Thermal Properties Measurements of Renatured Gelatin Using Conventional and Temperature Modulated Differential Scanning Calorimetry

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ABSTRACT: The thermal properties of amorphous gelatin films and gelatin films with renatured structural order were measured by using conventional and temperature modulated differential scanning calorimetry (DSC). Different amounts of gelatin structural order associated with a melting enthalpic change in the DSC measurement were prepared based on different gelatin drying conditions. Two consecutive heating and cooling DSC measurements on the gelatin films showed that there was no change in the glass-transition temperature (T_g) for the amorphous gelatin but there was a decrease in the T_g for the structural gelatin on the second DSC scan. This decrease was attributed to the plasticizing effect from the release of originally hydrogen-bonded water associated with the structural gelatin. In addition, a reversing endotherm observed upon melting of the structural gelatin during a temperature modulated DSC measurement indicated that the transition of bound water to free water occurred as the partial triple-helix gelatin melted.
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Key words: gelatin; triple-helix structure; drying; temperature modulated differential scanning calorimetry; bound water

INTRODUCTION

Gelatin is a denatured collagen, which consists of three extended polypeptide chains, each twisted in a left-handed helix (α chain), supercoiled together to form a right-handed triple helix.[1,2] The triple-helix structure is stabilized by the formation of interchain hydrogen bonds between C═O and N—H groups. During the denaturation process, the triple-helix structure is broken to form random-coil gelatins. In an aqueous solution, some gelatin chains reassociate with each other to form a partial collagen-like triple helix as the solution is cooled below its coil–helix transition temperature.[3,4] Gelatin with a partial collagen-like triple-helix structure is sometimes called "renatured" or "structural" gelatin. It is known that the presence of bound water plays an important structural and functional role in many proteins such as gelatin,[5] collagen,[6,7] and synthetic polypeptides.[8–14] In these proteins, the bound water forms bridges of hydrogen bonds with the triple helix.[15] Direct evidence of the existence and coordination of the bound water in these proteins has been verified using various structure-sensitive techniques including IR spectroscopy,[6] NMR spectroscopy,[5,16,17] circular dichroism,[17] and X-ray and neutron diffraction.[11,12] It is expected that the unfolding of the triple helix will be accompanied by the dissociation of the bound water with the structural gelatin. However, a standard thermal analysis technique like differential scanning calorimetry (DSC) shows only a single endotherm, which is often attributed to the melting of the triple-helix crystalline structure.[5,6,15] Here, we report that, by using a temperature modulated DSC technique (MDSC), the melting of the triple helix can be simultaneously observed with the transition from bound to free water from the structural gelatin.[18]

A new thermal analysis technique, step-scan MDSC (SS-MDSC) was recently introduced.[19–23] When using SS-MDSC, the tested sample follows a series of short temperature steps or modulations. The temperature modulation produces a series of heat flow peaks. The basic theory of SS-MDSC was presented by Reading[19] and Boller et al.[20] They used a simple equation derived from thermodynamics to describe the principle of SS-MDSC in which

$$dH/dt = C_p(dT/dt) + f(T, t)$$

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where dH/dt is the total heat flow; C_p is the sample's heat capacity, which stands for the reversing part of the thermal response; dT/dt is the applied heating rate; and $f(T, t)$ is the kinetic or "irreversing" response. The terms reversing and irreversing are used because the response time scale is small compared with that of the modulation. When the sample is held under isothermal conditions, the heating rate (dT/dt) becomes zero and the sample's heat flow is purely described by the kinetic term $f(T, t)$. When the sample is heated, the total heat flow is taken to be the average of the heat flow response. Because the reversing and irreversing responses of the sample are separately observed in the heating and isothermal portion of the cycle, the total heat flow is divided into the two parts.

In the present study both conventional DSC and SS-MDSC were used to measure the thermal property of amorphous and partially renatured gelatin films in their solid state. We demonstrate that, as the partially renatured structure melts, the originally hydrogen-bonded water with a partial triple-helix structure is released, which gives a distinct endotherm in the reversible response using SS-MDSC. In addition, once the bound water is released,[5] it becomes free and plasticizes the amorphous phase of the gelatin, which leads to a depression of the glass-transition temperature during the second heating run of the DSC measurements.

EXPERIMENTAL

Gelatin powder was purchased from Showa Chemical Co. and used as received. All gelatin coatings were prepared from 10 wt % gelatin solutions. The gelatin powder was first added to doubly distilled and deionized water and swelled at room temperature for at least 1 h to prevent the formation of gel particles in the solution. The mixture was then heated to 50°C so that the powder completely dissolved in water to obtain a homogenous solution. The gelatin solution was then coated onto a poly(ethylene terephthalate) substrate using a coating blade with different coating gap thicknesses to control the wet thickness of the coatings. The coated samples were dried in a temperature and moisture controlled oven to produce gelatin films with different amounts of renatured triple-helix structures. The effect of the drying condition on the amount of structural gelatin is discussed in the Results and Discussion section. For the MDSC measurement, two different types of gelatin coatings were prepared, depending on how the gelatin coating was dried. One type of gelatin film was dried in an oven at 50°C and 30% relative humidity (RH). The other type was dried at 23°C and 50% RH. When dried at 50°C and 30% RH, the gelatin is completely amorphous. When dried at 23°C and 50% RH, a certain amount of gelatin chains are renatured and form partial triple-helix crystalline structures. The amount of the renatured gelatin was measured from the enthalpy change associated with the structural gelatin melting in the standard DSC thermogram. Because gelatin is known for its humidity sensitivity,[1,2] moisture serves as a plasticizer to affect the glass-transition and melting temperatures of the gelatin coating when the coating is exposed to different RHs. Therefore, after drying, both types of gelatin films were placed in the temperature and humidity oven at 23°C and 50% RH for at least 24 h before each DSC measurement to equilibrate their moisture content.

The thermal properties of the gelatin films were measured using a PerkinElmer Pyris Diamond DSC. The temperature and enthalpy scales were calibrated using standard samples of indium and zinc. The gelatin sample was sealed in a hermetic pan to prevent any loss of moisture during DSC measurement. The approximate 9–11 mg gelatin sample was sealed in the pan in an environment of 23°C and 50% RH. Two consecutive temperature scans from 5 to 110°C at a 10°C/min heating rate were made to measure the thermal behavior of the gelatin films. Upon completing the first heating scan, the sample was quickly cooled at a rate of 40°C/min to 5°C and a rerun of the cooled sample was conducted. Ultrapure nitrogen was used as a purge gas at a rate of 20 mL/min during the measurement. The baseline subtraction was made to correct any heat capacity difference between the sample and the reference furnace. All samples are prepared for SS-MDSC in the same manner as for the conventional DSC. For SS-MDSC measurements, the experimental conditions used throughout the investigation consisted of a heating rate of 5°C/min, a heating step of 1°C, and an isothermal period of 60 s from 0 to 100°C. The baseline subtraction mirrored that of the conventional DSC. The data were subsequently analyzed to determine the reversing and irreversing response of the sample.

RESULTS AND DISCUSSION

The glass-transition temperature, melting temperature, and melting enthalpy of the renatured gelatins were measured by using conventional DSC. For a structural gelatin dried and equilibrated at 23°C and 50% RH, Figure 1 shows the first heating scan programmed from 5 to 110°C at a heating rate of 10°C/min, followed by a rerun of the subsequently cooled sample. As shown in the Figure 1, the midpoint of the specific heat change associated with the glass transition was taken as the glass-transition temperature (T_g) and the peak temperature of the melting endotherm was taken as the melting temperature (T_m) of the transition. The area underneath the endotherm divided by the total sample weight (ΔH/gelatin, J/g) indicates the percentage of structural order in the

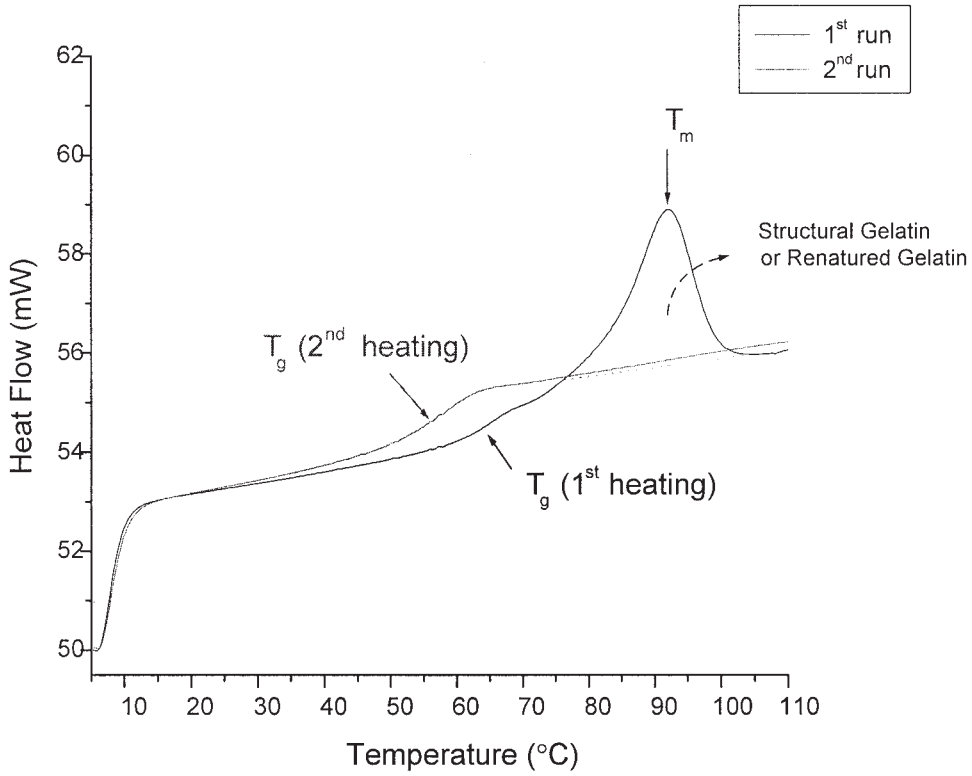

Figure 1 A characteristic DSC scan for a structural gelatin and a rerun of the subsequently cooled sample.

gelatin. In order to prepare gelatin coatings with different amounts of renatured structural order, gelatin films were coated onto a poly(ethylene terephthalate) substrate followed by drying and equilibrating the samples at different temperatures and relative humidities. Figure 2 shows the effect of the drying temperature on the percentage of structural gelatin prepared under a drying humidity of 50% RH. The amount of structural gelatin decreases monotonically with increasing drying temperature from 5 to 50°C. For a

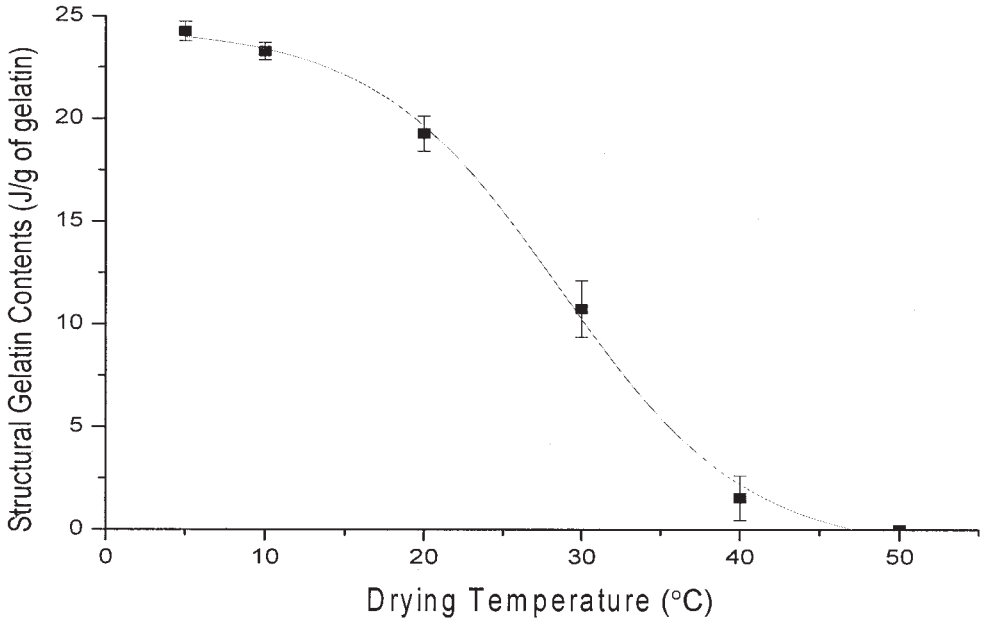

Figure 2 The effect of the drying temperature on the amount of structural gelatin that is generated.

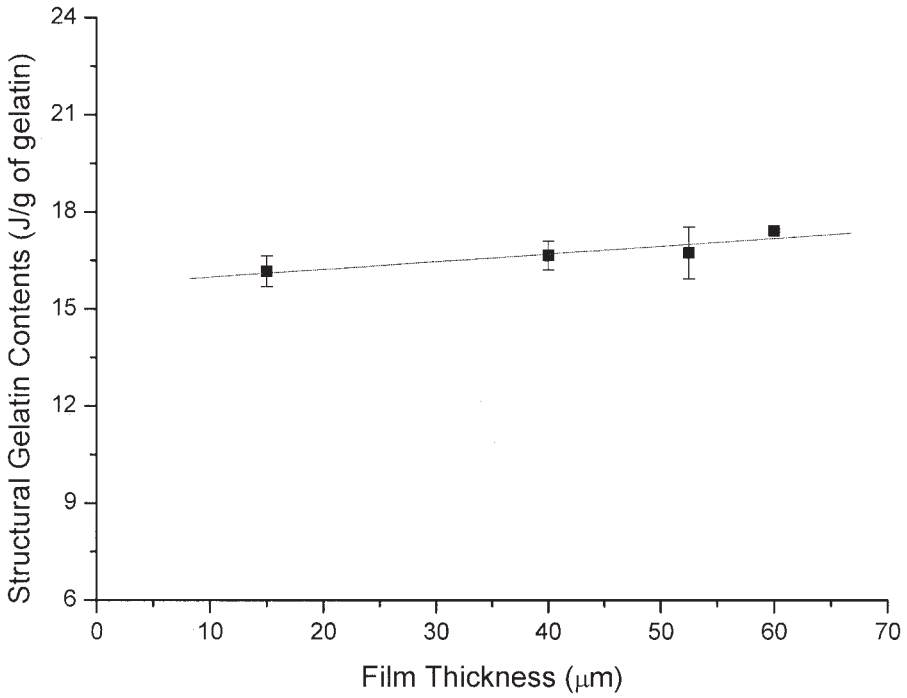

Figure 3 The effect of the coating thickness on the amount of structural gelatin for gelatin dried at 21°C and 50% RH.

drying humidity from 30 to 65% RH, the amount of structural gelatin generated was not affected by more than a 5% difference in the structural gelatin. The effect of the dried coating thickness on the amount of structural order generated in the samples was also studied. Figure 3 shows that the amount of structural gelatin generated during drying is relatively independent of the coating thickness from 10 to 60 μm. The result indicates a uniform structural gelatin distribution throughout the thickness direction in the coating.

The effect of the moisture content on the thermal properties of the gelatin was studied. Gelatin coatings dried at both 50°C and 30% RH (condition A) and at 23°C and 50% RH (condition B) were tested with conventional DSC and SS-MDSC measurements. Figure 4(a,b) shows the conventional DSC thermograms of the gelatin sample made from the two conditions. The first and second heating traces are shown. It can be seen by comparing Figure 4(a,b) that the drying conditions significantly affect the thermal properties of the gelatin film. Figure 4(a) shows that the gelatin made under condition A is completely amorphous without any structural enthalpy and has a T_g at about 70°C for the first heating and at about 72°C for the second heating. However, the gelatin made from condition B shows an endothermic melting peak (T_m) at 85°C and a T_g at 60°C in the first heating scan. Because the structural gelatin is completely melted in the first scan, there is no endothermic melting peak related to the partial triple-helix structure on the second heating scan. On the second scan in Figure 4(b), the gelatin shows a T_g at 50°C, which is 10°C lower than that in the first scan. The characteristics of the thermograms for both types of gelatin are listed in Table I.

Table I shows that for the gelatin originally with a structural order, the T_g on the second scan is 10°C lower than in the first scan, whereas for the amorphous gelatin there is essentially no change in the T_g between its first and second DSC scans. Ramachandran et al.[1] found that the moisture content in gelatin greatly affected the T_g of the gelatin. The reduction of the T_g for gelatin with the structural order indicates that the bound water in the structural gelatin was released at the end of the first heating scan. On the second heating scan, the released water plasticizes the now completely amorphous gelatin, which leads to the reduction of the T_g. For the amorphous gelatin, because there is no structural order to form hydrogen bonds with the bound water, the T_g between the first and the second scans remains the same.

We examined the bound–free water transition using the SS-MDSC technique. Figure 5(a,b) shows SS-MDSC thermograms of the two types of gelatin films. For the completely amorphous gelatin film, there is no endothermic peak in the nonreversible response, as expected, and a T_g at 68°C is shown in the reversible heat capacity component. However, for the gelatin film with structural order, in addition to a T_g at about 60°C in the reversible component of the specific heat capacity, there is an endothermic peak associated with the T_m at about 75°C in the nonreversing component. Furthermore, we found an additional endothermic

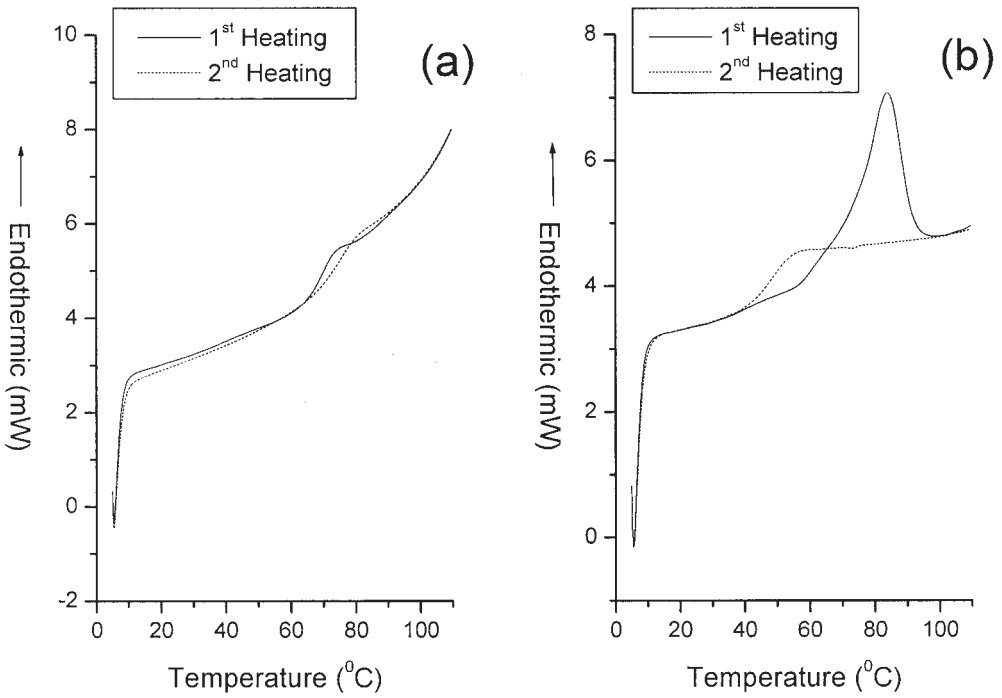

Figure 4 The first and second heating scans of DSC thermograms for a gelatin film (a) dried at 50°C and 30% RH or (b) dried at 20°C and 50% RH.

peak in the reversible component of the scan at 76°C, which coincides with the melt peak in the irreversible response at roughly the same temperature. For the completely amorphous gelatin, no additional endothermic peak was observed in the reversible component of the scan. Table II summarizes the results from the SS-MDSC measurements.

Bound water exists in a collagen or collagen-like triple-helical structure.[5,6,10–12] Bella et al. found that each triple helix is surrounded by a cylinder of bound water, with an extensive hydrogen-bonding network between the water molecules and collagen-like peptide acceptor groups.[11,12] They proposed the involvement of the bound water in stabilizing the triple helix by forming interchain water bridges.[11,12] Upon a heating treatment, the partially renatured gelatins unfold to form completely amorphous gelatin chains. This unfolding characteristic is believed to comprise two steps: the separation of the triple helices into individual ones, and the unfolding of the individual helices into random coils. The first step involves the disruption of water bridges (hydrogen bonds) between the three polypeptide chains of the collagen-like molecule, whereas the second step involves the disruption of intrahelical hydrogen bonds of the α chains. The intermolecular hydrogen bonding in the first step is the typical bound–free reversible process. Recent studies[24–26] demonstrated that a reversing endotherm during an MDSC measurement may correspond to the melting of the crystalline structure in the polymer. The just melted polymer may recrystallize rapidly because of a templating effect of the existing crystal on the just melted polymer. If they recrystallize, an exothermic enthalpic peak should be observed. However, no reversing exotherm during the SS-MDSC measurement was observed. We concluded that the additional endothermic peak in the reversible component of the SS-MDSC measurement for the structural gelatin was the bound–free water transition in which the bound water is released by breaking the equilibrium hydrogen bonding between water molecules and triple helix as the structural gelatin is melted.

TABLE I
Conventional DSC Measurement of T_g and T_m of Gelatins Dried Separately and Equilibrated at 50°C and 30% RH or at 23°C and 50% RH

	T_g		
	1st scan	2nd scan	T_m of 1st scan
Amorphous gelatin	70°C	72°C	None
Structural gelatin	60°C	50°C	85°C

The results of the T_m of the gelatin with the structural order observed by standard DSC [Fig. 5(b)] versus that of SS-MDSC [Fig. 4(b)] were compared. Note that the the T_m value in Figure 5(b) is 10°C lower than that in Figure 4(b). This T_m depression phenomenon is similar to the T_g depression phenomenon shown previously. During the heat-hold temperature program in the SS-MDSC measurement, the oscillation of the heat

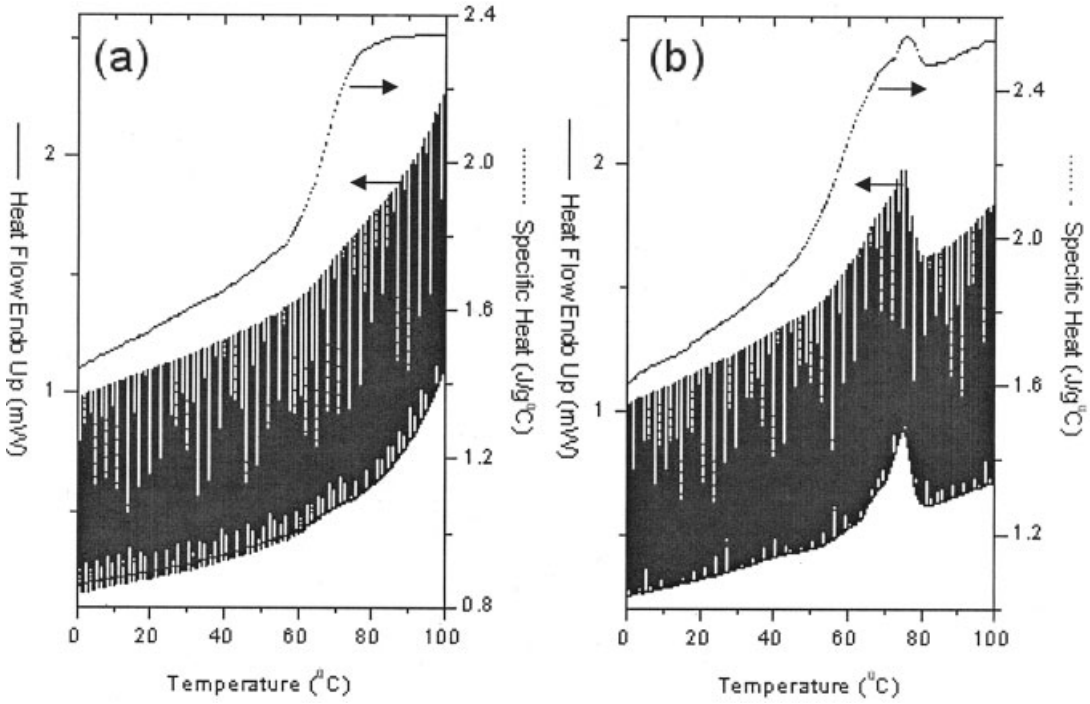

Figure 5 The (—) modulated heat flow and (—) nonreversible and (···) reversible components of step-scan MDSC thermograms of (a) amorphous gelatin film and (b) gelatin film with a structural gelatin content of ~18 J (g gelatin)$^{-1}$.

flow induces the release of bound water, which reduces the thermal stability of the partial triple-helix structure. This reduction in turn leads to a lower T_m in the SS-MDSC results compared to that from the conventional DSC results.

CONCLUSION

We used DSC and MDSC techniques to verify the existence of bound water in structural gelatins generated during gelatin coating and drying processes. From the conventional DSC measurement, we found that bound water released from the structural gelatin can plasticize the amorphous gelatin, which leads to a lower T_g. From the SS-MDSC measurement, we observed a reversible endothermic reaction that corresponds to the reversible bound–free water transition during which hydrogen bonds between bound water and the triple-helix-like structure breaks. The evidence suggests that the release of originally bound water from renatured gelatin occurs simultaneously with the melting of the partial crystalline structure.

TABLE II
SS-MDSC Measurements of Characteristic Transitions of Amorphous and Structural Gelatins

	T_g	T_m	Reversing endotherm
Amorphous gelatin	68°C	None	None
Structural gelatin	60°C	75°C	76°C

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