

Structural Modifications in Bivoltine Silk Fiber Under Thermal Treatment

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SYNOPSIS

The microstructural changes of semicrystalline bivoltine silk fibers annealed between 27 to 260°C were measured using positron annihilation lifetime spectroscopy (PALS). Measured lifetime and intensity as a function of temperature reveals the removal of water molecules until 60°C. Present results indicate a T_g of 140°C, which is further confirmed by differential scanning calorimetry (DSC). The structural changes like random-coil to alpha and then to beta form are observed in the crystalline and amorphous domains at 100 and 205°C, respectively. Degradation of the fibers occur around 240°C. As an extension of the present measurements, we have estimated the activation energy of 8.0 KJ/mol in the disordered domain of the fiber, based on Goldanskii's kinetic relations. © 1997 John Wiley & Sons, Inc.

INTRODUCTION

Natural polymers like silk fibers are a continuous high molecular weight fibrous protein consisting of many kinds of amino acids. These are produced by different species of silkworms, of which the principal species is *Bombyx mori*, or the mulberry silkworm. It has been shown that, depending on the conditions of the sample preparation, silk fiber in solid state can exist in the following three different conformations: i.e., alpha form (silk I),^{1,2} random-coil,³ and beta-form (silk II).^{1,2} Silk I and silk II are the crystalline phases, whereas the random-coil conformation refers to the amorphous phase. A recent study indicates that random-coil is not wholly amorphous but a mixture.⁴ From the application point of view, these are excellent biomaterials for enzyme-immobilized membranes used as biosensors. This property is due to the insolubility of the silk and immobilization of enzyme. These factors are mainly due to the conformational transition of the silk fibroin from water-soluble random-coil to water insoluble silk II in

the absence of any chemical reagents.⁵ Differential scanning calorimetry (DSC) and X-ray diffraction (XRD) measurements have shown that the beta form can be obtained from the random-coil form when the latter is dried after immersion in polar solvents like methanol.⁶ This transformation can also be effected by drawing the film or by the heat treatment of the solid.⁷ The nuclear magnetic resonance (NMR) technique has also been extensively used in the study of silk fibers, but a recent study reflects that it is not sensitive enough to detect the difference between silk I (alpha form) and random-coil conformation as there is no difference in the measured chemical shifts for the two conformations.⁸

In the present investigation, we have made use of the positron lifetime technique to look into the structural changes in terms of conformational transformations in silk, particularly bivoltine fibers (a popular Indian variety) for the first time. Positron lifetime measurement is a powerful technique in comparison to other conventional methods, mainly because of its sensitivity to crystalline defects and free volume holes. Furthermore, lifetime measurement also provides the glass transition temperature (T_g) of the material under investigation.^{9,10}

When positrons enter a condensed medium,

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from a radioactive source, they thermalize in a very short time and then annihilate via a free annihilation or from a trapped state or from a bound state called the Positronium atom (Ps). Depending upon the spin of the composite particles, Ps can exist in two forms: para-Ps and ortho-Ps. In materials like polymers, if Ps is formed, it annihilates through a fast channel (*o*-Ps pick-off process). Each of these have a characteristic lifetime. *o*-Ps lifetime is an important parameter as it is related to free volume holes.¹¹ Positron lifetime results on semicrystalline polymer PEEK proves beyond doubt that Ps is formed and annihilates in the free volume holes present in the amorphous domains.¹²

According to Goldanskii's kinetic theory of positron annihilation, positron and Ps in a polymer matrix selectively probe the crystalline and amorphous domains, respectively.¹³ Using this concept, we expect that the lifetime results as a function of temperature would detect the conformational transitions more clearly, and heat treatment can be used to effect the conformational changes. With these ideas in view, we have investigated the silk fiber bivoltine using positron annihilation lifetime spectroscopy (PALS) and DSC as a function of annealing temperature. These results are presented and discussed in the light of available information on silk fibers.

EXPERIMENTAL

Bivoltine silk fiber strands were wound on a centrally holed (1.5 cm diameter) aluminum sheet to a thickness of 1.5 mm. Two of this kind are used for positron lifetime measurements in the sandwich geometry. The annealing of the samples was done using a tubular furnace with temperature controller having an accuracy of $\pm 1^\circ\text{C}$, and the samples were quenched into the ice water to preserve its structure. XRD measurement showed that the silk fiber is not fully amorphous. This indicates that the fibers have random-coil conformation in conformity with the recent prescription.⁴ Lifetime spectra were measured using a fast-fast coincidence system having a time resolution of 340 ps. All spectra contained a minimum of one million counts. The details of the experimental procedure can be found in one of our earlier communications.^{9,10} A ^{22}Na source of strength 15 μCi deposited on a kapton foil was used as a positron source. Using the computer program RESOLUTION, the source correction terms and

the instrumental time resolution were obtained.¹⁴ The accumulated spectra were analyzed with PATFIT-88.¹⁴ The DSC measurement was done using Mettler DSC system (Model No. FP 900).

RESULTS AND DISCUSSION

All the measured spectra were resolved into three lifetime components, which yielded better chi square values compared to two- and four-component analysis. The attribution of different lifetime components to various states of positron annihilation is similar to the one described earlier.^{9,10} The first lifetime τ_1 (with intensity I_1) is due to the contribution from free annihilation and *p*-Ps self annihilation. The origin of the second lifetime component τ_2 (with intensity I_2) is related to annihilation of positrons trapped at the defects in the crystalline domains. The third longest lifetime component τ_3 (with intensity I_3) is attributed to annihilation of *o*-Ps in the free volume holes in the amorphous domains of the fiber. The *o*-Ps lifetime is related to the radius of the free volume hole size according to a model first developed by Tao for molecular liquids¹⁵ and later modified by Nakanishi et al.¹² for polymers. This relation can be written as follows:

$$\tau_3^{-1} = 2[(1 - R/R_o) + 0.159 \{\text{Sin}(2\pi R/R_o)\}] ns^{-1} \quad (1)$$

where R is the free volume hole radius and $R_o = R + \Delta R$ with $\Delta R = 0.1656$ nm. By fitting the above equation with the measured τ_3 values, we have evaluated R , and the free volume size is calculated as $V_f = (4/3)\pi R^3$. The fractional free volume (F_v) can then be calculated as follows:

$$F_v = CV_f I_3 \quad (2)$$

where C is a structure constant that can be evaluated by using the coefficient of thermal expansion.⁹ The value of C for silk fiber turns out to be $191 (\text{\AA})^{-3}$.

In Figure 1(a,b), the second lifetime component and its intensity are plotted as a function of annealing temperature, respectively. Figure 2(a,b) shows the variation of *o*-Ps lifetime and its intensity as a function of annealing temperature, while Figure 3(a,b) depicts the variation of free volume and fractional free volume. Earlier experiments on silk fibers have shown that water

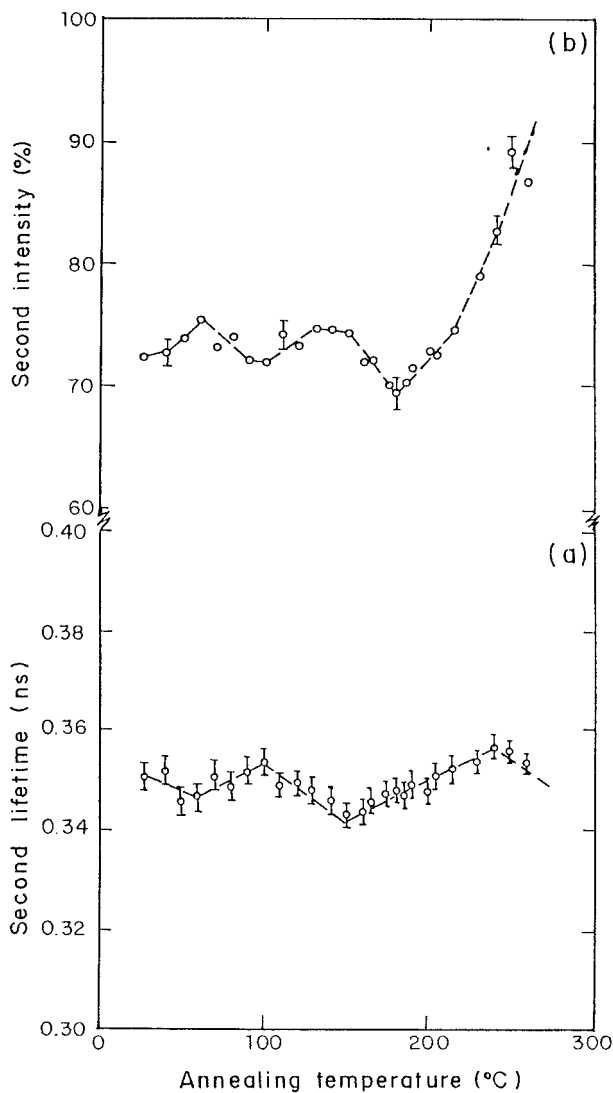


Figure 1 Positron lifetime τ_2 and its intensity I_2 as a function of annealing temperature. Dashed lines drawn through the points is to guide the eye.

molecules are mostly embedded in the amorphous domains of the fiber matrix and its removal results in the contraction of the fiber.¹⁶ The contraction of the fiber leads to different effects on the amorphous and crystalline domains. In the light of these observations, the present results can be interpreted as follows: With the increase in annealing temperature, removal of the water molecules takes place. As it can be seen from Figures 1(a) and 2(a), τ_2 and τ_3 show an opposite trend in their variation. If the water molecules are removed from free volume holes in the amorphous domains, we expect an increase in the average free volume size and, hence, an increase in the *o*-Ps lifetime during this initial stages of annealing.

Figures 2(a) and 3(a) support this, and it is also clear that this process continues until 60°C. Secondly, this contraction of the fiber has an effect on the free volume such that some of the free volume holes coagulate to yield bigger size free volume holes; and, as such, the number density of these holes decreases, which is reflected in Figure 2(b).

The presence of water molecules in the amorphous domains gives rise to strain in the crystalline domains.¹⁷ When the removal of water molecules starts, the strain on the crystalline domains will be withdrawn, and the molecular chains get some freedom to rearrange. This rearrangement may lead to the splitting of large size crystalline defects to smaller size defects, which should result

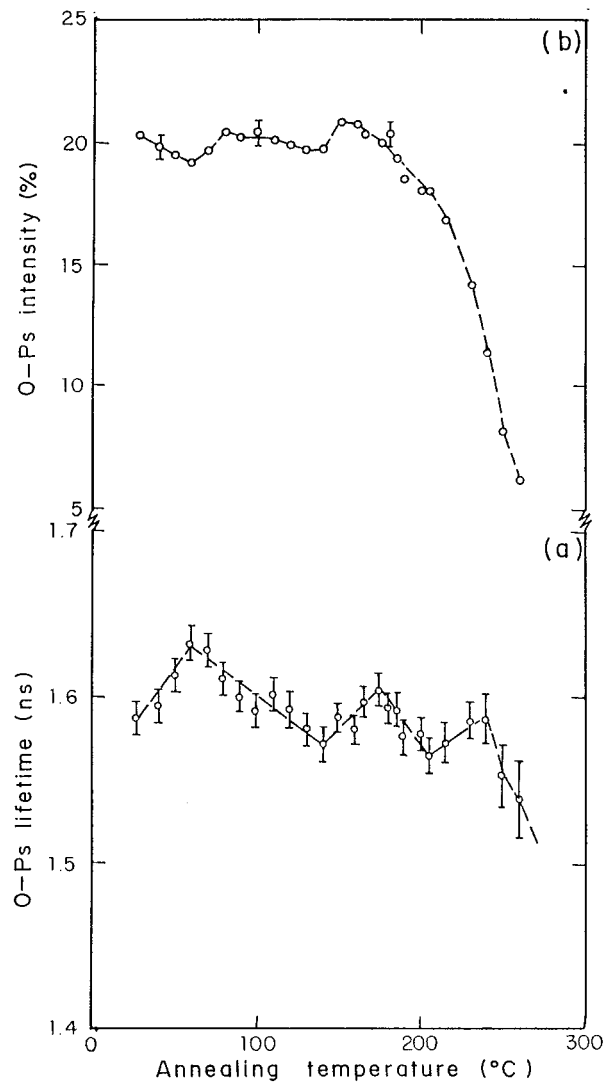


Figure 2 *o*-Ps lifetime τ_3 and its intensity I_3 as a function of annealing temperature. Dashed lines drawn through the points is to guide the eye.

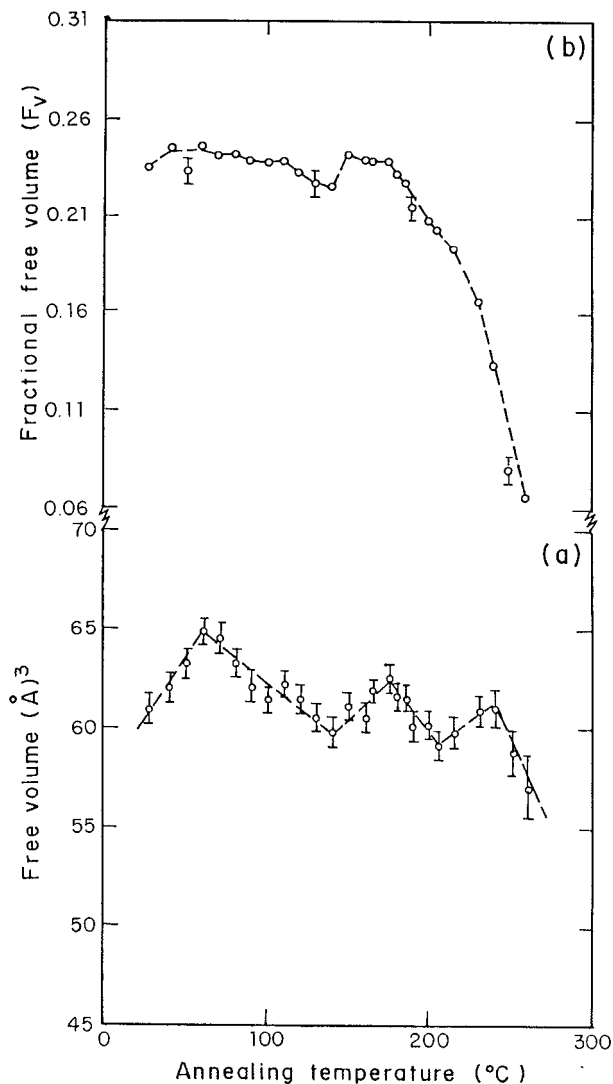


Figure 3 Average free volume size V_f and fractional free volume F_v as a function of annealing temperature. Dashed lines drawn through the points is to guide eye.

in a decrease of τ_2 and an increase in I_2 . This is what we are observing until 60°C in Figure 1.

After 60°C, the *o*-Ps lifetime (τ_3) and free volume (V_f) [Figs. 2(a) and 3(a)] begins to decrease, whereas I_3 [Fig. 2(b)] remains constant in this region. Normally, in a polymer, the glass transition is characterized by the onset of cooperative motion of the segments of the molecular chains, more in the amorphous domains. Below the glass transition temperature, only limited local motions of a small fraction of the molecules are possible.¹⁸ In the present case, it appears that this type of motion actually starts from 60°C, and at each annealing temperature, some free volume holes allows the molecular segments to diffuse into

them leading to a reduction in the average free volume size.

In the crystalline domain due to this type of limited local motion, some of the defects may coalesce, leading to an increase in τ_2 value and a decrease in I_2 . Above 100°C, the random-coil conformation seems to change into a mixture of alpha and beta form.⁷ NMR studies have shown that the alpha form (silk I) exists in loose helix structure and is less ordered compared to more ordered beta form. In the alpha form, the helical chains may get twisted in a manner such that the average crystalline defects size becomes smaller, and their number increases. In accordance with this, we observe a decrease in τ_2 and an increase in I_2 in this temperature region.

At 140°C, the *o*-Ps lifetime, its intensity, and free volume changes slope and starts increasing. Even τ_2 and I_2 also indicate a change in slope at 140°C. The mean lifetime ($\bar{\tau}$) and fractional free volume (F_v), which remained constant until 140°C, also shows an increase at this temperature. All these variations indicate that the T_g of this silk fiber is 140°C. The DSC scan shown in Figure 6 supports this secondary transition.

After T_g , the segmental motion of the fiber increases. This leads to increase in the average free volume size, and hence a raise in τ_3 is observed. The increased molecular motion of the fibroins above T_g was interpreted as due to the intramolecular and intermolecular hydrogen bond dissocia-

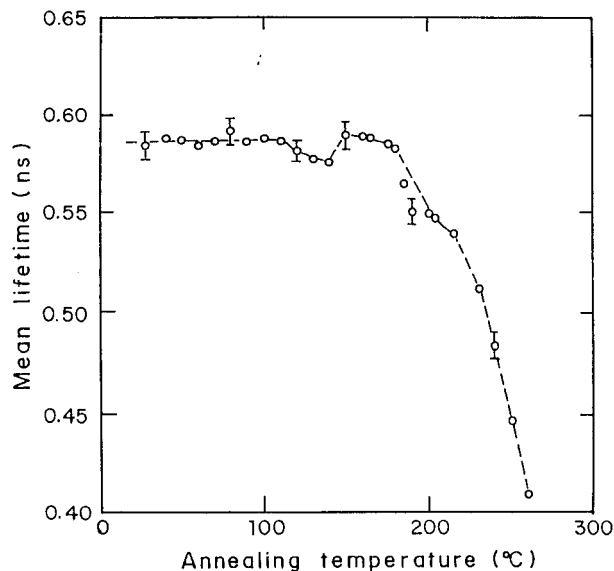


Figure 4 Mean lifetime ($\bar{\tau}$) as a function of annealing temperature. Dashed lines drawn through the points is to guide eye.

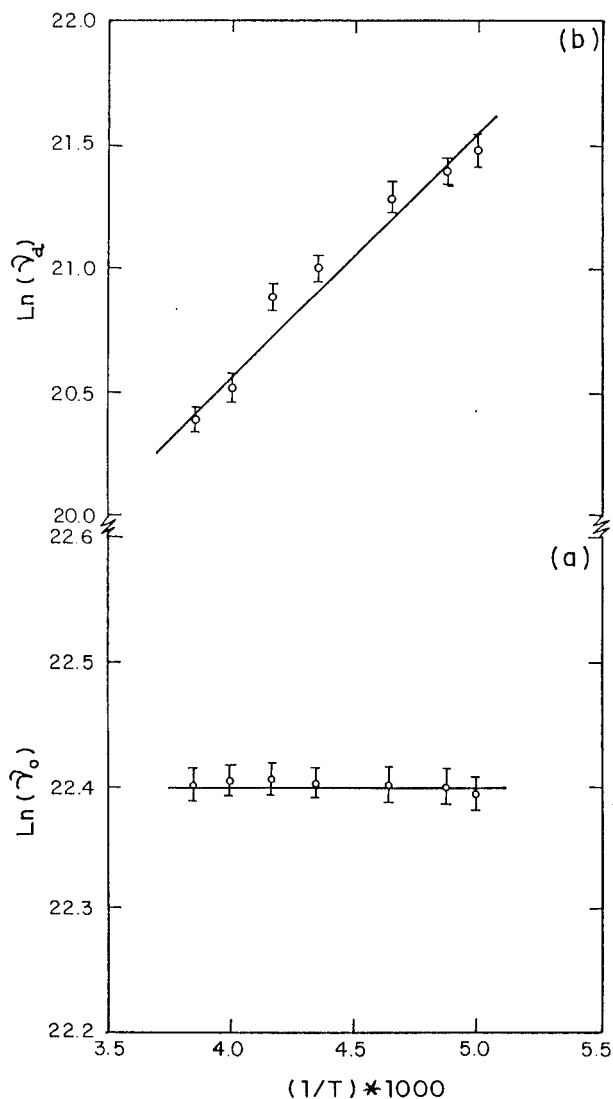


Figure 5 Positron trapping rate (ν_o) and positronium trapping rate (ν_d) as a function of $(1/T)$. Solid line drawn is the linear fit to the experimental points.

tion in the random-coil structure.⁷ Above 175°C, the conformational change is towards a more orderly beta form. This ordering results in the decrease of average free volume size, and hence τ_3 value decreases. This process continues until 205°C, indicating the completion of thermally induced crystallization to the beta form. This is very well reflected by the rapid decrease in I_3 and increase in I_2 .

Beyond 240°C, thermal degradation of the fiber takes place as reflected by the annihilation parameters. The DSC scan (Fig. 6) also shows two transitions at 205 and 240°C, supporting the lifetime results in respect of these changes.

As it can be seen from Figure 4, the mean lifetime ($\bar{\tau}$) remains almost a constant up to T_g ; thereafter, it shows a change. This reveals that the transformation from random-coil to alpha (silk I) does not affect the overall fiber structure, whereas the transformation to silk II affects the fiber structure very much as evidenced by the dramatic decrease in the mean lifetime value. The interesting point here is that the transformation from random-coil to alpha form is visible in the crystalline part of the silk matrix but not pronounced. Interestingly, the conformational change to beta form is visible in the amorphous part more predominantly.

In a previous study,⁹ we have shown that the activation energies in the ordered (E_o) and disordered (E_d) domains of a polymer matrix can be evaluated. This can be done by calculating the trapping rates for the ordered (ν_o) and disordered (ν_d) domains using the kinetic equations of Goldanskii et al.¹³ For the present case, the Arrhenius plots of trapping rates as a function of $1/T$ for the ordered and disordered domains are shown in Figures 5. From these figures, it is clear that E_o is almost zero and $E_d = 8.0$ KJ/mol. This activation energy corresponds to the temperature region above T_g . In this high temperature region, earlier investigations support the present results to infer that there occurs a conformational change to more orderly beta form.⁷ This highly ordered structure results through hydrogen bond dissociation, which needs an energy of roughly around 8.4 KJ/mol. The activation energy of 8.0 KJ/mol obtained

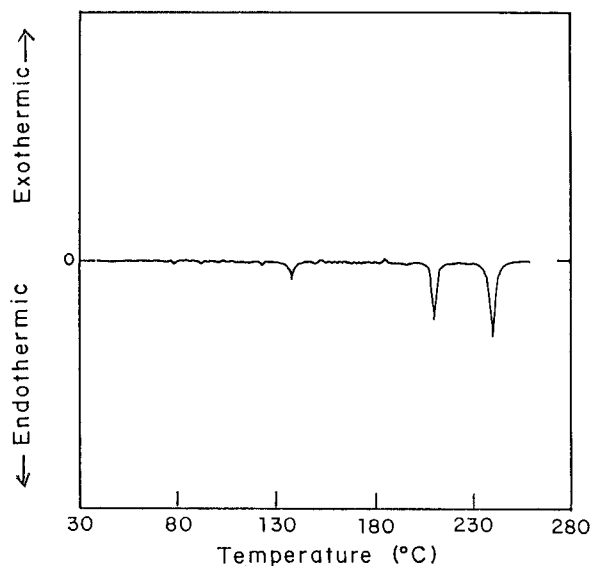


Figure 6 DSC curve of bivoltine silk fiber.

in the present case is comparable. Since this energy corresponds to the amorphous domains, we conclude further that hydrogen bond dissociation takes place mainly in the amorphous domains as the measured activation energy in the crystalline domain of the matrix is almost zero.

CONCLUSIONS

The thermal response of the *o*-Ps lifetime and its intensity for the bivoltine silk fibers are interpreted in terms of conformational changes in the fiber matrix. Present positron and DSC results measure the glass transition temperature (T_g) as 140°C. The conformational change from random-coil to alpha form has a mild effect on positron lifetime and its intensity in the crystalline domains. By contrast, the conformational change to beta form has a pronounced effect on the free volume number density rather than its size in the amorphous domains, indicating increased segmental mobility associated with this transition. Further, the activation energy of 8.0 KJ/mol determined for the disordered domain is comparable to hydrogen bond dissociation energy. The zero activation energy corresponding to the ordered domain indicates no such bond dissociation.

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