Development of Regenerated Protein Fibers from a Collagen-polyvinyl Alcohol Complex

by

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Abstract

Regenerated protein fibers were wet-spun by collagen protein extracted from chrome shavings. A "pure" collagen protein extract of molecular weight ≥100,000 Da and chromium content <5 ppm was produced. To increase the spinning stability and physical strength of the fibers, the aqueous collagen solution was mixed with polyvinyl alcohol containing ≤0.3 percent glutaraldehyde in a subsequent unique treatment. Good spinnability was obtained when the viscosity was ≥1,000 cps. Optimal spinnability conditions were obtained at water temperature of 30~35°C in a coagulating bath containing a mixture comprising a minimum of 40 percent Na_2SO_4 and 1 M H_3BO_3. A water-resistant regenerated protein was eventually produced by chemical crosslinkage using 15 percent Na_2SO_4, 0.5 percent glutaraldehyde, and 0.25 percent formaldehyde. The regenerated protein fiber had a specific gravity of 1.3 g/cm^2, tensile strength of 1.32~2.0 g/d, and elongation of 29~38 percent.

Resumen

Fibras de proteína regeneradas fueron rotadas en húmedo a partir de proteínas del colágeno extraídas de las virutas del cromo. Un extracto "puro" de proteína de colágeno de peso molecular ≥100,000 Da y contenido de cromo <5 ppm fue producido. Para aumentar la estabilidad de la rotación y la resistencia física de las fibras, la solución acuosa del colágeno fue mezclada con alcohol de polivinilo que contenía ≤0.3 por ciento de glutaraldehido en un tratamiento único posterior. Una buena habilidad de rotación fue obtenida cuando la viscosidad fue ≥1,000 cps. Las condiciones de rotación óptima fueron obtenidas con una temperatura de 30~35°C en un baño de coagulación que contenía una mezcla que abarcaba un mínimo de 40 por ciento de $Na2_SO_4$ and 1 M H_3BO_3. Una proteína regenerada hidrófuga fue producida eventualmente por un producto químico que reticula empleando 15 por ciento de Na_2SO_4, 0.5 por ciento de glutaraldehido, y 0.25 por ciento de formaldehído. La fibra de proteína regenerada tuvo un peso específico de 1.3 g/cm^2, resistencia a la tracción de 1.32~2.0 g/d, y elongación de 29~38 por ciento.

Introduction

Large quantities of organic wastes are generated from the leather making process that includes splitting, shaving, trimming, and buffing operations. Collagen protein was from the leather wastes that do not contain the heavy metal chromium and processed into glue or gelatin or used as animal feed for a long time. In order to increase its economic value, collagen is being used to manufacture foodstuff and in food packaging[1-5]. Recently, studies were conducted on the manufacture of membranes, fibers, and sponges by chemically treating collagen protein solution with reagents, such as hydrazine, that destroy its fine structure. Active research was conducted on the development of surgical sutures, artificial skin, blood vessels, and artificial organs by enzymatically removing antigenic determinants from the collagen solution[6].

Since collagen has the zwitterion property of bearing either the "+" or "-" charge depending upon pH conditions, it can be subject to zwitterion or zero-net-charge reactions. In the natural state, the isoelectric point of the collagen contained in raw skin is maintained at pH 7.0~7.8, which moves toward acidic if treated with acid and toward basic if treated with alkali. Collagen protein swells in water due to the change in its pH value causing a change in its thickness and strength. Although no swelling is observed at neutral pH 5~9, collagen protein has a property of swelling either under acidic conditions of pH 2~3 or under basic conditions of pH 10 and higher.

Polyvinyl alcohol (PVA) is generally known to possess crystallization properties due to its hydroxyl group, and the mechanical strength of the fiber is markedly affected by its crystallization characteristics[7]. It has also been reported that PVA is highly sensitive to the arrays of chains and free spaces in the crystallization boundary and that the influence on the propagation of solute becomes linear by the degree of crystallization[8,9]. PVA fibers were commercially since the 1950s

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in textiles as a silk substitute[10]. The high tensile strength and modulus of PVA[6] have also led to many industrial applications of this fiber such as manufacture of tire cords, belts, and ropes.

In this study, collagen protein of high molecular weight was extracted from the chrome shavings of leather wastes by treating with acid and alkali to remove chromium. In order to improve the physical properties and spinnability of the fiber, collagen protein was treated with soluble high polymer PVA and a crosslinking agent glutaraldehyde. Optimum spinnability conditions were obtained by blending the types and conditions of the coagulating agent in varying ratios.

EXPERIMENTAL

Materials

The chrome shavings were obtained from a commercial tannery and were kept at room temperature. The following reagents were used for the extraction of collagen protein: MgO (Sigma-Aldrich Co., St. Louis, MO) and Na_2CO_3 (Sigma-Aldrich Co., St. Louis, MO) both of reagent grade; PVA for preparing the spinning solution (Poval 217, MW 1,700 Da, saponification 99 percent; Kuraray); and glutaraldehyde (Sigma-Aldrich Co., St. Louis, MO) used as a 25 percent solution. Industrial Na_2SO_4 and H_3BO_3 were also used in the coagulation bath.

Apparatus

The apparatus used for the extraction of protein and mixing of the spinning solution comprised Pyrex four-necked flasks attached to a recirculating cooler. An attached thermostat (±0.5°C) facilitated control of the reaction temperature. While the suitable reagents were being added from a certain height, the stirring speed was adjusted in order to prepare a uniform chrome shavings mixture. A Minitan II ultrafiltration system (Millipore, USA) was used for the filtration of collagen and a thin film concentration & vapor system (Eyela, Japan) was used for the concentration process. The protein content was measured using an auto Kjeldahl system (Büchi 302, Switzerland), and gel permeation chromatography (150C; Waters, U.S.A) was used for measuring the molecular weight. For measuring the viscosity, a Brookfield viscometer DV-II+ was used. An inductively coupled plasma spectrophotometer (Atomscan 25; TJA, U.S.A) was used for measuring the chromium content in the collagen protein. As a fiber spinning apparatus, a laboratory-scale wet spinning M/C was constructed by the authors as shown in Figure 1. This apparatus comprises a storage tank, spinning nozzle, coagulation bath I, coagulation bath II, oil & crosslinker bath, and winding M/C.

Extraction of collagen protein from chrome shavings

Many studies have been conducted on the methods for extracting chrome-free collagen protein from chrome shavings. According to Taylor *et al.*[1-5], since due to its chemical and physical properties chrome shavings are structurally relatively more stable than limed pelt scrap, extracting collagen protein from it is difficult and, even if extracted, deterioration in the physical properties of the protein may occasionally be observed.

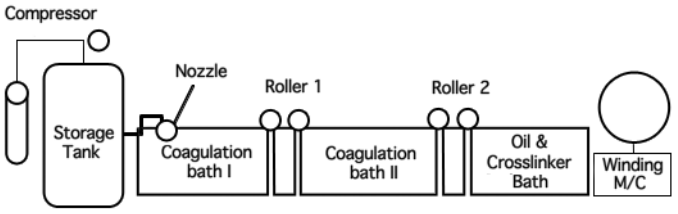

Figure 1. Wet spinning system for processing regenerated protein fiber.

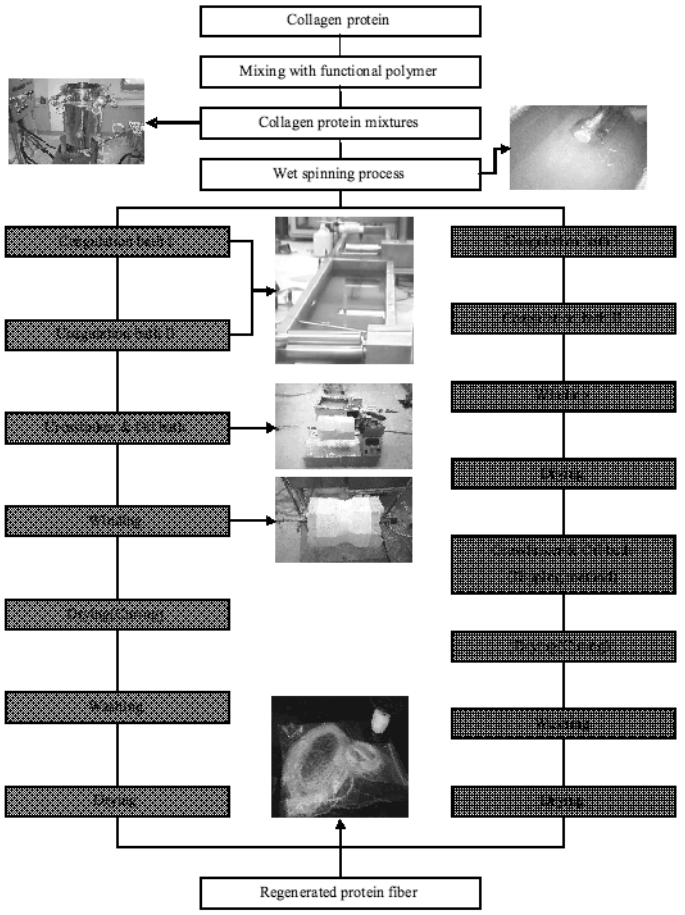

Figure 2. Flow sheet for regenerated protein fiber processing.

The physical properties of protein fibers typically depend upon their molecular weight; a molecular weight of at least 100,000 Da is required for satisfactory use.

Preparing and spinning collagen protein-PVA-glutaraldehyde mixtures

In order to obtain satisfactory spinnability and to improve the physical properties of collagen protein, a series of experiments were performed for preparing spinning solutions using the collagen protein extracted from chrome shavings. High polymer PVA was mixed in a number of mixing ratios, and the dissolution was made in an acid aqueous solution adjusted to pH 2.5~3.0 by organic acids, such as formic acid, acetic acid, and lactic acid in order to prevent coacervation due to the difference in the concentrations of the colloids. In order to increase the

TABLE I
Viscosity and Spinnability by Blending Rate of Collagen Protein, PVA, and Glutaraldehyde

Sample	H_2O (g)	Collagen protein (g)	PVA (g)	Glutaraldehyde (g)	Viscosity (cps)*	Spinnability
1	500	150	0	0.24	325	B
2	500	100	50	0.12	578	B
3	500	100	50	0.24	724	B
4	500	100	50	0.40	N.M	-
5	500	50	100	0.12	1024	G
6	500	50	100	0.24	1138	G
7	500	50	100	0.40	N.M	-
8	500	75	75	0.24	984	G
9	500	75	75	0.30	1075	G
10	500	75	75	0.40	N.M	-

* Viscosity at 60°C, PVA - Polyvinyl alcohol, B - Bad, G - Good, N.M - Not measurable

TABLE II
Coagulation Capacity of Species and Concentration of Coagulants

Sample	Na_2SO_4 (%)	$Al_2(SO_4)_3$	H_3BO_3 (g/mol)	Temp.(°C)	Coagulation capacity
1	10	-	-	30 ± 0.5	+
2	20	-	-	30 ± 0.5	+
3	30	-	-	30 ± 0.5	++
4	40	-	-	30 ± 0.5	+++
5	-	10	-	30 ± 0.5	+
6	-	20	-	30 ± 0.5	+
7	-	30	-	30 ± 0.5	+
8	-	40	-	30 ± 0.5	+
9	-	-	0.1	30 ± 0.5	++
10	-	-	0.5	30 ± 0.5	++
11	-	-	0.8	30 ± 0.5	++
12	-	-	1.0	30 ± 0.5	+++
13	40	40	-	30 ± 0.5	++
14	-	40	1.0	30 ± 0.5	++
15	40	-	1.0	30 ± 0.5	++++

+ very poor, ++ poor, +++ good, ++++ very good

viscosity of the spinning solution and the molecular binding force, 25 percent glutaraldehyde, a crosslinking agent, was added in varying concentrations. The effect of increasing viscosity on spinnability was accordingly observed.

Crosslinking regenerated protein fiber

Collagen and PVA have a high proportion of hydrophilic groups; however, their application is limited due to their ease of dissolution in water. In an effort to find a method for improving this property, after being discharged from the spinning solution into the coagulating bath and being fiberized, experiments have been conducted to observe the water resistance property, shape stability, and resistance to yellowing with varied concentrations and conditions of crosslinking agents and conditions in the crosslinking bath. Although the mechanism of crosslinking between collagen protein and glutaraldehyde is not clearly defined, it is assumed that the binding of glutaraldehyde to the carboxyl group of the collagen protein enhances the fiber's water and heat resistance properties.

Physical properties of the regenerated protein fiber

The tensile strength (dry & wet) and elongation of the non-crosslinked and crosslinked collagen fibers were measured and compared. The specific gravity, tensile strength (dry & wet), and elongation of the regenerated protein fibers were also measured and compared to those of 3 major natural fibers (wool, cotton, and silk) and 3 artificial fibers (nylon, polyester, and polyacrylonitrile).

RESULTS AND DISCUSSION

Extraction of collagen protein

The experiments in this study were carried out in accordance with the methods described by Taylor *et al.*,[1-5] for the extraction of collagen protein by treatment with MgO and Na_2CO_3; this method is relatively straightforward compared to the extractions using other alkaline reagents. The optimum process conditions for obtaining insoluble chromium and for the extraction of collagen protein were as follows: addition of 2 percent Na_2CO_3, reaction for 2 h at room temperature, addition of 2 percent MgO, and reaction for 6 h at 70°C for the reagents to react. Collagen protein having a molecular weight of more than 100,000 Da was then extracted.

Preparation and spinnability of collagen protein-PVA-glutaraldehyde mixtures

As shown in TABLE I, the amount of solution was fixed at 150 g for a given amount of solvent in order to facilitate easy agitation, and collagen protein, PVA, and glutaraldehyde were added in varying ratios. After adding the crosslinking agents to an amount that would not affect the agitation of the collagen protein and PVA mixtures, we attempted to enhance the water resistance property through drying after spinning. The viscosity of the spinning solution formulated in this way was measured and compared to the spinnability obtained by using the setup shown in Figure 1.

The viscosity increased with an increase in the amount of the crosslinking agent added into the collagen protein and PVA mixtures; however, when more than 0.3 g was added, agitation and measurement of the viscosity became impossible due to intense intermolecular entanglement. Since the viscosity of high polymer material is lowered at a high temperature, the viscosity of the sample processed by the spinning machine at the actual storage tank temperature of 60°C was measured. When the viscosity was lower than 1,000 cps, it was difficult to obtain spinnability because of the failure of smooth dehydration due to the low intermolecular stress and weak viscosity. A stable spinnability was obtained in the coagulation bath after the spinning solution passed the spinning nozzle, and was maintained at a viscosity lower than 1,000 cps.

Coagulation force of regenerated protein fiber

TABLE II shows the results of the comparative experiment for coagulation forces. The results depended upon the concentration of the generally known coagulants, including Na_2SO_4, H_3BO_3, and $A_l2(SO_4)_3$, for collagen and PVA mixtures. The optimum temperature for the coagulation bath required to prevent the re-dissolution of the collagen fibers coagulated by the coagulant, and for the easy separation of the fibers was found to be 30~35°C. The optimum coagulation force was observed at the saturation point, 40 wt percent and 1 M, of the solubility of the coagulants Na_2SO_4 and H_3BO_3 at a water temperature of 30~35°C. However, $Al_2(SO_4)_3$ exhibited an unsatisfactory coagulative force even at the saturation point of 40 wt percent.

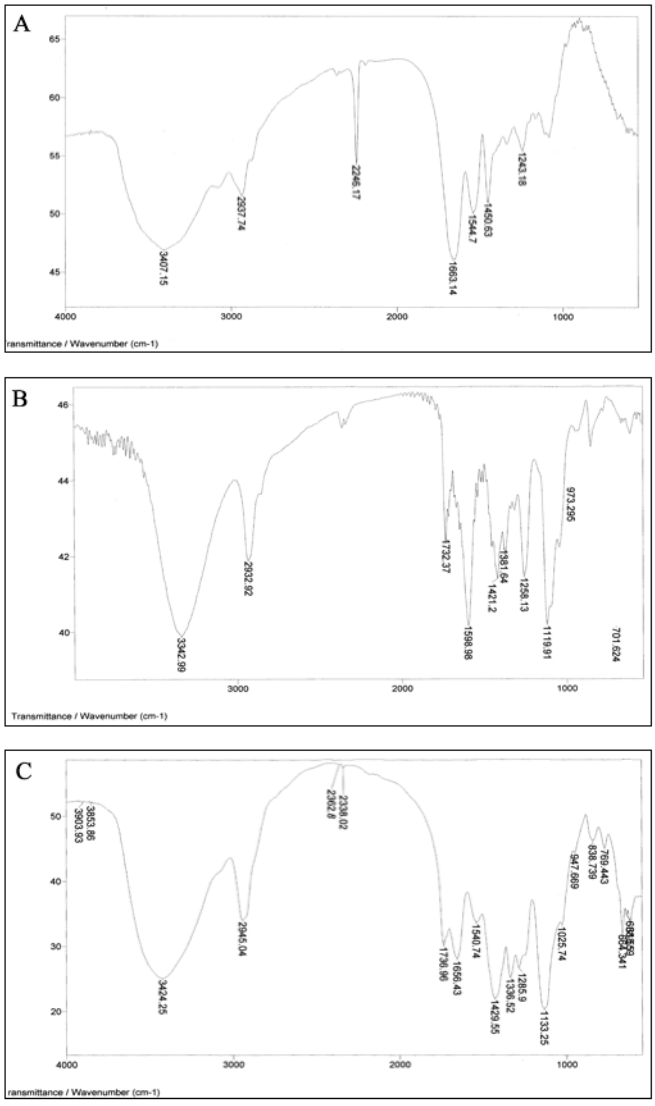

Figure 3. FT-IR results for collagen protein, Polyvinyl alcohol (PVA), and crosslinked regenerated protein fiber. (A) Collagen protein, (B) Polyvinyl alcohol (PVA), (C) Crosslinked regenerated protein fiber.

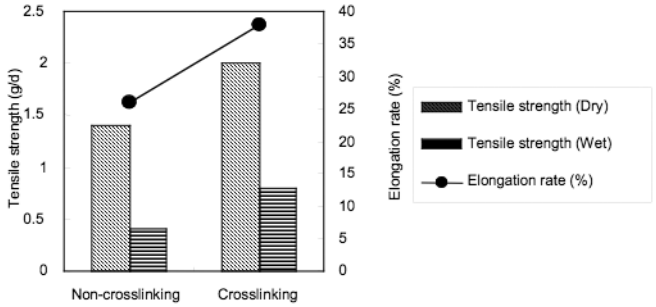

Figure 4. Comparison of the physical properties of non-crosslinked regenerated protein fiber and crosslinked regenerated protein fiber.

In conclusion, regenerated protein fibers could be processed from the spinning solution containing mixtures of 40 wt percent of Na_2SO_4 and 1 M H_3BO_3 in the coagulation bath at a water temperature of 30~35°C.

TABLE III
Properties of Regenerated Protein Fiber after Crosslinking Treatment

Sample	Na_2SO_4 (%)	G.A (%)	F.A (%)	Temp.(°C)	Structure stability	Water resistance	Yellowing resistance
1	-	3	-	R.T	-	-	-
2	-	6	-	R.T	-	-	-
3	-	12	-	R.T	-	-	-
4	-	24	-	R.T	-	-	-
5	15	0.5	-	R.T	+	+	+
6	15	1	-	R.T	+	+	+
7	15	1.5	-	R.T	+	+	+
8	15	2	-	R.T	+	+	+
9	15	0.5	0.25	R.T	+	+	-
10	15	1	0.5	R.T	+	+	-
11	15	1.5	0.75	R.T	+	+	-
12	15	2	1	R.T	+	+	-

G.A - 25% Glutaraldehyde, F.A - Formaldehyde, R.T - Room temperature + good, - poor

TABLE IV
Physical Strength of Regenerated Protein Fiber

Fiber	Specific gravity (g/cm^2)	Tensile strength (g/d)		Elongation rate (%)
		Dry	Wet	
Regenerated protein fiber	1.39	1.3~2.0	0.5~0.8	29~38
Wool	1.32	1.0~1.7	0.7~1.6	25~35
Silk	1.33~1.45	3.0~4.0	2.1~2.8	15~25
Cotton	1.54	3.0~4.9	3.3~6.4	3~7
Nylon	1.14	4.7~6.7	3.9~5.7	38~50
Polyester	1.38	4.4~5.5	4.4~5.5	40~50
Polyacrylonitrile	1.14~1.17	2.5~4.5	2.0~4.5	27~48

g/cm^2-grams per square centimeter, g/d-gram force per denier

Regenerated protein fiber crosslinking

As shown in TABLE III, the regenerated protein fiber coagulated in the crosslinking bath lacking the Na_2SO_4 coagulant dissolved in the water of the coagulating bath. Experiments were therefore conducted with Na_2SO_4 at ≥15 wt percent with the addition of glutaraldehyde and formaldehyde as supplementary crosslinking agents for maintaining the shape and for preventing yellowing.

As a result, excellent shape stability was obtained during the crosslinking reaction, compared to the treatment conditions without the coagulant, in which re-dissolution of the coagulated fiber occurred. In the case where formaldehyde was not added as a supplementary agent, the fiber was rendered water resistant by the addition of 0.5 percent or more glutaraldehyde; however, yellowing was observed in the crosslinked regenerated protein fiber. On the other hand, an improvement in the water resistance and resistance to yellowing was observed in the crosslinked fiber when 0.5 percent or more glutaraldehyde and 0.25 percent or more formaldehyde were added. Therefore, considering the toxicity of aldehyde compounds, it is concluded that 0.5 percent glutaraldehyde and 0.25 percent formaldehyde are the optimum amounts to be added. Based on this experiment, the crosslinking process could be performed in 2 ways after the coagulation of the collagen protein spinning solution in the coagulation bath as shown in Figure 2. Of the 2 methods-continuous crosslinking after coagulation and crosslinking as a process following fiber preparation-the former has been found to be the more advantageous considering cost and production efficiency.

The results of Fourier-transform infrared (FT-IR) spectroscopic analysis of the regenerated protein fiber crosslinked by glutaraldehyde are presented in Figure 3. The infrared spectra of collagen (Figure 3A.), PVA (Figure. 3B.), and crosslinked regenerated protein fiber (Figure. 3C.) were compared. Collagen exhibited characteristic amide absorption bands

appearing at approximately 1650, 1550, and 1240 cm^{-1} (Figure. 3A.). In the other FT-IR spectrum of PVA (Figure. 3B.), the absorption bands at 3340, 1446, and 1120 cm-1 characteristic of a hydroxyl group were observed. The spectrum for the crosslinked regenerated protein fiber (Figure 3C.) exhibited absorption bands that were characteristic of both amide and hydroxyl groups; however, the amide II band of >NH that is directly related to the crosslinking reaction with glutaraldehyde did not appear at 1550 cm^{-1}.

Physical properties of regenerated protein fiber

A comparison of the tensile strength (dry & wet) and elongation of the collagen fibers that were not crosslinked with those that were crosslinked using 0.5 percent glutaraldehyde and 0.25 percent formaldehyde is shown in Figure 4. The physical properties of the crosslinked collagen protein generally improved compared to that of the non-crosslinked collagen protein. This can be interpreted as an improvement resulting from the formation of a reticulate structure due to the strong binding of the crosslinking agent with the amino groups of the collagen protein.

The specific gravity, tensile strength (dry & wet), and elongation of the regenerated protein fiber were compared with those of the 3 major natural fibers (wool, cotton, and silk) and 3 artificial fibers (nylon, polyester, and polyacrylonitrile). The results are presented in TABLE IV. Similar to the other natural fibers, the regenerated protein fibers have a higher specific gravity than the artificial fibers, and their tensile strength in the dry state and elongation were similar to those of wool. However, due to the high affinity of the hydroxyl base of collagen, it has the drawback of low tensile strength in the wet state. Considering these physical properties, the regenerated protein fiber has the possibility of being used for absorbing humidity and for biodegradation purposes.

CONCLUSIONS

This study was conducted in order to find a method for processing regenerated protein fiber by wet spinning the chrome shavings generated from the leather making process. As a new method for utilizing the chrome shavings, this study focuses on extracting collagen protein by the physical and biochemical treatments introduced by Taylor *et al*[1-5]. When collagen protein was extracted from the chrome shavings and made spinnable for producing fiber, it was found that the collagen extraction using alkali was a superior method compared to the use of an acid or enzyme solution, and resulted in the effective removal of chromium and improvement of the physical properties of the fiber. When MgO and carbonate-series compounds were used together as a mixture, a higher extraction rate was observed and the collagen obtained was of a higher molecular weight.

In the case of water-soluble high polymer PVA, which is used for increasing the spinning stability and improving the physical properties during fiber fabrication, it was observed that using a PVA of 1,700 Da or higher mixed with the collagen protein is recommended. This was due to the fact that mixtures of PVA of 1,500 Da or lesser and the protein tend to lower the physical properties during spinning. When 0.4 percent or more glutaraldehyde was used, poor agitation-ability of the collagen protein and PVA mixtures was observed due to excessive crosslinking. As for the viscosity of the processed spinning solution, spinning was difficult when the viscosity was lower than 1,000 cps due to low strength of the spinning solution and lack of dehydrating power because of a high water content. It is, therefore, concluded that the viscosity of the spinning solution should be higher than 1,000 cps. Spinning was good in a coagulating bath containing 40 percent or more Na_2SO_4 and 1 M or more H_3BO_3 at 30~35°C water temperature. Further, a water resistant regenerated protein fiber having a specific gravity of 1.3 g/cm^2, tensile strength of 1.3~2.0 g/d and elongation of 29~38 percent could be obtained by the chemical crosslinking reaction occurring in the crosslinking bath containing 15 percent Na_2SO_4, 0.5 percent glutaraldehyde, and 0.25 percent formaldehyde. In the dry state, the regenerated protein fiber exhibited physical properties that were similar to those of wool, a typical natural fiber. However, the physical properties of the regenerated protein fiber in the wet state, exhibited a marked decline.

Further studies are required in order to solve this problem; however, it may be concluded that at present the regenerated protein fiber has made possible the utilization of a harmful scrap, which has been difficult to dispose off, for medical and industrial purposes; thus, preventing environmental pollution.

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