Structure Regulation of Silk Fibroin Films for Controlled Drug Release

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ABSTRACT: Silk fibroin (SF) films were prepared using a casting method and the change of secondary structure and crystal structure owing to treatment with ethanol–water mixtures of different concentrations was investigated by Fourier transform infrared spectroscopy and X-ray diffraction. The results were used to reveal the influence of structure of SF matrix on the release kinetics of drug compounds incorporated. It was found that the content of silk II structure, constituted by β-sheet, first increased and then decreased with increasing ethanol concentration ranging from 50 to 90%, reaching a maximum around 70–80%. The release kinetics of Rhodamine B, a model compound for small molecule drugs, was described by Peppas equation. The results indicated that Rhodamine B released from SF films via Fickian diffusion mechanism and the diffusional exponent n increased with increasing β-sheet content in the matrix. The experimental data had a good linear fit, suggesting that the silk II crystal could be used as a natural regulator for drug release from SF material. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 000: 000–000, 2012

Key words: silk fibroin; controlled release; structure; films; infrared spectroscopy

INTRODUCTION

Silk, a natural fiber produced by the silkworm, has been used traditionally in the form of threads in textiles for thousands of years. In the past decades, much attention have been paid to the structure and properties of fibroin, the major component of silk, with an extension of application from traditional textile to biomedical field. Because of their impressive mechanical properties as well as the biocompatibility and biodegradability, the silk fibroin (SF) has been used as an important set of material options in the fields of controlled release and scaffolds for tissue engineering. The special porous structure and chemical component of SF make it easy to incorporate the drug or bioactive compound using a variety of physical or chemical methods suited to the structure and property of the drug molecule or the application conditions, and achieve an enhancement of cell attachment, spreading, differentiation, proliferation, or specific therapeutic effects, when applied as scaffolds for tissue engineering.[1–4]

Currently, the preparation and application of SF as biomaterials has been greatly developed and efforts are aimed at adjusting the structure and properties of the materials to their required functions to address a broad range of biomedical needs,[5,6] in which secondary structure regulation is one of the most important works. Dissolution of SF is often required in nontextile applications. In general, aqueous SF solution is obtained by dissolving SF in the concentrated neutral salts, such as calcium chloride, lithium bromide. SF dissolved in water usually presents a random coil conformation in diluted solution; however, SF in random coil is unstable in thermodynamic sense and prior to transfer to β-sheet upon various kinds of treatment. Organic solvent has proved to be highly effective to induce conformation transition of SF from random coil to β-sheet. Alcohols, such as methanol and ethanol, are the commonly used solvents for the post-treatment of SF materials.[1–4,7–12] Such an attribute allows SF to make into different formats, such as film, gel, powder, fiber, or 3D porous scaffold with controllable level of crystallinity (β-sheet content). The control of secondary structure or crystal structure has been utilized to regulate the mechanical properties and morphology of SF materials to meet a

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specific biomedical application.[7–9] Unfortunately, little is known about the influence of structure on the release kinetics of incorporated compounds from SF matrix.

To make an understanding of the influence of structure on drug release from SF materials, SF films were prepared through a casting method and utilized as carriers to incorporate drug model compound. Organic dye, Rhodamine B was used as model compound for small molecule drugs, which could be easily traced visually as well as assayed spectrophotometrically owing to their characteristic absorbance spectra. The primary focus of this study was to determine the feasibility of controlling the drug release kinetics via control of the structure of SF films. The structure change of SF films owing to treatment with ethanol–water mixtures of different concentrations was investigated by Fourier transform infrared spectroscopy (FTIR) spectra deconvolution and confirmed by X-ray diffraction (XRD). UV–vis spectroscopy was employed to quantify the release of the model compound of which the kinetics was described by Peppas equation and discussed together with structure change of the matrix.

EXPERIMENTAL

Materials

Bombyx mori

silk used in this study was kindly provided by the Nanning Municipal Bureau of Quality and Technical Supervision. Sodium carbonate (AR, Shantou Xilong Chemical, Shantou, Guangdong Province, China), ethanol (AR, Shantou Xilong Chemical), lithium bromide (CP, Shanghai Chemical Regent, China Medicine Group, Shanghai, China), Rhodamine B (AR, Shanghai Chemical regent, China Medicine Group, Shanghai, China), and phosphate buffered saline (PBS, pH 7.4, Fuzhou Manqing Biotech, Fuzhou, Fujian Province, China) were used as received. Stock solution of Rhodamine B of 4 $g \cdot L^{-1}$ was prepared by dissolving the solid products in water and stored at 0–5°C. Double-distilled deionized water was used throughout the experiment.

Preparation of regenerated SF

Regenerated SF was prepared using a common procedure.[1,2,4] Briefly, raw silk was degummed twice with 0.5% (wt/wt) Na_2CO_3 solution at 100°C for 30 min and then washed thoroughly with double-distilled water. Degummed silk was dried at 50°C and dissolved in 9.5 $mol \cdot L^{-1}$ LiBr solution. After dialysis against double-distilled water with dialysis membrane (MWCO = 12,000–14,000) for 3 days with successive water changes, the solution was centrifuged at 3500 rpm to remove solid impurities and stored at 0–5°C. The concentration of SF was determined by weighting the solid mass after drying at 60°C. Part of SF solution was frozen at −20°C overnight and freeze dried in a freeze-dryer (Genesis 25-LE, Virtis).

Preparation of SF films

Films with an amount of SF of 2.0 $mg \cdot cm^{-2}$ were prepared by a casting method.[13] Aqueous solution containing SF of the amount required was poured into a polystyrene plate and dried at room temperature for 24 h. The SF films obtained were cut into approximately 2 cm × 2 cm squares and further dried in a desiccator for 48 h. The films were treated with ethanol–water mixtures for 30 min at about 25°C, dried in the air, and kept in the desiccator for further spectroscopic analysis.

FTIR analysis

FTIR measurement of SF films was directly performed with an accumulation of 20 scans and a resolution of 4 cm^{-1} on a Shimadzu FTIR-8000S Fourier transform infrared spectrometer by the method of transmission. Freeze-dried SF was ground with KBr and compressed to a pellet before the performance. FTIR spectra were recorded for three individual samples treated at the same condition.

X-ray diffraction

XRD spectra of the freeze-dried SF and the SF films were obtained with a Rigaku D/Max-2500V diffractometer by the method of reflection. Ni-filtered Cu-Kα radiation (λ = 1.5405 Å) was used as X-ray source at 30 KV and 20 mA. All scans were performed in the range of 2θ = 5–35° at a speed of $2 \cdot min^{-1}$, with a step size of 0.02.

Preparation of model compound incorporated films

SF films incorporating model compound were prepared by the method similar to that of SF films.[13] SF solution was mixed with Rhodamine B stock solution at weight ratio 50 of SF to Rhodamine B. The mixed solution was added to weighting bottles and dried at room temperature. After a further drying in a desiccator for 48 h, the samples were treated with ethanol–water mixtures of different concentrations for 30 min and then used for the drug release study. The ethanol–water mixtures used were collected and the Rhodamine B containing was evaluated by absorbance at 555 nm using a Shimadzu UV-2501PC UV–vis spectrometer and compared to a standard curve. The results were used to calculate the total

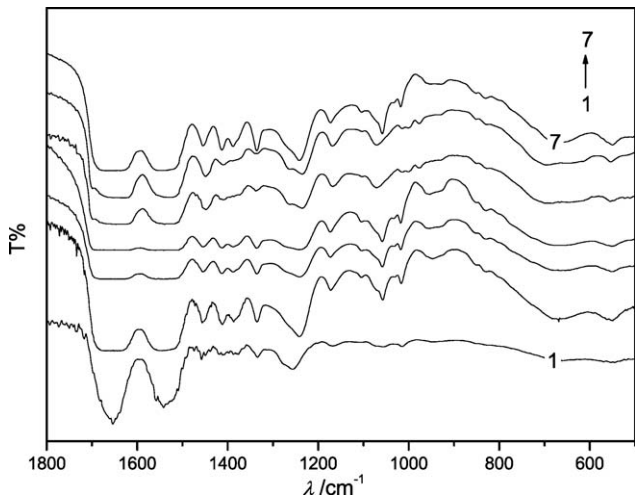

Figure 1 FTIR spectra of silk fibroin films and freeze-dried silk fibroin. (1) Freeze-dried silk fibroin; (2) film untreated; (3)–(7) films treated with ethanol–water mixtures of different contents, φ_{EtOH}/%: (3) 50; (4) 60; (5) 70; (6) 80; (7) 90.

amount of Rhodamine B incorporated in the samples.

Drug release from SF films

The release of the model compound from SF films was investigated by incubating the Rhodamine B-incorporated samples containing in the weighting bottles in 2 mL PBS at 37.5°C. At preset time intervals, the supernatant was collected and 2 mL fresh PBS was then added. The supernatant was analyzed for the amount of the released model compound by absorbance at 555 nm on a Shimadzu UV-2501PC UV–vis spectrometer. The amount of released compound from each sample was summed with the amount at each previous time point and divided by the total amount to obtain cumulative release value.[14] Experiments were run in triplicates and the data in the graphs represent the average±standard deviation.

RESULTS AND DISCUSSION

Secondary structure of SF films

FTIR is a powerful method to determine secondary structure of proteins, as the spectral characteristic of amide group is sensitive to the hydrogen bond which plays a key role in the formation of secondary structure of proteins.[15–17] To investigate the difference in secondary structure of SF films owing to treatment with ethanol–water mixtures of different content, FTIR spectra were recorded and the representative spectra in the wavelength range of 1800–500 cm^{-1} are shown in Figure 1. FTIR spectra of the films untreated and the freeze-dried SF were also recorded and shown in Figure 1 as controls. All of the SF samples show absorption band in the range of 1700–1600 cm^{-1} (amide I), 1600–1500 cm^{-1} (amide II), 1350–1200 cm^{-1} (amide III), and 800–600 cm^{-1} (amide V), respectively.[5]

Among the spectral regions arising from coupled and uncoupled stretching and bending modes of amide bonds, amide I is considered the most valuable to investigate the secondary structure of protein. However, the strong absorption of water in 1650–1640 cm^{-1} causes a certain degree of ambiguity. Although the intensity of amide II is relatively strong, it is not sensitive to secondary structure changes of proteins and strongly overlapped by bands originating from amino acid side chain vibration.[18,19] Also, it was found that spectra in amide I and amide II were prone to truncation owing to the strong absorption (Fig. 1). To make the analysis of spectra in amide I to determine the secondary structure content, the films are required to be prepared with the amount of SF no more than 1.0 $mg \cdot cm^{-2}$. However, such a kind of SF film is subject to break down in the post-treatment with ethanol–water mixtures, which make it impossible to use in spectral measurements. Compared with amide I and amide II bands, amide III in the region 1200–1350 cm^{-1} is relatively weak, but very sensitive to the secondary structure. Additionally, there is no water interference in this region.[18,19] In a study by Xie and Liu,[18] the component bands of secondary structure of β-sheet, random coil, β-turn, and α-helix in amide III were assigned as 1220–1250, 1245–1270, 1265–1295, and 1290–1330 cm^{-1}, respectively. The authors determined the peak position and bandwidth of the component bands of secondary structures using a Fourier deconvolution method and second-derivative spectra, and made a nonlinear fit to spectra of several proteins in amide III band. The secondary structure content of the proteins was evaluated by the area percentage of the component bands, according to central position by using the assignments proposed. The results were found to be consistent with those from the analysis of XRD and FTIR spectra in amide I band.

The secondary structure content of freeze-dried SF and SF films treated or untreated with ethanol–water mixtures was evaluated using a method similar to Xie and Liu's.[18] It is noticed that the samples exhibit weak absorption around 1335 cm^{-1} (Fig. 1). If the analysis was made only to the spectra in the region considered by Xie and Liu[18] (1220–1330 cm^{-1}), the α-helix content would be negligible, which was inconsistent with the reports by others.[5,17] On the other hand, it can be observed that all samples show absorption above 1200 cm^{-1} (Fig. 1). Thus, spectra analysis in amide III in this study is extended to 1200–1350 cm^{-1}, which is the same as that in the

TABLE I
Curve-Fitting Results of FTIR Spectra in Amide III Region of SF Films Treated With Ethanol–Water Mixtures and Assignment of Band Position

Assignment	Band position (cm^{-1})	Area[a] (%)						
		Freeze dried	Untreated	$\varphi_{EtOH} = 50\%$	$\varphi_{EtOH} = 60\%$	$\varphi_{EtOH} = 70\%$	$\varphi_{EtOH} = 80\%$	$\varphi_{EtOH} = 90\%$
β-Sheet	1220	4.4 ± 0.6	10.3 ± 1.6	11.1 ± 0.9	14.3 ± 1.1	14.8 ± 5.3	12.2 ± 4.4	10.3 ± 1.9
β-Sheet	1230	1.6 ± 0.3	6.6 ± 0.2	7.8 ± 0.4	6.6 ± 0.4	6.8 ± 0.7	7.8 ± 0.6	7.9 ± 0.5
β-Sheet	1240	14.9 ± 2.1	15.7 ± 1.3	15.5 ± 0.3	15.5 ± 1.1	20.1 ± 3.1	18.3 ± 4.2	15.6 ± 0.8
Random coil	1252	17.5 ± 1.4	12.6 ± 1.9	11.0 ± 2.5	7.9 ± 1.6	7.7 ± 2.5	9.7 ± 1.5	12.1 ± 1.8
Random coil	1256	10.6 ± 0.9	10.1 ± 1.1	9.8 ± 2.0	8.2 ± 0.9	9.9 ± 2.5	8.9 ± 1.8	8.7 ± 0.7
Random coil	1260	8.7 ± 2.2	9.3 ± 1.6	3.1 ± 2.7	4.7 ± 1.9	10.3 ± 1.5	9.7 ± 2.8	4.9 ± 2.0
β-Turn	1275	12.0 ± 2.4	0.2 ± 1.6	8.6 ± 1.4	12.2 ± 3.4	13.0 ± 2.5	8.5 ± 2.5	9.4 ± 3.5
β-Turn	1280	7.5 ± 1.3	11.1 ± 0.4	11.0 ± 2.8	7.8 ± 0.8	4.1 ± 2.2	4.6 ± 2.0	6.0 ± 2.3
β-Turn	1285	4.0 ± 0.7	9.2 ± 3.2	1.8 ± 2.9	2.4 ± 2.5	0.1 ± 0.1	3.4 ± 2.8	4.2 ± 4.1
α-Helix	1310	4.9 ± 1.2	2.5 ± 1.3	4.7 ± 1.5	6.1 ± 0.4	4.3 ± 0.9	4.6 ± 0.9	5.0 ± 0.9
α-Helix	1315	4.3 ± 0.5	2.0 ± 1.3	2.6 ± 0.9	2.4 ± 0.4	0.7 ± 1.1	1.5 ± 1.3	2.3 ± 0.4
α-Helix	1335.5	9.7 ± 0.3	10.2 ± 0.1	12.9 ± 0.4	12.1 ± 0.1	8.1 ± 2.5	10.8 ± 2.1	13.5 ± 0.8

[a] The area percentage of component bands of FTIR spectra was calculated by three individual samples in each case and the values in the table were represented as the average ± standard deviation.

study by Cai and Singh.[19] Following the instruction of Xie and Liu,[18] the FTIR spectra in the region of amide III were smoothed by a nine-point Savitzky–Golay function to remove the possible noise and a two-point baseline correction was performed. A nonlinear fit to the spectra in amide III was then made using 12 Gauss functions with central positions identified according to second-derivative spectra. The area percentage of the component bands was obtained (Table I) and was utilized to determine the content of secondary structure including β-sheet, random coil, β-turn, and α-helix, according to central position by using the assignments proposed by Xie and Liu,[18] and the results are shown in Figure 2. Typical curve fitting of FTIR spectrum in amide III of the SF film treated with ethanol–water mixture at $\varphi_{EtOH} = 60\%$ is shown in Figure 3. It is found that percentage of β-sheet, random coil, β-turn, and α-helix is 20.9, 36.7, 23.5, and 18.9 % for the freeze-dried SF and that for the SF film untreated is 32.6, 32.1, 20.6, and 14.8 %, respectively. After treating with ethanol–water mixtures with increasing ethanol concentration, β-sheet content in the films first increases and then decreases, whereas that of random coil, α-helix, and β-turn exhibit different behaviors, revealing the mechanism of conformation transition owing to solvent treatment.

The conformation transition of SF is actually a process of hydrogen bond rearrangement including the breaking down of original hydrogen bonds and the building up of the new ones, in which the movement of the polypeptide chain is inevitable. Chen[20,21] suggested that swelling of the film was

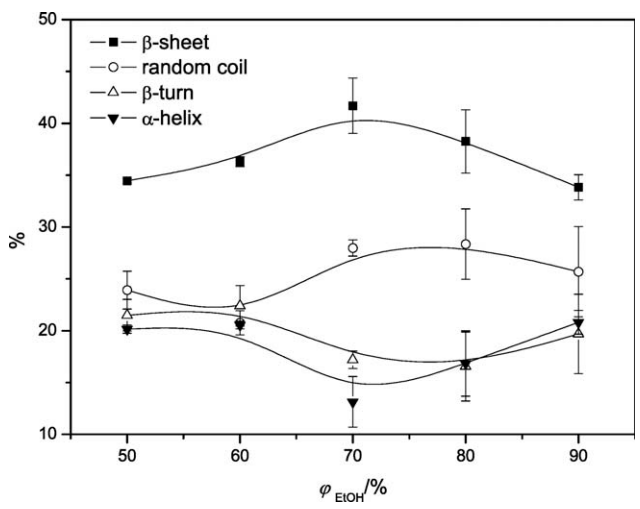

Figure 2 Secondary structure content of silk fibroin films treated with ethanol–water mixtures.

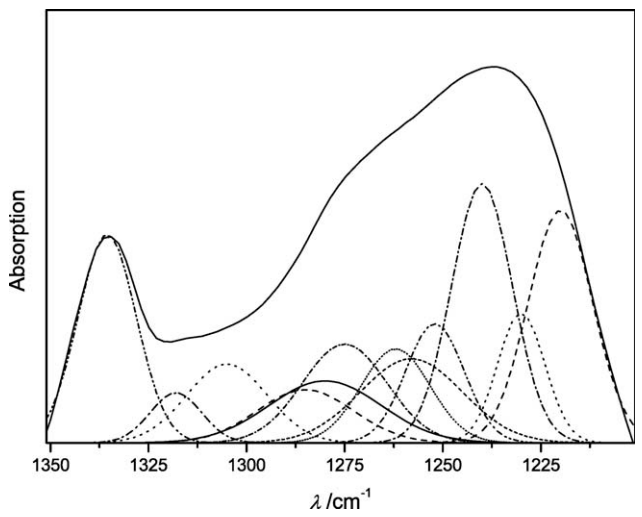

Figure 3 Curve fitting result of FTIR spectrum in amide III region of silk fibroin film treated with ethanol–water mixture at $\varphi_{EtOH} = 60\%$.

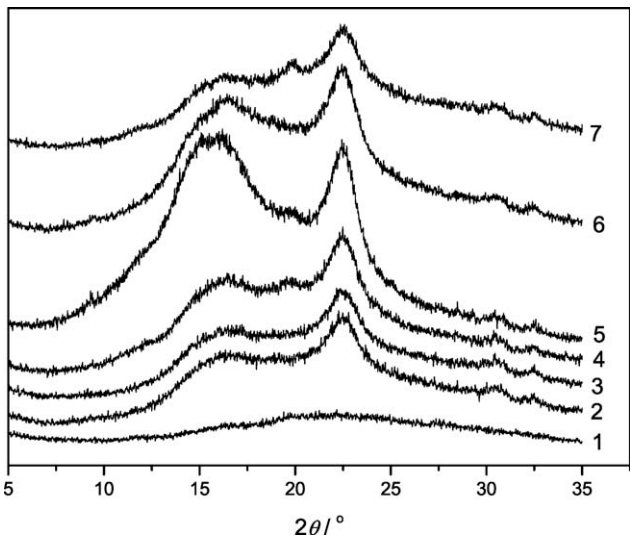

Figure 4 XRD spectra of silk fibroin films and freeze-dried silk fibroin. (1) Freeze-dried silk fibroin; (2) film untreated; (3)–(7) films treated with ethanol–water mixtures of different contents. φ_{EtOH}/%: (3) 50; (4) 60; (5) 70; (6) 80; (7) 90.

prerequisite for the conformation transition when contacting with solvent. In the case of alcohol–water mixtures, water molecule, which is of smaller size compared to alcohol, is ready to squeeze into the free space of the film and makes it swollen to support the movement of the polypeptide chain and the diffusion of alcohol molecules into the film, which will break down the original hydrogen bonds and induce the conformation transition of SF. According to the study by Chen, both the diffusion of water and alcohol molecules and SF–solvent interactions have impacts on the conformation transition of SF in the films. In the present study, the conformation transition of SF in the films treated with ethanol–water mixtures of different concentration depends on the competition between the diffusion of water and the ethanol molecules. As shown in Figure 2, compared to that with 50% ethanol–water mixture, the treatment using a mixture of 60% ethanol causes an increase of β-sheet and a decrease of random coil, however, does not have significant influence on β-turn and α-helix in the SF film, suggesting that the mixtures at low ethanol concentration are not capable of breaking down the original β-turn and α-helix, but inducing a transition from random coil to β-sheet in SF film. With increase in ethanol concentration above 60%, the percentage of β-turn and α-helix decreases at first and then increases, reaching a minimum around 70–80%, which is almost opposite to the behavior of random coil and β-sheet. The results indicate that, in the mixtures at ethanol concentration of 70–80%, the water content is still enough to make the SF films swell sufficiently to support the movement of the SF polypeptide chain, meanwhile the relatively high ethanol content also benefits the breaking down of the original β-turn and α-helix into random coil and the formation of β-sheet from random coil. A further increase in ethanol concentration causes a deduction in the conformation transition, maybe owing to the limited swelling of SF films resulting from the lack of water molecules.[20,21]

X-ray diffraction

XRD is an important method to investigate the crystal structure. To confirm the conformation change of SF in the films treated with ethanol–water mixtures, XRD measurements were performed and the results are shown in Figure 4.

Two crystalline forms, silk I and silk II, have been reported as the dimorphs of SF. Silk II is characterized by an antiparallel β-sheet structure. However, structure of silk I is not clearly understood. It is generally accepted that silk I is a combination of random coil, α-helix, and β-turns.[22,23] As there is no regular crystalline structure in SF solid, it is difficult to characterize the diffraction pattern of the two crystalline forms. Additionally, the difference in quality of raw material and preparation process may have influence on the XRD spectra, resulting in a great ambiguity in the XRD analysis by different

TABLE II
Curve-Fitting Results of XRD Spectra of SF Films Treated with Ethanol–Water Mixtures

Central position/°	Area/%						
	Freeze-dried	Untreated	φ_{EtOH} = 50%	φ_{EtOH} = 60%	φ_{EtOH} = 70%	φ_{EtOH} = 80%	φ_{EtOH} = 90%
12.0	1.9	2.8	2.8	5.7	16.2	4.3	4.8
16.2	17.0	27.6	27.2	28.7	41.8	36.9	22.6
19.8	15.6	12.0	11.0	13.2	10.4	12.8	13.5
22.5	5.5	12.2	12.6	13.1	11.3	14.0	11.6
24.0	43.3	19.3	16.9	16.9	17.6	20.1	14.3
29.0	13.9	24.7	28.3	21.7	0.9	10.0	32.7
30.5	0.9	1.1	0.9	0.5	1.1	1.4	0.5
32.5	1.9	0.3	0.3	0.2	0.7	0.3	0.1

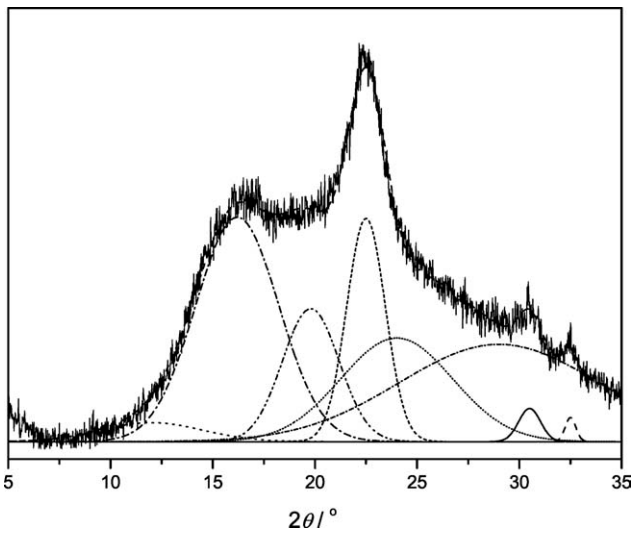

Figure 5 Typical curve fitting result of XRD spectrum of silk fibroin film treated with ethanol–water mixture at $\varphi_{EtOH} = 50\%$.

authors.[24–28] A widely accepted strategy is to make a comparison between the XRD spectra of the sample and the control, together with FTIR analysis.[25–28] For the sake of comparison, XRD spectrum of SF obtained by freeze drying, which was thought to be a good drying method to preserve the original structure of the material, was recorded and shown in Figure 4. The freeze-dried SF exhibits a broad peak at $2\theta = 20°$, which is a typical characteristic pattern of amorphous silk I structure.[29,30] For SF films, two major peaks around $2\theta = 16.2$ and 22.5° appear. As indicated by secondary structure analysis of FTIR spectra, β-sheet content of SF films, even untreated with solvent, is much higher than that of the freeze-dried SF. Thus, the diffraction peak around $2\theta = 16.2$ and 22.5° can be attributed to the silk II structure, similar to the assignment proposed by Kweon.[25]

Similar to secondary structure analysis from FTIR spectra, a deconvolution is performed directly to XRD spectra by using eight Gauss functions with central positions identified. The area percentage of the component bands is obtained and listed in Table II. Typical curve fitting of XRD spectrum of SF film treated with ethanol–water mixture at $\varphi_{EtOH} = 50\%$ is shown in Figure 5. As shown in Figure 4 and Table II, the crystal structure of SF film treated with ethanol–water mixture at $\varphi_{EtOH} = 50\%$ is almost the same as that of the untreated one. Taken the total area percentage of component bands with central position of 16.2 and 22.5 as an indication of silk II crystal structure content, the results summarized in Table II show that the silk II crystal structure content of SF films first increases and then decreases with increasing ethanol concentration in the mixtures, reaching a maximum around 70–80%. As silk II is characterized by β-sheet structure, the results suggest that the mixture of higher ethanol concentration does not favor the conformation transition of SF, which is consistent with the conclusion from FTIR analysis (Fig. 2) and gives further support to the mechanism of conformation transition of SF films in ethanol–water mixtures aforementioned.

In vitro drug release from SF films

The effect of structure of SF films on drug release was investigated. The release behaviors in PBS of the model compound, Rhodamine B, from SF films treated with ethanol–water mixtures are shown in Figure 6. All the curves follow a similar profile, an initial burst followed by a slower and steadier release.

In general, drug release from a sustained system is controlled via a mechanism of either diffusion of the drug molecule, swelling or degradation of the matrix, or the infiltration of the solvent molecules.[31] As semi-crystallized SF is rarely dissolved in water and difficult to degrade without proteolytic enzymes,[24,32] the release of drug molecules from SF films is considered to be controlled by diffusion of the drug molecule and swelling of the matrix, which is commonly described by Peppas equation,[33]

$$M_t/M_0 = kt^n \quad (1)$$

where M_t/M_0 is the fractional amount of the drug released at time t, k is a characteristic constant of the system, and n is diffusional exponent to describe the kinetics and the release mechanism, which depends on the geometry of the system. In the case of pure Fickian diffusion, the exponent n has the values of

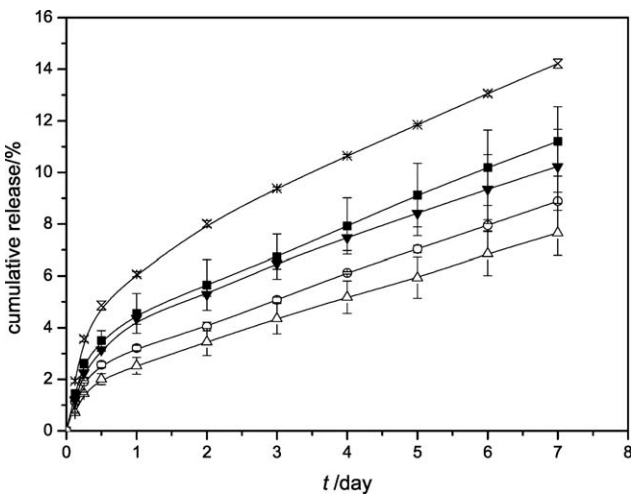

Figure 6 Release profiles of Rhodamine B from silk fibroin films treated with ethanol–water mixtures. φ_{EtOH}/% (■) 50; (○) 60; (△) 70; (▼) 80; (×) 90.

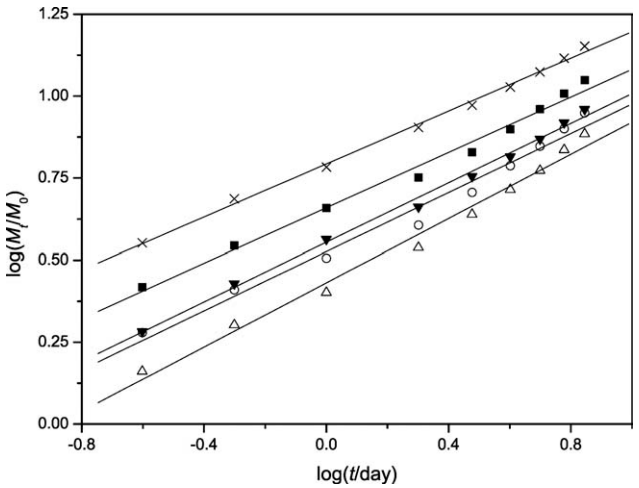

Figure 7 Experimental data based on Peppas equation for Rhodamine B release from silk fibroin films treated with ethanol–water mixtures. φ_{EtOH}/% (■) 50; (○) 60; (△) 70; (▼) 80; (×) 90.

0.5, 0.45, and 0.43 in the slab, cylinder, and sphere, respectively. On the other hand, when n values are 1.0, 0.89, and 0.85 for the slab, cylinder, and sphere, a case II transport is suggested. Other values for n indicate anomalous transport kinetic, a combined mechanism of pure diffusion and case II transport.[33,34] The above equation can be modified as follows:

$$\log(M_t/M_0) = \log k + n \log t \quad (2)$$

The values of k and n can be obtained from a linear fit to the plot of $\log(M_t/M_0)$ vs. $\log t$. In this study, an attempt to use eq. (2) was made to analyze the release profile excluding the burst release at the first point. It was found that the experimental data had a good linear fit for the release of Rhodamine B from the SF films treated with ethanol–water mixtures of different concentrations (Fig. 7). The values of release coefficient k, diffusional exponent n, and correlation coefficient R are summarized in Table III. It is apparent that the diffusional exponent n is within the limiting value of 0.5 in all the cases, suggesting a Fickian diffusion mechanism. However, the release is somewhat different from the diffusion from a typical slab, cylinder, or sphere system.

A fully understanding the mechanism of release requires considering the structure of SF films and its evolution as the solvent condition change. SF is characterized by the special semi-crystalline structure, which is considered as silk II crystals dispersing in a continuous and amorphous matrix constituted by silk I.[35] As shown in Figure 8, in the sustained release system based on SF, drug molecules may exist in both the noncrystalline region and the space between β-sheets in crystalline region. It has been

TABLE III
Parameters Obtained from Peppas equation for Rhodamine B Release from Silk Fibroin Films Treated with Ethanol–Water Mixtures

φ_{EtOH} (%)	k	n	R
50	4.56	0.42	0.994
60	3.36	0.45	0.991
70	2.70	0.49	0.994
80	3.58	0.45	0.998
90	6.21	0.40	0.998

known that silk II structure is highly stable, but silk I is subject to swelling in water.[36] In aqueous environment, when water molecules penetrate into the film and make it swollen, drug molecules existing in the noncystalline region release owing to the concentration gradient and are sustained by the amorphous polypeptide chain and silk II crystals. However, the rigidity of the silk II structure sets great barrier for the drug molecules to release from crystalline region, which may be attributed to the fact that the exponent n for the release of Rhodamine B from SF films is <0.5. As shown in Figure 8, the influence of an increase in silk II structure on the drug release from SF films would be separated into: (1) deducing the hindrance by the amorphous polypeptide chain; (2) increasing the free space in the amorphous region around the silk II crystals; (3) increasing the barrier to drug release by silk II crystals, and (4) decreasing the swelling of the matrix. A positive contribution to drug release is expected from the former two impacts, whereas that from the later two is negative. A further comparison of the results summarized in Table III and Figure 2 shows that the diffusional exponent n first increases and then decreases with increasing ethanol concentration in the mixture for post treatment, similar to that of β-sheet content. It is interesting that the diffusional exponent has a good linear fit to the β-sheet content (Fig. 9, correlation coefficient $R = 0.968$), indicating

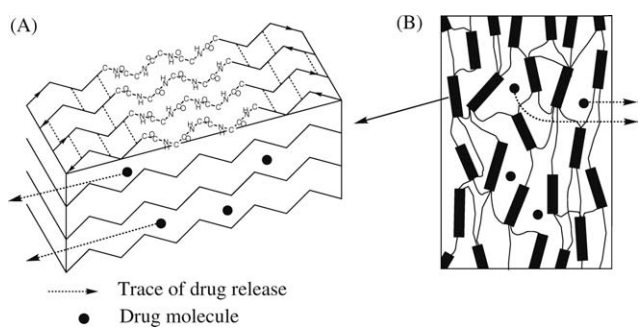

Figure 8 Schematic description of drug releasing from silk fibroin films. (A) Drug releasing from silk II crystal region into amorphous region; (B) Drug releasing from amorphous region into environment.

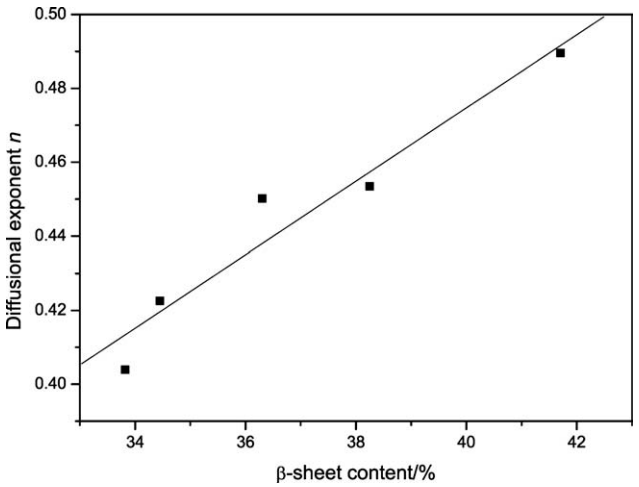

Figure 9 Plot of diffusional exponent *n* for the release of Rhodamine B vs. β-sheet content of silk fibroin films.

that the increase in silk II crystal favors the release of the model compound and that the influence (1) and (2) described above is predominant. Thus, the silk II crystal can be taken as natural regulator for the SF-based release system. Our results provide valuable information about the SF film system and are of great significance to explore the controlled release of other drugs.

CONCLUSIONS

The post-treatment by ethanol–water mixtures is a feasible method to prepare SF films of different secondary structure content. Both the diffusion of water and alcohol molecules and the SF–solvent interactions have impacts on the conformation transition of SF. In the ethanol concentration ranging from 50 to 90%, the content of silk II crystalline structure, constituted by β-sheet, first increases and then decreases with increasing ethanol concentration in the mixtures, reaching a maximum around 70–80%.

The special semi-crystalline structure, constructed by the crystalline silk II and amorphous silk I, plays a key role in drug release kinetics from SF-based system. The silk II crystals turn out to be a natural regulator for the drug release. The approach reported here offers an attractive framework for controlled release form SF-based biomaterials.

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