Synthesis and Characterization of New Acrylic Polymer Containing Silk Protein

Zhongmin Chen*, Matsumi Kimura*, Masahiro Suzuki*, Yoshiyuki Kondo*, Kenji Hanabusa*, Hirofusa Shirai*

*Department of Functional Polymer Science, Faculty of Textile Science and Technology, Shinshu University, Ueda Nagano 386-8567, Japan
*Graduate School of Science and Technology, Shinshu University, Ueda Nagano 386-8567, Japan

Abstract: New acrylic polymer containing silk protein was synthesized by copolymerization of acrylonitrile and silk fibroin peptide with vinyl groups, and its solubility, thermal property, and moisture absorption were investigated. This polymer has a covalently cross-linking structure, and its excellent thermal stability and better moisture absorption were indicated.

(Received 9 July, 2002; Accepted 24 October, 2002)

1. Introduction

Acrylonitrile (AN)-based polymers are widely used for plastics, rubbers, fibers because of their relatively cheap price and good mechanical properties. However, AN-based polymers are showing low dyeing affinity, uncomfortableness and low moisture absorption, thus there is a limitation of the applications. On the other hand, Bombyx mori silk fibroin fibers show excellent luster, tensility, handling, and moisture absorption, but they have some demerits such as expensiveness, photo-yellowing, poor wrinkle recovery and rub resistance, leading to narrow application ranges [1]. To improve the demerits of each polymer, new functional polymers consisting of combination of silk fibroin (SF) and AN-based polymers have been developed. The synthetic strategies reported are mainly classified into two methods: (i) blending of SFs and AN-based polymers [2] and (ii) graft-polymerization of acrylonitrile onto SFs [3][4]. These polymers show very poor adhesive properties, and the mechanical properties are inferior to those of polyacrylonitrile (PAN) homopolymer. In this paper, we describe the synthesis and characterization of new acrylic polymer containing silk protein. Moreover, the thermal properties, solubility in DMF and DMSO, and moisture absorption of the acrylic polymer are examined, and these properties are compared with those of polymers prepared by conventional methods, blending of PAN and SF and grafting of AN onto SF fiber.

2. Experimental

2.1 Materials

Acrylonitrile was purified by distillation just before use. Polyacrylonitrile (PAN) was purchased from Aldrich. N,N-Dimethylformamide (DMF) was purified by the method of vacuum distillation. α-Chymotrypsin and the other chemicals were of the highest commercial grade and used without further purification.

2.2 Synthesis of random copolymer of SFP-AC and AN (Polymer 1)

\[
\text{CH}_2 = \text{CH} \xrightarrow{\text{SFP-\text{NH}_2}} \text{CH} \quad \text{CON-CI} \quad \xrightarrow{(\text{C}_2\text{H}_4)\text{N/DMF}} \quad \text{CH} \quad \xrightarrow{\text{CON-H-SFP}} \quad \text{CN}
\]

\[
\text{CH}_2 = \text{CH} \xrightarrow{60\%\text{ZnCl}_2 \text{aq}} \text{CH} \quad \xrightarrow{\text{CON-H-SFP}} \quad \text{CN}
\]

(m: 0.001–0.002 n: 0.999–0.998 by elementary analysis)

Fig. 1 Synthesis procedure of polymer 1.

Polymer 1 was prepared according to Fig. 1.

2.2.1 Preparation of Silk fibroin (SF) powder

Silk fibroin (SF) was obtained by removal of needless components such as cocoon waxes, carbohydrates, lipids, and sericin from Bombyx mori cococon shell.

Cocoon waxes were removed by extraction with ether
using a Soxhlet extractor for 48 hours. Carbohydrates and lipids were extracted with ethyl alcohol. Sericin was removed by washing in boiling soapsuds. Pure SF powder was obtained by dialysis in a mixed solution of distilled water and methanol (1:5) containing LiBr (0.01 mol/l).

2.2.2 Preparation of silk fibroin peptide (SFP)

SF powder (1.0 g) was dispersed in a distilled water (100 ml) at 80°C for 5 minutes. The solution was adjusted by a 25% ammonia solution to ca. pH 7.5. α-chymotrypsin (0.1 g) was added and the solution was stirred at 37°C for 1 hour. After was deactivated by the reaction by cooling, the SFP was filtered, washed with distilled water, and dried.

2.2.3 Preparation of vinyl monomer containing SFP (SFP-AC)

To a dry DMF solution of SFP (1.0 g) and triethylamine (10 ml), acryloyl chloride (3.0 ml) was slowly added on an ice-bath with vigorously stirring. The resulting solution was stirred at room temperature for 1 h. The product was filtered, washed with distilled water, and dried in vacuum at 50°C for 24 h.

2.2.4 Random copolymerization of SFP-AC and AN (Polymer 1)

SFP-AC (0.1 g) was stirred in 60 wt% ZnCl2 aqueous solution at room temperature for 48 hours, and insoluble compounds were filtered off. To the filtrate, AN (10 g) and aqueous solution (5 ml) of ammonium persulfate (0.1 g) were added, and the mixed solution was heated at 55°C for 1 h with stirring. The resulting solution was poured into a dilute HCl aqueous solution. White precipitate obtained by filtration was washed with distilled water and acetone, and then dried in vacuum at 40°C overnight.

2.3 Blend Polymer of SF and PAN (Polymer 2)

SF fiber (0.1 g) and PAN (3.23 g) were mixed with aqueous solution containing 60 wt% ZnCl2 (120 g), and the resulting solution was stirred at room temperature for 24 h. After filtration of insoluble compounds, the blend polymer was reprecipitated into dilute HCl aqueous solution. The polymer was filtered, washed with water and acetone, and then dried in vacuum at 40°C overnight.

2.4 Graft-polymerization of AN onto SF fiber (Polymer 3)

SF fiber (0.1 g) was stirred in 60 wt% ZnCl2 aqueous solution (120 g) at room temperature, and insoluble compounds were filtered off. To the filtrate, AN (10 g) and aqueous solution (5 ml) of ammonium persulfate (0.1 g) were added, and the mixed solution was heated at 55°C for 1 h with stirring. The resulting solution was poured into a dilute HCl aqueous solution, and white precipitate was filtered, washed with water and acetone, and then dried in vacuum at 50°C overnight. The dried samples were then immersed in 9.5 mol L⁻¹ LiBr aqueous solution at 80°C for 3 h under constant stirring to dissolve out the unreacted SF, and don’t extract PAN homopolymer. Finally, the polymer 3 was filtered, washed, and dried.

2.5 Measurements

2.5.1 Apparatus

Infrared (IR) spectra were measured using a JASCO FS-420 spectrophotometer. Thermal analyses were performed in the temperature range between 25°C and 600°C under nitrogen atmosphere using a SEIKO DSC 15200 instrument system, consisting of thermogravimetric analysis, differential thermal analysis (TG/DTA 220) and differential scanning calorimetry (DSC 220), with a heating rate of 10°C min⁻¹.

2.5.2 Solubility

Solubility test of these polymers (1 to 3) was examined in DMF, DMSO, benzene, chloroform, acetone, 60 wt% ZnCl2 aqueous solution, and then mixture of H2O and methanol (1:5) containing LiBr (0.01 mol/l).

2.5.3 Moisture absorption

The moisture absorptions experiments were performed using an ENVIROS KCL-1000 (EYELA) Environmental chamber. Polymer samples (dried at 50°C for 1 week) were allowed to stand in an environmental chamber that was thermostated at 20°C and humidified at 65% of relative humidity for 1 week. The moisture absorption (%) of these polymers was defined by the following equation:

\[ \text{Moisture absorption} \% = \left( \frac{W_f - W_i}{W_i} \right) \times 100 \]

where \( W_f \) and \( W_i \) represent the weight of the dried sample and the moisture-absorbed sample, respectively.

2.5.4 Hydrolysis of polymer 1

Polymer 1 (0.05 g) was dissolved in DMF (3 ml) with stirring. After gelation, concentrated HCl (3 ml) was added and heated at ca. 110°C for 3 h.

3. Results and discussion

3.1 IR Spectrum

Figure 2 and 3 show the IR spectra of polymer 1 to 3, SF and PAN. The IR spectrum of SF shows peaks at 1645 cm⁻¹ (ν C=O, amide I), 1539 cm⁻¹ (δ N-H, amide II), and that of the PAN shows peaks at 2243 cm⁻¹ (ν C≡N) and 1454 cm⁻¹ (δ CH₂). The typical IR peaks of polymer 1 appear at 1645 cm⁻¹ (ν C=O, amide I), 1540 cm⁻¹ (δ N-H, amide II), 2243 cm⁻¹ (ν C≡N), and 1455 cm⁻¹ (δ CH₂), and the IR spectrum was almost the same.
as the combination spectrum of SF and PAN and that of polymer 2 (polymer blend), polymer 3 (graft-polymer). These results indicate that polymer 1 is copolymer consisting of SFP-AC and AN.

![FT-IR spectra](image)

**Fig. 2** FT-IR spectra in the range of 600 to 2500 cm⁻¹ of (a) SF (b) polymer 1 (c) PAN.

 containing 60wt% ZnCl₂. Table 1 summarizes the results of solubility test for polymer 1 to 3, SF, and PAN. Although polymer 1 was insoluble into DMF and DMSO,

<table>
<thead>
<tr>
<th>Poly.</th>
<th>Poly.</th>
<th>Poly.</th>
<th>SF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>DMF</td>
<td>Dissol</td>
<td>Gel</td>
<td>Dissol</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dissol</td>
<td>Gel</td>
<td>Dissol</td>
</tr>
<tr>
<td>Benzene</td>
<td>Insol</td>
<td>Insol</td>
<td>Insol</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Insol</td>
<td>Insol</td>
<td>Insol</td>
</tr>
<tr>
<td>Acetone</td>
<td>Insol</td>
<td>Insol</td>
<td>Insol</td>
</tr>
<tr>
<td>60% ZnCl₂</td>
<td>Dissol</td>
<td>Swell</td>
<td>Gel</td>
</tr>
<tr>
<td>H₂O</td>
<td>Insol</td>
<td>Insol</td>
<td>Swell</td>
</tr>
<tr>
<td>LiBr</td>
<td>Insol</td>
<td>Insol</td>
<td>Swell</td>
</tr>
</tbody>
</table>

![Chemical structures](image)

**Fig. 4** Tentative illustration of chemical structures of polymers 1 to 3.

3.2 Solubility test
It is known well that the PAN is soluble into DMF, DMSO very well, and SF is soluble into aqueous solution it swelled and formed a transparent gel. In 60wt% ZnCl₂ aqueous solution, polymer 1 was also insoluble and only swelled (not gelled). And also, when the 60wt% ZnCl₂ aqueous solution of polymer 1 is filtered, no SFP is found in filtrate. In contrast, polymers 2 and 3 had a good solubility for DMF and DMSO. Moreover, they were insoluble into 60wt% ZnCl₂ aqueous solution and formed a gel followed by swelling. Such different solubility among polymers 1 to 3 could be explained by their structures.

Considering the preparation procedure, the chemical structures of polymers 1 to 3 can be illustrated as shown in figure 4. Polymer 1 has a covalently cross-linking structure by SFP, and the PAN chains in polymer 3 are elongated from the SF as a center. Polymer 2 has a structure that the SF is dispersed PAN. The solubility of polymer 2 and polymer 3 into DMF and DMSO is...
affected by PAN chains (soluble), while polymer 1 is insoluble and forms a gel because of its crosslinked structure.

3.3 Hydrolysis

Fig. 5 Photograph of (a) polymer 1 before decomposition (b) decomposed polymer 1.

In order to prove the molecular structure of polymer 1, polymer 1 was hydrolyzed by 12 M HCl. Figure 5 shows the photographs of polymer 1 in mixture of DMF and 12M HCl before (a) and after (b) heating. After heating at ca. 110°C, the flocculent solution of polymer 1 was completely separated into a transparent liquid and precipitated polymer, indicating that the hydrolysis was achieved. Figure 6 shows the IR spectrum of polymer 1 precipitated by the hydrolysis. The IR spectrum was similar to that of PAN. It is assumed that the amide bonds and SFP in polymer 1 were hydrolyzed, namely the crosslinked paste is decomposed. This result indicates that polymer 1 has a cross-linking structure by SFP.

Fig 6 FT-IR spectra of decomposed polymer 1.

3.4 Thermal behavior

Figure 7 shows the TG curves of PAN, SFP, and polymers 1 to 3. The TG curve of SFP showed that the weight loss began above 250°C. For PAN, the weight also decreased over 300°C. And the weight always loss began above 292°C for polymer 1 to 3. Then Polymer 1 to 3, which is prepared by random copolymerization, blending and graft-polymerization, has good thermal stabilities compared with SFP. As shown in figure 7, the weight residues of Polymer 2 and polymer 3 are larger than PAN, because of that coexistence of amine and PAN cause the cyclization easily [5], namely the polymer 2 and polymer 3 have good thermal stabilities compared with PAN. The weight residue of polymer 1 is larger than that of SFP, namely, polymer 1 is more thermally stable than SFP. These results indicate that the thermal stability of silk fibroin protein was improved by graft-polymerization, blending and random copolymerization with PAN.

Figure 8 and figure 9 show the DTA profiles of these polymers. The DTA curve of SFP demonstrated an endothermic profile in the range of 250°C to 320°C with the peak at 282°C [6]. Such the thermal behavior reveals that the SFP is thermally decomposed, being supported by
the TG curve. For other polymers, the DTA profiles showed endothermic peaks around 290°C that the weight began to lose in the TG curves. Furthermore, the exothermic peaks appeared at 429°C for polymer 2 and 450°C for polymer 3 in the DTA curves, but not for polymer 1. It is well-known that PAN undergoes thermally intramolecular cyclization between 400°C and 600°C. Considering the fact, the PAN chains in polymer 2 and polymer 3 would be partially cyclized. In contrast, an exothermic peak was not observed around 450°C in the DTA curve of polymer 1; the thermally cyclization between AN segments does not occur. As shown in figure 4, polymer 1 is the random copolymer of SFP-AC and AN, and SFP segments seem to inhibit cyclization between AN segments.

3.5 Moisture absorption

Table 2 summarizes the moisture absorption of these polymers. The SF showed the highest moisture absorption (8.0%), and that of PAN is lowest (1.5%). Polymer 1 to 3 showed higher moisture absorption than PAN. (Moisture absorption of polymer 1 increased to 2.3% and moisture absorption of polymer 2 and polymer 3 increased to 3.0%). By blending, graft-polymerization, and random copolymerization with SF protein, the moisture absorption of PAN was improved slightly.

4. Conclusion

In this paper, new acrylic polymer containing silk protein was synthesized by copolymerization of AN and SFP with vinyl groups.

And first of all, by the typical IR peaks of polymer 1, it is indicated that polymer 1 is a copolymer consisting of SFP-AC and AN.

Second, we checked the solubility of polymer 1 and polymer 2 to 3, SF, PAN. Through such the different solubility among polymer 1 and other, we could explain by their structures, polymer 1 has a covalently cross-linking structure by SFP.

Third, we have investigated thermal property of polymer 1 and other polymers, and found that the thermal stability of SF was improved by graft polymerization, blending, and random copolymerization with PAN.

Finally, the moisture absorption of polymer 1 and other polymers showed that the moisture absorption of PAN was improved slightly after the PAN is combined with SFP segments.

Acknowledgments

This work was supported by a Grant-in-Aid for COE research (10 CE 2003) by the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References