

Silk Fibroin/Cellulose Blend Films: Preparation, Structure, and Physical Properties

GIULIANO FREDDI,^{1*} MARIA ROMANÒ,¹ MARIA ROSARIA MASSAFRA,¹ and MASUHIRO TSUKADA²

¹Stazione sperimentale per la Seta, via G. Colombo, 81, 20133 Milano, Italy; ²National Institute of Sericultural and Entomological Science, Tsukuba City, Ibaraki 305, Japan

SYNOPSIS

This article deals with the preparation and characterization of silk fibroin (*Bombyx mori*)/cellulose blend films. Following dissolution with a metal complex solution, the average molecular weight of silk fibroin slightly decreased, while cellulose was almost unaffected. After coagulation and washing, transparent films were obtained by blending fibroin and cellulose in all proportions. The crystalline structures of regenerated fibroin and cellulose were β -form and cellulose II, respectively, as shown by the characteristic x-ray diffraction profiles. Density values increased with cellulose content, though less than expected from a pure additive behavior. Moisture regain increased following the addition of a small amount of cellulose to silk fibroin. The mechanical properties showed that both strength and elongation at break of silk fibroin films were improved by blending with cellulose. IR spectra exhibited changes in the skeletal frequencies of silk fibroin, suggesting the occurrence of intermolecular interactions between fibroin and cellulose through hydrogen bond formation.

© 1995 John Wiley & Sons, Inc.

INTRODUCTION

Silk fibroin (*Bombyx mori*) is a fibrous protein whose chemical composition is characterized by the presence of few types of amino acid residues with small side chains, the sum of the three simplest amino acids (glycine, alanine, and serine) accounting for more than 80 mol %. The primary structure arising from this characteristic amino acid composition contains many $-(\text{gly-ala})_n-$ repeats, which form the highly specific secondary structure, known as antiparallel β -sheet structure.¹

Besides its textile uses, silk has recently been investigated as a starting material for the preparation of naturally based polymeric materials potentially interesting for applications in the biotechnological and biomedical fields. Silk fibroin can be prepared in the form of powder, gel, and film from either fibers, after dissolution with concentrated salt solutions (regenerated fibroin), or liquid silk taken directly from the mature silk gland (native fibroin).

Silk membranes have proved to be an excellent substrate for enzyme immobilization,^{2,3} thanks to their good physical and mechanical properties, thermal stability, microbial resistance, and absence of interactions with the enzyme immobilized. Asakura et al.⁴ prepared a glucose biosensor by immobilizing glucose oxidase (GOD) within silk fibroin membranes. A noticeable increase in biosensor sensitivity has recently been reported concerning GOD immobilized on the surface of nonwoven fabrics by means of silk fibroin gel.⁵ Silk fibroin membranes can be used to separate water from water-methanol solutions by pervaporation.⁶ The high oxygen permeability in the wet state, similar to that of other synthetic hydrogel membranes currently used to produce contact lenses, makes silk fibroin attractive as a biomaterial.^{7,8} Moreover, the good *in vivo* blood compatibility of silk fibroin has recently been reported.⁹

Crystallization of silk fibroin films cast from aqueous solution is promoted by suitable thermal, mechanical, and chemical treatments, which induce the conformational transition from random coil to β structure.¹⁰ Fibroin films in the dry state are very brittle and almost unsuitable for practical use,^{7,11,12}

* To whom correspondence should be addressed.

while in the wet state the elongation is considerably higher,⁷ in such a way that they can be applicable as biomaterials in the medical fields. The inferior tensile properties of silk fibroin films can be improved by blending with other natural or synthetic polymers. Blends of silk fibroin with chitosan,¹³ sodium poly-glutamate,¹⁴ sodium alginate¹⁵ and polyvinyl alcohol¹² have been reported. Water absorption, mechanical properties, and thermal stability were improved by blending silk fibroin with sodium alginate.¹⁵ Silk fibroin/syndiotactic-rich poly(vinyl alcohol) blend films showed an increased permeability to neutral salts, the permeability being influenced by the hydration radius of the ions.¹²

Among the large number of polymeric materials potentially suitable for blending with silk fibroin, we think that natural polymers should be preferred, due to the favorable impact of naturally based polymer blends in various kinds of applications. Our interest has focused on cellulose, a widespread natural macromolecule readily available in various forms. It is characterized by a highly regular chemical structure, being a homopolymer made of anhydro- β -glucose units, bonded together by 1,4 oxygen bridges. Like silk fibroin, cellulose exhibits a strong tendency to crystallization, forming crystals where interchain hydrogen bridges are the basic interactions responsible for structural stability. The use of both cellulose and cellulose derivatives for the preparation of different kinds of biomaterials has been reported by various authors.¹⁶

The aim of this article is to study the conditions for dissolving and blending fibroin and cellulose obtained from cocoon fibers and cotton linters, respectively. Fine structure, physical properties, mechanical behavior, and morphology of the resulting blend films will be discussed in relation to the blending ratio.

EXPERIMENTAL

Materials

Silk fibers were obtained from fresh cocoons by alkaline degumming with an aqueous solution of olive oil soap (0.7% w/vol), at 98°C for 60 min. The fibroin fibers were rinsed with warm deionized water and dried at room temperature before use.

Cellulose powder (cotton linters) was purchased from Fluka and used without any preliminary treatment.

Pure and blend films with different blending ratios (80/20, 60/40, 40/60, and 20/80 fibroin/cellulose,

wt/wt) were prepared from fibroin and cellulose, separately dissolved with a metal complex system ($[\text{Cu}(\text{NH}_3)_4](\text{OH})_2$).¹⁷ Solutions were spread over a glass plate and coagulated in an acetone-acetic acid (4 : 1 vol/vol) bath in order to remove the metal complex. The films obtained were washed with glycerine-water (7 : 13 vol/vol), then with water and dried at room temperature. Film thickness ranged from 10 to 30 μm .

Measurements

The intrinsic viscosity $[\eta]$ of silk fibroin was determined by means of the SNV 195595 standard method. Silk fibroin was dissolved with a saturated LiBr aqueous solution at 60°C for 3 h. After dilution with water (1 : 1 vol/vol), the viscosity was measured at 20°C with a capillary viscometer. Intrinsic viscosity $[\eta]$ was calculated from the relative viscosity, i.e., the ratio between the flowing time of the polymer solution and that of the solvent.

The degree of polymerization of cellulose was estimated by measuring viscosity with the UNI 8282-1982 standard method. Cellulose was dissolved with an aqueous solution of cupriethylenediamine (0.5M) at room temperature. The viscosity of the solution was measured with a capillary viscometer at 25°C. The degree of polymerization (DP) was calculated as follows:

$$\text{DP} = 1.50[\eta]$$

The molecular weight of silk fibroin was determined by size exclusion chromatography with a 1 \times 60 cm Superformance (Merck) column packed with Fractogel TSK HW 65 (Merck). Tris-HCl buffer 0.02M, pH 8, containing urea 5M and KCl 0.1M, was used as mobile phase at a flow rate of 1 mL/min. Eluates were detected at 276 nm. Silk fibroin from both fibers and films was dissolved with saturated LiSCN, dialyzed against water, and then against Tris-HCl buffer. The calibration was carried out with the Gel Filtration Molecular Weight Marker Kit (Sigma).

Wide-angle x-ray diffraction (WAXD) profiles were obtained by means of a Siemens D-500 diffractometer with a Siemens FK 60-10, 2000 W Cu tube at a scanning rate of 0.5 2θ /min. Samples were mounted on a rotational specimen carrier; the specimens were spun with a rotational speed of 30 rpm. The degree of crystallinity was calculated according to the Hermans' method.¹⁸

Density was measured by the floatation method under standard conditions of temperature (20°C)

and relative humidity (65% RH) with xylene-carbon tetrachloride solutions.

Moisture regain was determined at 20°C and 65% RH after drying at 105°C for 2 h.

Stress-strain measurements were performed in a standard atmosphere 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. The maximum load was 270 gf. Each value reported is the average of 20 measurements.

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

The morphology of fractured surfaces was examined by scanning electron microscopy (Cambridge, mod. Stereoscan 100) after gold coating. Films were fractured in liquid N₂.

RESULTS AND DISCUSSION

Preparation of Fibroin/Cellulose Blend Films

Dissolution of fibroin and cellulose fibers requires swelling of the structure by means of reagents capable of penetrating between adjacent chains and breaking hydrogen bonds, thus leading to complete dispersion of the constituent polymer chains.

The most common solvents for silk fibroin are concentrated solutions of Li salts (LiSCN, LiBr).¹⁹ LiSCN is usually preferred because the solution is neutral, is active at room temperature, and is unlikely to cause peptide bond hydrolysis. Aqueous solutions are obtained by dialysis. Mineral acids, alkalis, formic acid, and cupriethylenediamine can be used for dissolution, though degradation is likely to occur.

Mineral acids, strong bases, concentrated solutions of inorganic salts (ZnCl₂), metal complex solutions (with Cu, Ni, etc.) and nonaqueous systems containing nitrogen compounds can all be used to dissolve cellulose.²⁰ Some of these solvents may cause variable extent of degradation, with depolymerization of cellulose chains.

The conventional method for preparing silk fibroin films consists of casting aqueous solutions at room temperature.²¹ Because aqueous solutions of cellulose are difficult to obtain, a common solvent system was chosen that permitted mixing of fibroin and cellulose solutions and subsequent film preparation by casting. Jayme and Broschinski¹⁷ suggested the use of various metal complex solutions to prepare films with cellulose, silk fibroin, and other proteins. The most interesting results were obtained

by using the [Cu(NH₃)₄](OH)₂ solvent system (Cuoxam).

Cuoxam (cuprammonium hydroxide solution) is one of the most widely used solvents for preparation of cellulose filaments (Bemberg) and membranes (Cuprophane process)¹⁶ on a technical scale, as well as for viscosimetric determination of the degree of polymerization (DP).²⁰ In the experimental conditions adopted here, cellulose from cotton linters dissolves very easily, at room temperature, without significant degradation of polymer chains, as confirmed by the very slight decrease of the DP value (Table I).

Metal complex solutions are known to cause more or less extensive degradation of silk fibroin.¹⁹ We studied the influence of several parameters, such as reagent concentration and dissolution conditions, in order to optimize the procedure for preparing regenerated silk fibroin films by using Cuoxam. Silk fibroin dissolves quickly at room temperature. Times of solution preparation and drying (after casting, before coagulation) should be kept as short as possible, in order to avoid the possible degradation by alkali. Changes in the concentration of ammonia and copper hydroxide did not have any significant effect on the chemical structure of fibroin. As expected, the most critical parameter is NaOH concentration. The greater the amount of NaOH, the higher the rate of dissolution but at the cost of a pronounced decrease in average molecular weight (Fig. 1). We found that the minimum NaOH concentration necessary to attain fibroin dissolution is around 0.5–0.6 N. The corresponding intrinsic viscosity value (Table I) is lower than that of the fiber but acceptable for the integrity of silk fibroin structure, because such a value is normally attained during silk fiber processing on an industrial scale (after degumming, dyeing, etc.).

After coagulation and washing, pure fibroin and cellulose films are transparent. The presence of small bubbles has been observed in fibroin films.

Table I Degree of Polymerization of Cellulose (DP) and Intrinsic Viscosity of Silk Fibroin [η] before and after Film Preparation

Cellulose	DP
Cotton linters	396
Film	385
Silk fibroin	[η] (dl/g)
Fiber	0.72
Film	0.50

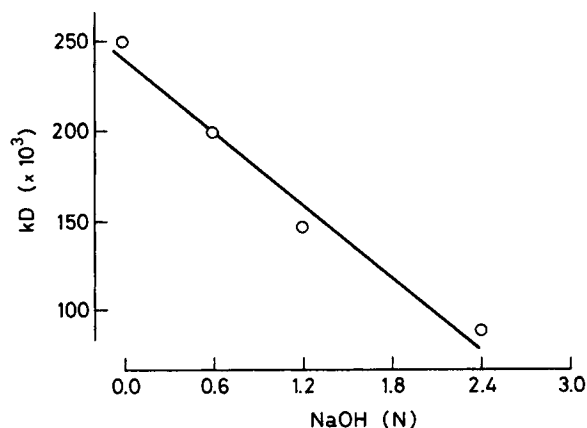


Figure 1 Changes in the molecular weight of silk fibroin as a function of the NaOH concentration in the solvent system.

Their formation can be partially limited by degassing the solution before casting. The same comments apply also to fibroin/cellulose blends, the films being transparent at all blending ratios examined, and the bubbles being more visible at higher fibroin content.

X-Ray Diffraction Curves

The solvent system used for the coagulation bath is known to induce conformational changes, leading to crystallization of silk fibroin.¹⁰ Crystallization of cellulose is also promoted, due to dehydration and subsequent drying. The crystalline structure of pure films was determined by means of x-ray diffraction spectroscopy (Fig. 2). After dissolution and regeneration in film form [Fig. 2(b)], silk fibroin crystallizes to β -sheet structure,²³ with a major and broad 2θ peak at 20.5° , corresponding to the crystalline spacing of 4.39 \AA . The same crystalline structure is present in the original silk filament [Fig. 2(a)].

The wide-angle x-ray diffraction profile of cotton linters [Fig. 2(c)] is characterized by the $(\bar{1}10)$, (110) , and (200) reflections, typical of Cellulose I crystal structure present in native celluloses.²¹ On regeneration from metal complex solution, the cellulose film exhibits 2θ peaks at 12° and 20° , corresponding to the $(\bar{1}10)$ and (110) reflections (crystalline spacings of 7.37 and 4.44 \AA , respectively). This diffraction pattern is typical of the Cellulose II crystalline form.²¹ The overlapping of the major crystalline reflections of both fibroin and cellulose in the x-ray spectra of the blend films did not allow to evaluate the contribution of each component to the crystalline structure.

The degree of crystallinity was calculated from x-ray spectra by means of the Hermans' method.¹⁸

The crystallinity of pure fibroin films is about 19%, a value larger than that reported for silk fibroin films regenerated by casting from aqueous solution (13%),⁸ though lower than that of the original silk filament (22.5%).²² This result confirms that conditions used for film preparation are favorable for crystallization of silk fibroin. On the contrary, the degree of crystallinity of pure cellulose films (20%) is rather low compared to the values reported for different regenerated cellulosic materials.²⁰

Density and Moisture Regain

The fine structure of fibroin/cellulose blend films was further investigated by density measurements. The density value of pure fibroin films is 1.345 g/cm^3 , slightly lower than that reported for degummed silk fibers (1.35 g/cm^3).²² Pure cellulose films are

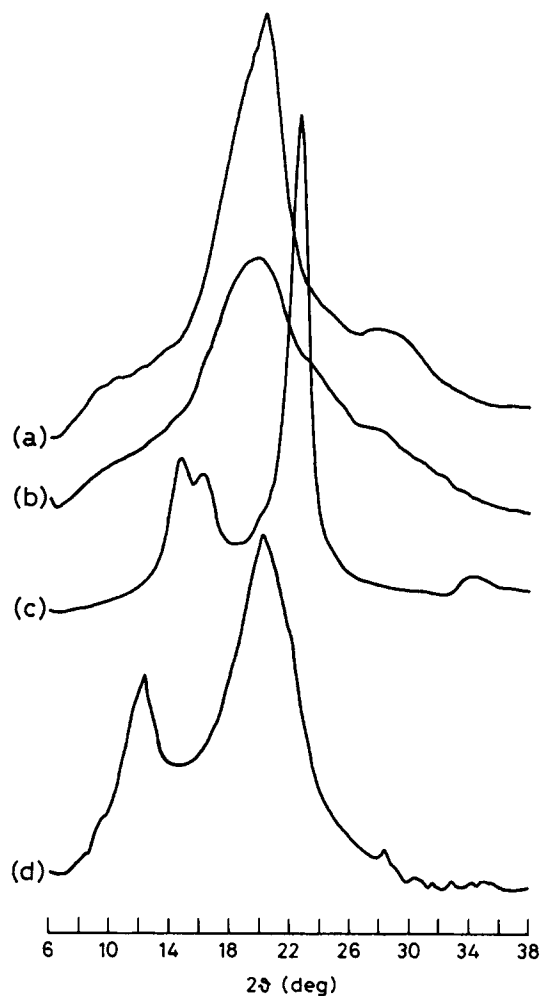


Figure 2 Wide-angle x-ray diffraction profiles of silk fibroin fiber (a) and film (b), and of native cellulose (cotton linters) (c) and film (d).

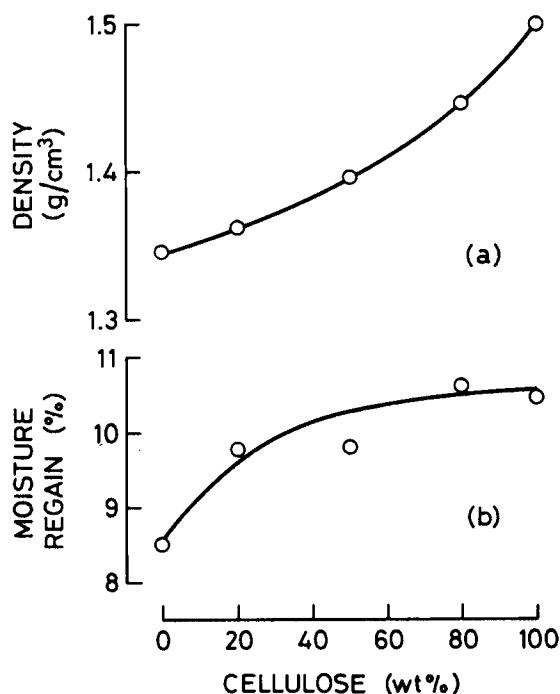


Figure 3 Behavior of density (a) and moisture regain (b) of fibroin/cellulose blend films as a function of the blending ratio.

characterized by a density of 1.497 g/cm^3 , a value considerably lower than that of most cellulose fibers ($> 1.54 \text{ g/cm}^3$), but close to the lower limit of the density range reported for viscose rayon and other kinds of regenerated celluloses ($1.50\text{--}1.54 \text{ g/cm}^3$).²⁰ The results of both fibroin and cellulose films are consistent with the above crystallinity data, showing a quite high degree of crystallinity for regenerated silk, while cellulose is prevalently amorphous.

The relation between density and fibroin/cellulose blending ratio is shown in Figure 3(a). Densities of blend films are intermediate between pure components, the values tending to be lower than one would expect from a mere additive behavior. The shift from the ideal behavior might suggest the occurrence of interactions between the two polymers, leading to a slight expansion of volume, as shown by the shape of the curve.

Because density reflects the structure of both amorphous and crystalline phases of the material, equilibrium moisture regain measurements were used as a probe to study the changes in the amorphous regions as a function of the blending ratio [Fig. 3(b)]. The results show that absorption of moisture increases sharply when 20% cellulose is added to silk fibroin, beyond which value a plateau is attained. The raise of equilibrium moisture regain

induced by a small amount of cellulose is only partly attributable to the higher hygroscopicity of the material added to silk fibroin. The addition of a prevalently amorphous material, as well as the interaction between fibroin and cellulose chains in the amorphous regions of the blend, might have changed both structure and behavior of these fiber domains, resulting in a higher uptake of water molecules. The behavior of density as a function of the blending ratio seems to support this hypothesis.

Mechanical Properties

Because polymeric materials, such as membranes, may be subjected to various kinds of stress during use, the determination of the mechanical properties involves not only scientific, but also technological and practical aspects. The stress-strain curves of pure and blend films were determined and the values of tensile strength and elongation at break plotted as a function of the increasing cellulose content (Fig. 4).

Pure fibroin films are characterized by very low values of both tensile strength and elongation. The tensile strength is about 1.5 kg/mm^2 , somewhat lower than that reported for native silk fibroin films.^{7,11,14} This result should be mainly due to the decrease in molecular weight induced by the solvent system used in our work to prepare the regenerated silk fibroin solution. The extremely low value of elongation is typical of a very brittle material and is consistent with the results reported by Minoura et al.,⁷ showing that silk membranes in the dry state

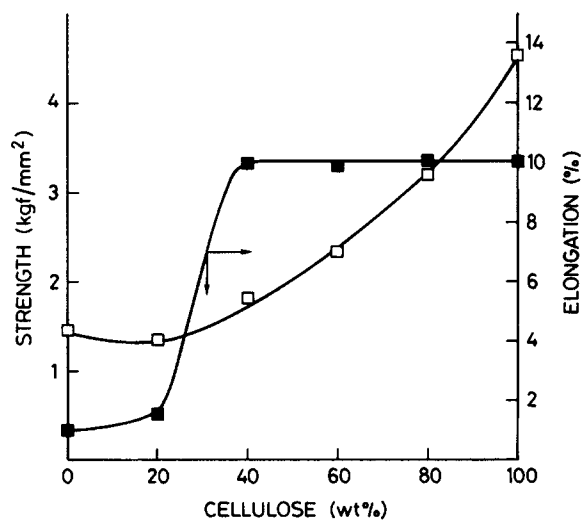


Figure 4 Tensile properties (tensile strength and elongation at break) of fibroin/cellulose blend films as a function of the blending ratio.

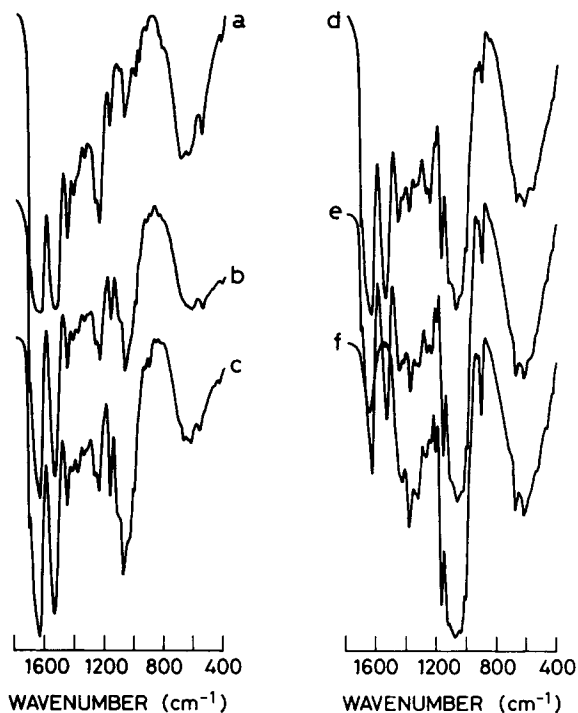


Figure 5 IR spectra of pure and blend films. (a) silk fibroin; (b) 80/20 fibr./cell.; (c) 60/40 fibr./cell.; (d) 40/60 fibr./cell.; (e) 20/80 fibr./cell.; (f) cellulose.

are almost unsuitable for practical uses, due to their high degree of brittleness.

The addition of cellulose to silk fibroin is effective in inducing significant changes in the mechanical properties of the resulting blend films. The behavior of tensile strength seems to vary according to the cellulose content, because this parameter increases almost linearly with the amount of cellulose from the 80/20 blend upwards. Elongation at break shows a sharp increase at 60/40 blending ratio, and then remains constant.

The blend film containing 40% of cellulose is 10 times more extensible than that made of pure fibroin. This result might be attributed to various factors, such as the intrinsic properties of the material added to silk fibroin, the higher hygroscopicity of the blends, the plasticizing effect of the additional water molecules absorbed, etc. However, the possibility that adjacent fibroin and cellulose chains might interact with each other in the amorphous region should be taken into account, in order to explain the sharp raise of elongation occurring within a narrow range of blending ratio.¹⁵

The mechanical properties of fibroin/cellulose blend films are quite interesting from the practical point of view. Though the ultimate value of rupture strength holds a certain interest in evaluating the

performance of a polymeric material, the ability of absorbing stresses by plastic deformation, as well as the possibility of elongating without breaking, are often of higher importance. In this context, the addition of cellulose to silk fibroin can be exploited as a useful tool for improving mechanical properties of silk fibroin films. Similar results have been reported for silk fibroin membranes blended with sodium alginate¹⁵ and sodium polyglutamate.¹⁴

IR spectroscopy

Conformational characterization of pure and blend films was carried out by means of infrared spectroscopy. The IR spectrum of silk fibroin film [Fig. 5(a)] shows the characteristic absorption bands at 1630 cm^{-1} (amide I), 1530 cm^{-1} (amide II), 1265 cm^{-1} (amide III), and 700 cm^{-1} (amide V), attributed to the crystalline β -sheet structure, and the bands at 1660 cm^{-1} , 1540 cm^{-1} , 1235 cm^{-1} , and 650 cm^{-1} , assigned to the random coil form.²³ The strong intensity of the β -sheet bands confirms that crystallization of silk fibroin occurred under the experimental conditions adopted. The IR spectrum of the cellulose film [Fig. 5(f)] is typical of cellulose II,²⁶⁻²⁸ as expected from the conditions used for sample preparation and in agreement with x-ray diffraction results.

The IR spectra of fibroin/cellulose blend films [Fig. 5(b-e)] are characterized by the presence of absorption bands typical of the pure components, whose intensities are roughly related to the blending ratio. However, significant changes can be noted in the spectral region 1250–800 cm^{-1} , which is characteristic of the primary structure of polypeptides. As concerns silk fibroin, the band at 1015 cm^{-1} arises from the —(gly—gly)— sequence, while those at 1000 cm^{-1} and 980 cm^{-1} are attributed to the —(gly—ala)— periodic sequence.²⁷ All these ab-

Table II IR Crystallinity Indexes of Silk Fibroin and Cellulose in Pure and Blend Films

Fibroin/Cellulose Blends	IR Crystallinity Indexes	
	Fibroin ^a	Cellulose ^b
100/0	0.66 ± 0.01	—
80/20	0.63 ± 0.02	(2.97 ± 0.16)
50/50	0.68 ± 0.01	0.90 ± 0.06
20/80	0.84 ± 0.01	0.64 ± 0.02
0/100	—	0.54 ± 0.04

^a Ratio between the absorption bands at 1265 and 1235 cm^{-1} .

^b Ratio between the absorption bands at 1420 and 900 cm^{-1} .

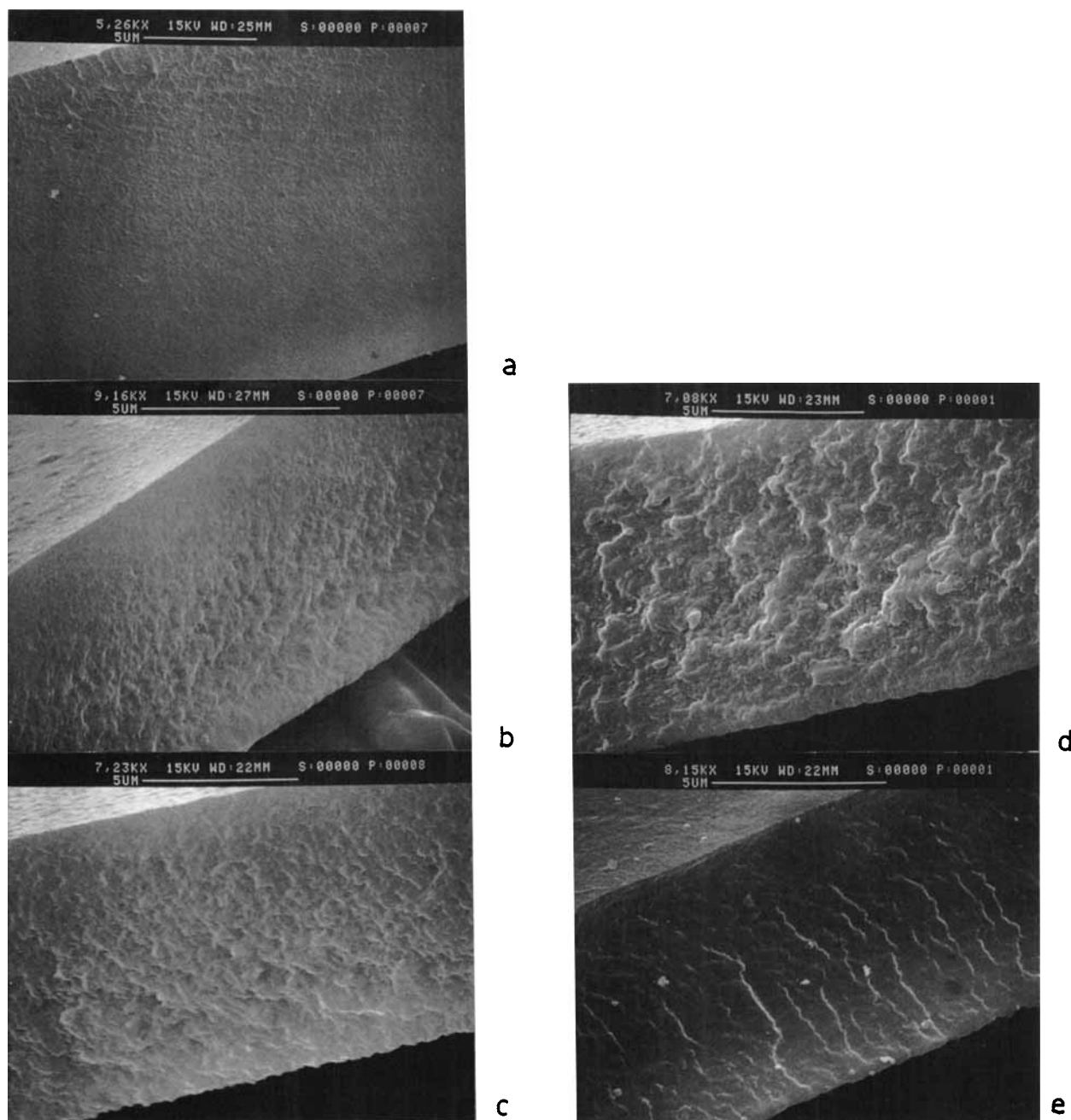


Figure 6 Scanning electron microscopy photographs of pure and blend films. (a) silk fibroin; (b) 80/20 fibr./cell.; (c) 50/50 fibr./cell.; (d) 20/80 fibr./cell.; (e) cellulose.

sorption bands appear modified by blending with cellulose. The modification of skeletal frequencies leads us to think that the presence of cellulose probably induces changes in the interchain alignment of polypeptide chains. These findings can be attributed to the occurrence of intermolecular interactions between the two polymers, mainly due to hydrogen bonding between hydroxyl groups of cellulose and amide groups of fibroin.¹⁵ Changes observed in the

OH— stretching region ($3500\text{--}3300\text{ cm}^{-1}$) seem to confirm this hypothesis. Further studies are in progress on this subject.

Both fibroin and cellulose IR spectra present absorption bands selectively attributed to the crystalline and amorphous structures. These bands, at 1265 and 1235 cm^{-1} for fibroin,²⁸ 1420 and 900 cm^{-1} for cellulose,²⁹ have been used to calculate crystallinity indexes for both polymers in pure and blend films

(Table II). The results indicate that the crystallinity of the minor component seems to be selectively enhanced by blending. This might be due to the fact that, during casting, the molecular mobility of the minor component is affected by the other in such a way that crystallization is promoted. Referring to silk fibroin films, it has been reported that the lower the drying rate, the higher the crystal content.³⁰ We suppose that the presence of hydrophilic cellulose molecules optimizes the rate of fibroin crystallization. The high crystallinity index calculated for cellulose in the 80/20 fibroin/cellulose blend is probably affected by a certain degree of uncertainty, due to overlapping of absorption frequencies of both polymers, especially around the 1420 cm⁻¹ region.

Morphological characteristics

Cryogenically fractured surfaces of fibroin/cellulose blend films are shown in Figure 6. The pure fibroin film [Fig. 6(a)] exhibits a dense and uniform microstructure, with a very fine texture. As the cellulose content increases [Fig. 6(b-d)], the morphology of fractured surfaces changes from smooth to rough, assuming a lamellar structure. The latter is typical of the pure cellulose film [Fig. 6(e)].

The transition from fibroin-like to cellulose-like morphology is induced as soon as a small amount of cellulose is added to silk fibroin. The results of blend morphology are consistent with the above-reported observations concerning the changes in physical properties and mechanical behavior, occurring when an amount of cellulose ranging from 20 to 40% is added to silk fibroin. In particular, the less compact morphological structure exhibited by the 80/20 blend film is in agreement with density and moisture absorption behavior, which seem to indicate a higher accessibility of the amorphous regions. It is interesting to note the considerable swelling of the lamellar phase of the blend films, compared to that of the pure cellulose film.

CONCLUSIONS

The results reported confirm that cellulose can be usefully added to silk fibroin for the preparation of blends by using natural polymers.

The chemical structure of both components is not significantly affected by the solvent system used. However, the possibility of using a solvent less degradative towards silk fibroin and with a lower environmental impact will be investigated. Moreover,

the recycling of waste products from both silk and cellulose processing cycles is under study.

Blend films are transparent and homogeneous from the morphological point of view. Physical properties of the blend films largely depend on the blending ratio. It is noteworthy that the addition of cellulose to silk fibroin permits the preparation of membranes with excellent elastic behavior.

Changes in shape and intensity of IR absorption frequencies characteristic of either silk fibroin or cellulose have been attributed to the occurrence of intermolecular interactions between the two polymers within the amorphous regions. These interactions should mainly depend on the ability of both cellulose and fibroin to establish interchain hydrogen bonds.

The authors express their thanks to Dr. Alberto Seves and his co-workers (Stazione sperimentale per la Cellulosa, Carta, Fibre Tessili Vegetali e Artificiali) for the x-ray diffraction analyses, and to Mrs. Silvia Beretta and Miss Francesca Boniardi (Stazione sperimentale per la Seta) for FT-IR spectra and fruitful discussion.

REFERENCES

1. R. D. B. Fraser and T. P. MacRae, *Conformation in Fibrous Proteins*, Academic Press, New York, 1973, pp. 293-343.
2. A. Kuzuhara, T. Asakura, R. Tomoda, and T. Matsunaga, *J. Biotechnol.*, **5**, 199 (1987).
3. M. Demura, T. Komura, T. Hirade, and T. Asakura, *Sen-i Gakkaishi*, **46**, 391 (1990).
4. T. Asakura, H. Yoshimizu, A. Kuzuhara, and T. Matsunaga, *J. Seric. Sci. Jpn.*, **57**, 203 (1988).
5. T. Asakura, M. Kitaguchi, M. Demura, H. Sakai, and K. Komatsu, *J. Appl. Polym. Sci.*, **46**, 49 (1992).
6. T. Hirotsu, S. Nakajima, S. Kitamura, K. Mizoguchi, and Y. Suda, *Sen-i Gakkaishi*, **44**, 72 (1988).
7. N. Minoura, M. Tsukada, and M. Nagura, *Biomaterials*, **11**, 430 (1990).
8. N. Minoura, M. Tsukada, and M. Nagura, *Polymer*, **31**, 265 (1990).
9. H. Sakabe, H. Ito, T. Miyamoto, Y. Noishiki, and W. S. Ha, *Sen-i Gakkaishi*, **45**, 487 (1989).
10. J. Magoshi, Y. Magoshi, and S. Nakamura, *J. Appl. Polym. Sci., Appl. Polym. Symp.*, **41**, 187 (1985).
11. C. X. Liang and K. Hirabayashi, *Sen-i Gakkaishi*, **46**, 181 (1990).
12. K. Yamaura, N. Kuranuki, M. Suzuki, T. Tanigami, and S. Matsuzawa, *J. Appl. Polym. Sci.*, **41**, 2409 (1990).
13. C. X. Liang and K. Hirabayashi, *Sen-i Gakkaishi*, **47**, 334 (1991).
14. C. X. Liang and K. Hirabayashi, *Sen-i Gakkaishi*, **46**, 535 (1990).

15. C. X. Liang and K. Hirabayashi, *J. Appl. Polym. Sci.*, **45**, 1937 (1992).
16. A. F. Turbak, Ed., *Membranes from Cellulose and Cellulose Derivatives, Applied Polymer Symposia*, No. 13, Interscience Publishers, John Wiley and Sons, New York, 1970.
17. G. Jayme and L. Broschinski, *Cellulose Chem. Technol.*, **10**, 655 (1976).
18. P. Hermans and H. A. Weidinger, *J. Appl. Phys.*, **19**, 491 (1948).
19. F. Lucas, J. T. B. Shaw, and S. G. Smith, *Adv. Protein Chem.*, **13**, 107 (1958).
20. A. Grobe, in *Polymer Handbook*, J. Brandrup, E. H. Immergut, Eds., Wiley, New York, 1989, pp. V/117.
21. S. H. Zeronian and H. S. Ryu, *J. Appl. Polym. Sci.*, **33**, 2587 (1987).
22. H. Ishikawa, *Zoku Kenshi no Kozo (Structure of Silk Proteins)*, N. Hojo, Ed., Shinkyō Publishing Co., Nagano, 1980, pp. 209–224.
23. J. Magoshi, M. Mizuide, Y. Magoshi, K. Takahashi, M. Kubo, and S. Nakamura, *J. Polym. Sci., Polym. Phys. Ed.*, **17**, 515 (1979).
24. H. J. Marrinan, and J. Mann, *J. Polym. Sci.*, **21**, 301 (1956).
25. R. H. Marchessault and C. Y. Liang, *J. Polym. Sci.*, **43**, 71 (1960).
26. J. Blackwell, *Cellulose Chemistry and Technology*, ACS Symposium Series, 48, Washington, DC, 1977, p. 206.
27. M. Asai, M. Tsuboi, T. Shimanouchi, and S. Mizushima, *J. Phys. Chem.*, **59**, 322 (1955).
28. N. V. Bath and G. S. Nadiger, *J. Appl. Polym. Sci.*, **25**, 921 (1980).
29. R. T. O'Connor, E. F. DuPré, and D. Mitcham, *Text. Res. J.*, **28**, 382 (1958).
30. M. Tsukada, *J. Polym. Sci., Polym. Phys. Ed.*, **24**, 457 (1986).

Received July 15, 1994

Accepted October 9, 1994