature was enhanced by blending. The 40/60 SF/PAAm blend [Fig. 1(e)] resulted in a rather complex DSC pattern, with a series of broad endotherms at 221, 258, and 277°C, and a new weak endotherm starting at above 300°C, which was detected also in pure PAAm film.

Overlapping of the characteristic thermal transitions of silk fibroin and PAAm seems the main feature emerging from the above DSC results. However, some changes appearing in the DSC pattern of blend films with low PAAm content (≤25%) may suggest that a certain degree of interaction was established between SF and PAAm. We mainly refer to the shift to lower temperature of the peak of β-sheet crystallization of silk fibroin. This effect can be interpreted as a decrease of the threshold temperature for accomplishing the crystallization process, as though the presence of PAAm favored the mobility of the fibroin chains at above T_g and their rearrangement leading to formation of β-sheet crystals. As it will be shown later (see SEM results), the two polymers formed separate domains in the blends. However,

Figure 2 TG curves of silk fibroin, PAAm, and SF/PAAm 40/60 films.

this feature does not exclude that a certain degree of molecular mixing occurred, especially at the boundary between the nucleating structures. Moreover, the SF–PAAm interchain interactions might have been enhanced during heating, when chain mobility was sufficiently high to allow further mixing.

TG Curves

The results of TG measurements are shown in Figure 2. The TG curve of the pure SF film [Fig. 2(a)] showed two zones of weight loss. The initial weight loss (ca. 8%) at approximately 100°C was due to loss of moisture. The second step of weight loss started at about 210°C and attained 24 and 39% at 300 and 350°C, respectively. It is associated with the beginning of chemical transformations leading to thermal degradation of silk fibroin. Data obtained by studying the pyrolysis products of silk fibroin during burning (unpublished results) showed that low molecular weight gases (H_2O, CO_2, NH_3), resulting from the breakdown of side chain groups of amino acid residues, as well as from cleavage of peptide bonds, are evolved at above 200°C.

PAAm [Fig. 2(b)] showed three steps of weight loss, located at about 100, 200, and 300°C. The former is attributable to loss of moisture, while those at higher temperature coincided with the broad DSC endotherms of PAA-rich blends [Fig. 1(d) and (e)]. The weak drop of the TG curve at 200°C can be related to thermal processes involving both melting of the PAAm chains and onset of degradation. On the other hand, the sharp drop at above 300°C is indicative of the occurrence of more extensive thermal degradation processes. The values of weight loss at 300 and 350°C were

16 and 28%, lower than pure SF film. The 40/60 SF/PAAm blend [Fig. 2(c)] showed an intermediate behavior, with weight loss values of 20 and 35% at 300 and 350°C, respectively. These results account for a slightly higher thermal stability attained by silk fibroin by blending with PAAm.

TMA Curves

The TMA curves of pure and blend films (Fig. 3) were characterized by an initial gradual contraction from room temperature until about 100°C, which was essentially due to moisture evaporation during heating. After this phase, the pure SF film [Fig. 3(a)] remained steady until about 180°C, when it started extending under the applied force, due to exceeding of the softening temperature.[25] Sample failure occurred slightly above 200°C. PAAm film [Fig. 3(g)] displayed a strong contraction from 160°C until about 210–220°C. The onset of the thermal shrinkage coincided with the value of glass transition temperature reported for PAAm.[27] Afterwards the film extended and finally broke at 290°C. It is interesting to note that the strong TMA shrinkage, which is indicative of a high level of molecular motion of the polymer chains, coincided with the DSC endotherm attributed to melting of PAAm.

The TMA pattern of the blend films changed as a function of the blending ratio. The addition of a small amount of PAAm (5%) to silk fibroin resulted in a significant increase of thermal stability of the blend film compared to pure SF, as shown by the TMA response, which covered a

Figure 3 TMA curves of silk fibroin (a), PAAm (g), and blend films. SF/PAAm composition: (b) 95/5; (c) 85/15; (d) 75/25; (e) 60/40; and (f) 40/60.

wider temperature range, reaching about 300°C [Fig. 3(b)]. The film showed a weak extension at above 200°C, which may account for a specific contribution of silk fibroin to the thermal behavior of the blend. Almost the same trend was observed for the blend films with 15 and 25% PAAm content [Fig. 3(c) and (d)]. When the amount of PAAm increased further [Fig. 3(e) and (f)], some thermomechanical features typical of pure PAAm became evident, such as a shrinkage in the range 160–200°C. However, it was much weaker in intensity, and its onset temperature shifted upwards with increasing the content of silk fibroin.

The above results show that the thermal expansion and contraction properties of both silk fibroin and PAAm can be modulated by varying the blending ratio. At low PAAm content ($\leq 25\%$), the thermal stability of silk fibroin was significantly increased, the limit of the final breaking shifting upwards of about 100°C. On the other hand, the addition of 40–60% silk to PAAm was effective in reducing its tendency to shrink at above T_g, therefore conferring a higher degree of stiffness on the blend films. These observations suggest that SF and PAAm could probably establish some interaction in the blend, which resulted in an improved thermal behavior compared to the pure polymers, in good agreement with the above discussed DSC results.

It is of particular interest to note the positive influence of the addition of a small amount of PAAm on the thermal stability of silk fibroin. Although almost the same effect can be achieved when pure silk fibroin films with random coil structure are subjected to physico-chemical treatments, such as immersion in organic solvents (unpublished results), the blending technique still appears more attractive because the original flexibility and elasticity of silk films is usually maintained. These findings are of primary importance when potential applications of silk films are considered, because functional properties, such as the dimensional stability over a wide temperature range, the resistance to shrinkage and extension, and the ability to absorb stresses by deformation are important in determining the performance of a material.

Dynamic Mechanical Behavior

The dynamic storage (E') and loss (E'') modulus curves of pure and SF/PAAm blend films were recorded in the temperature range from −30 to 260°C (Fig. 4). The pure SF film [Fig. 4(a)] dis-

Figure 4 DMA curves of silk fibroin (a), PAAm (d), and blend films. SF/PAAm composition: (b) 75/25; and (c) 40/60.

played the characteristic DMA pattern of amorphous films with random coil conformation.[21,25] The value of storage modulus increased from room temperature, owing to loss of moisture and consequent strengthening of the intermolecular interactions within the film matrix, which made it stiffer. Beyond T_g, the E' curve exhibited a sharp fall, accompanied by the appearance of a prominent relaxation peak in the imaginary part of the modulus (loss peak at 193°C), in response to strong motional transitions exhibited by the fibroin chains at this temperature. This peak has been attributed to the thermally induced segmental motion of the fibroin chains in the amorphous regions.[25]

The storage modulus of the PAAm film [Fig. 4(d)] showed a gradual decrease until about 50°C afterwards it remained steady or increased slightly before the final fall, which occurred as soon as the polymer entered the rubbery region and large chain segments underwent diffusional motion. Accordingly, the loss modulus curve exhibited two dispersion peaks, one weak and rather broad appearing below 50°C, and another stronger in intensity, which attained its maximum at 174°C. The blend films [Fig. 4(b) and (d)] showed a DMA pattern intermediate between the pure components, the main feature being represented by a gradual shift to lower temperatures of the peak of loss modulus as the amount of PAAm in the blend increased. This behavior can be explained, assuming that the presence of PAAm chains contributed to enhance the molecular motion of the fibroin chains in the blend. The hypothesis that the addition of PAAm to SF perturbed the hydrogen bond network characteristic of pure

Figure 5 FTIR spectra of silk fibroin (a), PAAm (c), and 50/50 SF/PAAm blend film (b).

silk fibroin and that new intermolecular interactions were established between the two polymers seems to be supported by the presence of an individual dispersion peak at above T_g, as well as by the results of DSC and TMA measurements previously discussed. It is reasonable to assume that these intermolecular interactions are mainly based on hydrogen bonding between the primary amide group of PAAm and various side chain groups of SF.

FTIR Spectroscopy

The conformational characterization of pure and blend films, as well as the study of specific interactions between SF and PAAm was carried out by means of infrared spectroscopy. The FTIR spectrum of silk fibroin [Fig. 5(a)] showed strong absorption bands at 3300 cm^{-1} (NH stretching), 1660 cm^{-1} (amide I, CO, and CN stretching), 1540 cm^{-1}, and 1244 cm^{-1} (amide II and III, respectively, predominantly NH bending and CN stretching, plus other minor vibrational modes), and 662 cm^{-1} (amide V, CN torsion, and NH

bending). Medium–weak bands could also be observed in the CH bending region (1500–1300 cm^{-1}) and in the skeletal stretching region (1100–900 cm^{-1}). In particular, the band at 1016 cm^{-1} arises from the —(gly-gly)— sequence of the silk fibroin chain.[28] As it is well known, amide I–V are conformationally sensitive bands for polypeptides and proteins.[29] From their position and intensity, we can desume that random coil was the prevailing molecular conformation of pure SF film.[30] The FTIR spectrum of the PAAm film [Fig. 5(c)] exhibited strong bands at 3400 and 3200 cm^{-1} (NH stretching), 1670 cm^{-1} (CO stretching), 1620 cm^{-1} (NH bending), and 625 cm^{-1}, attributed to the vibrational modes of the primary amide group. A CH stretching band at 2934 cm^{-1} and variuos CH bending bands in the spectral region 1500–1300 cm^{-1} were also detected. The FTIR spectrum of the 50/50 SF/PAAm blend film [Fig. 5(b)] was characterized by the presence of the absorption bands typical of the pure components, with an intensity roughly proportional to the blending ratio. The conformationally sensitive bands of silk fibroin did not show significant changes, suggesting that the addition of PAAm did not induce structural transitions, such as those reported for the blend of silk fibroin and sodium alginate.[18]

To study the interactions between SF and PAAm in more detail, difference spectra were calculated by subtracting the spectrum of pure PAAm from those of the blends. By comparing these spectra with that of pure SF film, we observed that amide and skeletal vibrational modes of silk fibroin were essentially unchanged, as far as position and intensity of the various bands is concerned. On the other hand, the NH stretching region showed significant changes (Fig. 6). The major SF band centered at 3300 cm^{-1}, which is the stretching vibration of the NH moiety of the amide group involved in both inter- and intramolecular hydrogen bonds [Fig. 6(a)], broadened, and its intensity decreased by blending, the extent of decrease being related to the blending ratio [Fig. 6(b)–(d)]. Moreover, a new band appeared at higher wavenumbers (3391 cm^{-1}). These features imply that part of the hydrogen bonds in which the amide groups of silk fibroin are involved were broken by addition of PAAm, and that free amide groups and/or new hydrogen bonds were formed. A broad distribution of local —CO—NH— environments of silk fibroin in the blends, i.e., the formation of various kinds of hydrogen bonds differing in length and strength,

Figure 6 FTIR spectrum of theNH stretching region of silk fibroin (a) and difference spectra of blend films. SF/PAAm composition: (b) 75/25; (c) 50/50: and (d) 25/75.

may, therefore, account for band broadening in the NH stretching region. These findings confirm that, despite the phase separation structure of the blend films detected by SEM, a partial molecular mixing occurred and molecular interactions, mainly based on hydrogen bonding, were established between SF and PAAm chains, as indicated by the results of thermal analyses.

Mechanical Properties

The study of the mechanical properties is of primary importance for determining the performance of a material that is expected to undergo various kinds of stresses during use. The values of rupture strength and elongation of SF/PAAm blend films were plotted as a function of the blending ratio (Fig. 7). The pure SF film displayed the typical behavior of brittle materials, with low strength (2.1 kg/mm^2) and elongation (0.7%) values, in good agreement with published results.[14] The addition of PAAm to silk fibroin was effective in inducing a slight improvement of the mechanical properties of blend films. In fact, both

strength and elongation increased with increasing the PAAm content, the optimum concentration for reaching the desired effect being in the range 20–25%. However, the changes in the mechanical properties were of small entity, much smaller than those observed by blending silk fibroin with other polymers, such as cellulose,[19] sodium alginate,[18] and sodium polyglutamate.[17] As shown by SEM observations (see Fig. 8), the SF/PAAm blend films displayed a sea–island morphology. For this kind of material, the mechanical properties should be strongly influenced not only by the performance of the individual components, but also by the adhesion at the interphase between them. From our results it appears that the interaction was weaker than that brought about by the other polymers,[17–19] which showed a higher degree of blending miscibility and could establish stronger intermolecular interactions by hydrogen bonding when mixed with silk fibroin.

Morphological Characteristics

Air surfaces of pure and blend films were examined by SEM (Fig. 8). The pure SF film [Fig. 8(a)] showed a smooth surface structure, as expected from the technique used for preparing the film, which leads to the formation of homogeneous membranes with densely packed fibroin chains. Also, pure PAAm films exhibited a uniform surface morphology (photo not shown) The presence of roundish particles immersed in a homogeneous matrix was observed in the blend films with 25 and 50% PAAm content [Fig. 8(b) and (c), respectively], as well as in the other blends prepared in this study. In the 50/50 SF/PAAm blend film [Fig. 8(c)] it is possible to observe nucleating structures

Figure 7 Strength and elongation at break of SF/PAAm blend film as a function of the blending ratio.

Figure 8 SEM photographs of silk fibroin (a) and SF/PAAm blend films with 75/25 (b) and 50/50 (c) blend composition.

with large variation in size, the shape being always round. From these results we can conclude that the two polymers gave rise to separated phases in the blend films. However, the possibility that smaller nuclei of one or both components might be included in the round structures observed by SEM would be worth of further inves-

tigation, for example by TEM. In fact, the presence of nucleating structures at different dimensional levels may bring about a great increase in contact area between the two polymeric phases, therefore favoring mixing and interaction at the boundary, with predictible improved performance of the blend material.

CONCLUSIONS

The results reported in this study show that SF/PAAm blends could be easily prepared by using the conventional casting method, and that the resulting films exhibited some interesting properties. It is noteworthy that blend films exhibited increased thermal stability and slightly improved mechanical properties compared to the pure SF film. These effects were attributed to the formation of specific molecular interactions between SF and PAAm. The nature of these interactions was elucidated by means of FTIR spectroscopy, which showed that the pattern of hydrogen bonds characteristic of pure SF was perturbed by addition of PAAm, and that new intermolecular hydrogen bonds were formed between the primary amide group of PAAm and the amide and various side chain groups of SF. Although phase separation was the main morphological feature emerging from the SEM analysis of the blend films, molecular mixing between SF and PAAm should have occurred to a certain extent, especially at the boundary zones between the nucleating structures.

Blend films with low PAAm content ($\leq 25\%$) were of special interest, because their thermal and mechanical properties significantly improved compared to pure SF films. If suitable improvements of the blending technique are implemented, aiming at favoring the formation of finer PAAm nuclei and their better dispersion in the silk matrix, an increase in the number and extension of the contact areas between the two polymers can be expected. Therefore, this blending system may become attractive for manufacturing biomedical and pharmaceutical devices in which the good biocompatibility of both PAAm[24] and silk fibroin (in preparation) is combined with the enhanced functional performance of the resulting blend material.

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