

Chemical Modification of *Bombyx mori* Silk Using Isocyanates

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ABSTRACT: *Bombyx mori* silk fibers were chemically modified with various kinds of isocyanates and diisocyanates, including phenyl isocyanate and hexamethylene diisocyanate. The reactivity of these modifying agents toward silk fibers was examined as a function of the reaction solvent, temperature, time, and isocyanate chemistry. The use of DMSO as the solvent, bifunctional isocyanates, such as hexamethylene diisocyanate, and a temperature of 75°C resulted in higher weight gains of modified silk fibers. The physical and chemical properties of the modified silk fibers were studied as well. The moisture regain tended to decrease as the weight gain increased, the extent depending on the kind of isocyanate used. The resistance of silk toward attack by hot acid and alkali was generally enhanced by a reaction with isocyanates, the only exception being phenyl isocyanate, which induced a drop of alkali solubility. The tensile strength remained almost unchanged, regardless of chemical modification, while elongation at break decreased. The optical properties and the thermal stability of the modified silk fibers were not influenced by the reaction with isocyanates, suggesting that the fine structure of silk remained unchanged, regardless of chemical modification. © 2001 John Wiley & Sons, Inc. *J Appl Polym Sci* 79: 1756–1763, 2001

Key words: *Bombyx mori* silk; chemical modification; isocyanate; diisocyanate; solubility; tensile properties

INTRODUCTION

The chemical reaction of certain chemical reagents with silk fibers is particularly attractive and interesting from the view of improving textile performance, as well as imparting to the silk new

properties for extending its application in the biomedical field.¹ Reagents for the chemical modification of silk fibers include epoxide compounds and dibasic anhydrides; but grafting of the fiber is also possible using vinyl monomers such as methyl methacrylate, methacrylamide, and 2-hydroxyethyl methacrylate.^{2,3} The chemical reagents of the first category react selectively with the reactive sites of the protein fibers while the grafting agents penetrate the silk fiber matrix and copolymerize with the protein fibers. Currently, chemical modification with these reagents

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is mostly confined to the textile field, where they are effectively used in protecting silk against photoyellowing and in improving crease recovery, dyeability, use and maintenance performance, comfort, and so forth.

Fraenkel-Conrat et al.⁴ studied the reaction of aromatic isocyanates in anhydrous media toward proteins, including silk fibroin fibers, and showed that in the presence of pyridine the basic groups, including amino, guanidyl, and imidazole, the carboxyl, the phenolic, and the hydroxy groups of amino acid side chains are reactive sites for isocyanates. Farnworth⁵ first showed that phenyl isocyanate reacts extensively with wool in pyridine solution through modification of carboxyl and amino groups. Other isocyanates also react with wool, the reaction proceeding much faster in solvents other than pyridine [DMSO, *N,N*-dimethylformamide (DMF), etc.].⁶ Moore and O'Connell showed that high levels of acylation of wool with both mono- and bifunctional isocyanates increase the resistance of wool to damage by acid and alkali and confer shrink resistance.^{7,8} Kuwamura and Negishi⁹ first studied the reactivity of isocyanate and diisocyanate toward silk fibroin and were able to achieve a maximum weight gain of 5.6% for silk fibers modified with methylene diisocyanate in benzene at 50°C for 20 h. Further studies were conducted by Marzona,¹⁰ who obtained a weight gain of 10% using 0.5–2.0*M* hexamethylene diisocyanate in DMF at 40°C. Lysine was the most reactive amino acid toward hexamethylene diisocyanate. He also showed that the tensile strength of modified silk fibers increased while alkali and acid solubility decreased, suggesting that the reactive agent formed crosslinking between fibroin chains.¹⁰

Isocyanates and diisocyanates react selectively with various amino acid side chains in proteins, and the resulting bond between the cyanates and the silk fiber is thought to be much more stable than that formed after the reaction with epoxide compounds. Furthermore, the modification with diisocyanates forms a free cyanate group for each modified amino acid, which can influence the dyeing properties of the silk fiber, as well as allowing for the possibility of hydrogen bonding between the free cyanate and the amide groups of the silk fibroin backbone. It can be expected that these compounds lead to substantial modifications of the functional properties of silk fibers. Hence, a detailed investigation of the reaction conditions and mechanisms is important in developing new modification techniques applicable to silk. The present study focuses on the optimum reaction

conditions for the modification of silk fiber from *Bombyx mori* silkworms by different kinds of isocyanates and diisocyanates. Moreover, the physical and chemical properties of the modified silk fibers are discussed.

EXPERIMENTAL

Sample Preparation

Reagent grade phenyl isocyanate (catalog no. 165-01743), hexamethylene diisocyanate (082-02822), and dodecyl isocyanate (586-47491) were purchased from Wako Pure Chemical Industries, Ltd. Allyl isocyanate (24,327-2) and 1,3-phenylene diisocyanate (30,823-4) were purchased from Sigma-Aldrich Japan K.K. Silk fibers from *B. mori* were treated with isocyanate or diisocyanate according to the procedure described by Kuwamura and Negishi.⁹ Silk fibers were first immersed for 10 min in 60% methanol at room temperature and then immersed 3 times in methanol (dehyd.). The fibers were then placed in either DMF (dehyd.) or DMSO (dehyd.). Immediately after removing the fibers from DMF or DMSO, the silk fibers were treated with isocyanate or diisocyanate compounds in DMF or in DMSO at 60–75°C for various periods of time. The reaction system was connected to a reflux condenser and held in a thermostatic bath. The material/liquor ratio was 1:30. At the end of the reaction, the silk samples were first washed twice with DMF or DMSO at 55°C, then placed in acetone at 55°C for 2 h in order to remove any unreacted isocyanate or diisocyanate, and finally washed with water. The weight gain was calculated from the increase in weight of the original silk fibers before and after the reaction.

Moisture Regain

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

Alkali and Acid Solubilities

The alkali or acid solubility was determined by treating silk samples with a solution of 0.1*N* NaOH or 3*N* HCl at 65°C for 60 min. The values were calculated on the basis of the dry weight of the undissolved residue remaining after treat-

Table I Weight Gain of Silk Fibers Modified with 2*M* Hexamethylene Diisocyanate at 75°C for 3 h in Different Solvents

Solvent	Weight Gain (%)
<i>N,N</i> -Dimethylformamide	14.8
DMSO	34.0

ment and were expressed as a percentage of the original dry weight.

Tensile Properties

The tensile properties of the modified silk fibers were measured with a Tensilon UTM-II (Toyo Baldwin Co.) using the standard technique at 20°C, 65% RH, a gauge length of 100 mm, and a strain rate of 40 mm/min.

Refractive Indices

The refractive indices were measured using the Becke's line method with a polarized microscope under monochromatic (Na) light at 20°C and 65% RH. The measurement procedure is described in detail elsewhere.¹¹

Differential Scanning Calorimetry (DSC)

The 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 an open aluminium pan, which was swept with N₂ gas at a flow rate of 200 mL/min.

RESULTS AND DISCUSSION

Solvent Effects

The effect of the solvent on the reactivity of hexamethylene diisocyanate toward *B. mori* silk fibers was examined using DMF or DMSO as the solvent for chemical modification. Table I summarizes the weight gain of the silk fibers after the reaction.

The reaction efficiency of DMSO was 2 times higher than that of DMF. This behavior may be related to the physical properties of the solvents. Although the solubility parameters (δ) for DMF and DMSO do not differ too much, the latter is characterized by a lower dielectric constant.

Moreover, the ability of the solvent to swell the silk fiber matrix and to enhance the diffusion of the reagent toward the reactive sites must be taken into account. In fact, it was shown that the volume swelling induced onto silk by DMSO at 30°C is 4 times larger than that of DMF.¹² We therefore used DMSO as the solvent throughout the present study.

Temperature Effects

The reaction temperature is an important factor for the control of the reaction efficiency. Table II shows the effect of the reaction temperature on the acylation of silk fibers with hexamethylene diisocyanate and phenyl isocyanate in DMSO at 60 and 75°C.

The results show that the higher the temperature, the higher the overall efficiency of the reaction, which agrees with the results reported for wool.⁵ They furthermore suggest that hexamethylene diisocyanate attained higher weight gain than phenyl isocyanate. This probably reflects the differences in the physicochemical properties and reactivity between the two modifying agents (i.e., the presence of a bulkier aromatic group in the molecule of phenyl isocyanate, as well as the different number of cyanate groups).

Rate of Acylation with Isocyanate and Diisocyanate

To investigate the difference in the reactivity of isocyanate and diisocyanate, *B. mori* silk fibers were treated with 3% (w/v) phenyl isocyanate or hexamethylene diisocyanate in DMSO at 75°C over various reaction times (Fig. 1).

The weight gain of silk fibers modified with hexamethylene diisocyanate increased rapidly during the first 30 min and then proceeded at a slightly lower rate, while the weight gain of the

Table II Weight Gain of Silk Fibers Modified with 5% (w/v) Phenyl Isocyanate or Hexamethylene Diisocyanate in DMSO for 3 h at Different Temperatures

Isocyanate Compound	Weight Gain (%)	
	60°C	75°C
Phenyl isocyanate	10.9	19.1
Hexamethylene diisocyanate	7.3	11.3

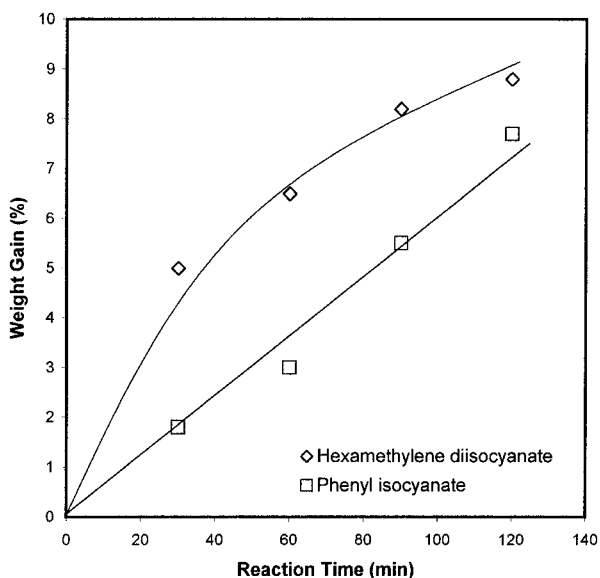


Figure 1 The weight gain of silk fibers modified with 3% (w/v) hexamethylene diisocyanate and phenyl isocyanate in DMSO at 75°C over various reaction times.

sample modified with phenyl isocyanate increased linearly over the course of the experiment. The apparent difference in reaction orders is indicative of a significant difference in the chemical reactivity and reaction mechanism of the reactants. Although hexamethylene diisocyanate has two reactive sites that can both potentially interact with the silk fiber or another hexamethylene diisocyanate molecule, phenyl isocyanate carries only one reactive site. Additionally, the different rates of reaction between the two modifying agents may account for the different rates of penetration and diffusion of the molecules within the fiber matrix,⁸ as well as for the kind and strength of interactions established with the fibroin chains.

Reactivity of Various Isocyanates

The theoretical and experimental weight gains of silk fibers modified with different acylating agents are compared in Table III.

The theoretical weight gain was estimated on the basis of the assumption that the reagents react completely with basic amino acids, such as arginine and lysine, and with hydroxy amino acids, such as serine, tyrosine, and threonine, in the amorphous regions of the fiber. Although basic amino acids are mostly present in the amorphous regions, a significant part of the hydroxy amino acids are blocked in the ordered crystalline regions and are not accessible to reagents.^{13,14} Therefore, a correction was made assuming that the amorphous regions constitute about 40% of the total fibroin fiber and the resulting amount of potentially reactive sites accounted for 10.2 mol/10⁴ g of silk.⁹

From the results listed in Table III it is possible to see that the experimental weight gains of phenyl isocyanate and dodecyl isocyanate were very close to the corresponding theoretical values. Allyl isocyanate attained about 70% of the theoretical saturation value, and the reactivity of 1,3-phenylene diisocyanate was considerably lower than expected. The yield of hexamethylene diisocyanate was higher than that calculated on the basis of the number of potentially reactive sites of the silk fibers. This behavior can be explained by either admitting that other amino acid residues (i.e., acidic amino acids) react with the modifying agent or considering the possibility of self-condensation of the bifunctional isocyanate molecules. The latter hypothesis is partly confirmed by the results given in Table I, wherein the presence of a large excess of reactant (2*M*) silk attained a weight gain (34%) much larger than that ex-

Table III Theoretical and Experimental Weight Gain of Silk Fibers Modified with Different Isocyanates and Diisocyanates (5% w/v) in DMSO at 75°C for 3 h

Isocyanate Compound	MW (g/mol)	Weight Gain (%)	
		Theoretical	Experimental
Phenyl isocyanate	119.12	12.2	11.3
1,3-Phenylene diisocyanate	160.13	16.3	4.8
Allyl isocyanate	83.09	8.5	6.1
Dodecyl isocyanate	211.35	21.6	20.2
Hexamethylene diisocyanate	168.20	17.2	19.1

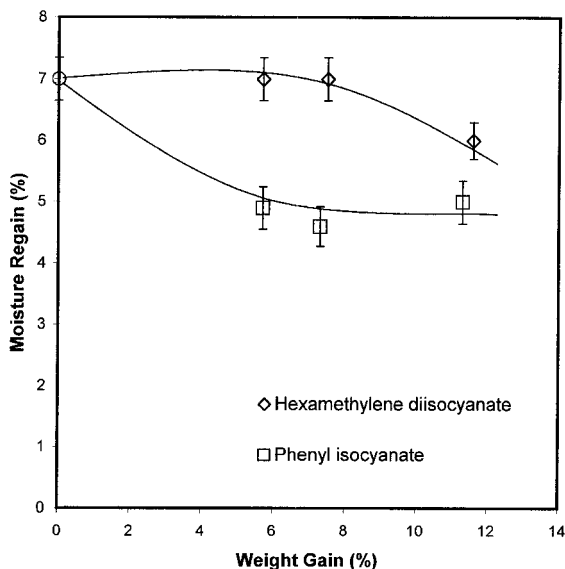


Figure 2 The moisture content versus the weight gain of silk fibers modified with hexamethylene diisocyanate and phenyl isocyanate.

pected, even considering the contribution of acidic amino acids (23.9%).

Moisture Content

The moisture content values of silk fibers modified with phenyl isocyanate and hexamethylene diisocyanate are plotted in Figure 2 as a function of weight gain.

Following the reaction with phenyl isocyanate the moisture content of silk fibers decreased, even at low weight gain, while the effect of hexamethylene diisocyanate became detectable only at above 8% weight gain. As expected, the reaction with isocyanates altered the balance of the electrostatic charges of the silk fiber, replacing existing hydrogen bonds and introducing additional hydrophobic interactions. The amount of water molecules at saturation may therefore decrease, especially as a consequence of the introduction of bulky hydrophobic groups. In this respect the aromatic group of phenyl isocyanate was particularly effective, which was in accordance with the results reported for wool.^{7,8}

Alkali and Acid Solubility

The modification of protein fibers (silk and wool) with isocyanates is known to enhance their resistance to damage by acids and alkalis.^{7,8,10} Figure 3 shows the behavior of the acid and alkali solu-

bility of silk following a reaction with hexamethylene diisocyanate as a function of weight gain.

The alkali and acid solubility both decreased with increasing weight gain. The alkali solubility decreased gradually, but the acid solubility decreased sharply, even at relatively low weight gain, indicating that acylation resulted in a marked protection of silk against solubilization in hot acid. The increased chemical resistance of silk can be attributed either to the higher hydrophobicity imparted on the fiber by the modifying agent or to the introduction of relatively stable crosslinks into the silk structure by reaction with the bifunctional reagent.^{8,10} However, the fact that monofunctional isocyanates are also effective in improving the chemical resistance of protein fibers⁷ led us to suggest that the increased fiber hydrophobicity might play the major role.

The acid and alkali solubility of silk acylated with phenyl isocyanate showed quite peculiar features (Table IV).

While acid solubility decreased with a trend very similar to that observed for hexamethylene diisocyanate, the alkali solubility behavior was significantly different. In fact, the amount of alkali soluble silk material abruptly increased from 27.3% (control sample) to 80.9% for the sample with 5.66% weight gain and then tended to decrease with increasing weight gain. However, it remained significantly higher than that of the control sample, indicating the occurrence of a

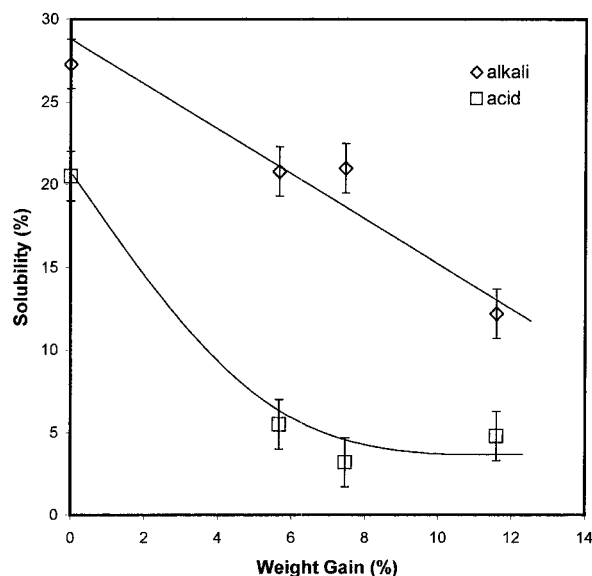


Figure 3 The acid and alkali solubility of silk fibers modified with hexamethylene diisocyanates as function of weight gain.

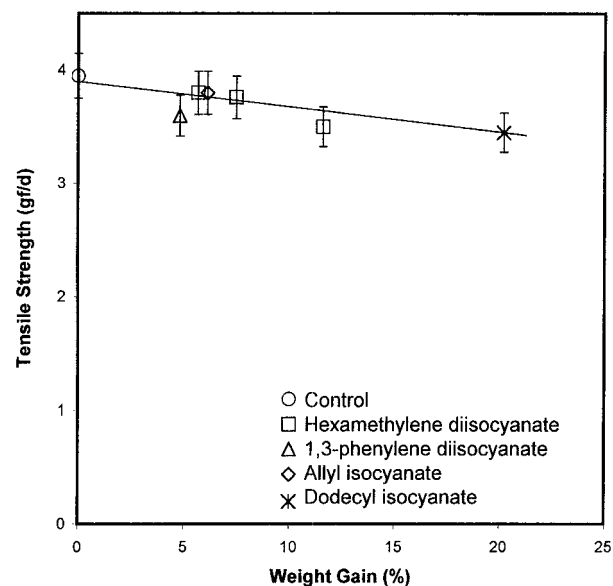
Table IV Acid and Alkali Solubility of Silk Fibers Modified with Phenyl Isocyanate as Function of Weight Gain

Weight Gain (%)	Solubility (%)	
	Alkali	Acid
Untreated	27.0	19.5
Control	27.3	20.5
5.7	80.9	5.4
6.7	68.8	7.0
11.3	42.9	4.6

more or less extensive depolymerization of the fiber. This conclusion was also confirmed by the physicochemical measurements (Table V). The different behaviors against acid and alkali can be explained by taking into account the different mechanisms of their hydrolytic action toward silk. Mineral acids preferentially attack the peptide bonds involving serine and threonine, while the alkali attack is thought to take place starting from the ends of the peptide chain.¹⁵ The hydroxy amino acids, which are extremely labile toward acids, were probably protected against the acid attack through binding with the modifying agent. Their chemical microenvironment probably became less accessible to the hydrolytic agent. On the other hand, the alkali was less hindered from displaying its hydrolytic action against the degraded fibers, which became slightly more resistant only at high weight gains.

Tensile Properties

The tensile properties such as strength, elongation at break, and energy are important fiber characteristics that determine the functional performance of textile materials. The tensile strength and elongation at break of silk fibers

**Figure 4** The tensile strength of silk fibers modified with different kinds of isocyanates and diisocyanates as function of weight gain.

modified with various kinds of isocyanates were measured in the dry state as a function of the reaction time. The tensile strength decreased only slightly as a function of the weight gain (Fig. 4).

The decrease mostly depended on the gradual increase of the transverse fiber dimension due to filling with isocyanates. In fact, the values of breaking load remained almost unchanged over the weight gain range explored. The elongation at break decreased as well (Fig. 5).

The lower extensibility of the fibers can be attributed to changes in the range and strength of the chemical interactions within the fiber matrix. The introduction of new alkyl and aryl groups probably increased the level of hydrophobic interactions, making the modified fiber stiffer than the original one.

Table V Tensile Properties of Silk Fibers Modified with Phenyl Isocyanate as Function of Weight Gain

Weight Gain (%)	Breaking Load (gf)	Strength (gf/d)	Elongation at Break (%)	Energy (gf/mm)
Untreated	439 ± 21	3.95 ± 0.19	18.7 ± 1.2	3302 ± 576
Control	437 ± 20	3.93 ± 0.18	24.6 ± 1.0	3385 ± 373
5.7	210 ± 31	1.75 ± 0.26	4.0 ± 0.5	458 ± 113
7.5	214 ± 16	1.79 ± 0.13	4.5 ± 0.4	476 ± 115
11.3	327 ± 101	2.80 ± 0.87	14.0 ± 2.1	2148 ± 794

The tensile properties of silk modified with phenyl isocyanate as a function of weight gain are listed in Table V.

A dramatic drop of all the tensile parameters was observed after modification with the isocyanate, even at low weight gain. The partial recovery of fiber strength at 11.6% weight gain can be related to stiffening induced by the reactant filled within the fiber matrix. The trend of the tensile properties was closely related to the alkali solubility data discussed above, confirming the occurrence of degradation phenomena during the reaction.

Refractive Indices

In order to elucidate the structural changes induced by modification with isocyanates, the birefringence (Δn) and the isotropic refractive index (n_{iso}) of modified silk fibers were determined (Table VI).

The variation of the birefringence and isotropic refractive index as a function of weight gain was very small, suggesting that the fine structure of silk was not significantly affected by the reaction. This conclusion agrees well with the results reported for other acylating agents, such as acid anhydrides.²

Thermal Properties

The thermal properties of silk modified with different isocyanates were analyzed by DSC. The

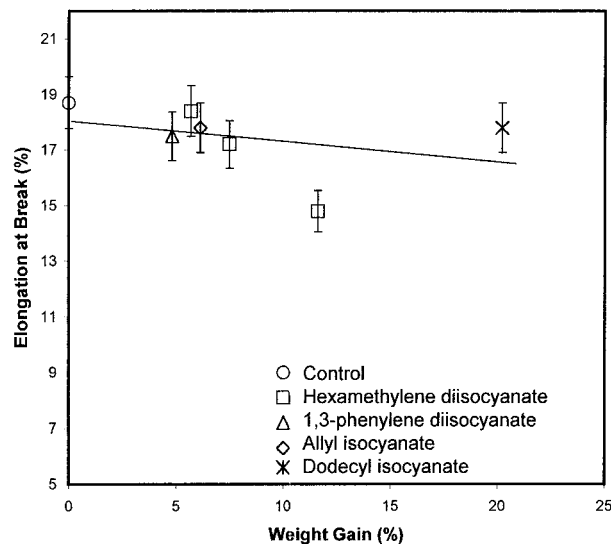


Figure 5 The elongation at break of silk fibers modified with different kinds of isocyanates and diisocyanates as function of weight gain.

Table VI Birefringence (Δn) and Isotropic Refractive Index (n_{ISO}) of Silk Fibers Modified with Hexamethylene Diisocyanate as Function of Weight Gain

Weight Gain (%)	Δn	n_{ISO}
Untreated	0.050	1.552
5.7	0.048	1.550
7.5	0.047	1.549
11.6	0.045	1.550

DSC profiles of the untreated and modified silk fibers were almost identical. Neither changes in the shape and intensity of the main decomposition endotherm of silk fibroin nor additional thermal transitions were detected as a function of the weight gain. The peak temperature of the major endotherm attributed to the thermal decomposition of the silk fibroin with an oriented β -sheet crystalline structure was located at 318°C for untreated silk and at $317 \pm 3^\circ\text{C}$ for silk fibers modified with different kinds of isocyanates. These findings confirm that silk retained its thermal stability, regardless of the chemical modification.²

CONCLUSIONS

This work showed that different isocyanates are absorbed and bound by silk fibers at a different rate and extent, depending on the reaction conditions (solvent, time, temperature) and chemical structure of the reactant. Hexamethylene diisocyanate displayed the highest reactivity toward silk in DMSO at 75°C, which was probably due to the presence of two reactive groups in the molecule, partly leading to self-condensation. Other aliphatic isocyanates showed weight gains very similar to the theoretical ones, while the reaction with aromatic isocyanates had some drawbacks. 1,3-Phenylene diisocyanate displayed low reactivity in spite of its bifunctional character, and phenyl isocyanate induced a marked degradation of silk attributable to oxidative phenomena occurring during the reaction.

The most striking result of the chemical modification of silk with isocyanates was the enhanced resistance toward solubilization by hot acids and alkalis. This effect, which was mainly attributable to increased fiber hydrophobicity and to local changes in the chemical environment of

labile amino acid residues,^{6,15} could be successfully exploited for technological applications. The substantial preservation of the intrinsic tensile properties and thermal stability of silk, together with the negligible effect on the fine structure of the fiber, makes this chemical modification technique potentially attractive for the development of new textile and nontextile materials.

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