

Effect of Moisture Absorption on the Thermal Properties of *Bombyx mori* Silk Fibroin Films

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ABSTRACT: Films of regenerated *Bombyx mori* silk are strongly affected by absorbed moisture, a phenomenon studied here by differential scanning calorimetry (DSC). Exposure of previously dried films to environments of controlled relative humidity produces test samples of well-defined equilibrium moisture content. Ultimate moisture uptake is as high as 20–23% (by weight) at 75% relative humidity. The glass transition temperature, T_g , drops by 40°C at moisture uptakes as low as 2%, and T_g depressions as large as 140°C are observed at higher relative humidity. The moisture-induced decrease of T_g is completely reversible, as a film remoistened and then redried possesses an unchanged T_g . Trends in T_g with water uptake correspond reasonably well to predictions of a classical thermodynamic theory, indicating that the plasticization effect of moisture on the combined silk-water system can be satisfactorily explained from macroscopic properties of the constituents without any reference to specific interactions. © 1997 John Wiley & Sons, Inc. *J Appl Polym Sci* **63**: 401–410, 1997

Key words: silk fibroin; moisture absorption; thermal properties; relative humidity; glass transition

INTRODUCTION

The unusual physical and chemical properties of silk fibroin have recently captured considerable attention from the polymer community, with much of the interest stemming from the unique *in vivo* processing of silk polymers by organisms such as *Bombyx mori* and *A. Penyi*.^{1–4} These organisms process fibroin, a moderately concentrated mixture of silk and sericin, a coating protein, at mild temperatures, producing fibers with many desirable mechanical properties. During the *in vivo* processing, silk molecules undergo a morphological transition from a soluble, random coil/ α -helix conformation to an insoluble, β -sheet

structure, the transition presumably driven by the loss of water and/or the application of shear and tensile stresses.^{5,6} Among the useful properties of silk from silkworms and spiders are high modulus (1–30 GPa), high strength ($\sim 10^8$ Pa), high elongation to break (10–30%), and high energy to break ($\sim 10^4$ J/g).⁵ Inherent fiber strength and toughness, combined with solvent-free and low-energy processing, provide ample motivation for detailed mechanistic studies of silk spinning; researchers have probed the natural spinning process^{5,7–9} and devised *in vitro* methods to spin controlled monofilaments from regenerated silk solutions.¹⁰

Moisture exerts a significant, and often deleterious, effect on the physical properties of hydrophilic polymers. For example, as moisture content increases from 0 to 20% by weight, the glass transition temperatures of collagen, elastin, and cellulose decrease by approximately by 200, 145, and

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260°C, respectively.¹¹ Gelatin, another hydrophilic polymer, absorbs as much as 22% moisture at 75% relative humidity, with both the tensile modulus and yield strength dropping with moisture content and the elongation to break rising.¹² If a sample of any of these materials is volumetrically constrained during moisture uptake, large mechanical stresses develop, and catastrophic failure may ensue. Likewise, exposure of unconstrained samples to variable ambient moisture can produce large dimensional changes. The dimensional change of human hair, for example, permits the deployment of hair as a sensory element in humidity measuring instruments. Exposure to moisture affects the properties of epoxies and nylons in a comparable manner.¹³⁻¹⁷

Given the similarity in composition between silks and nylons, analogous alterations of physical properties in response to moisture uptake might be expected. Although the existing literature provides a large sampling of data on the properties of silk fibroin, the influence of absorbed moisture has not been addressed in as much detail as for nylons. Our aim in this study is to quantify the effect of moisture on the thermal properties of regenerated silk. We examine the moisture sensitivity of the glass transition temperature in detail and compare measured trends to the predictions of a phenomenological theory derived from classical thermodynamics.

BACKGROUND

Several qualitative models for the interaction of water with hydrophilic polymers have been reported in the literature.¹² These models generally hypothesize that water molecules are either bound to specific polymer chain sites or free to disperse themselves homogeneously throughout the amorphous polymer matrix. York¹⁸ listed three mechanisms by which water could be held in a polymer system: (1) as monomolecular layers bound to the surface (adsorbed moisture), (2) as isolated molecules distributed internally, either bound or free (absorbed moisture), and (3) as multimolecular layers (condensed moisture). Recently, Jelinski et al.¹⁹ demonstrated via quadrupole echo deuterium nuclear magnetic resonance (NMR) the presence of a specific interaction between water and an epoxy. Water molecules were observed to jump from site to site at a characteristic frequency. Although unable to pinpoint the

type of interaction involved, Jelinski et al. did not find evidence for either bound (residence time greater than 10^{-3} s) or free (spin-lattice relaxation time comparable to pure water) water.

These sorts of studies, although valuable for their insights into the water-matrix interaction, offer light guidance toward a quantitative understanding of the influence of moisture on physical properties. Therefore, to interpret the current thermal data, we resort to a classical thermodynamic theory for the compositional dependence of T_g in a system of polymer and diluent. The glass transition of polymers has often been modeled as a second-order thermodynamic transition obeying the relations developed by Ehrenfest.²⁰ Although arguably not a true second-order transition due to kinetic limitations, discontinuities in the specific heat and thermal expansion coefficient at T_g are reminiscent of such transitions. The ad hoc assertion of a second order transition at T_g greatly simplifies the interpretation of data collected in this study.

Several semiempirical expressions predict the compositional dependence of T_g .²¹⁻²³ In most cases, these expressions emerge from analyses that assume additivity of both volume and entropy. One of the more widely applied expressions is that by Couchman and Karasz,^{24,25} as follows:

$$\ln\left(\frac{T_g}{T_{g1}}\right) = \frac{X_2\Delta C_{p2} \ln\left(\frac{T_{g2}}{T_{g1}}\right)}{X_1\Delta C_{p1} + X_2\Delta C_{p2}} \quad (1)$$

where subscripts 1 and 2 refer to the polymer and diluent, respectively, while X is the weight fraction, and ΔC_p is the incremental heat capacity change at T_g . If the two pure component transition temperatures do not differ greatly, the above relation simplifies, as follows:

$$T_g = \frac{X_1\Delta C_{p1}T_{g1} + X_2\Delta C_{p2}T_{g2}}{X_1\Delta C_{p1} + X_2\Delta C_{p2}} \quad (2)$$

When these equations have been tested against experimental data,^{14-17,26} satisfactory agreement has generally been noted. Based on this success, we analyze our results using eqs. (1) and (2).

EXPERIMENTAL

Materials

Untreated silk cocoons were obtained courtesy of Prof. Y. H. Huang (Guangzhou Institute of Chem-

istry, China) and stored in a refrigerator prior to use. Hydrated crystalline lithium thiocyanate ($\text{LiSCN}\cdot x\text{H}_2\text{O}$), sodium carbonate, and sodium dodecyl sulfate (SDS) were purchased from Aldrich Chemical, Fisher Scientific, and Polysciences Inc., respectively. Solution dialysis was performed in Spectrapor® dialysis tubing from Thomas Scientific.

Sample Preparation

Guided by a literature procedure, regenerated silk films were prepared from untreated cocoons via the following steps.

Degumming of Silk

Cocoons were cut by scissors into pieces approximately $\frac{1}{8}$ in. long, and approximately 5 g of these pieces were boiled for 1.5 h in 100 mL of distilled water containing 55 mg sodium carbonate and 300 mg SDS. The insoluble silk was then removed from the boiling liquid, wrung out, and rinsed thoroughly in warm distilled water, steps that facilitated the removal of the more water-soluble sericin fraction. Next, the recovered and still damp silk was boiled in 100 mL of distilled water containing 21 mg sodium carbonate for 1 h. The silk was again removed and rinsed with warm distilled water. Finally, the silk was soaked in 30 mL of methanol for 0.5 h. The purified silk was left to dry overnight in a laboratory fume hood.

Preparation of Silk Solution

The product of the first procedure was solubilized in aqueous 9.0M LiSCN solution. Although initially turbid, this mixture became clear after 3 h when kept in an oven at 60°C. Returning the solution to room temperature, the clear silk solution was dialyzed against distilled water for three days, with the dialysate replaced every 24 h. Finally, the solution was filtered through 0.45 μm syringe filters from Millipore.

Film Casting and Drying

A known volume of dialyzed silk solution at 2 wt % was deposited in a polystyrene dish and dried overnight in an oven at 50–60°C. Subsequently, the dish was transferred to a vacuum oven for additional drying at 50°C for 2 h. Cut into small pieces and placed in aluminum pans, the silk was again dried in a vacuum oven for 2 h, in this in-

Table I Relative Humidities Generated by Saturated Salt Solutions in the Given Temperature Range

Salt Solution	Relative Humidity (%)	Temperature Range (°C)
Lithium chloride	12.9 \pm 2.4	15–90
Potassium acetate	16.5 \pm 3.2	25–40
Magnesium chloride	31.7 \pm 1.9	25–70
Potassium carbonate	41.5 \pm 3.6	15–70
Magnesium nitrate	54.0 \pm 2.0	15–25
Sodium chloride	75.9 \pm 1.3	15–100

stance, at 150°C. Though not independently confirmed, we believe that the final dried films had a negligible moisture content. Films were translucent, yellowish white, void-free but brittle, and easily redissolved in water.

Measurements

Dry film samples were exposed to controlled relative humidity (RH) in homemade proximity equilibration cells (PECs),^{27,28} using saturated salt solutions to regulate RH in the vapor phase above the films.^{12,29} Table I lists the specific salt solutions and humidities examined, and Figure 1 provides a schematic of the PEC design. Silk films deposited in the cells were equilibrated at constant RH for one to two weeks, with weight change periodically monitored by a Sartorius balance accurate to 0.01 mg. The system was considered to have reached a steady state when the weight change was within $\pm 3\%$. This was also the maximum spread in the moisture content values. The temperatures of the PECs fluctuated with the local laboratory temperature, approximately in the range of 23–26°C, well within the applicable span of RH values for the salt solutions.

After hermetic sealing of the aluminum sample pans, differential scanning calorimetry (DSC) thermograms were recorded at 20°C/min, unless otherwise mentioned, using a Du Pont DSC2910 calorimeter. The DSC cell was maintained under nitrogen flow (70–80 mL/min) during these scans, and temperature calibration relied on an indium standard. To estimate the glass transition temperature T_g , a half-width method was applied to a plot of heat flow versus temperature. After cell calibration with a sapphire standard,³⁰ the same plots yielded the specific heat change ΔC_p

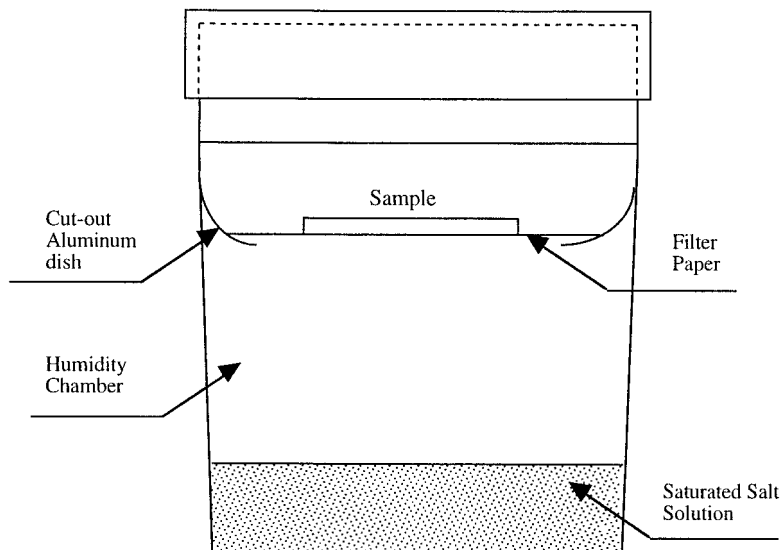


Figure 1 Schematic sketch of proximity equilibration cell (PEC).^{29,30}

associated with T_g . For fully dried silk, preliminary DSC runs ascertained the following reference values for T_g and ΔC_p : 179.18°C and 0.66 J g⁻¹ K⁻¹, respectively. Corresponding values for water, 134 K and 1.94 J g⁻¹ K⁻¹, were taken from the literature.^{17,31,32} Thermal degradation of the silk samples was examined by a Du Pont thermogravimetric analyzer TGA2950 operated at either 10 or 20°C/min. The furnace and balance sections of the TGA2950 were continuously purged with nitrogen. Finally, a Du Pont thermomechanical analyzer TMA2940 provided the thermal expansion coefficient of silk across the relevant temperature ranges.

RESULTS AND DISCUSSION

Figures 2–4 present the DSC, thermogravimetric analysis (TGA), and thermomechanical analyzer (TMA) thermograms for a fully dried silk film. The DSC thermogram reveals three thermal transitions: a glass transition at 179°C, a small exotherm at 220°C, and a broad degradation peak roughly centered at 264°C. These transition temperatures agree with literature values for silk.² The exothermic peak has been ascribed to conformational changes accompanying silk's transition from random coil/ α -helix to β sheet. The 5% weight loss inferred by TGA as the film is heated beyond 260°C supports identification of the corresponding DSC endotherm as a degradation fea-

ture. As indicated in Figure 4, TMA profiles from successive heating and cooling cycles below T_g provide an average thermal expansion coefficient of 54.4 $\mu\text{m}/\text{m}^\circ\text{C}$. These curves have been vertically shifted by an arbitrary value to enhance clarity. During a final heating to higher temperature, an abrupt increase in this coefficient is noted at 172°C, a value consistent with the T_g measured by DSC.

Figure 5 shows the moisture absorption isotherm for dry silk films. Steady-state moisture uptake is as large as 20.5% for a sample kept at 75% RH. Error bars summarize inaccuracies that can be traced to the weight measurement and/or film nonhomogeneity. These films clearly have a high affinity for water, with uptake rising almost exponentially in RH.

As demonstrated in Figure 6, the general appearance of the DSC thermograms depends strongly on RH. The curves are again shifted vertically along the y -axis by an arbitrary value. Exposure of a film to 13% RH increases the moisture content to 1.8% and lowers T_g by approximately 40°C. As expected, the same trend in T_g continues at higher RH. The transitions at 19% RH and 33% RH are broader than at other RH values, and a careful examination of the two relevant curves detects a weak exothermic fluctuation near the center of the transition range. Although the other data sets also display a weak exotherm (in these cases, however, immediately after T_g), their transitions remain sharp and pronounced, with clear onset and end points. Possible ambiguities in the

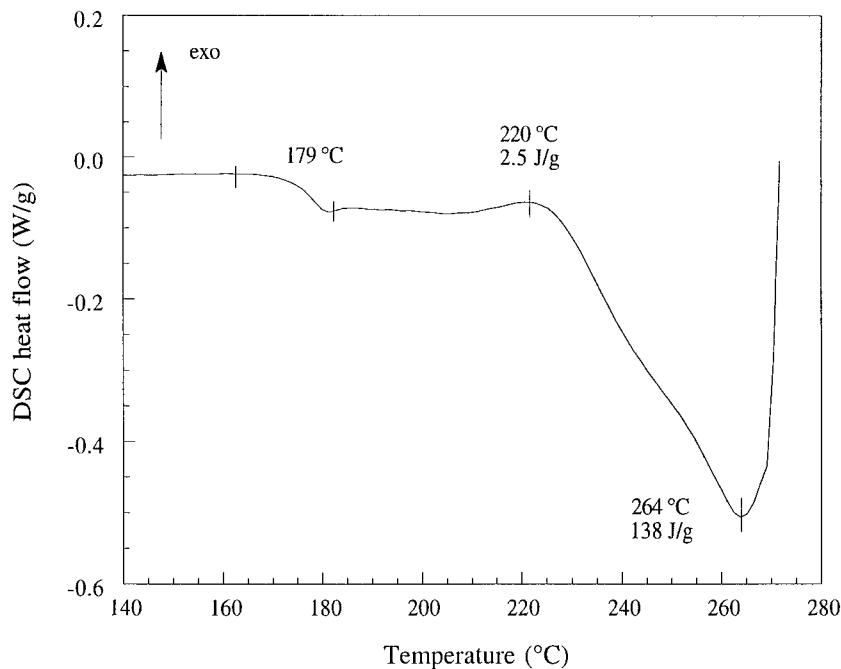


Figure 2 DSC thermogram of dry silk film at 10°C/min under nitrogen.

onset and end points, especially at the two troublesome RH values, account for the size of the T_g error bars in the next figure. The shapes of the thermograms are entirely reproducible, implying that the differences in thermogram shape displayed in Figure 6 can be traced solely to RH. Above T_g , the relatively high specific heat of water

explains the noticeable endothermic trend. Indeed, an endothermic peak can be discerned for the sample with the highest moisture content (20.5%, at 75% RH) due to evaporation of water. This sample possesses a T_g of 39°C, nearly 140°C lower than that of the dry sample.

A comparison of the experimentally observed

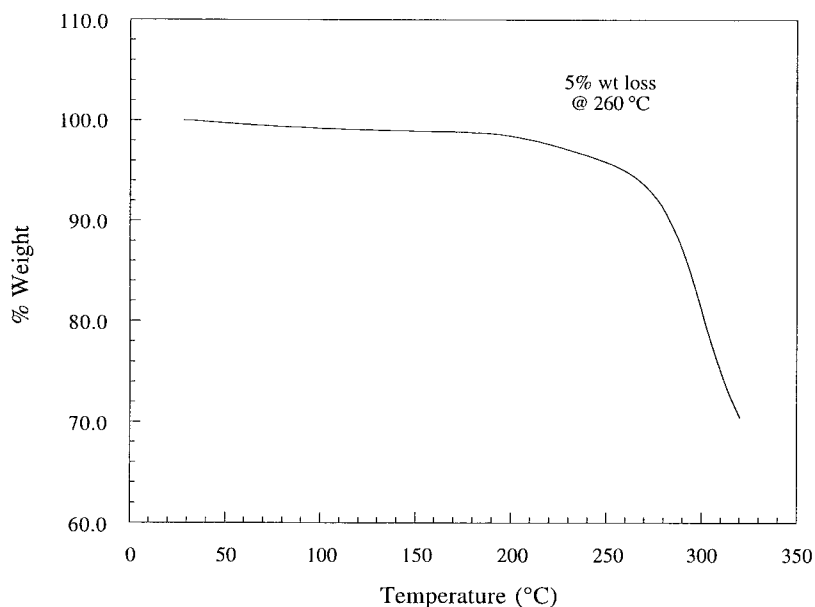


Figure 3 TGA thermogram of dry silk film at 20°C/min under nitrogen.

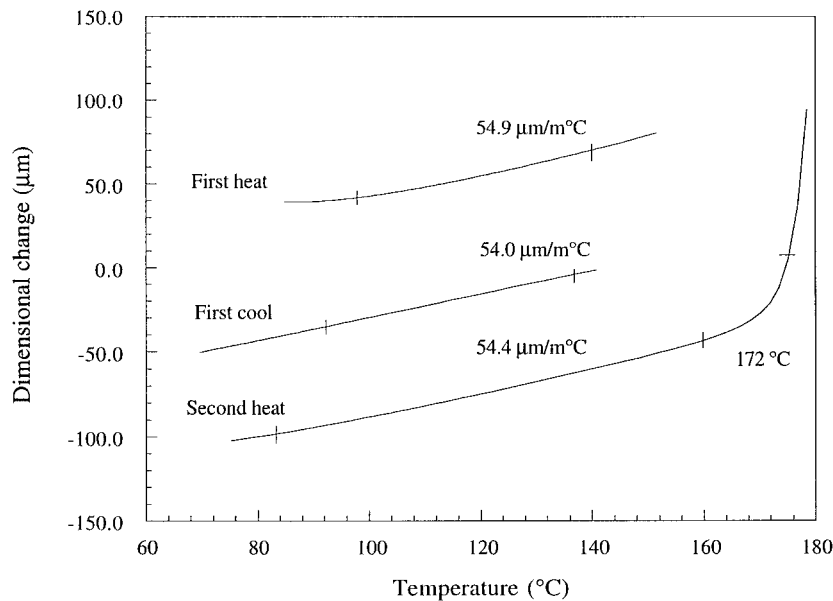


Figure 4 TMA thermogram of dry silk film at 10°C/min under nitrogen.

T_g behavior with that predicted from eqs. (1) and (2) is presented in Figure 7. To within experimental error, the data correspond reasonably well to the theoretical predictions of eq. (1) (curve A). As expected, eq. (2) (curve B) does not predict the experimentally observed results as adequately because of the large T_g difference between the two components. A closer examination of the data sug-

gests that T_g drops and then saturates with moisture content somewhat more rapidly than the Couchman and Karasz expression suggests. However, given the simplicity of the model and the wide range of moisture contents of the silk films, the overall accord between theory and experiment is quite satisfactory.

Effects of moisture on these samples are com-

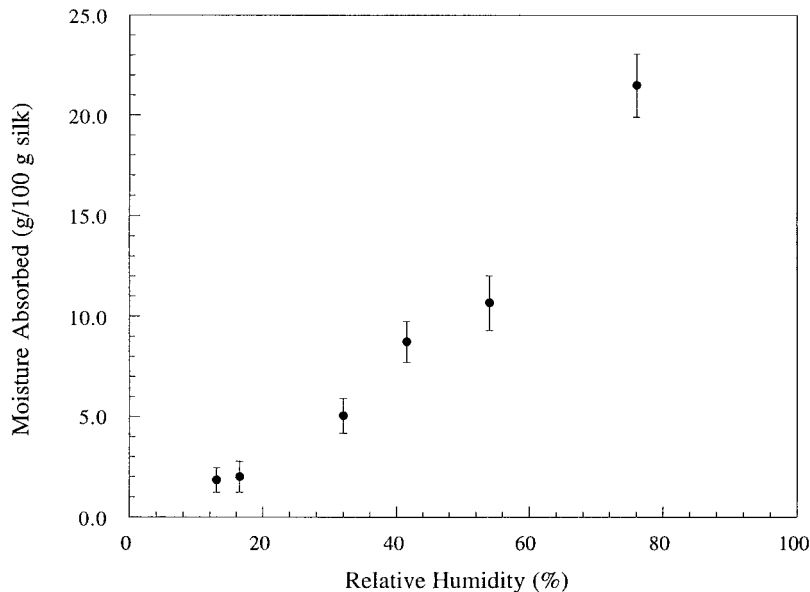


Figure 5 Moisture absorption isotherm for silk films under laboratory conditions: (23–26°C, 26% RH).

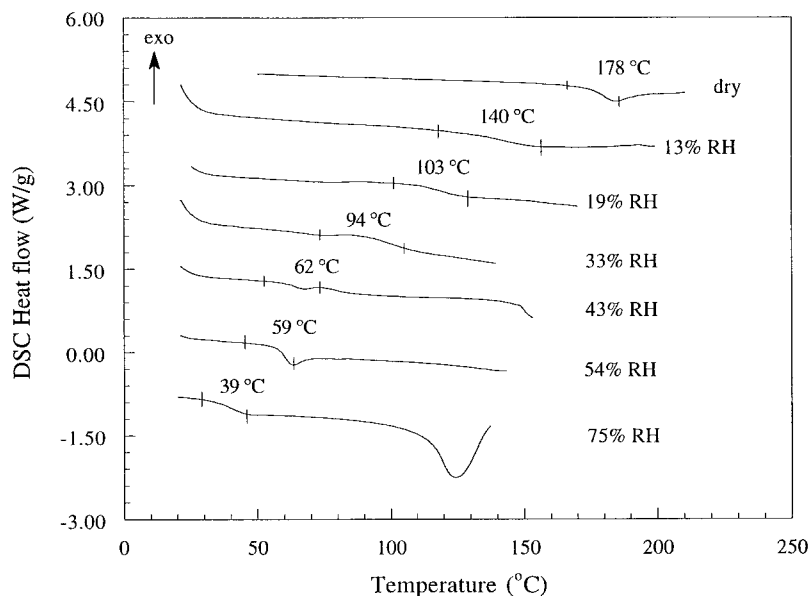


Figure 6 DSC thermograms of silk films exposed to different relative humidity (RH) conditions.

pletely reversible, as shown with DSC thermograms in Figure 8. The film exposed to 75% RH, a wet film, provides a T_g of 39°C and an endothermic water evaporation peak. A second sample of the same wet film, dried at 150°C for 2 h, exhibits a T_g close to that of the original dry film (175°C versus 178°C).

Figure 9 presents the TMA thermograms for a film exposed to 75% RH. Once again, individual thermograms are vertically translated for clarity. Substantial sample contraction results from the loss of moisture during the first heating to 150°C, yielding a negative overall thermal expansion coefficient as the water loss exceeds the normal

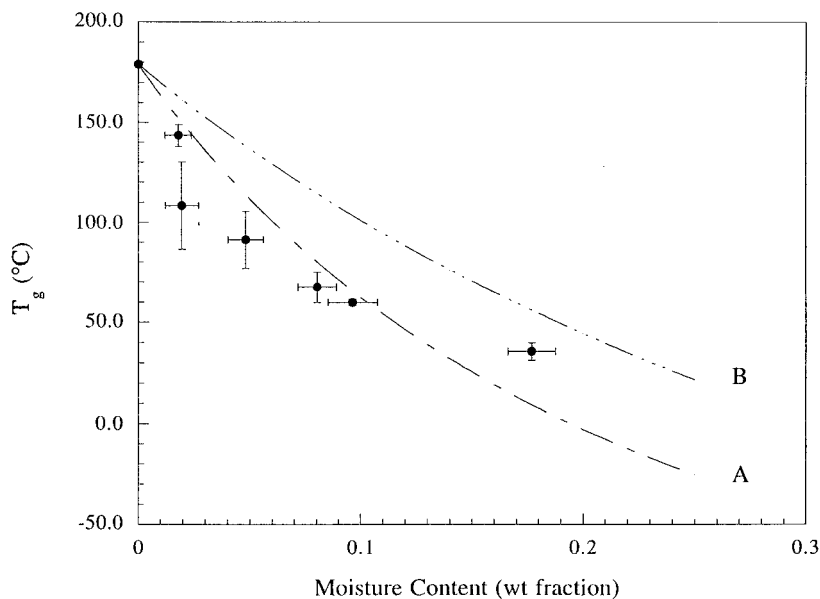


Figure 7 Effect of moisture absorption on the glass transition temperature (T_g) of silk films compared with theoretical predictions. Curve A: eq. (1); curve B: eq. (2).

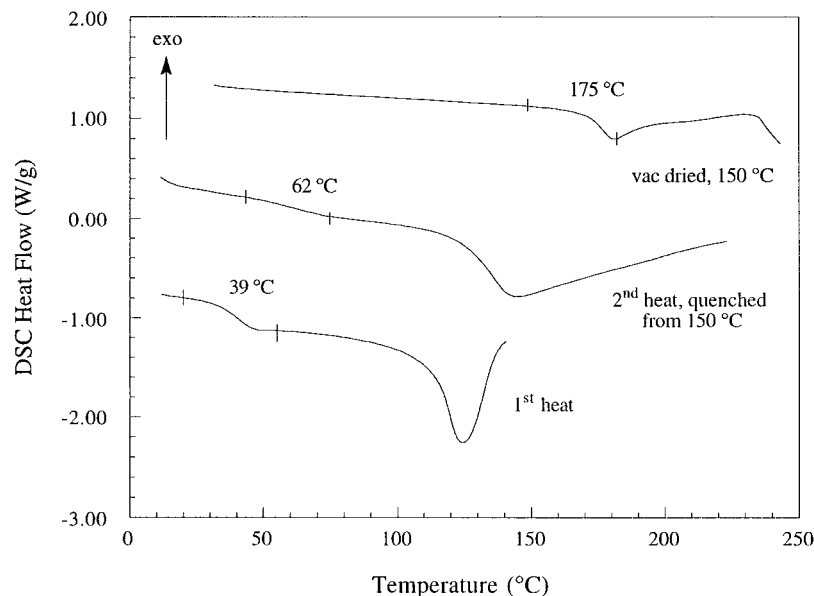


Figure 8 DSC thermograms of silk film exposed to 75% RH.

thermal expansion of silk. The measured expansion coefficient on a subsequent run becomes positive, reflecting the loss of water, and much smaller, decreasing to $46.1 \mu\text{m}/\text{m}^\circ\text{C}$ during a second heat to 150°C . The product film is essentially dry at this stage. However, to effect complete moisture removal, the sample can be held at this temperature for a longer period, up to 1 h. When

this fully dry sample is subjected to another cooling and heating cycle, the expansion coefficient is unchanged, as noted from the lower curve. During this third and final heating to 200°C , the expansion coefficient abruptly rises beyond 158°C . This rise confirms that the effect of moisture on thermal properties is completely reversible.

These experiments show that a significant, al-

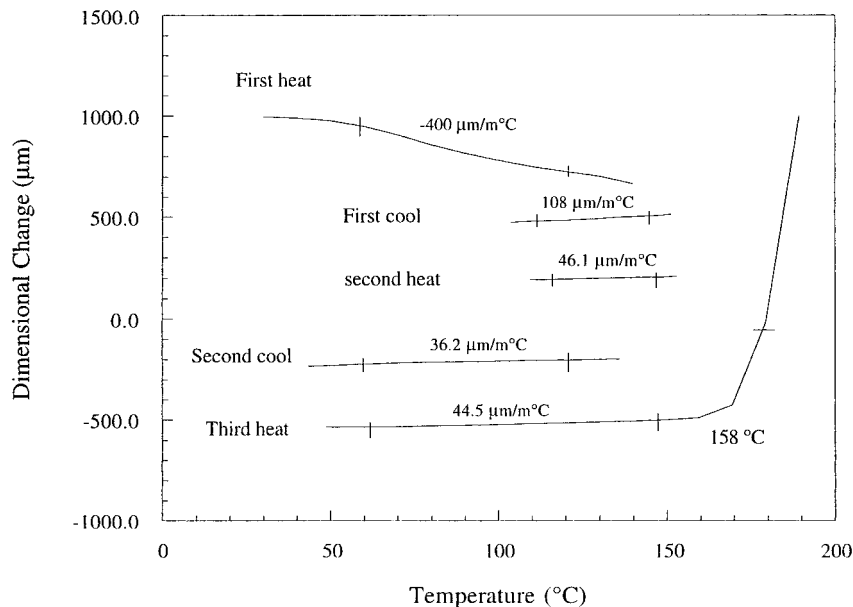


Figure 9 TMA thermograms of silk films exposed to 75% RH. Sample held at 150°C for 1 h after the second cooling cycle and then reheated a third time beyond the T_g .

beit entirely reversible, depression in T_g can be caused by the presence of moisture in silk films. The nature of the interaction between water and silk molecules remains unclear, since this study has measured only macroscopic film properties. Although there are clearly specific interactions in this system, and possibly disruption of hydrogen bonds by water, comparable studies on nylon-4, which contains polar moieties similar to silk, have shown that the role of water in T_g suppression is no different than that of a miscible diluent in a nonpolar polymer.¹⁷ All of these results are in accord with further studies^{14-17,26} that have concluded that the depressed T_g s of polymer-diluent systems can be fully explained by the constituent T_g differences regardless of specific interactions or structure. Morphological features, such as crystallinity and crosslinking, remain important only to the degree that they affect diluent distribution.^{17,26} Even in this case, the basic Couchman and Karasz expression needs little modification to explain experimental results. In the present study, morphological features remain unimportant as samples are always amorphous; care has been exercised to keep temperatures below those necessary to induce crystallization to the β -sheet structure.

These conclusions do not imply that T_g for moisture laden polymers can be properly explained without an accounting for the specific interactions of water with the matrix. Indeed, these types of interactions are responsible for the substantial moisture uptakes observed in hydrophilic polymers. These polymers generally provide generous opportunities for water to hydrogen bond with the matrix material. Nonetheless, the impact of these specific interactions is concentrated in the moisture uptake alone; knowing this uptake, the T_g behavior can be subsequently explained without further reference to specific interactions or even the existence of these interactions.

CONCLUSIONS

We have characterized the water-induced plasticization of regenerated *Bombyx mori* silk films, probing the depression of T_g with increasing moisture content. These films have a strong affinity for moisture, and the final moisture content is as high as 20–23% (by weight) at 75% relative humidity. The reversible depression of T_g can be adequately explained by a classical thermody-

amic theory, indicating that the plasticization effects need not be ascribed to specific interactions between water and silk molecules once the equilibrium moisture level is ascertained.

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