

Chemical Modification of Silk with Itaconic Anhydride

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SYNOPSIS

Bombyx mori silk fibers were chemically modified by acylation with itaconic anhydride. The reactivity of the modifying agent toward silk fibroin was investigated on the basis of the amino acid analysis. We examined the physical properties, the structural characteristics, and the thermal behavior of modified silk fibers as a function of the weight gain. Silk fibers with a weight gain of 9%, corresponding to an acyl content of 68.9 mol/10⁵ g, were obtained at the optimum reaction conditions for silk acylation (75°C for 3 h). The amount of basic amino acid residues (Lys, His, and Arg) decreased linearly as the weight gain increased. The alkali solubility increased proportionally with the weight gain, probably due to the dissolution of the modifying agent reacted with silk fibroin, and not to the degradation of the fibers induced by the chemical modification. The birefringence value, related to the molecular orientation, slightly decreased when the weight gain increased. The isotropic refractive index, associated with the crystallinity, increased when the weight gain ranged from about 5 to 7% and then remained unchanged. The moisture regain did not change regardless of the chemical modification, and the crease recovery behavior of modified silk fabrics did not show significant improvement. The thermal behavior of silk fibers was affected by the modification with itaconic anhydride. The decomposition temperature shifted up to 322°C, 10°C higher than the control silk fibers, suggesting a higher thermal stability induced by chemical modification.

INTRODUCTION

The reactivity¹⁻³ and the physico-chemical properties⁴⁻¹⁰ of *Bombyx mori* silk fibers have recently attracted a considerable academic and practical interest. As a textile fiber, silk is highly appreciated for its outstanding characteristics, but suffers from some inferior performance, which could be improved by suitable chemical treatments. Moreover, new textile materials have been recently studied and developed by blending silk with other natural and/or synthetic fibers. These technical and brilliant developments opened up the possibility of changing the physico-chemical properties of silk by reaction with selected modifying agents.⁹⁻¹² Besides the traditional use as textile fiber, silk fibroin has been

recently exploited as substrate for enzyme immobilization¹³ because of its chemical stability, good mechanical strength, and the presence of several free functional groups that can be used in different activation processes for enzyme immobilization.¹³

The good thermal stability is another attractive characteristic that makes silk fibers suitable for several applications. In fact, it has been reported that the thermal decomposition of silk occurred at 310–320°C, about 100°C higher than wool (230–240°C), another protein fiber. Therefore, the purpose of improving the thermal resistance of silk by suitable treatments should be considered a new approach for the research in the field of structural proteins in order to make new materials and expand their utilization.

The reactivity of silk toward external chemical agents is largely dependent on the functional groups of the amino acid residues forming the sequence of

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the fibroin chains. The amino acid composition of silk fibroin is characterized by the presence of glycine and alanine in very large amount (about 75 mol %).^{1,2} Hydroxy-amino acid residues comprise about 17 mol %, while the content of acidic and basic amino acid residues accounts for about 4 mol %.^{1,2} It has been elucidated that most of the amino acid residues with polar and chemically reactive side groups are located in the more accessible amorphous regions of the fiber.

Amine, carboxyl, and hydroxyl groups have been exploited as potentially reactive sites for the chemical modification of silk. The dyeing with reactive dyes is a significant example of modification by formation of covalent bonds with an external chemical agent, i.e., the reactive dye. Among the modifying agents, epoxides^{1-3,14} have been proved to be markedly effective in improving some intrinsic properties of silks. The reactivity of mono and bifunctional epoxides toward silk fibroin and the physical properties of epoxide-treated silk fibers have been extensively studied.¹⁻³ The acylation of silk with different mono-carboxylic acid anhydrides has been reported by Shiozaki et al.¹² They investigated the effect of different substituent on the reactivity of anhydrides toward silk fibroin and elucidated some physical properties and the transfer printing behavior of chemically modified fibers.¹⁵ We have recently reported the chemical modification of silk with aliphatic¹⁰ (succinic and glutaric) and aromatic¹⁶ (phthalic and *o*-sulfobenzoic) acid anhydrides. We¹⁷ investigated the optimum conditions for silk acylation, the physical and thermal properties and the dyeability with acid and cationic dyes.

The acylation of silk with acid anhydrides appeared particularly attractive because a limited chemical modification resulted in significant changes of the properties of silk fibers. The newly introduced carboxyl (or sulfonic) functions greatly changed the balance of ionized groups in the silk fiber, causing a drastic decrease of acid dye absorption. Moreover, the anhydride molecules interacted with the fibroin chains in the amorphous regions, influencing the moisture content, the crease recovery, the antiyellowing behavior, and the thermal properties of modified silk fibers.

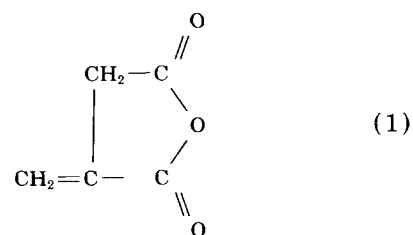
In this study we report the chemical modification of silk by acylation with itaconic anhydride. The reactivity of silk toward the modifying agent was investigated by means of amino acid analysis. The physical properties of modified silk fibers were evaluated by measuring the alkali solubility, the refractive indices, the moisture content, and the crease

recovery. The thermal behavior was investigated on the basis of differential thermal analysis.

EXPERIMENTAL

Materials

Reagent grade itaconic anhydride (MW = 112.08) [Eq. (1)], purchased from Wako Pure Chemical Industries, Ltd., was used without further purification.



Samples of 0.5 g Habutae silk fabric (ca. 7.5 g/10 cm²) were treated with itaconic anhydride (10 g/100 mL) in dimethyl formamide (DMF) at 75°C for different periods of time. The reaction system was connected to a reflux condenser and held in a thermostatic bath. The material-to-liquor ratio was 1 : 30. At the end of the reaction, the silk samples were successively washed with DMF, then with acetone at 55°C for 1 h in order to remove the unreacted anhydride and finally with water. The weight gain was calculated from the increase in weight of the original silk fabric after chemical reaction. The acyl content was thus estimated from the weight gain values.

Measurements

Dried silk fibers treated with itaconic anhydride with different values of weight gain were hydrolyzed by heating at 110°C for 20 h in HCl 6*N* under vacuum. The hydrolyzed samples were filtered through a glass disk to remove small amounts of residues, dried in a rotary evaporator at 40°C, and dissolved in a buffer solution at pH 2.2 (1 mg/mL). The amino acid composition was determined by using a Hitachi L-8500 type rapid amino acid analyzer.

The alkali solubility was determined by treating silk samples with a solution of 0.5*N* NaOH at 65°C for 60 min. The values were calculated on the basis of the dry weight of the undissolved residue remained after treatment, and expressed in percentage referring to the original dry weight.

The refractive indices were measured with the Beche's line method using a polarized microscope under the monochromatic light (Na light) at 20°C and 65% relative humidity (RH) as previously described.¹⁶

The moisture regain was determined on dried samples kept at 20°C and 65% RH for 7 days and expressed as grams of moisture/100 g fibroin.

Conditioned crease recovery angles were measured by using the Monsanto method as described in a previous paper.¹⁰

The differential thermal analysis (DTA) and thermogravimetric analysis (TGA) were carried out under nitrogen on a Rigaku Denki Thermoflex system at a heating rate of 10°C/min. DTA range, sample weight, and DTA full scale were $\pm 10 \mu\text{V}$, 6 and 5 mg, respectively.

RESULTS AND DISCUSSION

Reactivity of Itaconic Anhydride

The effect of the reaction time on the weight gain of silk fibers treated with itaconic anhydride is shown in Figure 1. The weight gain steadily increased within the first 3 h. A further extension of the reaction time from 3 to 4.5 h did not result in a significant increase of weight gain. The optimum reaction temperature was 75°C and the concentra-

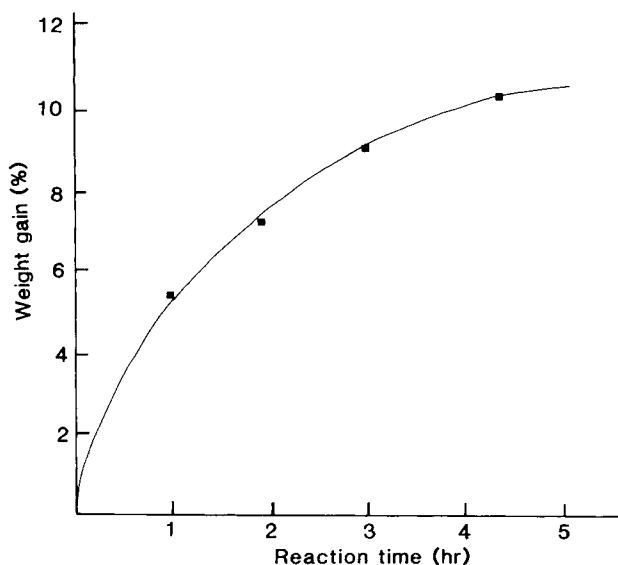


Figure 1 Weight gain of the silk fibers treated in dimethylformamide solution containing 10% (w/v) itaconic anhydride at 75°C for different periods of reaction time.

tion of modifying agent was 10% (w/v) in DMF. It is interesting to note that these conditions are the same as those reported for the acylation of silk with succinic and glutaric anhydrides,¹⁰ as expected from the fact that all these modifying agents have a very similar chemical structure. However, the maximum weight gain value attained by reaction with itaconic anhydride was slightly lower than that obtained by reaction with other aliphatic acid anhydrides.¹⁰ In this work silk samples with a weight gain of 9.7%, corresponding to an acyl content of 75.2 mol/10⁵ g, were obtained after 4.5 h of reaction time.

In order to evaluate the reactivity of itaconic anhydride toward silk fibroin, we determined the amino acid composition of modified silk fibers with different values of weight gain. Table I shows that the content of basic amino acid residues decreased by acylation. Figure 2 represents the relationship between the weight gain and the loss of basic amino acid residues. Lysine, arginine, and histidine exhibited almost the same reactivity and their contents decreased linearly as the weight gain increased. These data are in agreement with those already reported^{10,18} and give evidence that the amine groups of basic amino acid residues are important reactive sites for silk acylation. In addition, it is reasonable to consider that anhydrides should react also with the hydroxyl groups of tyrosine, serine and threonine. However, the amount of these amino acid residues remained unchanged, probably due to the fact that the ester bond once formed was broken during acid hydrolysis.

Alkali solubility is a useful parameter used to identify and estimate the occurrence of chemical modifications on silk fibers, such as hydrolytic degradation of peptide bonds of the fibroin backbone or crosslink formation. The former effect contributes to increase alkali solubility, while the latter usually decreases the amount of alkaline soluble fraction. The alkali solubility values of modified silk fibers as a function of acyl content are listed in Table II. The data show that alkali solubility of silk fibers modified with itaconic anhydride increased linearly with the acyl content. Since the covalent bonds of silk fibroin are sensitive to the alkaline attack, the above increase should be mainly attributed to the hydrolysis of these bonds and to the solubilization of the modifying agent. We can therefore estimate that the intrinsic alkali solubility of silk fibers remained unchanged and that the reaction with itaconic anhydride did not result in silk fiber degradation.

Refractive Indices

In order to elucidate the structural changes induced by reaction with itaconic anhydride, we determined the birefringence (Δn) and the isotropic refractive index (n_{iso}) of acylated silk fibers (Fig. 3). The birefringence values exhibited a slight but constant decrease as the weight gain of modified fibers increased. Birefringence may be regarded as a measure of the average orientation of the molecules in a fibrous polymer. As it has been already reported,^{14,16} birefringence generally decrease by graft copolymerization or chemical modification of silk fibers, owing to the changes in fibroin molecule arrangement induced by the presence of the polymer and/or monomer filled inside the amorphous regions of the fibrous substrate. However, we would like to point out that the amount of Δn decrease due to acylation with itaconic anhydride is very low, compared to that of grafted silks, suggesting that the intrinsic physical structure of silk fibers was not markedly affected by the reaction.

The isotropic refractive index (Fig. 3) showed a different behavior, exhibiting a quite remarkable increase in the range of weight gain from about 5 to 7%. A further increase of weight gain did not cause any change of n_{iso} , suggesting that an equilibrium value has already been attained. The isotropic re-

Table I Amino Acid Composition (mol %) of Chemically Modified *Bombyx Mori* Silks with Itaconic Anhydride with Different Values of Weight Gain

	Weight Gain (%)			
	0	5.5	6.7	9.0
Asp	1.65	1.65	1.61	1.63
Thr	0.89	0.90	0.89	0.90
Ser	10.63	10.71	10.64	10.79
Glu	1.21	1.28	1.20	1.18
Gly	45.10	45.31	45.22	45.22
Ala	29.21	29.37	29.32	29.22
Val	2.42	2.57	2.53	2.54
Met	0.14	0.20	0.15	0.15
Ile	0.71	0.68	0.66	0.65
Leu	0.54	0.52	0.50	0.50
Tyr	4.97	4.71	4.95	4.97
Phe	0.70	0.71	0.72	0.71
Lys	0.33	0.28	0.25	0.22
His	0.18	0.13	0.09	0.11
Arg	0.49	0.43	0.41	0.40
Pro	0.77	0.55	0.84	0.80

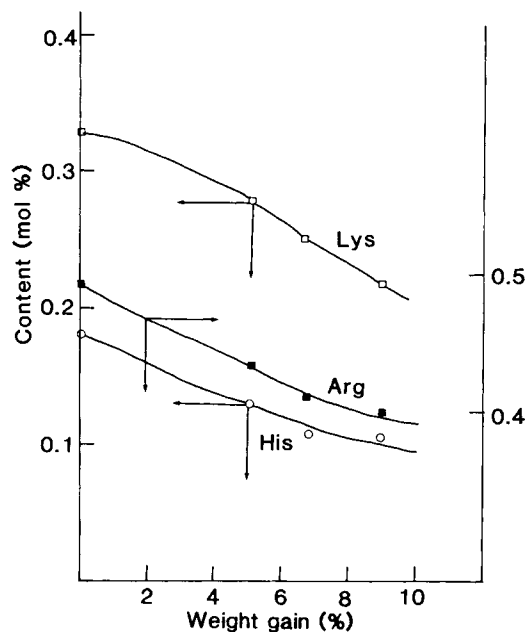


Figure 2 Change in amount of basic amino acid residues of the silk fibers after chemical modification with itaconic anhydride as a function of the weight gain.

fractive index is related to the degree of order and crystallinity of the fiber as well as to the density of the sample.^{14,16} As reported in previous studies, the reaction of silk with grafting and/or modifying agents did not directly affect the crystalline structure of silk fibers. Therefore, we can estimate that the increase of the n_{iso} value of silk acylated with itaconic anhydride should be partly related to the increase of fiber density. The anhydride molecules introduced into the fibers acted as an "interchain bridge" between adjacent fibroin molecules, mainly by formation of weak interactions (i.e., hydrogen bonds), and increased the interactions in the amorphous regions of silk fiber.

Table II Acryl Content and the Alkali Solubility of the Modified Silks with Itaconic Anhydride Possessing Different Values of Weight Gain

Reaction Time (h)	Acyl Content (mol/10 ⁵ g)	Alkali Solubility (%)
0	0	35
1	42.6	44
2	52.0	48
3	68.9	51
4.5	75.0	—

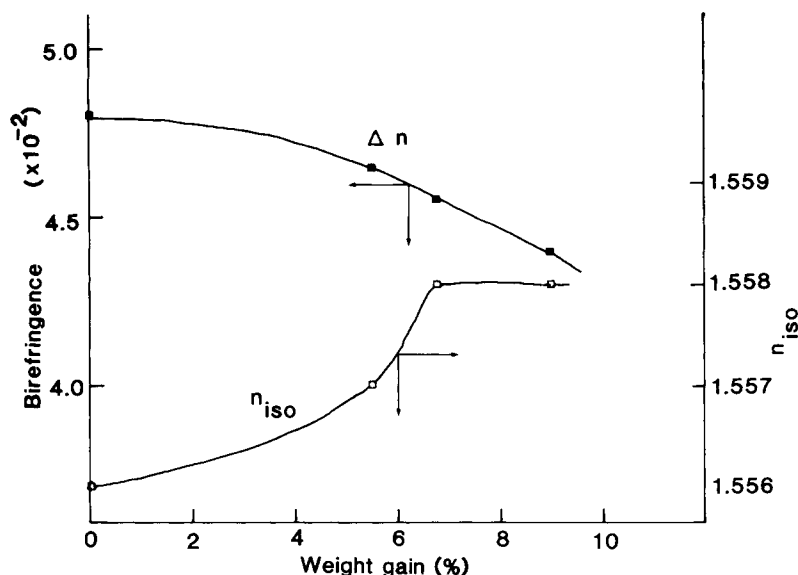


Figure 3 Birefringence and isotactic refractive index of the chemically modified silk fibers with itaconic anhydride as a function of the weight gain.

Physical Properties

Table III shows the changes in moisture content and crease recovery of acylated silk fibers with different values of weight gain. Silk fabrics modified with itaconic anhydride did not exhibit a noticeable variation of moisture regain. In addition silk did exhibit a significant increase of crease recovery in spite of the chemical modification. These results are in fair agreement with those already reported for succinic anhydride¹⁰ and highlight the influence of the chemical structure of the modifying agent (i.e., shape and size of the molecule) in the extent of modification of the physical behavior of silk fibers. Both itaconic and succinic anhydrides showed a very low effect on the crease recovery behavior of silk, mainly

Table III Moisture Regain and Crease Recovery of the Silk Fabrics Treated with Itaconic Anhydride in Dimethylformamide at 75°C with Different Values of Weight Gain

Weight Gain (%)	Moisture Content (%)	Crease Recovery (%)
0	9.3	77
5.5	8.5	79
6.7	8.6	80
9.0	9.2	81

because a relatively small molecule was introduced inside the fiber. As reported in a previous paper, a longer alkyl chain (glutaric anhydride)¹⁰ or a bulky benzoyl substituent (phthalic and *o*-sulfobenzoic anhydrides)¹⁶ were more effective in improving the crease recovery behavior of acylated silk fibers.

Thermal Properties

The differential thermal analysis (DTA) of modified silk has been carried out in order to evaluate the changes in thermal properties induced by silk acylation. Figure 4 shows the DTA curves of control (a) and silk fibers modified with itaconic anhydride (b) possessing a weight gain of 9.0%. Modified silk fiber exhibited major endothermic peak at around 322°C, attributing to the thermal decomposition of silk with β configuration.¹⁹ The position of the above endothermic peak was about 10°C higher than the control sample. These results indicate that the chemical modification with itaconic anhydride has a large effect upon the thermostability in terms of the thermal decomposition of silk fiber, in striking contrast with the data already reported for silk fibers acylated with succinic anhydride.¹⁰ Moreover, the shape of the decomposition endotherms of the grafted sample [Fig. 4(b)] became slightly narrower and more symmetric compared with that of the control silk fibers.

The thermal behavior of silk modified with itaconic anhydride was discussed on the basis of the

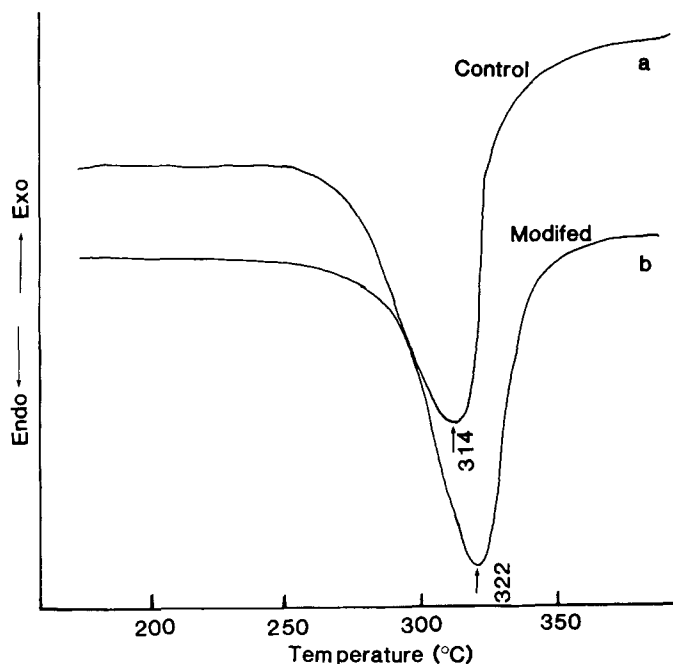


Figure 4 DTA curves of the (a) control and the (b) modified silk fiber with weight gain of 9%.

thermogravimetric analysis (TGA) measurement. The TGA curves indicated that the acylated silk samples exhibited a slightly higher thermal stability, especially in the temperature range from 270 to 330°C (Fig. 5). These results demonstrate that the

modifying agent, after reaction with the active sites in the fibrous substrate, interacted with the fibroin molecules and increased the thermal stability of the fibers. This explanation is also supported by the DSC measurement (Fig. 4).

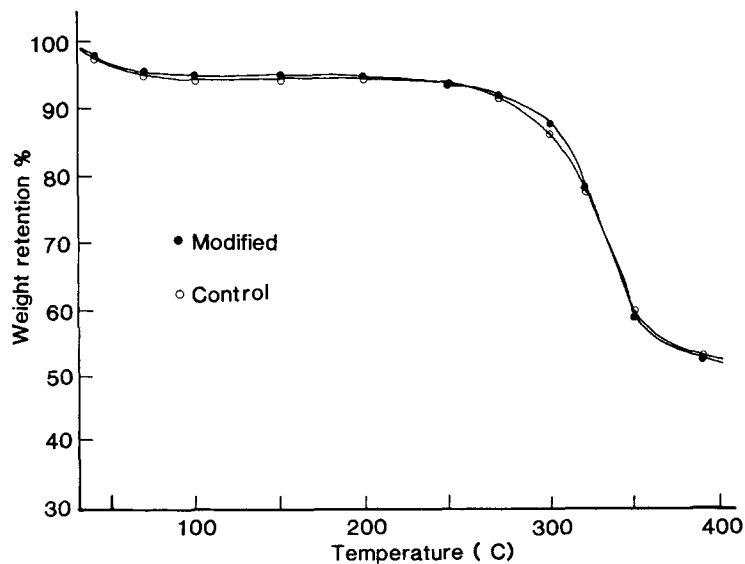


Figure 5 TGA curves of modified silk fiber with (●) weight gain of 9% and (○) control silk in nitrogen atmosphere. Heating rate 10°C/min; sample weight 6 mg.

From the results obtained it appeared that silk fibers modified with itaconic anhydride show some interesting new features. The structure and the physical properties of modified fibers were not essentially affected, although the refractive index values slightly changes (Fig. 3). Silk fibers maintained their excellent physical properties, even though their chemical characteristics were significantly modified. These experimental results, demonstrating the higher thermal stability of modified silk fibers, will provide new perspectives for the use as textile and nontextile material.

We have already reported the influence of the newly introduced carboxyl groups on the dyeing behaviors. In addition, we would like to emphasize that a silk fiber enriched in carboxyl groups could become a highly efficient substrate for enzyme immobilization because some activation processes are based on the reactivity of this functional group. Furthermore, the presence of the vinyl group could be exploited as a new reactive site for polymerization or other modification reactions.

REFERENCES

1. Y. Tanaka and H. Shiozaki, *Makromol. Chem.*, **129**, 12 (1969).
2. Y. Tanaka and H. Shiozaki, *J. Polym. Sci. Polym. Chem. Ed.*, **12**, 2741 (1974).
3. H. Shiozaki and Y. Tanaka, *J. Seric. Sci. Jpn.*, **45**, 74 (1976).
4. M. Tsukada, *J. Appl. Polym. Sci.*, **35**, 965 (1988).
5. M. Tsukada, *J. Appl. Polym. Sci.*, **35**, 2133 (1988).
6. M. Tsukada and H. Shiozaki, *J. Appl. Polym. Sci.*, **39**, 1289 (1990).
7. M. Tsukada, *J. Seric. Sci. Jpn.*, **53**, 380 (1984).
8. M. Tsukada and H. Shiozaki, *J. Seric. Sci. Jpn.*, **58**, 15 (1989).
9. M. Tsukada, H. Shiozaki, H. Urashima, and M. Yokozawa, *J. Text. Inst.*, **80**, 547 (1989).
10. M. Tsukada and H. Shiozaki, *J. Appl. Polym. Sci.*, **37**, 2637 (1989).
11. M. Tsukada, *J. Seric. Sci. Jpn.*, **56**, 157 (1987).
12. H. Shiozaki, M. Tsukada, and M. Matsumura, *J. Seric. Sci. Jpn.*, **57**, 165 (1988).
13. M. Demura and T. Asakura, *Biotech. Bioeng.* **33**, 598 (1989).
14. M. Tsukada, M. Nagura, H. Ishikawa, and H. Shiozaki, *J. Appl. Polym. Sci.*, **43**, 643 (1991).
15. H. Shiozaki, Y. Tanaka, H. Iida, and K. Nishina, *J. Seric. Sci. Jpn.*, **41**, 33 (1972).
16. M. Tsukada, G. Freddi, H. Shiozaki, *J. Appl. Polym. Sci.*, in press.
17. M. Tsukada, G. Freddi, M. Matsumura, H. Shiozaki, and N. Kasai, *J. Appl. Polym. Sci.*, submitted.
18. M. Tsukada and H. Shiozaki, *J. Appl. Polym. Sci.*, **41**, 1213 (1990).
19. H. Ishikawa, M. Tsukada, I. Toizume, A. Konda, and K. Hirabayashi, *Sen-i Gakkaishi*, **28**, 91 (1972).

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