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DSC STUDY OF MELTING AND GLASS TRANSITION IN GELATINS

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In the range from –50° to +130°C, the temperature dependence of the heat capacity for different kinds of gelatins with water contents of from 2 to 95% was studied by the DSC method. It was shown that, in all studied cases, metastable collagen-like structures are formed in gels or crystalline gelatins, with thermodynamic parameters depending on the formation conditions. The characteristic properties of the glass transitions in amorphous gelatins and crystalline gelatins with different melting heats and different contents of the ordered phase were established. Special attention is paid to the structural properties of free and bound water. The dependence of the glass transition temperature T_g on the bound water content was shown to be of general applicability for many denatured biopolymers. Free water in gelatins, in distinction to the bound water, does not act as a plasticizer, but forms a rigid matrix inhibiting the glass transition.

Keywords: DSC, gelatins, glass transition temperature

Introduction

On cooling, amorphous gelatin is transformed into the glassy state at a temperature depending on the water content in the sample [1–3]. However, the glass transition in gelatin has so far been studied for a relatively narrow range of concentrations and in a limited temperature range. Moreover, despite the fact that gelatin has long been used as a model substance for the study of gel formation in polymers [4], the thermal properties of its gels are not completely established. Not only the magnitudes of the melting heat for gels, but also the general mechanisms of gel formation are discussed [5–7].

It is natural to suppose that the characteristics of the glass transition in gelatin should depend on its supermolecular structure, and will differ for amorphous and crystallized samples. The purpose of the present work is to study the heat capacities of crystalline and amorphous gelatins of different concentrations by modern

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calorimetric techniques in a wide temperature range, including the glass transition and melting of both gelatin gels and free water.

We expected that a comparison of these data with the results of earlier studies of the heat capacities of native and denatured collagen [8–11], which is a parent substance for gelatin production, would give new information on the melting processes and glass transitions in such systems with complex supermolecular structure.

Experimental

For the study of heat capacity, we used a Setaram DSC-111 differential scanning calorimeter with a sensitivity of 3×10^{-5} J/sec. The samples had a mass of 50–100 mg. Temperature was controlled with a precision of ±0.2 deg. The error in the absolute values of melting heat and heat capacity was less than 3%. For determination of the bound water content, the samples were vacuum pumped at T=105°C. The values of the melting heat are related to the dry weight of the sample.

In the first part of the work, devoted to a study of the gel melting, we measured only the temperature dependence of the apparent heat capacity in the region of gel melting. In the second part, involving a study of the glass transition, the absolute values of heat capacity were measured in the temperature range from –50° to +130°C.

Since the number of cross-links strongly influences the processes of gel formation [4], we used three different kinds of gelatin samples (Gelatin I, II and III):

I. Gelatin samples produced by thermal denaturing of native collagen in the same hermetic calorimetric ampoule as further used for the study of gel formation. Such samples preserve all the native cross-links originally present in the collagen.

II. α-Gelatin consisting of completely isolated chains with a molecular mass of 92000 (the commercial product of Serva used as a calibration standard for determination of molecular masses).

III. Commercial gelatin from Sigma (Bloom number 225). These samples have a structure intermediate between the structures of the first two kinds of gelatin, with partly destroyed cross-links.

Results and discussion

Melting of gels

This part of the work is devoted to a study of gel formation in the gelatin samples of different kinds as functions of concentration and temperature. We shall discuss first some general characteristics of gel formation which we have found to be common for all the studied samples. As an example, we shall discuss these characteristics in detail for the samples of Gelatin I.

Figure 1 presents the temperature dependence of the apparent heat capacity for the samples of Gelatin I (concentration of gelatin c=20%) which after denaturing were kept at constant temperature T=22°C for different periods of time. The maxima in the curves correspond to the phase transition of gel melting. The results in Fig. 1 demonstrate the kinetics of gel formation. It is seen that increase of the formation time is accompanied by the characteristic transformation of the melting curves.

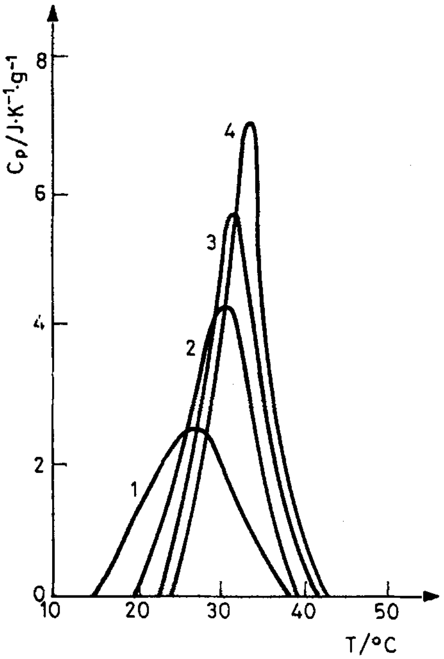

Fig. 1 Evolution of melting curves for Gelatin I in dependence on the time of gel formation at T=22°C. Curves 1, 2, 3 and 4 correspond to formation times of 30 min, 3 h, 18 h and 7 days, respectively. Heating rate V_h=1 deg/min

The melting heat Q_m and the melting temperature T_m increase, whereas the halfwidth of the melting curve ΔT decreases. Similar transformations of the melting curves as a function of the formation time were observed for all gelatin samples with concentrations of from 2 to 85% at temperatures of 15°–35°C. It can be concluded from our results that the structures which are formed in the gels are metastable, since the thermal stability of these structures changes with time.

We shall next discuss the influence of the concentration of the gelatin solutions on the thermodynamic parameters of the gels. We shall present separately the results for gels with a gelatin content of less than 20% and for more concentrated gels. The magnitudes of the parameters under discussion are the limiting values corresponding to the longest formation times.

It is seen from Fig. 2 that the limiting values of T_m are practically independent of concentration in the range from 2% to 20% and have a precision comparable to the measurement error for the samples of the three different kinds. The value of the melting temperature for the gels formed at 22°C is T_m=35.0°±2.0°C.

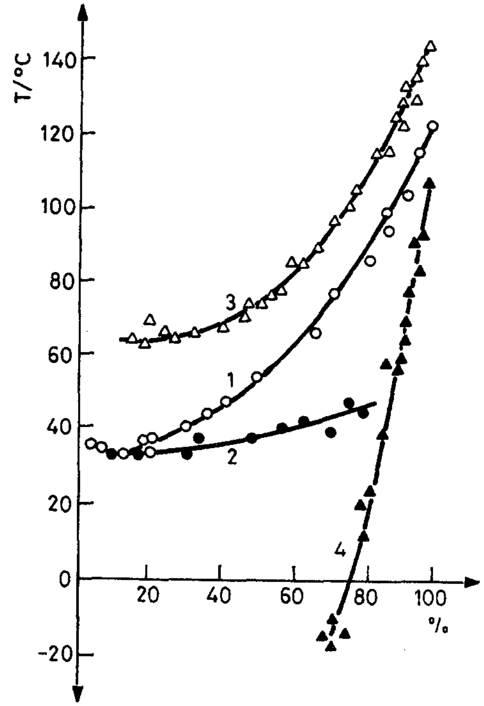

Fig. 2 Concentration dependence of melting (T_m) and glass transition (T_g) temperatures for Gelatin III and denaturation temperature (T_d) for native collagen. 1–T_m for Gel A, 2–T_m for Gel B, 3–T_d for collagen, 4–T_g for amorphous gelatin

The limiting values of the melting heat Q_m for Gelatins I and III are practically independent of concentration in the range 2–20%, but depend critically on the number of cross-links in the starting gelatin. In distinction to cross-linked gelatins, the melting heat in Gelatin II (α-gelatin) depends strongly on the concentration. With decrease of the concentration, the values of Q_m (related to the dry weight of gelatin) increase.

As shown in [12], in strongly diluted solutions (concentration less than 0.2%) the isolated gelatin molecules (the collagen chains) form three-chain collagen-like structures. It can be presumed that on the increase of concentration (up to 20%) these collagen-like structures serve as the elements which form the gel network. Thus, it can be supposed that the limiting (although practically not achievable) values of the thermodynamic parameters for the gelatin gels should be given by the denaturing heat and temperature of collagen in solutions of the same concentration. For a 20% collagen solution, these values are Q_d=78.6 J/g and T_d=42.0°C. The proximity of the real gel structure to the ideal native collagen structure (i.e. the perfection of the gel structure) depends on the type of gelatin. In strongly cross-linked gelatins in which the gel formation is similar to polymer crystallization in the presence of heterogeneous seeds [13], the melting heat of the gels increases with increasing cross-link density. At the same time, in α-gelatin the gel formation is controlled by homogeneous seed formation and leads to formation of the most perfect structures, although during much longer times.

In what follows, we shall present the results of the study of melting of highly concentrated gels (c >20%). It is known that such gels can be obtained in two different ways, either by drying of gels with a relatively low concentration (method A), or through gel formation in solutions of high concentration (method B).

The dependence of the thermodynamic parameters of gels on the gelatin concentration for the samples prepared by method A (concentration of the starting gel 5%) and method B (formation of gel at fixed concentration) is shown in Figs 2 and 3, respectively. It should be noted that, on decrease of the water content, the transition of water gels of gelatin into crystalline gelatins with different contents of bound water takes place. As can be concluded from curves 1 and 2 in Fig. 2 and curves 3a and 3b in Fig. 3, this transition is smooth and does not produce any irregularities as a function of the melting heats of temperatures versus the water content. Thus, it can be concluded that similar collagen-like structures are formed in both solutions of gelatin in water and solutions of water in gelatin.

For the samples obtained by method A, as can be seen from Fig. 2, the melting temperature increases with decrease of the water content. At the same time, the melting heat Q_m is practically unchanged on decrease of the water content down to 25%; it strongly diminishes on further drying. It should be noted that the concentration dependence of the thermodynamic parameters of melting for gels and crystalline gelatin is very similar to the concentration dependence for the parameters of collagen denaturing established previously [8, 9]. An essential difference, however, is that for gelatin the corresponding values are always lower than for collagen with the same water content. This is in good agreement with the supposition that the structures formed in gels of high concentrations and in crystalline gelatin are in essence collagen-like.

As in the case of collagen, the decrease in the melting heat observed for the gels with a small water content is caused by the loss of bound water stabilizing the triple helix structure [8, 9]. A simultaneous increase in the melting temperature most probably reflects the decrease in the entropy change S_m resulting from the decrease in molecular mobility on drying.

For the gels formed directly from the highly concentrated solutions by method B, in distinction to the previous case, on increase of the gelatin concentration one can observe only a slight increase in the melting temperature (curve 2 in Fig. 2), accompanied by a substantial decrease in the melting heat (curve 3b in Fig. 3).

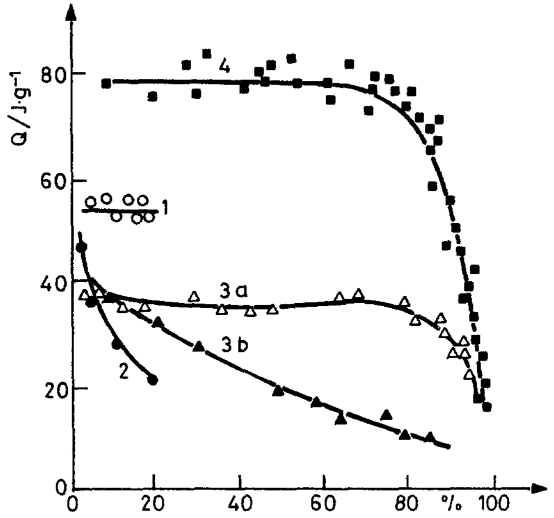

Fig. 3 Concentration dependence of melting heats for Gelatin I (1), Gelatin II (2), Gelatin III (3). Curves 3a and 3b correspond to the gels prepared by methods A and B, respectively. Curve 4 shows the denaturation heat for native collagen

The observed difference in the thermodynamic parameters for highly concentrated gels and crystalline gelatins prepared by the two methods can be understood if the well-known dependence of the melting parameters for small systems on the size and number of the defects is taken into account [14]. The parts of the collagen-like helix which form the gel network can definitely be regarded as small systems. The destruction of these small systems represents the process which is responsible for the transformation of the gel into the isotropic solution or for transition of the crystalline gelatin into the amorphous state. It seems quite natural that in the gels of high concentration used in method B the kinetic limitations due to the small water content will lead to the formation of structures with many more defects as compared with the moderately concentrated gels used in method A. As a result, the small structures formed in method B display a substantially decreased melting heat and temperature.

Glass transition in gelatin

In order to study the glass transition in gelatin, we started with measurements of the temperature dependence of the absolute values of heat capacity for the samples in which all the water is in a bound state.

We shall discuss first the results for the crystalline gelatins obtained by method A. As an example, Fig. 4 shows the temperature dependence of the heat capacity for the sample with a bound water concentration of 14%. Curve 1 corresponds to the first heating of the sample, while curve 2 shows the results for the second heating. The heating rate in both cases was V_h=3 deg/min. After the first heating, the sample was cooled at the same rate.

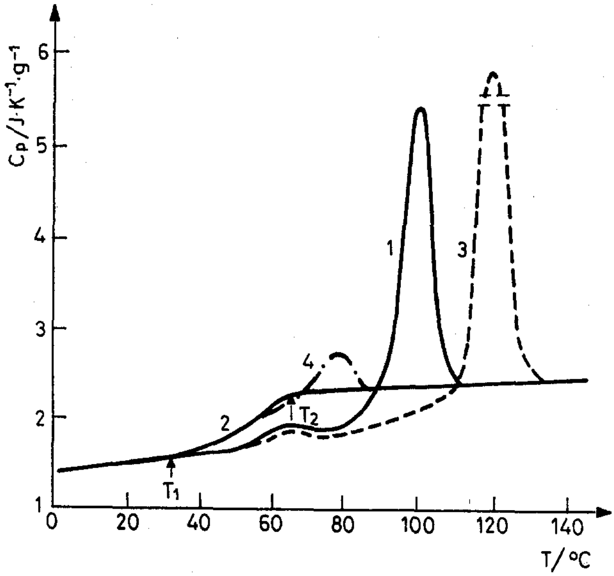

Fig. 4 Temperature dependence of heat capacity for crystalline (1, 4) and amorphous (2) Gelatin III and native collagen (3). Curve 1 is for the Gel A with Q_m=28.8 J/g, curve 4 for the Gel B with Q_m=6.5 J/g. Concentration of water is 14% for all samples, heating rate is 3 deg/min

A characteristic feature of the first heating curve is the maximum in the strong heat absorption, corresponding to the melting of the gel, which is superimposed on the practically linear temperature dependence of the gelatin heat capacity. Since all the water in the sample is in a bound state, the absorption peak corresponding to the melting of free water at T=0°C is completely absent.

Curve 2 in fact corresponds to the heating of the amorphous gelatin and shows an irregular dependence of the heat capacity in the range 30–70°C. The heat capacity changes linearly up to some temperature T_1. Above this temperature, the heat capacity abruptly increases up to some temperature T_2, after which the linear dependence of the heat capacity on temperature is restored, but with a slightly smaller slope. It can be concluded that there is a heat capacity jump for the amorphous gelatin. This jump can be characterized by the transition temperature T_g, which corresponds to the inflection point of the heat capacity curve, and by the magnitude of C_p, which is given by the difference in heat capacity values extrapolated to T_g along the linear parts of the temperature dependence from higher and lower temperatures.

For the amorphous gelatin with a water concentration of 14%, the value of C_p is 0.50±0.05 J/g deg. Below temperature T_1, amorphous and crystalline gelatins have the same value of heat capacity. Above this temperature, up to the melting range, the heat capacity for the amorphous gelatin is higher than that for the crystalline material.

The observed difference in the heat capacities of amorphous and crystalline gelatins in characteristic for amorphous and partly crystalline polymers in the temperature range of the glass transition. Thus, the anomaly in the temperature dependence of the heat capacity of amorphous gelatin can be ascribed to the heat capacity jump at the glass transition [13, 15].

We studied the influence of the heating rate and the thermal history of the sample on the behavior of the heat capacity in the jump region. With increase of the heating rate V_h from 1 to 5 deg/min, the transition temperature increases by 4–5 deg. The annealing of the amorphous samples at temperatures slightly below T_1 produces an additional maximum in the region of the jump in the temperature dependence curve. The temperature position of this maximum and its intensity increase with increase of the annealing time (Fig. 5).

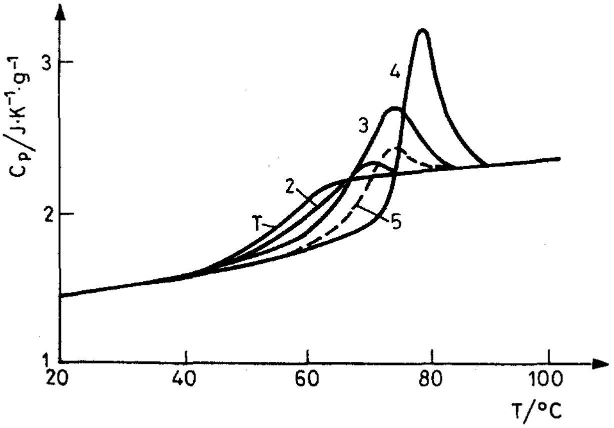

Fig. 5 Temperature dependence of heat capacity for the amorphous gelatin annealed at T=22°C during the different times: 1 – without annealing, 2–18 h, 3–3 days, 4 and 5 –10 days. Heating rate is 5 deg/min for curves 1–4 and 1 deg/min for curve 5. Concentration of water is 14%.

This additional maximum is known to have not a structural, but a relaxation origin. This means that the intensity of this maximum should decrease with lowering of the heating rate. In reality, we observed such a decrease when using the lowest heating rate of 1 deg/min. On the basis of our kinetic studies, we can conclude that the anomaly in the temperature dependence of the heat capacity of the amorphous gelatin reveals all the features characteristic for the glass transitions in amorphous polymers [13, 15].

We found that the temperature dependence of the heat capacity exhibits similar behavior for amorphous and crystalline samples of gelatin with the concentration of bound water varying form 5% to 30%. The magnitude of the heat capacity jump related to the dry weight of gelatin is practically constant, at ΔC_p= 0.50±0.05 J/g deg, whereas the glass transition temperature depends strongly on the water concentration (curve 4 in Fig. 2), especially for the samples with a small water content. On change of the water content by 1%, the shift in the glass transition temperature can amount to 5–7 deg.

It can be seen from Fig. 2 that the difference between the glass transition and melting temperatures depends on the method of preparation of crystalline gelatin: it reaches several tens of degrees for the samples of method A, whereas it is only several degrees for the method B samples with the same water content. It can be concluded that the bound water, which is an intrinsic element of the ordered structure of the gelatin, at the same time acts as a natural plasticizer, lowering the glass transition temperature.

We found that the dependence of the glass transition temperature T_g on the water concentration for the α-gelatin and denatured collagens of different origins coincides within the experimental accuracy with that for Gelatin III. Moreover, our experimental data on the glass transition tempera- tures as a function of the water content for a wide class of denatured proteins and nucleic acids [10, 11] fit curve 4 in Fig. 2 perfectly.

It can be supposed that the relation between the glass transition temperature and the water content established for gelatin in this work has a far more general applicability. The denaturation process destroys the unique native structure of biopolymers so that many denaturated polymers can be considered just as statistical copolymers composed of chains with different combinations of amino acid residues.

We also found the manifestation of the glass transition in crystalline gelatins. As can be seen from curve 1 in Fig. 4, the temperature dependence of the heat capacity of the starting crystalline gelatin prepared by method A does not show the jump corresponding to the glass transition. Such a jump is also absent for the native collagen with the same water content (curve 3 in Fig. 4). However, for both kinds of samples a small additional maximum can be observed in the temperature range of the glass transition. On subsequent heating this maximum is missing. However, after a long storage at room temperature the maximum is restored, with intensity proportional to the storage time. On change of the water content, the maximum shifts together with the shift in the glass transition temperature.

It can be concluded that the appearance of this additional maximum in the temperature dependence of the heat capacity of crystalline gelatin or native collagen is caused by the glass transition in the amorphous regions incorporated in both crystalline gelatin and native collagen [16]. The storage of the samples at room temperature most probably leads to the annealing of these amorphous regions and restores the additional maximum of relaxation origin.

It should be noted that the glass transitions in partly crystalline polymers do not always lead to the appearance of the jumps in the temperature dependence of the heat capacity. In some cases, particularly in fibers and oriented polymers with a high degree of crystallinity, the temperature de- pendence of the heat capacity shows no anomalies in the corresponding temperature range [17]. As an example, this is clearly demonstrated by the temperature dependence of the heat capacity of the native collagen which has a well-established fibrillar structure. The absence of the jump for the crystalline gelatins can serve as additional evidence in favor of the supposition that in samples prepared by method A, with high values of the melting heat, the collagen-like structures are formed.

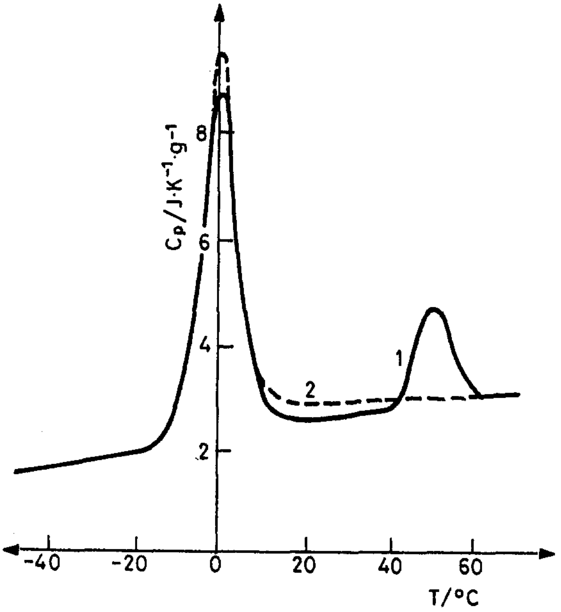

Fig. 6 Temperature dependence of heat capacity for crystalline (1) and amorphous (2) Gelatin III. Heating rate is 3 deg/min, concentration of water is 45%

At the same time, the crystalline gelatins with small melting heats prepared by method B demonstrate quite different behavior. Curve 4 in Fig. 4 shows the results for the gel formed from the sample with 14% water content during a 3-day storage at T=60°C. The heat capacity of this crystalline gelatin coincides with that of amorphous gelatin at all temperatures outside the melting range. The magnitude of the heat capacity jump and the temperature of the glass transition also have the same values as for amorphous samples. As follows from our results for the crystalline gelatins with small melting heats, the glass transition can be observed as clearly as for amorphous samples. It can be presumed that in these cases the structural relaxation of the amorphous phase is practically independent of the presence of crystalline collagen-like phase with a large number of defects.

The above results correspond to the cases when all the water present in the samples is in a bound state. We shall now consider the changes produced in the temperature dependence of the heat capacity by the presence of free water in the gelatin. We found that the amount of bound water in gelatin cannot exceed 30% of the total sample weight. All the excessive water exists in a free state, which is clearly evidenced by water freezing effects on cooling. Figure 6 shows the results for the crystalline (first heating) and amorphous (second heating) gelatin samples with 45% water content. In this case, 15% of the sample mass corresponds to the water in a free state. At around T=0°C, the melting of the frozen water can clearly be observed.

At temperatures above 10°C, the heat capacity of this sample is higher than that of crystalline samples, whereas at low temperatures of from –50° –5°C, the two values coincide within experimental accuracy. The temperature dependence of the heat capacity at this water content does not show any anomalies reflecting the glass transition. When the sample is cooled below T=0°C, the free water forms an ice matrix in which the gelatin chains are firmly fixed. As a result, the ice matrix inhibits the glass transition and only above its melting point does the heat capacity of amorphous gelatin exceed that of crystalline samples. Free water, in distinction to bound water, does not act as a plasticizer in gelatin, but forms a rigid matrix. The minimum temperature of the glass transition (–15°C) corresponds to the maximal content of water (30%) at which all the water molecules are still in a bound state.

Conclusions

It follows from our results that in water gels and crystalline gelatins collagen-like metastable structures are formed. The thermodynamic parameters of such structures are strongly dependent on the gelatin concentration and temperature of gel formation, and vary in a wide range. However, the melting heats and temperatures of gels never exceed the denaturing heat and temperature of the native collagen, which can serve as an indication that the collagen-like structures in gels are not completely perfect.

We have shown that the characteristic properties of the glass transition in gelatin are also dependent on the supermolecular structure and the degree of ordering. In amorphous gelatins and strongly disordered crystalline gelatins with small melting heats (Q <10 J/g) and low melting temperatures, the glass transition produces a heat capacity jump $\Delta C_p=0.50\pm0.05$ J/g deg. On the other hand, in crystalline gelatins with large melting heats (Q_m ~30 J/g) and high melting temperatures, the glass transition shows up only as a small additional maximum of a relaxation nature caused by the annealing of the amorphous phase.

The structural properties of gelatins are found to be strongly influenced by the state of the water present in the sample. The dependence of the glass transition temperature T_g on the bound water content is shown to be of general applicability for many denatured biopolymers. Free water in gelatins, in distinction to bound water, does not act as a plasticizer, but forms a rigid ice matrix inhibiting the glass transition.

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Zusammenfassung — Mittels DSC wurde im Bereich –50° bis +130°C die Temperaturabhängigkeit der Wärmekapazität für verschiedene Arten von Gelantine mit einem Wassergehalt von 2 bis 95 % untersucht. Es wurde gezeigt, daß in allen untersuchten Fällen metastabile kollagenähnliche Strukturen in Gelen oder kristallinen Gelantinen gebildet werden, deren thermodynamische Parameter von den Bildungsbedingungen abhängen. Es wurden die charakteristischen Eigenschaften der Glasumwandlungen in amorphen Gelantinen und kristallinen Gelantinen mit unterschiedlichen Schmelzwärmen und einem unterschiedlichen Gehalt an geordneter Phase bestimmt. Spezielle Aufmerksamkeit wurde den strukturellen Eigenschaften von freiem und gebundenem Wasser gewidmet. Es wurde gezeigt, daß die Ab-hängigkeit der Glasumwandlungstemperatur T_g vom Gehalt an gebundenem Wasser generell für viele denaturierte Biopolymere anwendbar ist. Im Unterschied zu gebundenem Wasser fungiert freies Wasser in Gelatinen nicht als ein Weichmacher, bildet aber eine starre Matrix, die die Glasumwandlung verhindert.