

Chemical Modification of *Tussah* Silk with Acid Anhydrides

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ABSTRACT: *Tussah* silk fibroin was chemically modified by acylation with aliphatic, aromatic, and hydrophobic acid anhydrides. The *tussah* silk fibers were pretreated by immersing them in a lithium thiocyanate (LiSCN) solution and then acylated in dimethylformamide (DMF) at elevated temperatures. Using this method, acylated *tussah* silk fibers with weight gains of 8–22% could be obtained. The pretreatment with LiSCN was necessary to promote the acylation. Without it, the reaction did not proceed. The optimum temperature and reaction time of the pretreatment was 55°C and 60 min, respectively. When examining the physical properties and the thermal behavior of both pretreated and acylated *tussah* silk, it was found that the mechanical properties and the position of the major DSC endothermic peak remained unchanged, regardless of pretreatment and acylation. The moisture regain of the pretreated *tussah* silk increased slightly while the moisture regain of the acylated silk decreased linearly with increasing weight gain. The chemical modification allows for a wide control of the *tussah* silk fiber's properties, making it possible to use *tussah* silk for the development and production of novel textile and biomaterials. © 2000 John Wiley & Sons, Inc. *J Appl Polym Sci* 78: 382–391, 2000

Key words: *tussah* silk; chemical modification; acid anhydrides; pretreatment; acylation

INTRODUCTION

Silk fibers are important textile materials. In general, two kinds of textile silk fibers can be distinguished: silk from the domesticated *Bombyx mori* silkworm and *tussah* silk, the silk fiber from the wild silkworm of the species *Anthearea pernyi*. Based on the amount of production and consumption, *Bombyx mori* silk fibers are more important than are *tussah* silk fibers in the textile industry. Besides its textile applications, however, *tussah* silk fibers are potentially interesting biomaterials with various applications in the biomedical field,

for example, *tussah* silk has been the focus of developing novel biomaterials for cell-adhesion matrices. For establishing the feasibility of *tussah* silk fibroin-based biomaterials, Minoura et al.¹ investigated the attachment and growth of fibroblast cells (L929) on matrices of silk fibroin from *B. mori* and *A. pernyi*. *B. mori* silk fibroin exhibited cell attachment and cell growth comparable to a collagen matrix. *Tussah* silk fibroin, which contains the tripeptide sequence Arg–Gly–Asp (believed to be a specific interaction site for cell attachment), displayed a much higher cell attachment and growth compared to *B. mori* silk fibroin. Minoura et al.² also examined the mechanism of cell attachment to silk fibroin. The cell attachment to silk fibroin matrices is most likely a result from electrostatic interactions between the cells and the substrate since cell attachment is

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closely related to the electrostatic charge of arginine, the major positively charged amino acid. Cell attachment could also be influenced by chemically modifying arginine residues. It was furthermore shown that cell attachment was at a maximum around a biopolymer basicity value of 9, indicating a close relation of cell attachment to the basicity of the substrate.³

So far, chemical modifications of *tussah* silk fibroin, except epoxide-based modifications for textile applications, have hitherto not been examined in detail. Other studies have investigated only basic physical properties of the fiber, such as changes in the molecular conformation by heat treatment⁴ and the physical and chemical properties of *tussah* silk fibroin films.⁵ Tsukada et al. also studied the structural changes of *tussah* silk fibers induced by heat treatment⁶ and the changes in molecular conformation of *tussah* silk fibroin films induced by methanol treatment.⁷ Tsukada et al. previously reported the chemical modification of *B. mori* silk with aliphatic acid anhydrides, such as succinic and glutaric anhydride,⁸ and aromatic acid anhydrides such as phthalic and *o*-sulfobenzoic acid anhydrides.⁹ This study examined the reactivity of several kinds of acid anhydrides toward *tussah* silk fibers and discusses some physical properties of acid anhydride-acylated *tussah* silk. It also focused on the importance of a preliminary treatment of *tussah* silk fibers using LiSCN. These novel chemical modifications of *tussah* silk are not only useful for textile applications of *tussah* silk but are crucial for the preparation of novel biomaterials based on this biopolymer.

EXPERIMENTAL

Materials

All chemicals used were reagent grade or better. Octadecenylsuccinic anhydride (catalog no. 41649), ethylenediaminetetraacetic acid dianhydride (33,204-6), and 4,4'-(hexafluoroisopropylidene)diphthalic anhydride (38,644-8) were purchased from Sigma-Aldrich Japan K.K. Succinic anhydride (198-04355), itaconic anhydride (098-01742), glutaric anhydride (070-01602), and phthalic anhydride (161-02485) were purchased from Wako Pure Chemical Industries, Ltd. *O*-sulfobenzoic acid anhydride (S0124) was purchased from the Tokyo Chemical Industry Co. Ltd. Lithium thiocyanate *n*-hydrate (LiSCN ·

*n*H₂O) (assay 60–79%) was purchased from Kanto Chemical Co. Inc.

Sample Preparation

Tussah silk fibers were preliminary treated with a lithium thiocyanate solution (LiSCN) and then acylated in dimethylformamide (DMF). An amount of 0.1 g silk fiber (ca. 7.5 g/10 cm²) was immersed in 0.929 g/mL LiSCN · *n*H₂O for 30–120 min at 40–75°C. The sample was then washed with running water for 5 min and allowed to stand in methanol at room temperature for 15 min. The sample was transferred to a fresh methanol bath for 15 min and then washed twice with DMF to remove any residual LiSCN, which was absorbed within the silk fibers. After removing from DMF, the silk fibers were acylated with 20 g/100 mL acid anhydride (0.78 mM) in DMF at 80°C for various periods of time. The reaction system was connected to a reflux condenser and held at a constant temperature in a thermostatic bath. The material-to-liquor ratio was maintained at 1:40. At the end of the reaction, the silk samples were washed first with DMF and then with acetone at 55°C for 1 h to remove any unreacted anhydride and, finally, with water.

Weight Gain

Fiber weight gain was calculated from the difference in weight of the silk fabric before and after the chemical reaction. The acyl content could thus be estimated from the weight gain.

Analytical Measurements

Moisture Regain

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 silk fiber.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) measurements were performed on a Rigaku Denki Co., Ltd., Instrument (Model DSC-10A) at a heating rate of 10°C/min. The DSC range and heating rate were 10.5 mJ/s/10 mA and 10°C/min, respectively. The sample was compressed in sealed aluminum pans under a N₂ atmosphere at a flow rate of 200 mL/min. The open aluminum cell was swept with N₂ gas during the analysis.

Tensile Properties

The tensile properties of the modified silk fibers were measured with a Tensilon UTM-II (Toyo

Table I Weight Loss of *Tussah* Silk Fibers Treated with LiSCN at Various Temperatures

Temperature (°C)	Reaction Time (min)	Fiber Weight Loss (%)
40	60	0
55	60	0.22
70	60	1.56

Baldwin Co.), using the standard technique at 20°C and 65% RH at a gauge length of 100 mm and strain rate of 40 mm/min.

Refractive Indices

Refractive indices were measured using the Becke's line method, using a polarized microscope under monochromatic (Na) light at 20°C and 65% RH. The measurement procedure was described in detail elsewhere.⁶

RESULTS AND DISCUSSION

Pretreatment with LiSCN

The acylation of *tussah* silk fibers did not proceed at all in a conventional reaction system as previously reported for *B. mori* silk. This behavior can be attributed to the high order of *tussah* fibroin compared with *B. mori* silk. *Tussah* fiber chains are linked together by intermolecular hydrogen bonds.¹⁰ Since *tussah* silk fibers are naturally occurring in this highly ordered arrangement, it is necessary to promote the breaking of the hydrogen-bond network in the fiber array, prior to acylation. The breaking of the hydrogen-bond network can be achieved by swelling the silk fibers when immersing them in neutral salt solutions such as LiSCN. The highly ordered fiber structure is subsequently released in the swelling process. Since LiSCN¹¹ can achieve breaking of the *tussah* silk fiber hydrogen bonds at moderate temperatures without inducing adverse reactions, such as hydrolytic decomposition or derivatization, it is a desirable substance for *tussah* fiber pretreatment. From a processing point of view, LiSCN is also a desirable additive since its solution shows neutral pH and it is active at room temperature, and, hence, unlikely to cause peptide bond hydrolysis or adverse effects on tools and machinery.

Reaction Conditions

The optimum conditions for the reaction temperature and time for pretreating *tussah* silk fibers with LiSCN were investigated first. Table I shows a weight loss of *tussah* silk fibers after treatment with LiSCN for 60 min at various temperatures. Maximum weight loss of 1.5% occurred at 70°C. This weight loss can be attributed to minor hydrolytic decomposition of the silk fiber backbone that occurs when the silk fiber is exposed to LiSCN at high temperatures. When investigating the acetylation reaction after pretreatment, it can be seen that the pretreatment reaction temperature is important for the subsequent chemical modification. Figure 1 shows the weight gain of silk fibers acylated with octadecenylsuccinic anhydride. The weight gain increased gradually when increasing the temperature of the preliminary treatment reaction. Since LiSCN can dissolve silk fibers at higher temperatures, 55°C was chosen for further pretreatment reaction studies in order to maintain the structural integrity of the silk fiber. Figure 2 shows the influence of pretreatment time on acylation. The weight gain increases slightly with pretreatment time, corresponding to increased structural decomposition of the fiber. To achieve satisfactory acylation while minimizing fiber damage, the pretreatment conditions chosen for further studies were 0.929 g/mL LiSCN · *n*H₂O (about 1M) at 55°C for 60 min.

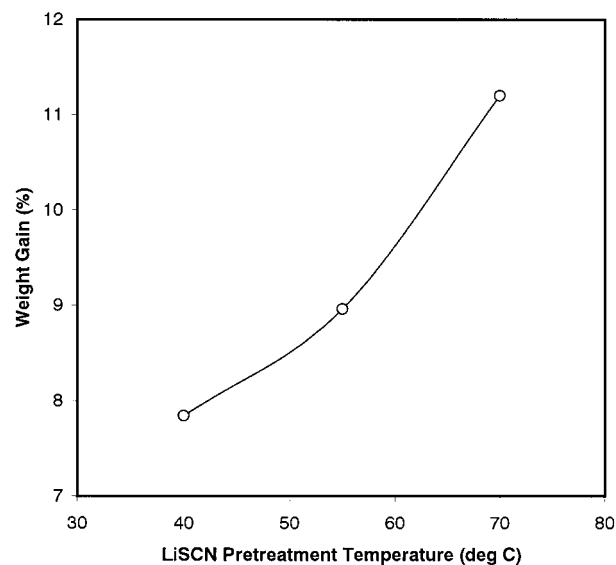


Figure 1 Weight gain of *tussah* silk fibers acylated with octadecenylsuccinic anhydride as a function of LiSCN-pretreatment temperature.

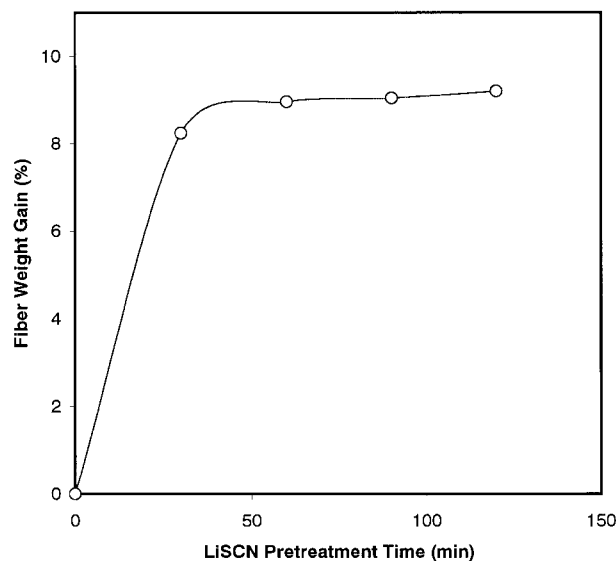


Figure 2 Weight gain of *tussah* silk fibers acylated with octadecenylsuccinic anhydride as a function of LiSCN-pretreatment time.

Tensile Properties

Tensile properties such as strength, elongation at break, and tensile modulus are important fiber characteristics, which determine the functional performance of textile materials. Tensile properties of LiSCN-pretreated *tussah* silk fibers were measured in the dry state as a function of pretreatment reaction time (Table II). It can be seen that the variation of the tensile properties is very small, suggesting that the pretreatment with LiSCN does not have a significant effect on the intrinsic tensile properties. This suggests that the original mechanical properties of silk fibers can be retained regardless of the LiSCN-induced swelling and associated hydrogen-bond loosening or breakage as long as peptide-bond formation is not compromised.

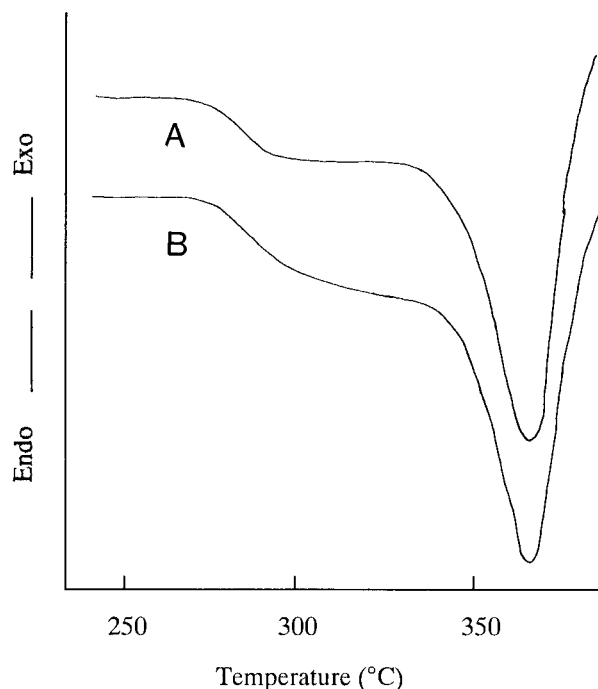


Figure 3 DSC curves of (A) untreated and (B) LiSCN pretreated (1M, 55°C, 1 h) *tussah* silk fibers.

Thermal Behavior

Differential scanning calorimetry (DSC) was carried out to evaluate the changes in thermal properties induced by LiSCN fiber treatment. The position of the major endothermic peak for both untreated and LiSCN-treated *tussah* silk samples is about 360°C. The thermal decomposition of both those fibers occurring at almost the same temperature indicates that the LiSCN-induced swelling does not compromise the oriented beta-form structure and the structural integrity of the fiber (Fig. 3).

Moisture Content

Moisture content is an important physical parameter, which can influence the functional behavior

Table II Tensile Properties of *Tussah* Silk Fibers Treated with LiSCN

Reaction Time (min)	Breaking Load (g)	Tensile Strength (g/d)	Elongation at Break (%)	Energy (g mm)
Control	182 ± 7.3	2.63 ± 0.11	31.8 ± 0.8	1901 ± 163
30	191 ± 20	2.96 ± 0.31	32.5 ± 1.3	2072 ± 166
60	197 ± 12	2.73 ± 0.17	32.5 ± 1.4	2286 ± 196
90	160 ± 14	2.56 ± 0.22	30.5 ± 1.3	1758 ± 149
120	200 ± 12	3.02 ± 0.18	33.1 ± 1.0	2202 ± 186

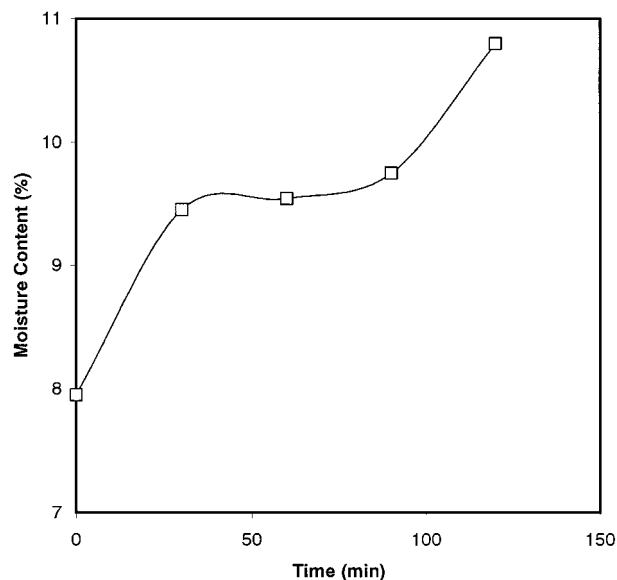


Figure 4 Moisture content of *tussah* silk fibers pretreated with LiSCN for different periods of immersing time.

of silk in textiles such as comfort and crease recovery. The moisture content of LiSCN-treated *tussah* silk fibers showed a linear increase with increasing reaction time (Fig. 4). The increasing moisture content of the *tussah* silk fibers after pretreatment is closely related to the hydrogen-bond breakage and slight hydrolysis, since these allow the penetration of water molecules into the fiber structure and the replacement of intra- and intermolecular hydrogen bonds with water association.

Refractive Indices

To elucidate the structural changes induced by reaction with LiSCN, the birefringence (Δn) and the isotropic refractive index (n_{iso}) of LiSCN-treated silk fibers were determined (Fig. 5). While the birefringence values exhibited increasing values with increasing reaction time, the isotropic refractive index did not show any significant changes, compared to the untreated sample. This increase of the birefringence suggests an increase of fiber anisotropy, probably due to shrinkage stress-induced in the course of pretreatment.

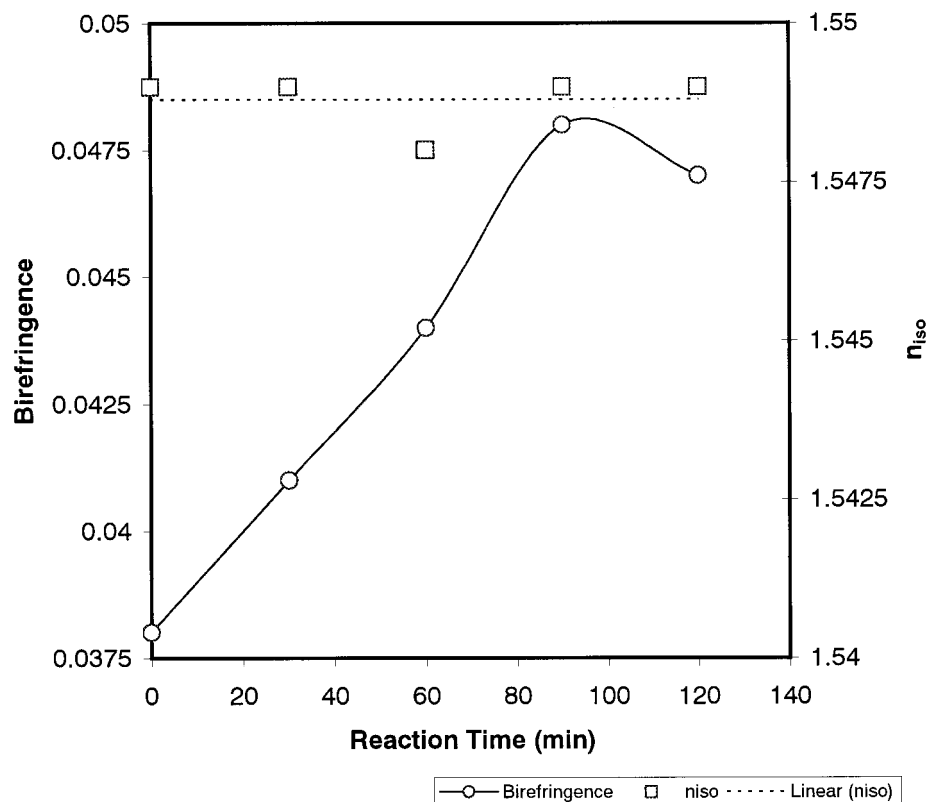


Figure 5 Birefringence and isotropic refractive index of pretreated *tussah* silk fibers with LiSCN for different periods of immersing time.

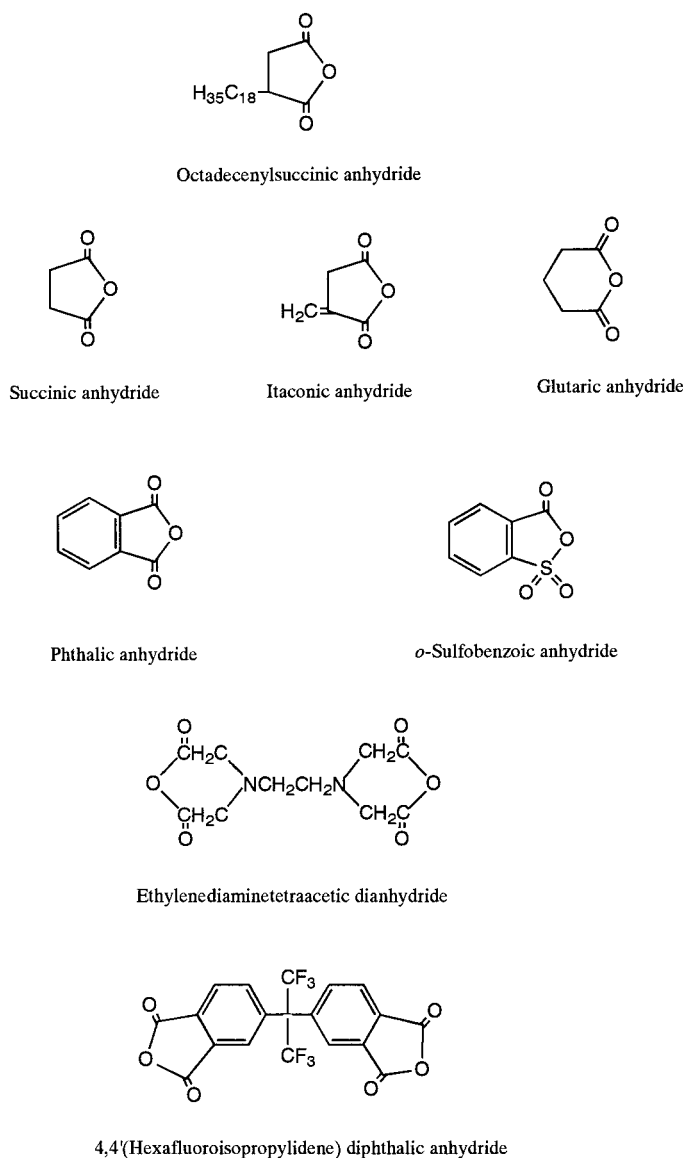


Figure 6 Chemical structure of various acid anhydrides.

Since the birefringence and isotropic refractive indices are measures of the average molecular orientation and crystallinity, respectively, it can be concluded that the LiSCN treatment induces some orientational changes in the fine structure of the silk fiber, due to swelling and hydrogen-bond breaking, but does not change the structural integrity of the fiber or its crystallinity (i.e., beta-sheet) content.

Acylation of *Tussah* Silk

Chemical Reactivities of Various Acid Anhydrides

Tussah silk fibers were modified in DMF with various acid anhydrides (Fig. 6) under identical reac-

tion conditions (75°C for 7 h) in order to analyze the specific chemical interaction. Figure 7 shows the weight gain attained for silk fibers using aliphatic (succinic, itaconic, and glutaric) and aromatic (phthalic and *o*-sulfobenzoic) acid anhydrides. Succinic, itaconic, and glutaric anhydrides showed similar reactivity toward the *tussah* silk fibroin, as expected from the fact that their chemical structure is very similar. However, the weight gain obtained with *o*-sulfobenzoic acid anhydride was much higher than that obtained using phthalic anhydride. Tsukada et al. previously reported that *o*-sulfobenzoic acid anhydride was more reactive toward *B. mori* silk fibroin than was phthalic anhydride.⁹ They concluded that this behavior could be

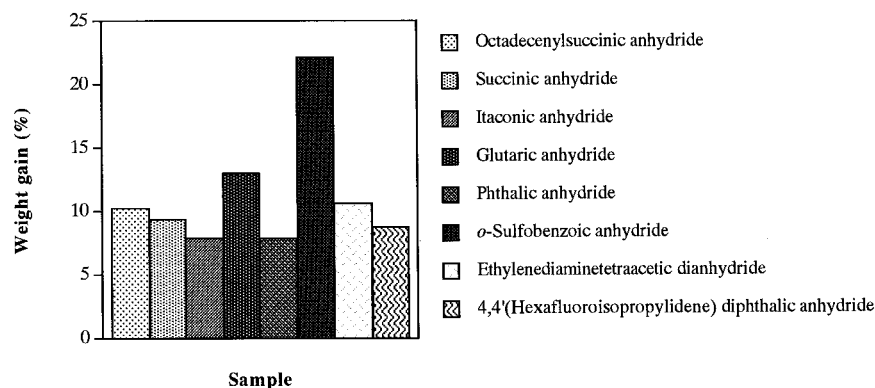


Figure 7 Weight gain of LiSCN-pretreated *tussah* silk fibers for different kinds of acid anhydrides.

attributed mainly to the presence of an electronegative sulfonic group. Besides the reactivity of monoanhydrides, the reactivity of some dianhydrides such as ethylenediaminetetraacetic acid dianhydride and 4,4'-(hexafluoroisopropylidene)diphthalic anhydride was examined. The weight gain obtained by the reaction of *tussah* silk fibers with dianhydride was similar to that of the monoanhydrides (Fig. 8).

Reaction Time of Acylation

To evaluate the conditions for the acylation reaction as well as the effect of the acylation on the fiber properties, the following studies were carried out using octadecenylsuccinic anhydride. Since this acid anhydride has a large aliphatic side chain (i.e., high molecular weight), even a small degree of acylation leads to a distinct change of the fiber properties, making it possible to investigate the reaction under any conditions.

The effect of the reaction time on the weight gain of silk fibers treated with octadecenylsuccinic anhydride is shown in Figure 8. The weight gain increased with increasing reaction time. *Tussah* silk samples with a weight gain of 10.3%, corresponding to an acyl content of $26.7 \text{ mol}/10^5 \text{ g}$, could be obtained after 7 h.

Mechanical and Physical Properties

Table III lists the results of the breaking load, tensile strength, and elongation at break of *tussah* silk fibers acylated with octadecenyl succinic anhydride with different amounts of weight gain. The differences among the sample are very small, suggesting the absence of any significant effect of the chemical treatments (LiSCN treatment and

acylation) on the intrinsic tensile properties of these fibers. Only the elongation value, 32.5% in the dry state, gradually decreased to 28.6% with increasing weight gain.

The octadecenylsuccinic anhydride acylated *tussah* silk fibers exhibited a linear decrease in moisture content with increased weight gain (Table IV). This can be attributed to the hydrophobic character of the long hydrocarbon chain introduced onto the fiber, playing a positive role in enhancing the water-repellent behavior of the modified fibers.

The decreased moisture content is known to cause changes in the silk fiber properties, with variable consequences not only on silk processing

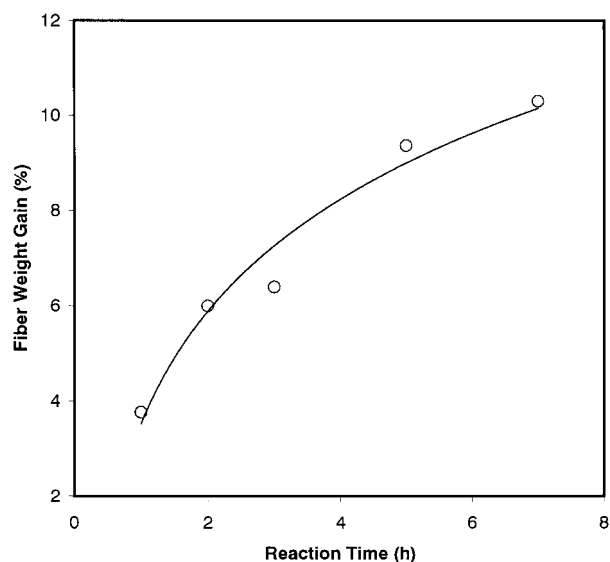


Figure 8 Weight gain of LiSCN-pretreated *tussah* silk fibers as a function of reaction time.

Table III Tensile Properties of *Tussah* Silk Fibers Acylated with Octadecenylsuccinic Anhydride

Weight Addition (min)	Breaking Load (g)	Tensile Strength (g/d)	Elongation at Break (%)	Energy (g mm)
Control	197 ± 12	2.73 ± 0.17	32.5 ± 1.4	2286 ± 196
3.76	188 ± 9.5	2.66 ± 0.14	30.1 ± 1.3	2026 ± 155
5.99	216 ± 11	3.01 ± 0.15	30.0 ± 1.1	2265 ± 131
6.39	210 ± 13	2.79 ± 0.17	28.5 ± 1.0	2242 ± 152
9.36	192 ± 17	2.59 ± 0.23	28.6 ± 0.8	2102 ± 117

(dyeing, printing, etc.), but also on the end use and maintenance of silk goods. While handle and comfort may be negatively influenced by a lower degree of moisture, absorption, crease recovery, water staining, and “wash-and-wear” may show improved performance.

CONCLUSIONS

Preliminary Treatment

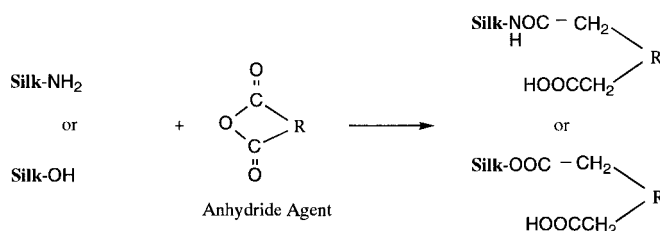
Contrary to *B. mori* silk fibers, chemical modification of *tussah* silk does not proceed without a preliminary treatment. Pretreatment of *tussah* silk with LiSCN is likely to cause a swelling of the silk fibers, allowing water to penetrate the fiber structure and to rupture the inter- and intrahydrogen bonds caused by alanine repeats, which constitute a crystalline structure embedded in the fiber. It has, however, been shown that *tussah* silk fibers possess a much higher internal porosity and longitudinal order than those of *B. mori* silk.¹² These features should favor internal diffusion of modifying reagents toward potentially reactive amino acid residues. Since the number of reactive amino acid residues is also higher compared to *B. mori* silk, one would expect that a chemical modification can be achieved easily. The necessity of the pretreatment might, hence, also indicate the presence of a surface barrier that prevents the penetration and diffusion of reagents into the fiber, but gets disrupted once penetration (swelling) occurs. It is important to notice that this pretreatment with LiSCN does not cause a change in the highly ordered *tussah* fibroin structure. Both the molecular orientation and the crystalline structure remain unchanged as shown by the refractive indices of untreated and pretreated *tussah* silk fibers. Thus, the structural integrity of the fiber is not compromised and

the inherent fiber properties remain unchanged during the chemical modification process, which is an important requisite for textile applications.

Complete removal of LiSCN and water was shown to be necessary prior to the acylation reaction. The swelling of the fiber structure and the associated breaking of the hydrogen-bond network of silk fibroin molecules allows for a penetration of the fiber structure and gives access to its reactive sites. For the reaction to proceed, the swelling has to be stopped to prevent hydrolysis and the water molecules have to be replaced by an organic solvent. In practice, this can be achieved by repeated washing first with running water and then with DMF prior to the acylation. During the final drying of the sample, the internal hydrogen bonds recombine, allowing for the fibrous structure to be reconstituted. The effect of the LiSCN pretreatment can thus be viewed as an accelerant for the acylation reaction toward *tussah* silk fibroin. For industrial applications, for example, for the acceleration of acylation, a pretreatment with 0.929 g/mL LiSCN (about 1M) at 55°C for 60 min can be considered sufficiently safe in order to achieve satisfactory acylation levels, making this pretreatment fast and economical.

Table IV Moisture Content of Octadecenylsuccinic Anhydride-acylated *Tussah* Silk Fibers at Different Weight Gains

Weight Gain (%)	Moisture Content (%)
0	7.95
3.76	6.76
5.99	6.60
6.39	6.33
9.56	6.19



Scheme 1 The mechanism of acylation toward *tussah* silk fibers using anhydrides. Silk-NH₂ denotes the amino side group of the *tussah* silk fiber's basic amino acids lysine, arginine, and histidine. Silk-OH represents the side chain of serine and tyrosine. R represents the hydrocarbon chains.

Chemical Modifications

After pretreatment with LiSCN, the rate of *tussah* silk fiber acylation increased significantly. *Tussah* silk fibers could be acylated with different kinds of anhydrides. The water-repellent behavior of *tussah* silk fibers could be modified using anhydrides having long alkyl carbon chains such as octadecenylsuccinic anhydride. This chemical modification and the performance of these water-repellent silk fabrics will be discussed in detail elsewhere. Anhydrides with sulfonic groups such as *o*-sulfobenzoic acid anhydride had the ability to balance ionized groups of the silk fiber samples. It is expected that the tensile properties of *tussah* silk fibers can be controlled by crosslinking the fiber backbone chains with succinic and glutaric anhydride⁸ as indicated by the acylation with these anhydrides. In using chemical modifications, a large number of *tussah* silk fibers with diverse biochemical and physical properties are feasible to obtain, thus adapting the fibers to various requirements in the textile industry as well as for biomedical applications.

Biopolymers and Textile Properties

Compared to *B. mori* silk fibers, *tussah* silk fibers contain a larger amount of basic amino acids such as arginine, lysine, and histidine,^{5,9} which are potentially chemical reactive sites. Due to this relatively high content of basic amino acids, acylation of *tussah* silk with acid anhydrides appears particularly attractive since a limited, controlled chemical modification would result in significant changes of the fiber properties. As shown in Scheme 1, lysine, histidine, arginine, tyrosine, and serine residues of the silk fibroin can be used as reactive sites for chemical modifications of silk.

It is expected that newly introduced functional groups, such as COOH groups, replacing the silk's

—OH or —NH₂ groups, could balance ionized groups in the silk fiber, resulting in changes of acid dye absorption levels toward *tussah* silk. In addition, the anhydride molecules should selectively interact with the molecular chains in amorphous regions, influencing the moisture content and the thermal properties of the silk fibers. Using a similar strategy, the acylation of silk fibers from *B. mori* silkworms with different monocarboxylic acid anhydrides has been shown to be of interest in regards to the chemical modification of the functional properties of these silk fibers. Since the degree of basicity of *tussah* silk fibroin can be controlled to a great extent using chemical-modification techniques, it should be feasible to control the extent of cell attachment and to adapt the fibroin-based matrix to environmental conditions.

The technique developed for the chemical modification of *tussah* silk is interesting not only from the view of producing textile materials originating from natural protein fibers exhibiting excellent dyeability, moisture content, and thermal stability. Using this method, it is also feasible to develop and produce novel biomaterials tailored for specific cell attachment, which have a wide array of applications in the medical fields.

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