Thermal and crystalline behaviour of silk fibroin/nylon 66 blend films

Yi Liu, Zhengzhong Shao*, Ping Zhou, Xin Chen

Department of Macromolecular Science, The Key Laboratory of Molecular Engineering of Polymers, Fudan University, Shanghai 200433, China

Received 13 July 2004; accepted 3 September 2004
Available online 21 September 2004

Abstract

A series of blend films of silk fibroin (SF) with nylon 66 were prepared by the common solution cast method. DSC analysis of every blend sample showed a large and broad endothermic peak around 150 °C. For SF/nylon 66 samples containing 10 and 30 wt% SF, large spherulites were observed and their melting processes were recorded using a polarizing microscope with hot-stage. Furthermore, a detailed study on 30 wt% SF sample indicated a distribution of two distinct spherulites: large spherulites in part A and much smaller ones in part B. In WAXD pattern, the large spherulites presented new peaks different from nylon 66 or silk’s characteristic peaks that were ever reported, while the small ones assumed triclinic form of normal nylon 66. SEM showed that the SF and nylon 66 were nearly miscible in all ratios except that of 50 wt% SF.

Keywords: Silk protein; Polyamide; Miscibility

1. Introduction

Ever since its debut in 1935, nylon 66 has been one of the most successful commercial materials. Its superior mechanical properties and well-understood synthesis lead nylon 66 to a key position in the market of synthetic materials. Apart from the basic research on its physical and chemical properties, new areas have emerged, focusing on promoting and altering nylon 66 properties. Of these researches, blending nylon 66 with other polymer to obtain multi-component polymer materials is a typical approach [1–3]. Among these, some remarkable studies have been carried out on crystallization into spherulites of miscible blends from the melt in which an amorphous diluent was added to a crystallizable host polymer. Keith et al. even found that startling morphological changes could happen when the diluent was at low concentration [4]. Clark et al. proved that semicrystalline polyamide could also develop into interesting structures when trace quantities of amorphous PVP were added in [5]. Parallel studies were conducted on other blends consisting of semicrystalline and amorphous components [6–10].

On the other side, as a member of nylon family, the silk fibroin (SF, considered as nylon 2) is a potential biomaterial for wide use due to its good biological compatibility as well as biodegradability [11,12]. As we know that the natural silk fiber is one of the strongest and toughest materials mainly because of the dominance of well orientated β-sheet structures of protein chains [13,14]. However, SF film, in which the random coil conformation is predominant, is brittle, which restraints further application. Efforts have been made to improve the performance of SF film [15–19]. Blending is probably the best alternative for its convenience and effectiveness. SF/chitosan film [15], SF/PAA film [16], SF/PVA film [17,18], SF/cellulose [19] have been studied before, and some blend films even showed satisfactory properties [19]. Yet under most conditions, SF and the other polymer component exhibited severe phase separation [17,18].

SF and synthetic nylon could be good candidates to modify each other. It is not only because SF’s similar chemical structure with synthetic nylon (e.g. both of them contain amide bonds) may lead to a possible miscibility between the two components, but also there is a chance that the molecular chain of SF and nylon might mutually aggregate to form a new ordered structure, which may then change the crystallization behavior or even improve the
properties of SF or nylon for various application purposes. However, blending cannot be carried out in the melt state because SF is unable to melt readily. On the other hand, nylon is a hydrophobic polymer. Thus finding a proper solvent is the key for proper SF/nylon 66 blending.

In this paper, we focused on blending SF with nylon 66 in a mixed solvent for cast films. Initial studies on a series of SF/nylon 66 films showed some notable phenomena. Amorphous SF did not simply act as diluent in semicrystalline nylon 66 at low concentration (10 and 30 wt% SF) but it probably induced co-crystallization with nylon 66. Also we could provide the evidence that indicates a certain strong interaction between SF and nylon 66, which greatly affects nylon 66’s crystallization behavior.

2. Experimental section

2.1. Samples and methods

2.1.1. Degumming of silk fiber

*Bombyx mori* silk was boiled in 0.5 wt% Na$_2$CO$_3$ aqueous solution for 40 min to remove the sericin, then rinsed three times successively in distilled water, and soaked in distilled water overnight. The degummed silk was dried in an oven under 60 °C for 7 h.

2.1.2. Preparation of SF/nylon 66 films

The degummed silk was dissolved in a LiCl/formic acid solution (10 wt% LiCl). To minimize the degradation of SF, the dissolution process was performed in an ice-water bath at a temperature of 5 °C, and the solution was kept at the same temperature before mixing with nylon 66.

Nylon 66 was thoroughly dissolved in formic acid at 60 °C, and then the solution was cooled to room temperature.

The SF and nylon 66 solutions were mixed at different weight ratios at room temperature with moderate agitation for 10 min. These limpid solutions were stored at 5 °C overnight, and then they were respectively poured into a large amount of acetone to form precipitates which contained different ratios of SF and nylon 66. The precipitates were rinsed with acetone and distilled water several times to remove the residual LiCl. After drying in vacuum for more than 10 h, the series of precipitates were stored in a desiccator. Prior to use, the precipitates were dissolved again in formic acid. The solutions were cast on plastic weighing boats and dried in vacuum for 10 h to remove the formic acid. Thus, the blend films of SF/nylon 66 were prepared. Pure nylon 66 and SF films were obtained by the same procedure. To testify the possible residual LiCl in the blend films, they were, respectively, soaked in 20 ml distilled water for 12 h, and then the 0.1 M AgNO$_3$ solution was added in. The transparent solutions turned slightly cloudy but no precipitate appeared. It suggests that only small amount of LiCl remains in the blend films if there is any.

The aqueous solution of lithium salts is a normal solvent to dissolve SF [20–22], and it is reported that the molecular decomposition of SF did not occur during the dissolution process in formic acid within 1–2 days of storage time at room temperature [23]. Furthermore, the viscosity of all our solutions seemed unchanged during the preparation of samples (within 24 h). Hence, we thought the molecular weight of SF should be stable in LiCl/formic acid solution under our experimental conditions.

2.2. Measurements

The blend films were fractured in liquid nitrogen and the morphology of their cross-sections was observed by a Philips XL30 scanning electron microscope (SEM) after gold coating.

DSC measurements were performed on a Setaram DSC 161 at a heating rate of 10 °C/min in nitrogen atmosphere. An Olympus BX-51 polarizing microscope equipped with temperature control stage was used to monitor the melting processes of crystals in the blend film at a heating rate of 3 °C/min.

Wide angle X-ray diffraction (WAXD) studies were carried out using a Rigaku D/max-rB diffractometer with CuKα radiation (40 kV, 60 mA). Digital data were collected in a continuous scan mode at a scan rate of 4.0 deg/min.
3. Results and discussion

3.1. Thermal behavior and spherulites of SF/nylon 66 blend

Fig. 1 showed the DSC curves of blends of SF/nylon 66. It was found in Fig. 1(a) that there was a glass transition around 10 °C, a temperature that was much lower than the $T_g$ of pure nylon 66 (around 50 °C). Those findings apply to blend films containing 10, 30 and 50 wt% SF. However, the $T_g$ of blend material disappeared when the SF content exceeded 70 wt%. It is worth noticing that a broad endothermic peak around 150 °C shows up in the DSC curve of each blend sample. A control experiment done with pure nylon 66 as well as pure SF confirmed that such a broad endothermic peak was not caused by the sample preparation procedure (Fig. 1(b)). Moreover, the samples containing 10, 30 and 50 wt% SF, respectively, showed a relative narrow endothermic peak above 200 °C in its DSC curve, which...
shifted to lower temperatures when the concentration of SF increased. Clearly, this phenomenon could be ascribed to a melting point depression of nylon 66’s crystals, caused by mixing with other polymers, or perhaps by the presence of unwashed LiCl [24,25].

The melting point of nylon 66 is 262 °C and SF is not expected to melt, which means the broad endothermic peak around 150 °C in the DSC curve of blended SF/nylon 66 film can hardly be explained as melting point depression of nylon 66s induced by simple blending or the possible residual of LiCl [24,25]. Therefore, it seems more reasonable to assume that SF and nylon 66 would co-crystallize or form certain compounds because of some strong interactions between these two components.

We investigated blend films which contained 10 and 30 wt% SF with a polarizing microscope and monitored the melting process of the spherulites utilizing the temperature control stage, as shown in Figs. 2 and 3.

In the polarizing microscope images, it could be seen clearly that there were two different crystals in the blend film containing 10 wt% SF. There are a few large spherulites, of which the diameter is about 120–150 μm; the others are small crystals, which are abundantly present, providing the background of the large ones. The images of the large spherulites started to change around 120 °C, and the spherulites melted completely at 145 °C, whereas the small crystals still remained. Because a slower heating rate was applied than that of the DSC experiment, we could confirm that the melting process of the large spherulites contributes to the large endothermic effect around 159 °C present in the DSC curve. However, some of the small crystals did not persist above 200 °C (images are not shown) and we assigned them to nylon 66’s crystals which are severely affected by the addition of SF and their melting might not correspond to the endothermic peak around 245 °C in the DSC curve.

A fairly few large spherulites could be observed in the sample containing 30 wt% SF (Fig. 3). As far as the size and the profile are considered, these spherulites resemble the large ones in 10 wt% SF blend film. Again, these spherulites started to fuse around 120 °C and melted at about 131 °C. This process could also be related with the broad endothermic peak around 145 °C in the corresponding DSC curve. Because these large spherulites are quite bright, no other crystals could be observed at lower temperatures. As the temperature increased, the large spherulites melted and their brightness faded away while some small crystals were revealed. Since most of these small crystals did not melt until about 220 °C (images are not shown), we assumed that their melting process corresponds to the endothermic peak around 228 °C shown in the DSC curve. So these small crystals were nylon 66 crystals whose melting behavior is affected to some extent by SF and/or residual LiCl [24,25].

Further study demonstrated that the blend films containing 10 and 30 wt% SF could be divided into two parts: one part includes the large spherulites (part A), the other part contains only the small nylon 66 crystals (part B).

Taken into consideration that the endothermic peaks around 150 °C in DSC curves were too large and broad to be attributed simply to the relatively sparse large spherulites’ melting, it is reasonable to suggest that in the samples containing 10 and 30 wt% SF, there also exist some amorphous compounds of nylon 66 and SF which will dissociate around 150 °C. We did not observe similar large spherulites in the sample containing 50 wt% SF and only some irregular crystals were found. In samples containing 70 and 90 wt% SF, no obvious crystals were detected under the polarizing microscope. However, the broad endothermic peak around 150 °C exists for every blended SF/nylon 66 film. So it implies that the endothermic effect taking place around 150 °C includes at least two possibilities: one is melting of the large spherulites; another is disassociation of the amorphous compounds. In samples containing either 10 or 30 wt% SF, both possibilities could take place, while in samples containing more SF, the former process did not happen.

3.2. Crystalline and morphological properties

The regular WAXD traces of blend film of SF/nylon 66 were recorded at room temperature, as shown in Fig. 4. When SF was present at low concentrations (10, 30 wt%), nylon 66 could readily crystallize to the triclinic from [26,27], however, once the SF ratio reached 50 wt%, no
obvious crystalline peaks could be observed. We found only amorphous haloes in the WAXD traces of samples containing 70, 90 wt% SF and pure SF. It suggests that SF hampers nylon 66 crystallization severely at high SF concentrations, which corroborated the observation by the polarizing microscope. Part A of the sample containing 30 wt% SF exhibited a remarkable WAXD profile. Table 1 lists the $d$ values of samples displaying strong or medium strong peaks in their WAXD profiles. It could be determined that there exists more than one type of crystal forms in part A of the 30 wt% SF sample. One is the triclinic form of nylon 66 (characterized by $d = 4.35$ and $3.67 \text{ Å}$), corresponding to the small crystals observed under polarizing microscope; other forms are not clear. For nylon 66, only two basic structures have ever been reported at room temperature: the triclinic form and the pseudohexagonal form [28]. The characteristic

<table>
<thead>
<tr>
<th>SF content(^a)</th>
<th>$d_1$ (Å)</th>
<th>$d_2$ (Å)</th>
<th>$d_3$ (Å)</th>
<th>$d_4$ (Å)</th>
<th>$d_5$ (Å)</th>
<th>$d_6$ (Å)</th>
<th>$d_7$ (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 wt%</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4.42</td>
<td>–</td>
<td>3.66</td>
<td>–</td>
</tr>
<tr>
<td>10 wt%</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4.46</td>
<td>–</td>
<td>3.73</td>
<td>–</td>
</tr>
<tr>
<td>30 wt% B</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4.44</td>
<td>–</td>
<td>3.67</td>
<td>–</td>
</tr>
<tr>
<td>30 wt% A</td>
<td>7.36</td>
<td>5.16</td>
<td>4.35</td>
<td>3.86</td>
<td>3.67</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>100 wt%</td>
<td>10.1</td>
<td>4.5</td>
<td>3.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

To avoid influence of background noise, only strong and medium strong peaks were considered.

\(^a\) 0, 10 wt%, 30 wt% B, 30 wt% A correspond to the curves $a$, $b$, $c$, $d$ in Fig. 5; 100 wt% refers to crystal of beta-sheet form of silk fibroin [29].

![Fig. 5. SEM images of blend samples.](image-url)
that of the latter is around 4.00 Å [26,27]. So some of the crystalline peaks cannot be assigned to those known crystalline peaks of either nylon 66 or SF. Those peaks may suggest co-crystallization of nylon 66 and SF, corresponding to the large spherulites observed under polarizing microscope. Because large spherulites were much scatter in the 10 wt% SF sample, we were not able to obtain the correspondent XWAXD profile of new crystals.

The ratio of SF and nylon 66 in the blend should be considered as a key factor to affect the crystal and/or possible co-crystal of the two components. At low SF concentrations, i.e. 10–30 wt%, the increasing SF content could yield more co-crystals. But when the SF content was increased to a certain degree (i.e. 50 wt% in our case), co-crystallization did not occur anymore, and even crystallization of nylon 66 was suppressed. It should be noticed that 50 wt% SF probably was a key ratio, because the SEM observation on the cross section of SF/nylon blend film showed that only the 50 wt% SF sample presented clear phase separated morphology although the composition of these phases was unclear (Fig. 5). Given the chemical structure of SF and nylon 66, hydrogen bonds between different molecules were supposed to account for a good miscibility in blends. Therefore, an explanation could be that the pure SF predominantly exists in an amorphous form while the pure nylon 66 is semicrystalline in the preparation of our blend film. Hence a nylon 66 rich sample can induce SF to co-crystallize and a SF rich sample can prevent nylon 66 from crystallizing because of the strong interaction between them. When the weight ratio of SF/nylon 66 is 1:1, neither nylon 66 nor SF could prevail, then the phase separation takes place. Nevertheless, this remains to be demonstrated in more details.

4. Conclusions

A blend film of silk fibroin and nylon 66 can be properly prepared by casting their co-solutions of formic acid. It is suggested that there is strong interaction between the molecular chains of SF and nylon 66, which may lead to the miscibility at specific ratios of the two components. Moreover, co-crystallization of SF and nylon 66 probably occurred during the preparation of blend films. However, it seems there is a trade-off between SF and nylon 66’s interaction. A small amount of SF is inclined to co-crystallize with nylon 66, while a large amount of SF may form an amorphous composite with nylon 66. In the situation of 50 wt% SF, the blend film shows a heterogeneous morphology, and there is no visible crystal in the blend. In all cases, the crystallization behavior of nylon 66 is dramatically influenced in the blends with SF.

Acknowledgements

The authors are grateful to Prof D. Grubb of Cornell University, Prof Jacquse G. H. Joosten of DSM (The Netherlands), Dr A. Terry and C. Dicko of Oxford University for helpful discussion. This project was initiated and funded by DSM Research (Netherlands), and further supported by the STF of Shanghai (China) and the National Natural Science Foundation of China.

References