Dissolution and Wet Spinning of Silk Fibroin using Phosphoric acid/Formic acid Mixture Solvent System

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ABSTRACT: Regenerated silk fibroin (SF) filaments could be prepared by wet spinning in common solvent/coagulation system. SF was directly dissolved in mixture solvent of phosphoric acid and formic acid (20/80–30/70 ratio) and coagulated in methanol bath. The concentration and stability of SF dope solution have been studied by varying the mixture ratios of these solvents in accordance with elucidating the role of formic acid in the mixture solvent system. Morphological structure as well as crystalline structure of the regenerated filament was examined using SEM and XRD analyses. As a result of tensile test, the regenerated SF filament, which was made by one-step dissolution and coagulation process, had good mechanical properties, 2.3 gf/d tenacity and 18% breaking strain. In this study, a simple wet spinning method which enables to apply to practical production has been reported for the preparation of the regenerated SF filament. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 105: 1605–1610, 2007

Key words: proteins; mechanical properties; crystal structure; fibers; solution properties

INTRODUCTION

Proteins can be regenerated into proper forms such as powder, film, membrane, sponge, filament, etc. by dissolving in proper solvents. Especially, silk fibroin (SF) has been mainly focused on because it has excellent functionalities and properties as a high performance fibrous protein.[1–4] However, it is not easy to dissolve the SF in common solvents for obtaining a true solution because of its high molecular weight and crystallinity. Inorganic acids such as phosphoric acid and neutral salts have been used for the dissolution but degradation or deterioration always occurred because of severe conditions.[5–7]

In general, wet spinning method can be applied to prepare the regenerated SF filament by coagulating the dope solution. There are several reports for dissolution/coagulation system and mechanical properties of the SF filament. In 1989, Ishizaka et al.[7] used ortho phosphoric acid (H_3PO_4) as a solvent and the dope solution was spun into ammonium sulfate and sodium sulfate as a coagulant. The as-spun regenerated SF was immersed in 90% methanol and then drawn. The obtained breaking strength and strain were about 2.11 gf/d and 10.1%, respectively. However, phosphoric acid has a very strong acidity and it may cause a severe decrease of molecular weight of SF. Lock[8,9] also produced the SF filament using 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and methanol as a solvent and a coagulant, respectively. Excellent mechanical properties could be obtained; breaking strength of 4.76 gf/d, and breaking strain of 8.9%. However, the HFIP is not only very harmful but also very expense, which makes limitations for its practical use.

Um et al.[10,11] had reported the regenerated SF filament using a formic acid/methanol system. Formic acid was not able to dissolve the SF due to its high molecular weight and crystallinity. Therefore, they first dissolved the SF in a ternary solvent system, a mixture of calcium chloride, water, and ethanol (molar ratio: 1/8/2). And then, the SF was regenerated in the form of sponge type via dialysis and lyophilizing. Finally, the regenerated SF, which is partially water-soluble, was dissolved once again in formic acid and the dope solution was spun into methanol coagulation bath. They reported that the mechanical properties were comparable to the results of Ishizaka et al.[7] and the formic acid could contribute an excellent stability of the dope solution.

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Unfortunately, this method is too complicated due to a two-step dissolution process.

Therefore, we suggest a simple wet spinning method using common solvent/coagulation system in this study. The SF (degummed cocoon) was dissolved in mixture solvent of phosphoric acid and formic acid directly and the dope solution was spun into coagulation bath of methanol in one-step, which makes possible the coagulation and crystallization to occur simultaneously. The structure and mechanical properties of the regenerated SF filament were examined together with the properties of dope solution by comparing the results of Ishizaka et al. and Um et al. for its feasibility of a practical use.

EXPERIMENTAL

Materials

Bombyx mori cocoons were degummed twice with 0.3% (o.w.f.) sodium carbonate and 0.5% (o.w.f.) marseillus soap solution at 100°C for 1 h and then dried for obtaining the SF. The chemicals, 85% phosphoric acid (Daejung chemicals, Korea), 98% formic acid (Kanto chemicals, Japan), and 99% methanol (Daejung chemicals, Korea), were used without further purification.

Preparation of dope solution

After formic acid and phosphoric acid were mixed with various volume ratios of these solvents, the degummed cocoons were dissolved in the mixture solvent until completely dissolved. Then, the solution was filtered and degassed for the dope solution of wet spinning. The processing time was strictly regulated to prevent an excessive degradation of the SF, which caused to drop off molecular weight and viscosity by phosphoric acid.

Wet spinning

The SF dope solution was spun into methanol coagulation bath using a syringe and syringe pump with a 22 gauge needle and 30 mL/h flow rate at room temperature. The range of the SF dope concentrations for wet spinning was 10–18% (w/v). The as-spun regenerated SF filaments in the methanol coagulant were left overnight for complete solidification and crystallization as well as complete elimination of formic acid. The SF filaments were immersed in distilled water bath at 60°C for 15 min and then drawn with 5.0× draw ratio constantly. And they were dried with tension to prevent the contraction during drying process.

Measurements and characterizations

To determine the stability of the dope solution, viscosity was measured with time progress at 25°C during 24 h using shear rate viscometer (DV-E, Brook field, USA) after dissolving the SF for an hour. Turbidity of the dope solution was determined at 700 nm with UV spectrophotometer (Uvicon 933, Kotron Instrument, Italy) as a function of the SF concentration using following equation;

$$\tau = -(\ln\ T)/c$$

where τ is turbidity, T is the transmittance at 700 nm, and c is a cell length. The cross section and surface of the regenerated SF filament were observed by scanning electron microscope (JSM-5410LV, JEOL, Japan) after gold coating. To investigate crystalline structure and orientation of the regenerated SF filament, wide angle X-ray diffraction (WAXD) analysis was performed with General Area Detector Diffraction System (GADDS, Bruker-Axs, Germany) using Cu K_α radiation. Radiation conditions were 45 kV and 40 mA. Mechanical properties of the regenerated SF filament were evaluated according to stress-strain curves. Tenacity and breaking strain were obtained using MINIMAT (Rheometric Scientific, USA). All samples were preconditioned in a standard condition and measured 10 times for each sample.

RESULTS AND DISCUSSION

Properties of SF dope solution

A dope concentration plays an important role in wet spinning because a high tenacity filament can be usually produced at a high concentration and it is often necessary to determine a maximum concentration of the dope solution to be spun. Table I lists maximum concentrations for dissolution and spinning of the SF in phosphoric acid/formic acid solvent system, which was determined after dissolving for 1 h at room temperature. The cocoons were hardly dissolved in formic acid. However, as the content of phosphoric acid increased, the solubility increased. When the ratio of phosphoric acid/formic acid was in the range of 20/

TABLE I
Solubility and Spinnability of Silk Fibroin Dissolved in Phosphoric Acid/Formic Acid Solvent Mixture

Mixture ratio of phosphoric acid/formic acid solvent	Maximum concentration (%)	
	Dissolution	Spinning
0/100	Not dissolved	–
10/90	10	10
20/80	18	16
30/70	20	18
40/60	20	18

80–30/70, the maximum spinnable solubility was 16–18% (w/v). Although the SF was dissolved completely at a higher concentration, the spinnability was very poor due to extremely high viscosity unable to be processed. Furthermore, a high content of phosphoric acid in the mixture solvent made the coagulation of the SF much harder in methanol coagulation bath. Even above 40% (v/v) of phosphoric acid, the coagulation did not occur and consequently the optimal mixture ratio of phosphoric acid/formic acid could be determined as 20/80 and 30/70 for wet spinning of the SF.

The stability of the dope solution is another important factor for the spinning process. The dope solution should be stable and its viscosity should be maintained for certain periods of spinning time. Otherwise, the spinning is not uniform. When acidic solvents are used for the dissolution, severe molecular chain scissions may occur in most cases due to acid-catalyzed hydrolysis. In the case of phosphoric acid used for the SF dissolution, the viscosity dropped off markedly within 1–2 h, indicating that this solvent could not be used by itself. Therefore, it is necessary to minimize a decrease of the dope viscosity and at least to maintain a spinnable viscosity during spinning process.

Figure 1 shows the effect of phosphoric acid/formic acid mixture ratio on the viscosity change of the SF dope solution. Even though the dope viscosity steadily decreased with time within 1–2 h, the addition of formic acid retarded a viscosity-drop and exhibited a positive effect on improving dope stability. Especially, when 30/70 ratio of phosphoric acid/formic acid mixture was used for the dissolution, the viscosity of the SF solution maintained a relatively high value for certain times, implying that the spinning could be carried out sufficiently for at least 2 h.

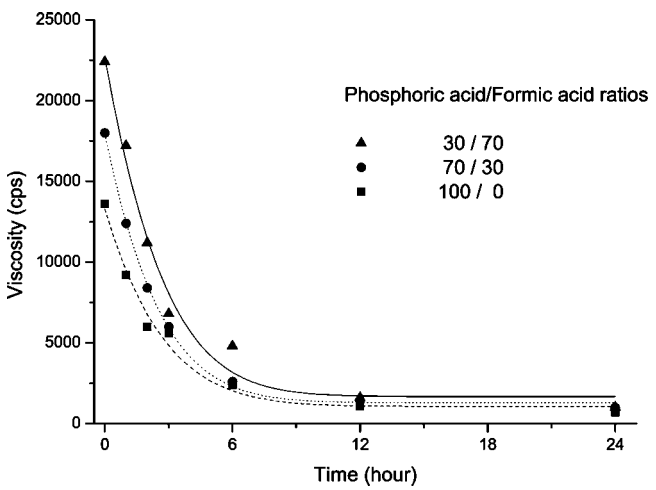

Figure 1 Viscosity changes of SF dope solution with time for phosphoric acid/formic acid mixture solvents.

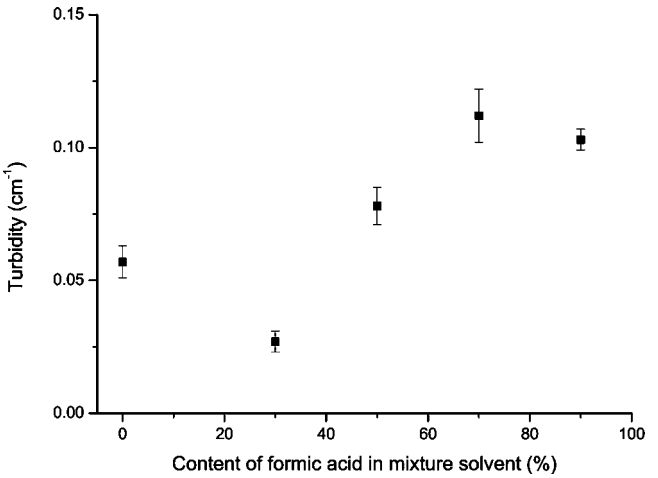

Figure 2 Turbidities of SF dope solution dissolved in different mixture ratios of phosphoric acid/formic acid solvent.

As a result of turbidity measurement of the SF solution, as shown in Figure 2, the lowest value was obtained at 30% formic acid content in phosphoric acid/formic acid mixture solvent while the turbidity was relatively high at high content of formic acid. A high turbidity means that the SF molecular chains are entangled more bulkily because formic acid by itself is a poor solvent for dissolving the SF. On the other hand, at a high content of phosphoric acid in the mixture solvent, the value was generally low. It seemed that phosphoric acid might be more effective solvent for dissolving the SF than formic acid. However, considering that the SF molecular chain scission was occurred severely by phosphoric acid and the coagulation of the SF solution does not occur in methanol bath when only phosphoric acid was used as a solvent, it can not be said that phosphoric acid is a good solvent for wet spinning process, either. Therefore, it can be expected that formic acid in the mixture solvent plays an important role of retarding the rate of molecular weight decrease and gives a positive effect on β-sheet transition and recrystallization of SF molecules in methanol coagulation bath. When a certain amount of formic acid (about 30%) was added, the solution showed rather lower turbidity. This indicates that formic acid is also playing an effective role as a cosolvent.

Judging from a point of view in wet spinning process, formic acid in the mixture solvent can make a stronger benefit for coagulation of the dope solution. Ishizaka et al.[7] reported that a concentrated salt solution was required for the coagulation when phosphoric acid was used for the dissolution of the SF. Meanwhile, in the case of phosphoric acid/formic acid mixture used, the SF dope solution could be coagulated in methanol bath in which the crystallization as well as solidification occurred at once although the crystallization of the regenerated SF is

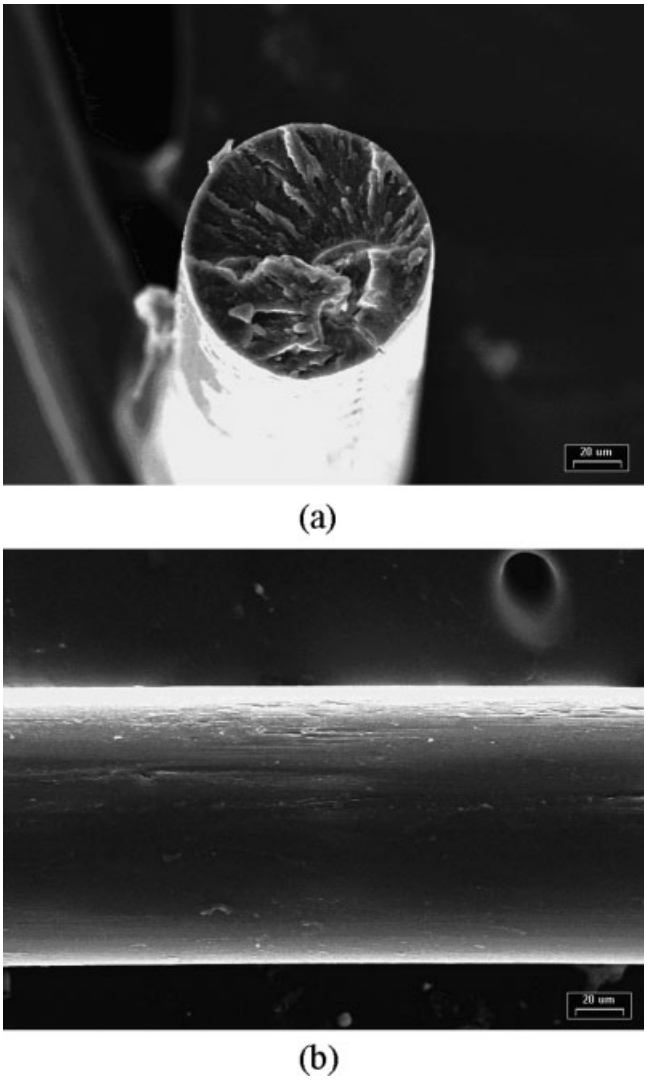

Figure 3 SEM micrographs of regenerated SF filament: (a) cross section and (b) longitudinal view.

usually carried out by the treatment of methanol separately. Therefore, formic acid can be considered as an excellent cosolvent with phosphoric acid for whole wet spinning process; direct dissolution, coagulation, and recrystallization of SF. The mixture solvent retards the rate of viscosity decrease of the dope solution, contributing to the spinnability in wet spinning process.

Structure and properties of regenerated SF filament

SEM micrograph of an example of the regenerated SF filament is shown in Figure 3. It shows very dense structure with a circular cross-sectional shape and a smooth surface. In wet spinning process, the important factor that largely affects the morphological structure is volumetric mass transfer rate difference of a solvent and nonsolvent. The circular shape means the mass transfer rate of coagulant inward the filament is faster than that of solvent outward the coagulant by very rapid coagulation. As a result, in the coagulation process, the pressure inside as-spun filament was maintained and this could make a circular and smooth morphology possible. The morphological structure of wet spun filament is closely related to its mechanical and physical properties. Generally, the filament, which has uniform structure, shows excellent mechanical properties superior to nonuniform structure. It is expected that the regenerated SF filament should have good mechanical performance due to its uniform circular structure.

X-ray diffraction pattern of the regenerated SF filament was examined for the crystalline structure and orientation. Figure 4 and 5 showed a 2D pattern and

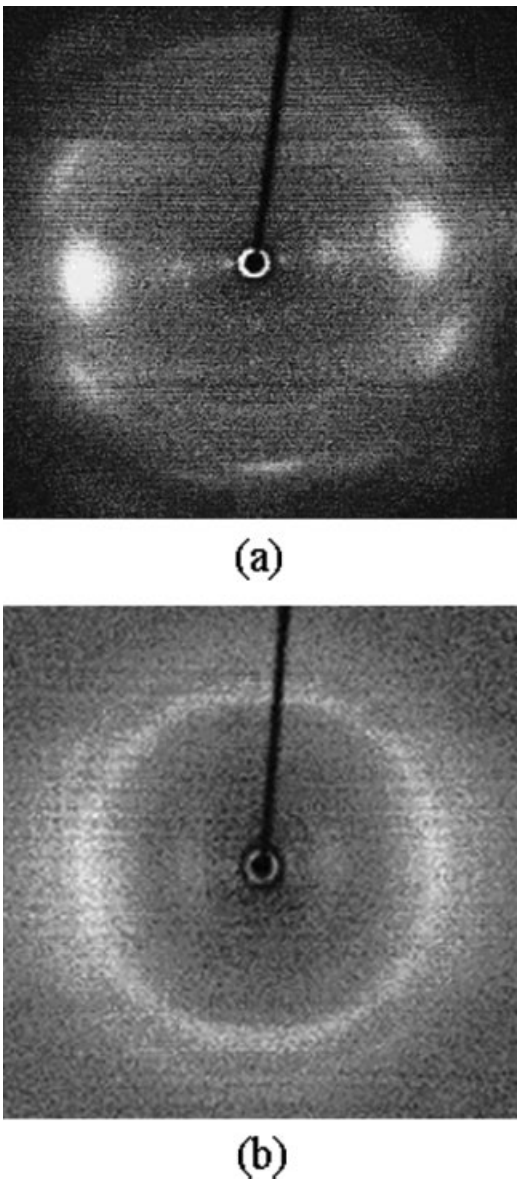

Figure 4 (a) X-ray fiber patterns of raw silk fiber and (b) regenerated SF filament.

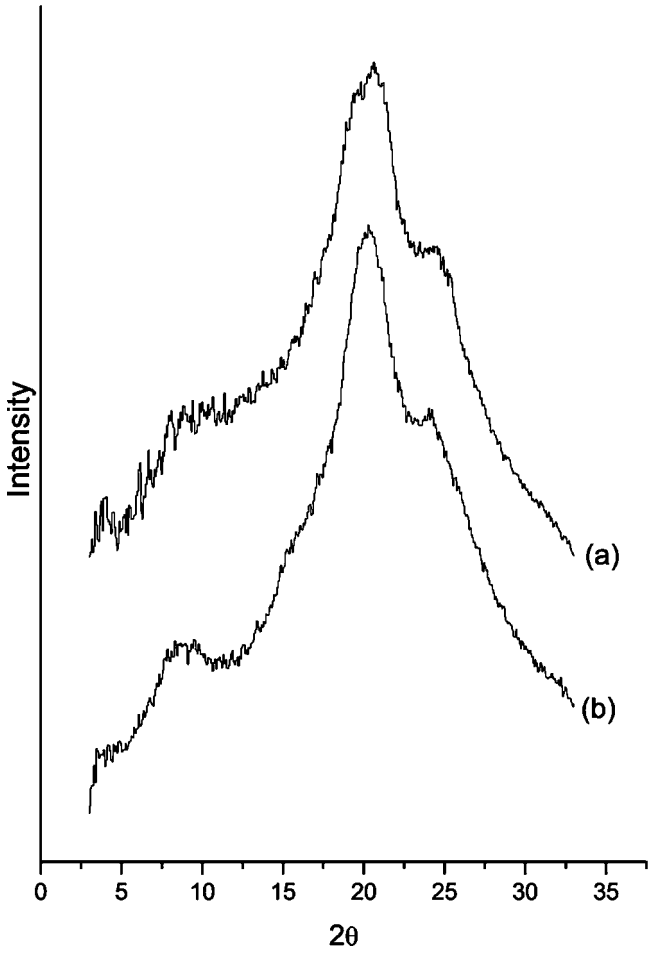

Figure 5 (a) X-ray diffractograms of raw silk fiber and (b) regenerated SF filaments.

X-ray diffractogram of SF filament, respectively. Compared with a natural raw silk fiber, molecular orientation of the regenerated SF filament was less developed due to its dull and diffused arc. However, a typical β-sheet crystalline structure was observed similar to raw silk fiber, as shown in Figure 5. The crystalline lattice spacing of 10.1, 4.5, and 3.8 Å appeared at $2\theta = 8.7^\circ$, 19.6°, and 23.3°, respectively.[10–12] Accordingly, the regenerated SF filament showed well developed crystalline structure with high crystallinity but somewhat low orientation because of a low draw ratio of the regenerated filament in wet spinning process (here, 5.0× draw ratio).

In most cases, mechanical properties of the regenerated SF filaments are worse than those of natural silk fiber because of limited and severe conditions of spinning process as well as regeneration. Especially, tensile strength of the regenerated SF filament, which has been reported mostly to be 1.5–2.5 gf/d, should be low due to the decrease of molecular weight, molecular orientation and crystallinity. Even though very high breaking strength of 4 gf/d was reported,[8,9] the main target has been to improve the tensile strength of the regenerated SF filament by adjusting appropriate spinning conditions.

Table II showed tensile properties of the regenerated SF filament prepared in this study. When the mixture solvent of phosphoric acid and formic acid, in the range of 20/80–30/70, was used, tenacity was 2.0–2.3 gf/d while breaking strain was 11–20% depending on the dope concentration and dissolution time. Although the brittleness of regenerated SF filament was concerned due to the molecular weight decrease and low crystallinity by severe process condition, the flexibility of the regenerated SF filament was comparable with that of the filament prepared by two-step dissolving process of Um et al. (data not shown). Anyway, the best result on tensile properties could be obtained for the sample which was dissolved in 30/70 mixture ratio of phosphoric acid and formic acid at 18% dope concentration for 2 h dissolution time. 2.3 gf/day tenacity and 18% breaking strain were comparable to those of Ishizaka et al. or Um et al.[7,10,11] However, their dissolution and spinning processes (solvent/coagulation/crystallization process) were very complex. We dissolved the silk fibroin directly in mixed solvent of phosphoric acid and formic acid, which was coagulated and crystallized simultaneously in methanol bath later. Therefore, the regenerated SF filament in this study, which has good mechanical properties, can be made by one-step dissolution process as well as one-step

TABLE II
Tensile Properties of Regenerated SF Filaments

Preparation conditions				Tensile properties	
Mixture ratio of phosphoric acid/ formic acid solvent	Concentration of dope solution	Dissolution time	Draw ratio	Tenacity (gf/d)	Breaking strain (%)
20/80	16	1	5.0	2.0	20
20/80	16	2	5.0	2.0	15
30/70	18	1	5.0	2.3	18
30/70	18	2	5.0	2.0	11
40/60	18	2	5.0	1.2	15

wet spinning process. It can be said that the preparation method of the regenerated SF filament is much simpler and more effective.

CONCLUSIONS

Common solvent/coagulation system for the wet spinning of the regenerated SF filament was successfully developed. The solvent system of phosphoric acid/formic acid mixture could be used for the preparation of dope solution by dissolving the SF directly. Formic acid may retard the degradation of the SF molecules caused by phosphoric acid and also enhance a solvating power of the mixture solvent. Furthermore, the coagulation and crystallization occurred simultaneously in methanol bath, enable to proceed by one-step. Therefore, formic acid acts as an excellent cosolvent for the preparation of the regenerated SF filament. By using this wet spinning process, the SF filament could be obtained with a tenacity of 2.3 gf/day and breaking strain of 18% at optimum preparation conditions, which makes favorable to a practical use.

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