

Preparation of Semi-Interpenetrating Polymer Networks Composed of Silk Fibroin and Poly(ethylene glycol) Macromer

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ABSTRACT: Semi-interpenetrating polymer networks (SIPNs) composed of silk fibroin (SF) and a poly(ethylene glycol) (PEG) diacrylate macromer were synthesized and characterized. The conformation of SF in SIPNs was changed from a random coil to a β -sheet structure by formation of SIPNs with PEG, while the crystalline structures of SF and PEG in SIPNs was not changed. The decomposition temperature of SF in the SIPNs was inner-shifted, an indication of SIPN formation between the SF and the PEG macromer. The tensile strength and elongation of the SIPNs were much higher than were those of SF itself or the SF/PEG blend and increased with an increase of the PEG content. The equilibrium water content of SF was remarkably increased by formation of SIPNs with PEG due to the hydrophilic property of PEG. © 2001 John Wiley & Sons, Inc. *J Appl Polym Sci* 80: 1848–1853, 2001

Key words: silk fibroin; PEG macromer; SIPNs

INTRODUCTION

Recently, silk fibroin (SF) as the natural protein polymer has been applied for biomaterials such as the matrix for enzyme immobilization,¹ mammalian cell culture,^{2,3} drug delivery systems,⁴ artificial skin,⁵ and so on. There are several advantages of SF for biomaterials, such as biocompatible natural polymers, biodegradability, minimal inflammatory reactions, and good water-vapor permeability. On the other hand, there are also disadvantages such as antigenicity and poor mechanical property for the biomaterials as the

sponge form. Much research has been done to improve the mechanical properties of SF by blending with other synthetic or natural polymers, such as poly(sodium glutamate),⁶ sodium alginate,⁷ chitosan,^{8,9} and cellulose.¹⁰ Moreover, the permeability of neutral salts was promoted by the addition of SF into syndiotactic-rich poly(vinyl alcohol)¹¹ and water vapor and the oxygen permeability of SF was increased by blending with chitosan.⁹

The poly(ethylene glycol) (PEG) macromer, having an acrylated-terminated PEG derivative,¹² was chosen to formulate semi-interpenetrating polymer networks (SIPNs). The physical properties were improved by independently crosslinking a hydrophobic network within the crosslinked hydrophilic network.¹³ This mixture

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of two independently crosslinked polymers cannot be physically separated into interpenetrating polymer networks (IPNs). If only one of the two polymers is crosslinked, the product is called an SIPN. It is usually made by polymerizing or crosslinking one component in the presence of the other. In this system, the PEG macromer is crosslinked in the presence of SF by UV. To make an SIPN, two polymers should dissolve in the same solvent. PEG, as the widely used and accepted biomaterial, is water-soluble and nonimmunogenic.¹⁴ The water-soluble PEG macromer used as a matrix for incorporation of other polymers, for instance, lysine and hydroxyethyl methacrylate,¹⁵ β -chitin,¹⁶ poly(lactic acid),¹⁷ and poly(ϵ -caprolactone),¹⁸ forms a crosslinked three-dimensional hydrogel.¹⁹

The aim of the present work was to report on the properties of SIPNs composed of SF and the PEG diacrylate macromer. The resulting SIPNs are expected to show a greater enhanced mechanical strength than that of SF, which can be used for biomaterials such as for artificial skin and wound dressing.

EXPERIMENTAL

Materials

Raw silk was degummed twice with 0.5% on the weight-of-fiber (o.w.f.) marseilles soap and a 0.3% o.w.f. sodium carbonate solution at 100°C for 1 h and then washed with distilled water. Degummed silk was dissolved in $\text{CaCl}_2\text{:H}_2\text{O:ethanol} = 1\text{:}8\text{:}2$ in volume. The fibroin solution was obtained after dialysis of a dissolved fibroin solution against distilled water for 4 days and then stored in a refrigerator prior to use.

PEG was purchased from Wako Pure Chemical Inc. (Tokyo, Japan) and was dried by azeotropic distillation with benzene. Acryloyl chloride and 2,2-dimethoxy-2-phenylacetophenone were obtained from the Aldrich Chemical Co. (Milwaukee, WI) and were used without further purification. All other chemicals were extrapure reagent grade and used as received.

Synthesis of PEG Macromer

Purified 12 g (1.6 mM) of PEG was dissolved in 150 mL of benzene in a 500-mL round-bottomed flask. A total of 0.89 mL (3.2 mM) of triethylamine

and 0.61 mL (7.5 mM) of acryloyl chloride were added to the flask, and the reaction mixture was stirred for 3 h at 80°C. The reaction mixture was filtered to remove triethylamine hydrochloride, and then the macromer was obtained by pouring the filtrate into an excess of *n*-hexane. Finally, it was dried at 40°C under reduced pressure for 24 h.

Preparation of SIPNs

The SIPNs were prepared by a simultaneous IPN method. Briefly, 25 μL of the initiator solution (100 mg of 2,2-dimethoxy-2-phenylacetophenone dissolved in 1 mL of *N*-vinylpyrrolidone) was added to the 2 wt % aqueous solution of silk fibroin and 2, 3, and 4 wt % of the PEG macromer. The mixture solution was irradiated for 5 min using a low-intensity LWUV lamp (Toshiba Chemical Lamp FL 20 LB; wave range 300–400 nm, maximum intensity 360 nm) and freeze-dried to obtain spongelike materials. Then, the prepared SIPNs were repeatedly washed with ethyl alcohol to remove the unreacted PEG macromer and photoinitiator.

Measurements

A Fourier transform infrared (FTIR) spectroscopy (Midac M series spectrometer, USA) was used to confirm the structure of the PEG macromer and SIPNs. Wide-angle diffractometry (Bruker AXS D5005) was used to investigate the crystalline structure of the SIPNs, with the following measurement conditions: scanning speed, 2°/min, and voltage and current, 35 kV and 20 mA, respectively.

The melting temperature (T_m) and the thermal decomposition temperature (T_d) were measured with a DSC 2910 differential scanning calorimeter (TA Instruments Co., USA). The measurements were carried out in the range from 30 to 340°C under nitrogen gas with a scanning rate of 10°C/min.

The tensile load and elongation at break was measured by using a Minimat (Rheometric Scientific, USA). The experimental conditions are as follows: initial load cell: 200 N; extension rate: 10 mm/min; and dimension of the sample: $1.5 \times 4 \times 0.5$ cm.

To measure the water content, preweighed dry samples were immersed in distilled water. After the excess surface water was removed with filter

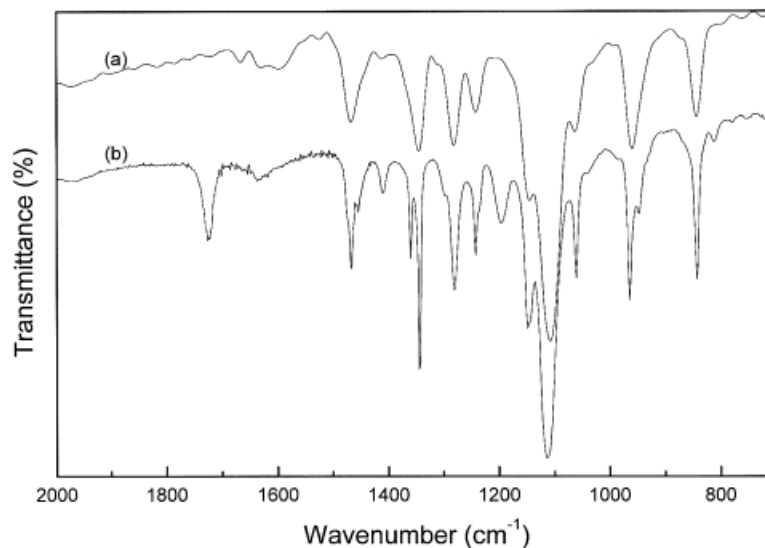


Figure 1 FTIR spectra of (a) PEG and (b) PEG macromer. MW of PEG = 1000.

paper, the weight of the swollen samples was measured at various time intervals. Water contents were calculated according to following equation:

$$\text{Water content (wt \%)} = (W_s - W_d) / W_d \times 100$$

where W_s is the weight of the swollen samples, and W_d , the weight of the dry samples.

RESULTS AND DISCUSSION

FTIR Spectra

Figure 1 shows FTIR spectra of (a) PEG and (b) the PEG macromer. The PEG macromer prepared by the reaction of PEG and acryloyl chloride showed new peaks at 1722 and 1409 cm^{-1} , attributed to the stretching vibration bands of carbonyl and the C=C group, respectively, indicating

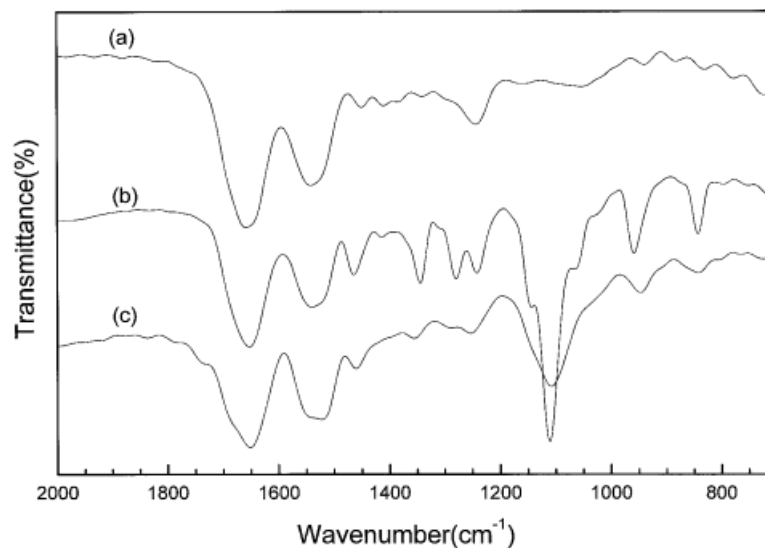


Figure 2 FTIR spectra of (a) SF, (b) SF/PEG (2/2) blend, and (c) SF/PEG (2/2) SIPNs. MW of PEG = 2000.

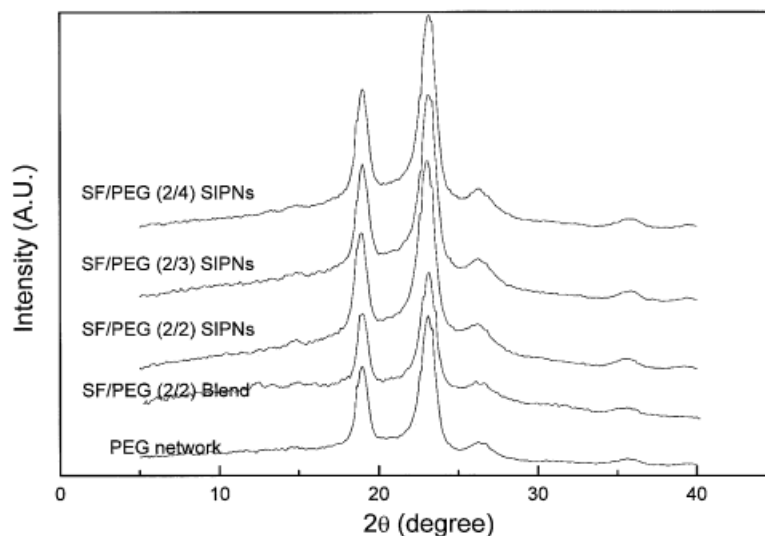


Figure 3 X-ray diffractograms of crosslinked PEG, SF/PEG blend, and SF/PEG SIPNs. MW of PEG = 8000.

preparation of PEG diacrylate. Figure 2 shows FTIR spectra of (a) SF, (b) the SF/PEG (2/2) blend, and (c) SF/PEG (2/2) SIPNs. SF exhibited an absorption band at 1655 (amide I), 1546 (amide II), and 1240 cm^{-1} (amide III), attributed to the random coil conformation. On the other hand, the spectrum of the polymer blend composed of SF and PEG (MW = 1000) is no more than an overlapping spectrum of SF and PEG. However, SIPN did not show the absorption band attributed to the C=C group at 1409 cm^{-1} and showed dull and smooth peaks due to the environmental complex induced by SIPN formation. The characteristic hydroxyl group band of PEG at 3300 cm^{-1} also became weak and dull after formation of the SIPNs (data not shown). Interestingly, the amide absorption band of SF in SIPNs was exhibited at 1530 cm^{-1} , assigned to the β -sheet structure. This result suggested that the conformational change of SF occurred from the random coil to the β -sheet structure due to the intermolecular hydrogen bonds between SF and PEG in the SIPNs formed.

X-ray Diffraction

X-ray diffraction curves of SIPNs are shown in Figure 3. SF showed a typical amorphous diffraction pattern (data not shown), whereas the crosslinked PEG network showed sharp diffraction peaks at $2\theta = 19.0, 23.1,$ and 26.2° , corresponding to the crystalline spacing of 4.67, 3.85,

and 3.40 Å, respectively.²⁰ The diffraction pattern of PEG in SIPNs is consistent with that of the PEG network itself, regardless of the formation of SIPNs. But the crystalline intensity of PEG in the SIPNs was higher than that of the SF/PEG blend.

SF chains were interpenetrated into the crosslinked PEG networks. It could be expected that the PEG macromer formed a gel network and silk fibroin chains were entangled through PEG networks resulting in SIPNs. The diffractograms show that the incorporation of silk fibroin does not affect the crystal structure of PEG. Liang et al.²⁰ reported that the crystalline structure of PEG did not change by blending with cellulose.

Thermal Properties

The T_m of PEG and the T_d of SF in the SIPNs are shown in Table I. The results showed that the T_m 's of PEG in the SIPNs and blend were higher than that of the PEG network itself and increased with increase of the PEG content in the SIPNs. The T_m of PEG networks was a little lower than that of PEG in the blend due to the crosslinking. But the T_m of PEG in the SIPNs was a little higher than that of PEG itself. It may be thought that the increased T_m of PEG in the presence of SF came from some intermolecular interaction between the SF and PEG such as hydrogen bonding. On the other hand, the T_d of SF in the SIPNs was lower than that of SF itself, an indication of

Table I Thermal Properties of SF/PEG SIPNs Measured by DSC

Sample (w/w)	T_d (°C)	T_m (°C)
PEG network	N.M.	51.0
SF	281.3	N.M.
SF/PEG (2/2) blend	274.4	53.1
SF/PEG (2/2) SIPNs	264.4	52.7
SF/PEG (2/3) SIPNs	268.7	53.3
SF/PEG (2/4) SIPNs	268.6	54.4

N.M.: nonmeasurable; MW of PEG = 8000.

either the formation of SIPNs with PEG or the existence of some degree of miscibility.

Mechanical Properties

The tensile strength and elongation at break of samples calculated from the load-extension curves in the dry state are shown in Table II. Both the tensile strength and elongation at break of SIPNs were much higher than those of SF itself or the SF/PEG blend. Also, those of SIPNs increased with increase of PEG in the SIPNs due to the increased crosslinking density of the PEG network. In particular, the tensile strength of SIPNs (SF/PEG = 2/2 in weight) was 41 times stronger and the breaking elongation was 7.5 times higher than those of SF/PEG (2/2 in weight) blend.

Water Content

The water content of the PEG networks according to the molecular weight of PEG are shown in Figure 4. The results indicated that the PEG networks swelled rapidly and reached equilibrium within 1 h due to the hydrophilic property of PEG. The equilibrium water content of the PEG net-

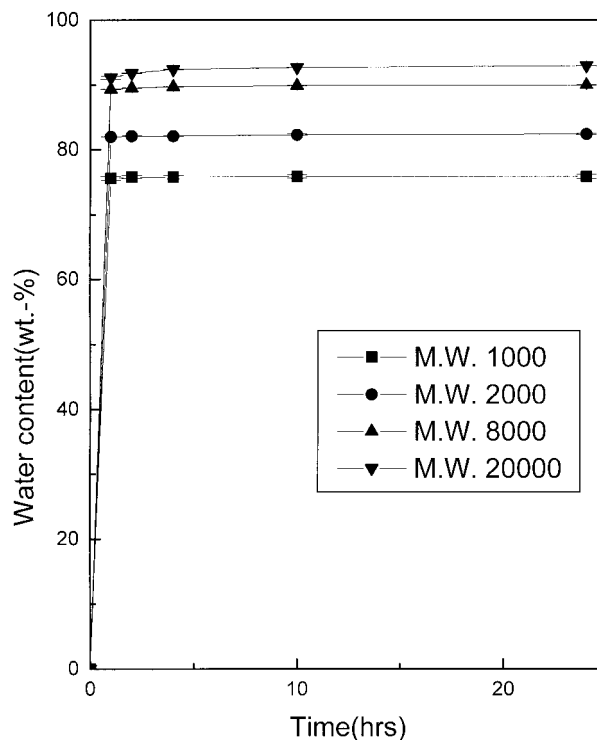


Figure 4 Water contents of PEG networks against time according to the molecular weight of PEG.

works increased from about 75 to 93 wt % with an increase of the molecular weight of PEG. The results suggested that the equilibrium water content of the PEG networks was dependent on the molecular weight of PEG.

The equilibrium water contents of SF and SF/PEG SIPNs against various ratios are shown in Figure 5. The water contents of SF itself increased with formation of SIPNs with PEG due to the hydrophilic property of PEG. But those of SF/PEG SIPNs were not much different with an increase of PEG content in the SIPNs. It may be due to the increased crosslinking density of the PEG network with an increase of PEG in the SIPNs.

Table II Tensile Strength and Elongation at Break of SF, SF/PEG Blend, and SF/PEG SIPNs

Sample (w/w)	Tensile Strength (N)	Elongation at Break (%)
SF	0.4	1.6
SF/PEG (2/2) blend	0.2	0.8
SF/PEG (2/2) SIPNs	8.2	6.0
SF/PEG (2/3) SIPNs	15	13
SF/PEG (2/4) SIPNs	26	48

MW of PEG = 8000.

CONCLUSIONS

PEG is accepted as a biomaterial owing to its unique properties such as water solubility, non-immunogenicity, and nontoxicity. Also, SF has been used as a biomaterial, including for the matrix for mammalian cell cultures and enzyme immobilization and the matrix for tissue engineer-

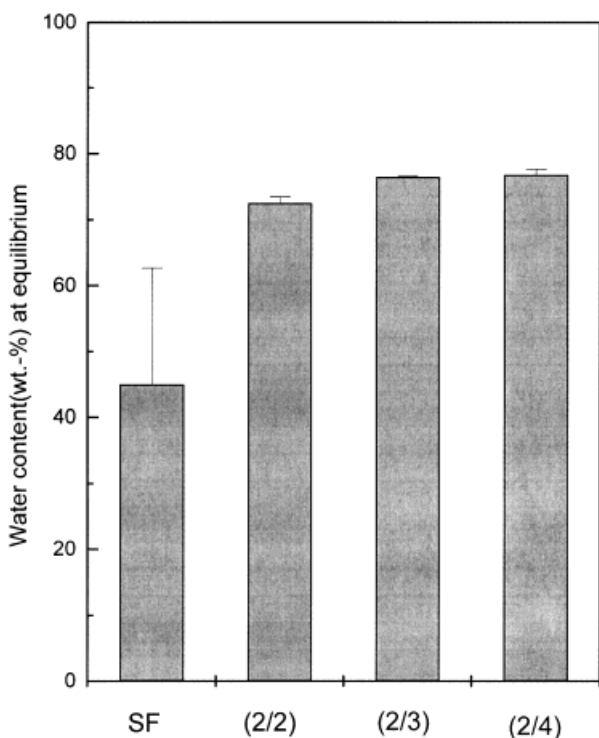


Figure 5 Equilibrium water content of SF/PEG SIPNs against various composition ratios. MW of PEG = 8000.

ing. Therefore, we prepared SIPNs composed of SF and the PEG macromer to enhance the functional properties and examined the structural characteristics and thermal and physical properties. The FTIR spectrum showed that the conformational change of SF occurred from the random coil to the β -sheet structure by formation of SIPNs. WAXD showed that the crystalline intensity of PEG in the SIPNs was higher than that of the SF/PEG blend, but the crystalline structure of PEG was not changed with the formation of SIPNs with SF. The T_d of SF in the SIPNs was lower than that of SF itself, an indication of either the formation of SIPNs with PEG or the existence of some degree of miscibility. The tensile properties of SIPNs depend strongly on the PEG content and are more improved than is SF itself by the formation of SIPNs. Also, the water content of SIPNs increased with incorporation of PEG into the SF due to the hydrophilic property of PEG. Therefore, it can be expected that the SF/PEG SIPNs will be used for artificial skin as the biomaterial.

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