Using Flash DSC for Determining the Liquid State Heat Capacity of Silk Fibroin

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Abstract

In this technical note, we report a study of the heat capacity of amorphous silk fibroin protein evaluated using the Flash DSC1 for fast scanning calorimetry. Nineteen amorphous thin films were obtained either after casting directly from water solutions, or after melting of previously crystalline films. Fibroin films were mounted onto Flash DSC1 sensors, dried free of bound water, relaxed just above the glass transition, T_g , then scanned in heating and cooling at ± 2000 K/s. The heat flow rate data were analyzed by finding the symmetry line between the heating and cooling scans, and by subtraction of the empty sensor heat flow rate, similarly corrected for its symmetry line. To evaluate the sample mass, two approaches were compared. First, the mass was obtained from known solid state heat capacity, $C_p^{solid}(T)$, at a temperature below T_g . Second, the mass was obtained from the known heat capacity increment at the glass transition, $\Delta C_p(T_g)$. The $C_p^{solid}(T)$ and $\Delta C_p(T_g)$ had been obtained previously from slow scanning differential scanning calorimetry. The use of either method for mass determination necessitated additional corrections to the heat capacity data to bring them into agreement with the literature values. After these corrections, the heat capacity of silk fibroin in the liquid state was evaluated over a wide temperature range above T_g . We find $C_p^{liquid}(T) = (1.98 \pm 0.06) \text{ J/gK} + T \cdot (6.82 \pm 1.4) \times 10^{-4} \text{ J/gK}^2$ in the temperature interval from 510 to 570 K with an uncertainty of about $\pm 5\%$.

Keywords: Fast scanning calorimetry; Flash DSC1; Silk fibroin protein; Liquid state heat capacity

1. Introduction

For non-adiabatic fast scanning calorimeters, operating at scan rates reaching 10^6 K/s, temperature calibration is reasonably well established [2-8] and results in uncertainties of ± 1 K at rates of 1000 K/s. At higher rates uncertainty increases slightly and reaches ± 10 K at 10^6 K/s. Uncertainty for the caloric measurements with non-adiabatic chip calorimeters, contrary to the quasi-adiabatic systems [1] are, to the best knowledge of the authors, not yet available. This is mainly due to the uncertainty of the primarily unknown, and indirectly determined, sample mass. For a specially constructed differential power compensated calorimeter using thin film chip sensors XI-296, Zhuravlev and Schick have estimated the uncertainty of specific heat capacity at 1000 K/s as $\pm 10\%$ [2,3]. All problems known from two-dimensional heat flux differential scanning calorimeters, DSCs, like heat flows to the surroundings not recognized by the measuring system [9], apply also to these calorimeters. They cannot be corrected for by a measurement of an empty sensor, as would be done by measuring an empty pan in conventional slow scan DSC, because these heat losses depend on the sample itself.

Additional difficulties arise from the nature of the sample. Sometimes the very first heating scan is the most important, because the sample changes irreversibly during heating and the initial state cannot be restored during cooling. Such samples include those which melt once and tend not to crystallize from the melt upon cooling (*e.g.*, silk fibroin protein [10]), samples which crosslink during heating (*e.g.*, epoxies [11]), or those losing mass during the scan as occurs during dehydration of lipids [12]. Other samples can only thermally crystallize upon holding above T_g for a long time, at which point they degrade (*e.g.*, sucrose [13], silk fibroin [14], resilin protein [15]). There are also special sample forms which are changed by heating and must be examined on the first scan only, including, for example, PLA electrospun fibers [16].

In all these cases, the sample mass cannot be found from a first heating to the liquid state above the melt (or above T_g for non-crystallizable materials) for comparison with C_p^{liquid} . In the case of materials for which the liquid state heat capacity data are unknown, or when the sample loses mass during heating above T_g due to degradation or removal of bound water [17-19], alternative methods must be used to determine the sample mass.

Fibrous proteins are classic examples where the secondary structure formation occurs upon lyophilization [19], exposure to solvent, water vapor [20], or by heating above T_g [21]. But once melting has occurred, the sample will not reform the secondary structures using heat treatment alone, because of the competition between crystallization and degradation processes. Crystallization above T_g by thermal means is slow, whereas degradation is fast. Use of fast scanning calorimetry is therefore an important advance in the study of these proteins [10]. The very fast heating rates allow the secondary structural transitions to be investigated without thermal degradation, a deleterious process that prevents observation of melting behavior in conventional temperature modulated instruments (TMDSCs) operating at slow rates. We have already shown that silk fibroin protein beta sheet crystals can be melted by very fast heating [10] (or also, by exposure to strong acids such as LiBr [14]), but crystals do not reform during cooling. In a purpose built chip calorimeter, heating at 2000 K/s was sufficient to avoid degradation and allow melting to be observed [10]. After the sample was melted, thermal means could not be used to restore a crystalline state. Only upon exposure of the now-disordered silk to MeOH would the sample recrystallize. Use of materials which can only be examined once in heating creates a situation in which great care must be taken in the sample preparation and the determination of the sample mass.

Here, we return to the study of silk fibroin protein using the Flash DSC1 commercial instrument, but concentrating on samples that are non-crystalline, either by virtue of their sample preparation or because they had been once-melted. We want to: 1. test the function of the Flash DSC1 at the same heating rate used before in the purpose-built calorimeter; and, 2. use this instrument to extend the range of the fast scanning as far as possible above T_g to establish the heat capacity in the liquid state over a wider range of temperature than had been possible before using slow scan DSC [22]. The challenges of this study include existence of about 5 wt. % bound water in silk, an unknown liquid state $C_p(T)$ (the previously published estimate was deduced for a short range of T only), unknown sample mass, and the necessity of obtaining high quality data on the first scan to high temperature.

2. Materials and Methods

2.1 Sample and Sensor Preparation

Silk fibroin protein was extracted from the cocoons of the *Bombyx mori* domesticated silk worm, using methods published previously [23]. A variety of fibroin thin films was prepared including amorphous films cast directly from water solutions, and semicrystalline films prepared by: 1. annealing in water at 25 °C (298 K) for 12 h; 2. annealing in water 37 °C (310 K) for 3 h or 12 h; 3. exposure to methanol (MeOH)-water solution (50:50 v:v) at 25 °C (298 K) for 15 h; or, 4. autoclaving at 120 °C (393 K) for 1 h. Several parent films of each type (about 2.5 cm x 2.5 cm) were prepared and weighed, and the areal densities calculated. Based on an assumed density of 1.348 g/cm³ for silk fibroin [24], we used the areal densities to estimate the film thicknesses which ranged from 6.6 to 10.4 μm. Recently, the importance of small film thickness was discussed by both Toda and Konishi [25] and Schawe [26]. With film thickness not

exceeding 10 μm the samples are suitable for measurements at rates of 2000 K/s. The treatments and areal densities of the films are listed in **Table 1**. Small samples were cut from the parent for placement on the Flash DSC1 sensors. Optical microscope images were taken on an Olympus BX41 microscope with 10x lens and DCM 510 camera in reflection mode before and after thermal scanning. The initial state of crystallinity was judged using Fourier transform infrared spectroscopy performed on larger pieces of the same samples. In Table 1, all samples except the As Cast were initially crystalline.

Scans were performed under an inert atmosphere of nitrogen with flow rate about 50 ml/min. The empty sensor was conditioned according to the manufacturer's procedure four or five times. The temperature of the ceramic support was set at 298 K. The empty sensor was scanned by heating and cooling at ± 2000 K/s to 450 K. Next, using a fine wire, a small droplet of low viscosity silicon oil was placed on the sample side of the sensor. Finally, the sample film of silk was mounted in the center of the sensor, on top of the silicon oil.

Table 1 goes here

2.2 Scan Procedure

The sample was heated at 2000 K/s to 440 K (167 °C), which is below the glass transition temperature of dry silk, 451 K (178 °C) [27, 28], and held there for 30 min to remove bound water [29,14]. At the same heating and cooling rate of 2000 K/s, the sample was heated to above the glass transition to remove the enthalpy relaxation peak which obscured the glass transition heat capacity step. The sample was exposed to a series of increasing temperatures ranging from 520 K to 533 K (247 °C to 260 °C), stopping when a clear glass transition step was observed

without any enthalpy relaxation peak. Finally, the sample was heated and cooled repeatedly at 2000 K/s from 343 K (70 °C) to temperatures in the range from 623 K to 653 K (350 °C to 380 °C) for observation of any thermal events occurring above T_g .

2.3 Data Analysis

The heat capacity is determined from heat flow rate, using a symmetry line correction to account for the heat losses during the heating and cooling scans. For this correction it is assumed that the heat losses are only temperature dependent [30, 31, 2, 3]. They should be the same for heating and cooling and independent of any transition in the sample. For temperature ranges outside any transition in the sample the distance between the symmetry line and the measured heat flow rate at cooling and heating is then a measure of sample plus addenda heat capacity.

The outcome of the symmetry line correction is to place the heating and cooling scans symmetrically about the x -axis (temperature axis). The symmetry line is found using the following approach [2,3]. The sample's glass and liquid heat flow curves, away from T_g , are fitted in first order. The fitted lines are used to construct the symmetry line, which is the arithmetic average of the fitted heating and cooling heat flow data, yielding two sections to the symmetry line, *i.e.*, below and above T_g . The entire symmetry line is then fitted in third order, interpolating through the region of T_g to connect the two sections, and then the heating and cooling heat flows are corrected by subtracting the symmetry line. The empty sensor heating and cooling scans, at the same rates, are separately corrected for their symmetry line to take care of the addenda heat capacity. The resulting corrected empty sensor heat flows are subtracted from the corrected sample heat flows. This correction is small since the addenda heat capacity of the UFS1 sensor is about 1.7×10^{-8} J/K while the sample heat capacity is $\sim 2.9 \times 10^{-7}$ J/K (estimated in the glassy state at 144 °C). Uncertainties regarding addenda heat capacity resulting from changes

of the temperature distribution in the sensor due to the presence of the sample are assumed negligible. Finally, symmetry-corrected and empty sensor-subtracted sample heating and cooling heat flows (in units of Watts) are converted to heat capacities, $C_p^{meas}(T)$ in J/K, using the heating or cooling rate (± 2000 K/s).

Sample mass is estimated using two approaches. First, the solid state mass, m_{ss} , was calculated from the ratio of the heat capacity (units of J/K) found in the fast scanning technique, to the specific heat capacity (units of J/gK) determined from slow scanning calorimetry:

$$m_{ss}(T) = C_p^{meas-solid}(T) / C_p^{solid}(T) \quad (1)$$

where we use:

$$C_p^{solid}(T) = 0.134 \text{ J/gK} + T \cdot (3.7 \times 10^{-3}) \text{ J/gK}^2 \quad (2)$$

a relationship previously determined from slow scan temperature modulated differential scanning calorimetry, TMDSC [14, 31]. The temperature chosen for the evaluation of the mass is 420 K (147 °C), which is well below the glass transition region observed in the silk samples during fast scanning. An alternative method would be to use the theoretical vibrational heat capacity curve [32] in the mass determination. At 420 K, the vibrational heat capacity, C_p^{vib} , is smaller than C_p^{solid} from Eqn. (2) by less than 0.3%. At 450 K, the difference increases with C_p^{vib} smaller than C_p^{solid} by 0.8%. The choice, to use C_p^{solid} rather than C_p^{vib} , introduces negligible error for determination of m_{ss} and was made to facilitate later processing steps. We note parenthetically that the difference between C_p^{solid} and C_p^{vib} will increase with temperature, and C_p^{vib} should be preferred especially if the temperature range of interest is extended from 500 K to 650 K.

The second approach to finding the mass is to use the heat capacity increment at the glass transition temperature, $\Delta C_p^{meas}(T_g=451 \text{ K})$. To obtain this value, the measured fast scanning solid and liquid heat capacity data were fitted linearly, and extrapolated to the glass transition

temperature, $T_g = 451$ K, determined from slow scan TMDSC [14,22]. The mass, m_Δ , was estimated from:

$$m_\Delta = \Delta C_p^{meas}(T=451 \text{ K}) / \Delta C_{p0} \quad (3)$$

where $\Delta C_{p0} = 0.478$ J/gK for 100% amorphous silk film at 451 K [14,22].

After the mass was evaluated, further corrections to the specific heat capacity data include matching the specific heat capacity at temperatures below the start of the glass transition relaxation process to the linear C_p^{solid} relationship given in eqn. (2).

3. Results and Discussion

Images of silk fibroin samples on the Flash DSC1 sensor are shown in **Figure 1a,b** for silk film water-annealed for 12 h at 310 K (37°C) and **Figure 1c,d** for silk film exposed to 50% methanol-water for 15 h at room temperature. Before heating, both films are semicrystalline (Fig. 1a, c) while after heating at 2000 K/s they are both amorphous (Fig. 1b, d). The water-annealed sample was heated a total of three times to 623 K (350 °C) and after heating (Fig. 1b), the sample maintains its overall shape, and the roughness of the cut edges has been smoothed after melting. Slight discoloration can be seen. The methanol-water treated sample was heated twice to a higher temperature of 653 K (380 °C). Now the sample (Fig. 1d) has undergone degradation, as shown by the brown coloration and appearance of bubbles. We found that heating silk in the Flash DSC1 to 623 K (350 °C) resulted in minimal changes in the sample, whereas degradation set in at about 643 K (370 °C), and was severe when the sample was heated to 653 K (380 °C) even though the total exposure time above 643 K was only 20 ms. In prior studies of silk using a purpose built calorimeter [2,3], silk samples were able to be heated under nitrogen protection gas to 650 K (377 °C) with minimal or no degradation [10]. In the prior study, a vacuum tight system

was used to enclose the sample. First, the sample was pumped to remove any air and back filled with pure nitrogen, and this procedure was repeated three times. Compared to the continuously purged Flash DSC1, the residual oxygen content in the gas was probably lower in the purpose built calorimeter which could account for the reduced degradation of the samples in the prior study.

Figure 1 goes here

Figure 1. *Optical microscopy images of silk fibroin samples on the sensor before (a, c) and after (c, d) heating at 2000 K/s. The diameter of the heated area (light circle) of the sensor for the Flash DSC1 is 500 μm . The scale bar in part (d) applies to all figure parts. a.) Silk water annealed for 12h at 310 K (37°C) before heating. b.) Same film as (a) after heating three times to 623 K (350 °C). c.) Silk exposed to 50% methanol-water solution for 15 h before heating. d.) Same film as (c) after heating twice to 653 K (380 °C).*

The first step of the data analysis is the symmetry line correction. **Figure 2a, b** gives an example of the symmetry line correction and final heat capacity scans for a silk film autoclaved for 1 h. The sample was heated according to the water removal protocol, and then heated once to 653 K (380 °C) to melt any crystals and obtain an amorphous sample. The first cooling and second heating scans are shown in blue. The cooling data are non-linear from the start of cooling to below 573 K (300 °C), and at the very tail end of the cooling some erratic behavior is noted below 363 K (90 °C). The symmetry line correction is implemented only for heat flow data in the linear regions just below and just above T_g (shown by heavy green and magenta lines, respectively). The resulting symmetry line is shown by the orange trace, and when subtracted from the sample heat flow data, the corrected heat flow is obtained (black curves). Also shown are the symmetry-corrected empty sensor traces (thin magenta and cyan, for heating and cooling,

respectively). Finally, the corrected empty sensor heat flows are subtracted from the corrected sample heat flows for evaluation of the heat capacity.

The resultant heat capacity data for the autoclaved sample are shown in Figure 2b. The heat capacities in the glassy state derived from the corrected cooling and heating heat flows match very well; the heat capacities in the liquid state match less well and both curves show more noise above T_g . The heat capacity from the second heating scan shows an enthalpy relaxation peak above T_g . The liquid and glassy states are fitted linearly, and the dashed lines show the extrapolation of these lines through the glass transition region. Where the extrapolated tangents intersect the vertical dashed line at T_g (451 K in the dry state, as determined from slow scan TMDSC [14]) the $\Delta C_p(T_g)$ is calculated and used to evaluate the m_Δ from eqn. (3). Below T_g , the heat capacity at 420 K is used to determine m_{ss} from eqn. (1).

Figure 2 goes here

Figure 2. Symmetry line correction applied to heat flow data (a), and resulting heat capacity (b), for silk fibroin film autoclaved for 1 h. a.) Sample heat flow data during first cooling and second heating (blue curves); regions used for symmetry line determination (heavy green and magenta); symmetry line (orange); empty sensor heat flow data (thin magenta and cyan); corrected sample heat flow after symmetry line and empty sensor subtraction (black curves); negative of the corrected sample heat flow in heating (red); line $y = 0$ (black). b.) Corrected sample heat capacity in cooling (black) and heating (red); extrapolated lines from high and low temperature tangents to the data (dashed); line marking the glass transition temperature from slow scan DSC (vertical dashed). The green circles show the intersection points at $T_g = 451$ K, at which the heat capacity increment is evaluated.

Figure 3 (and **Table 2**) show comparison of the masses evaluated using the two methods for all nineteen amorphous samples. The determination of m_{ss} relies on an absolute measurement of specific heat capacity whereas the determination of m_Δ relies on a relative measurement of

ΔC_p . In conventional slow scanning calorimetry, the relative measurement is less prone to error, and we assume the same to be true for fast scanning calorimetry. Depending on method used, the masses ranged from about 100-275 ng, though one heavier mass sample ($m \sim 400$ ng) was measured. Except for two points, all data lie above the line $y = x$, indicating that the masses, m_Δ , from $\Delta C_p(T_g)$, were almost always greater than m_{ss} . This is a systematic variation, not one that could be assigned to random error. The reason for the increasing difference between the two independently determined values with increasing sample mass in Fig. 3 is not yet known. In deciding which mass to use for further calculations of specific heat capacity, we first note that the value of m_{ss} was dependent upon the temperature chosen for its evaluation in the solid state.

Figure 3 goes here

Figure 3. Silk fibroin sample mass m_Δ , determined from the heat capacity increment at T_g , $\Delta C_p(451\text{ K})$ from eqn. (3), vs. mass m_{ss} , determined from $C_p^{solid}(420\text{ K})$ from eqn. (1). The dashed line represents $y=x$.

We examined a range of temperatures in the glassy state, from 380-430 K and calculated $m_{ss}(T)$ from eqn. (1). m_{ss} varied by about 2-4% over the temperature range, and always increased with temperature. While this is not a large variation, it does signal that there is some inherent difficulty in accurately determining the heat capacity from the heat flow data, due possibly to thermal losses from the sample still unaccounted for by the symmetry line correction. Therefore, we decided to use m_Δ instead of m_{ss} for further analysis. This is based on the assumption that while the numerical value of the heat capacity, $C_p^{meas}(T)$, might be slightly inaccurate due to uncompensated thermal losses, the value of $\Delta C_p^{meas}(T_g)$ might be less affected.

Figure 4a-c shows the specific heat capacity for 19 amorphous silk fibroin samples, after symmetry line correction of the heat flow data, subtraction of the empty sensor, and division by the mass, m_{Δ} . In (a) data are shown without y-axis calibration. The black dashed line in (a) shows the $C_p^{solid}(T)$ relationship known from literature [14], and the red dashed line shows the theoretical C_p^{vib} [32] for comparison. Division of the heat capacity data by m_{Δ} results in a set of curves whose values below T_g are widely spread and generally fall below the known solid state relationship as expected from Fig. 3. The data in Figure 4b have been corrected by vertically shifting to force a match between the literature value of C_p^{solid} at the arbitrary temperature point of $T = 420$ K. The final level of correction of the y-axis is shown in (c) where the data have been further corrected by rotation about the point $T = 420$ K, to bring the solid state slope into agreement with the known relationship [14].

The data sets were fit linearly in the liquid state above the enthalpy relaxation peak seen just above the inflection point signifying T_g . The average value of $C_p^{liquid}(T)$ is illustrated by the dashed line so marked in Figure 4c, and obeys the relationship:

$$C_p^{liquid}(T) = 1.98 \text{ J/gK} + T \cdot (6.82 \times 10^{-4}) \text{ J/gK}^2 \quad (4)$$

The standard deviation on the intercept is 0.098 J/gK and on the slope is 2.2×10^{-4} J/gK² when all 19 data sets are included. Two data sets (Water Annealed for 3h at 37 C, orange dot; Autoclaved 60 min, red x) appear to be outliers, but their inclusion does not affect the average value of $C_p^{liquid}(T)$ because of their positions, respectively, above and below the rest of the data. If the two outliers are removed, the standard deviations are reduced to 0.065 J/gK and 1.4×10^{-4} J/gK² on intercept and slope, respectively.

Figure 4 a-c go here

Figure 4. Specific heat capacity for 19 amorphous films of silk fibroin determined using the mass, m_{Δ} . a.) Data before y-axis calibration; b.) Data after vertically shifting to match literature relationship for C_p^{solid} (420 K); c.) Data after additional rotation to bring solid state slopes into agreement with eqn. (2). *The dashed red line in (a) shows the vibrational heat capacity from literature [32]; dashed black lines show the extrapolated C_p^{solid} relationship from literature [14] using equation (2), and, in (c), the extrapolated liquid state heat capacity above T_g averaged over all samples.* Samples are: As cast (blue $\square$, magenta dot, red o); MeOH 15h (blue dot, green o, magenta o); Autoclaved 1 h (cyan dot, red x, black o); Water Annealed 12h 25 °C (grey dot, green x); Water Annealed 12h 37 °C (black x, yellow x); Water Annealed 3h 37 °C (green dot, black dot, orange dot, magenta x, cyan x, blue x).

Our previous estimate of the liquid state heat capacity of silk fibroin was based on standard DSC heating and temperature modulated DSC heating at 2 K/min, and quasi-isothermal TMDSC [22]. That estimate was based on only a 10 degree temperature range above the enthalpy relaxation peak. The estimated linear relationship displayed a slope that was quite similar to the slope of the solid state heat capacity curve, *i.e.*, $3.47 \times 10^{-3} \text{ J/gK}^2$ (liquid) compared to $3.7 \times 10^{-3} \text{ J/gK}^2$ (solid). In light of the present research, using a much faster scan speed, it seems possible to conclude that the previous slowly scanned samples were subject to thermal degradation, which resulted in a slope that was too steep. The present data show the liquid state slope to be much shallower, at $6.82 \times 10^{-4} \text{ J/gK}^2$. Furthermore, the new data are derived based on a much wider 40 to 60 K temperature range (depending upon the sample) above the enthalpy relaxation peak.

The glass transition temperatures were evaluated from the inflection points of the heat capacity curves in Figure 4c, based on analysis of the peak position of the first derivative curves. The data are shown in **Table 2** for each sample. The glass transition temperatures were clustered from 478 K to 483 K (205 °C – 210 °C), and the as-cast silk has a slightly higher average value of T_g than the other samples. There was no correlation of the glass transition temperature with the mass, m_{Δ} , for the range of masses in this study. The location of the enthalpy relaxation peaks

in Figure 4c, determined from the zero crossing of the first derivative curves, was more varied and ranged from 486 K to 501 K (213 °C – 228 °C).

In a separate study, whose detailed results will be reported later, semi-crystalline silk fibroin samples were also examined using Flash DSC1 at the same heating rate. The glass transition temperatures of semi-crystalline silks were always greater than their amorphous counterparts, ranging from 494 K - 507 K (223 °C - 234 °C). This allows us the confidence to say that the 19 samples studied here are indeed fully amorphous, **whether prepared that way as cast, or after first melting of the initially crystalline sample.**

Table 2 goes here

4. Conclusions

1. Heat capacity of 19 amorphous silk fibroin samples was determined from the measured and corrected heat flow rates at heating and cooling at 2000 K/s in the temperature range from 343 to 650 K.
2. Sample mass was determined from the measured solid heat capacity at 420 K and the known experimental C_p^{solid} at that temperature. Similarly, sample mass was also determined from the heat capacity increment at the glass transition at 451 K. Why the sample mass determined from heat capacity at 420 K is systematically larger by about 11% compared to the sample mass determined from ΔC_p is not yet known. One possibility is that the empty sensor measurement does not provide the addenda heat capacity relevant for the sample measurement. Or, at the same sensor temperature the

sample surface temperatures, determining the heat losses, are different on heating and cooling. To clarify this is not an easy task but experiments are under way.

3. After further internal calibration of the measured specific heat capacities by using the temperature dependence of the solid specific heat capacity, the temperature dependent specific heat capacity of the liquid silk fibroin was obtained.

4. The following relation provides specific heat capacity of silk fibroin in the temperature interval from 510 to 570 K with an uncertainty of about $\pm 5\%$:

$$C_p^{liquid}(T) = (1.98 \pm 0.06) \text{ J/gK} + T \cdot (6.82 \pm 1.4) \times 10^{-4} \text{ J/gK}^2 \quad (5)$$

with T in Kelvin.

5. Reduction of the uncertainty seems possible but requires a better understanding of the addenda heat capacity contribution to the sample measurement and of the heat losses from the sample to the surrounding.

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Figure 1

Figure 2a

Figure 2b

Figure 3

Figure 4a

Figure 4b

Figure 4c

Table 1- Sample treatments, areal densities, and thicknesses for nineteen amorphous silk samples

Sample Treatment	Time and Temperature	Areal Density ($\mu\text{g}/\text{mm}^2 \pm .05$)	Thickness ^a (mm $\pm .0002$)	Number of Samples ^b
As Cast	na	14.05	0.0104	3
Water Annealed	12 h, 25 °C	13.6	0.0101	2
Water Annealed	3 h, 37 °C	8.85	0.0066	6
Water Annealed	12 h, 37 °C	11.30	0.0084	2
Autoclaved	1 h, 120 °C	12.98	0.0096	3
50% MeOH ^c	15 h, 25 °C	11.85	0.0088	3

^a Thickness was estimated from the measured areal density and an assumed sample density of 1.348 g/cm³ [24]

^b Samples of similar sample treatment were cut from the same parent film

^c MeOH-water 50:50 by volume

Table 2- Sample treatment, mass ^a, and glass transition temperature for nineteen amorphous silk fibroin samples, and average glass transition within treatment categories.

	Sample	m_{ss} / ng	m_{Δ} / ng	$(T_g \pm 0.5) / ^\circ\text{C}$ _b	$T_g^{avg} / ^\circ\text{C}$
1	As Cast	189	224	481.6	482
2	As Cast	355	402	481.7	
3	As Cast	152	170	483.1	
4	WA12h25C	222	241	478.7	481
5	WA12h25C	234	257	482.8	
6	WA3h37C	146	171	481.6	481
7	WA3h37C	115	130	481	
8	WA3h37C	171	165	481.8	
9	WA3h37C	124	145	479.8	
10	WA3h37C	107	130	479.7	
11	WA3h37C	230	264	479.7	
12	WA12h37C	166	170	477.5	478
13	WA12h37C	160	184	478.4	
14	AC 60 m	158	181	478.1	480
15	AC 60 m	204	206	481	
16	AC 60 m	210	247	480.9	
17	MeOH	154	175	477.9	479
18	MeOH	222	249	479.8	
19	MeOH	148	175	478.6	

^a m_{ss} determined from Eqn. (1) matching the solid state heat capacity with known value at 420 K;

m_{Δ} determined from Eqn. (3) matching the heat capacity increment with known value at $T_g = 451$ K.

^b Determined from the inflection point of the heat capacity curve in heating.