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Thermal stability of gelatin gels: Effect of preparation conditions on the activation energy barrier to melting

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ABSTRACT

10, 20, and 40 wt.% aqueous gelatin gels were prepared under isothermal (annealing at 15, 20, and 25 °C for 15 to 120 min) and nonisothermal (cooling at 1 °C min^{-1}) conditions. Isoconversional kinetic analysis of DSC data on gel melting (gel–sol transition) of all types of gels revealed significant variations in the activation energy throughout the process. Activation energy barrier to melting of isothermally prepared gels was in the range 160–190 kJ mol^{-1} and found to increase with increasing the annealing temperature that was the major effect discovered. Activation energy barrier to melting of nonisothermally prepared gels was determined to be around 120–140 kJ mol^{-1} and increase with increasing the concentration. Local reversibility of the gel melting was demonstrated by using temperature modulated DSC.

1. Introduction

Gelatin is a biopolymer produced by denaturing collagen, which is the major protein present in connective animal tissue. It is easily soluble in hot water in a wide range of concentrations. On cooling, gelatin solutions readily form gels. As other polymer gels, gelatin gels are soft solids or, in other words, are polymer solutions that lost their ability to flow because of crosslinking of polymer chains. Structurally, gelatin gels present an infinite network whose junctions are held by multiple hydrogen bonds. Gelatin gels have found numerous applications as food, pharmaceutical, cosmetic, photographic and other materials. Yet another novel application is in the area of tissue engineering materials that combine gelatin and various inorganic nanoparticles [1–14] One of the major factors defining the effectiveness of the applications of gelatin gels is their stability that is closely related to the gel structure (e.g., the number of junctions and their strength). Due to the non-equilibrium metastable nature of gels [15], their structure depends significantly on the temperature conditions of preparation. In particular gels can be prepared nonisothermally, i.e., by continuous cooling of a polymer solution, or isothermally, i.e., by cooling to a certain temperature and annealing at it for a period of time. The structure of nonisothermally prepared gels will differ from that of isothermally prepared gels, whose structures will also differ from each other depending on the temperature and time of annealing. For this reason, the temperature conditions of preparation are expected to affect stability of gels.

The present study is concerned with thermal stability of gelatin gels that is defined as their resistance to melting or, in other words, to the thermally stimulated transition from gel to sol. The specific goal of the study is to explore the effect of the concentration and the thermal conditions of preparation of gelatin gels on the activation energy barriers to the process of melting. The goal is accomplished by applying isoconversional kinetic analysis [16] to calorimetric data on the gel melting. As demonstrated in a recent review paper [17], this type of analysis has been instrumental in kinetic studies of a large variety of thermally stimulated process in polymers. Latest original applications of isoconversional kinetics involve the thermal denaturation of collagen [18], gelation of gelatin [19] and methylcellulose [20], physical aging of glass [21] and melting of polystyrene and polystyrene–clay gels [22]. The latter work [22] has initiated the application of isoconversional analysis to the gel melting kinetics, but did not touch upon the effect of the thermal conditions of the gel preparation on the activation energy, which is the specific topic of this study. The study focuses on the activation energy although it is not the only parameter that determines thermal stability. The reason is threefold. First, isoconversional methods evaluate the effective activation energy unambiguously, whereas the pre-exponential factor and reaction model are determined in a conjoint form so that their separation is associated with significant uncertainties. Second, unlike the two other parameters, the effective activation energy can

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allow for a relatively straightforward theoretical interpretation in terms of the energy barriers [16,17]. In the case of gel melting the effective activation energy is expected to represent the energy barriers to destruction of the network junctions in gel. Third, the activation energy defines the temperature sensitivity of the process rate that makes it the key parameter in practical kinetic predictions [16]. The novelty of this work consists in advancing the concept of the energy barrier as a means of characterizing the stability and melting of polymer gels.

2. Experimental part

Gelatin was purchased from Sigma–Aldrich (catalog number G9391). It was type B gelatin derived from lime cured bovine skin. According to the manufacturer, this gelatin has the Bloom number 225, the average molecular weight $\sim 50{,}000\ \mathrm{g\,mol^{-1}}$, and the isoelectric point 4.7–5.2. 10, 20, and 40 wt.% aqueous solutions were prepared by weighing the respective amounts of gelatin powder and deionized water in small vials and heating the mixtures at $\sim 60\ ^\circ\mathrm{C}$ in a water bath. The bath temperature was chosen to be $\sim 10\ ^\circ\mathrm{C}$ above the upper boundary of melting temperature range observed in the DSC experiments for the aforementioned gels. Since the formation of stable crosslinks (i.e., helices) is not possible at this temperature, the gelatin molecules must be present exclusively in the coil conformation. Attaining such initial state was considered essential for reproducible preparation of the gels. The hot solutions quickly gelled on cooling to a room temperature. The gel samples were stored in a refrigerator at 5 °C to slow down potential degradation. Refrigerated samples were used for less than a week, after which new samples had to be prepared. It was experimentally established that within a week of storage the samples exhibit reproducible behavior in terms of the heats and temperatures of both sol–gel and gel–sol transitions. The transitions were followed by regular (Mettler-Toledo DSC 823e) and stochastically modulated multi-frequency DSC (TOPEM® by Mettler-Toledo). Temperature, heat and tau-lag calibrations were performed by using indium and zinc standards. All measurements were conducted under nitrogen flow of $80\ \mathrm{ml\,min^{-1}}$. A typical sample size of gelatin gel was about 60–70 mg. Gelatin samples were cut from a single piece, put in 100 μl Al pans, and hermetically sealed. The absence of leaks (constancy of the sample mass) was confirmed by weighing samples after each DSC run. All DSC measurements were initiated by first erasing the thermal history that was accomplished by melting the refrigerated samples and holding them at 55–60 °C for 2 min. From this equilibrium state the gels were prepared under well controlled thermal conditions. Isothermal and nonisothermal conditions were used. Under isothermal conditions the solutions were cooled down at $20\ ^\circ\mathrm{C\,min^{-1}}$ from the equilibrium state to a given temperature of annealing and held at this temperature for a certain period of annealing time. The annealing temperatures were in the range 13–27 °C. The annealing times varied from 15 to 120 min. Under nonisothermal conditions the solutions were cooled down at $1\ ^\circ\mathrm{C\,min^{-1}}$ from the equilibrium state to $-10\ ^\circ\mathrm{C}$. Once the gels formed they were melted by heating at the rates 2.5, 5, 7.5, 10, and $12.5\ ^\circ\mathrm{C\,min^{-1}}$. Nonisothermally prepared gels were heated immediately after finishing the cooling segment. Heating of isothermally prepared gels immediately after completing the annealing segment could not produce a stable baseline before the melting effect was observed. To avoid this problem, on completion of the annealing segment isothermally prepared gels were cooled at $20\ ^\circ\mathrm{C\,min^{-1}}$ to $-10\ ^\circ\mathrm{C}$ and immediately after that reheated through melting at the aforementioned heating rates. Temperature modulated DSC runs were performed by using two different procedures. The first procedure involved heating of isothermally and nonisothermally prepared gels at $1\ ^\circ\mathrm{C\,min^{-1}}$. The second procedure was quasi-isothermal. It was designed by analogy with earlier quasi-isothermal measurements [23] performed on melting of crystalline polymers. The procedure included heating of isothermally and nonisothermally prepared gels in a series of isothermal steps that were performed in the range of temperatures from -8 to 61 °C. Each step lasted for a period of 15 min, on passing of which the temperature was raised by 3 °C. In either procedure the underlying heating program was overlaid with stochastic temperature pulses, whose amplitude was 0.5 K and the time between which was ranging from 25 to 50 s.

3. Isoconversional analysis

The DSC data have been treated by using an advanced isoconversional method developed by Vyazovkin [24,25]. The method allows one to reveal a variation in the effective activation energy (E_α) with the extent of conversion (α). The latter values are determined as partial areas of a DSC peak. The advanced method possesses two key advantages over simpler isoconversional methods such as those of Flynn and Wall [26] and Ozawa [27]. First, it is applicable to data obtained under arbitrary temperature program, $T(t)$ which provides the advantage of treating the data obtained on cooling as well as of accounting for temperature deviation due to self-heating/cooling. For a set of n runs conducted under different temperature programs, $T_\mathrm{i}(t)$, the effective activation energy is evaluated at each given α by finding E_α, which minimizes the function [24,25]

$$\Psi(E_\alpha) = \sum_{\mathrm{i}=1}^{n} \sum_{\mathrm{j}\neq i}^{n} \frac{J[E_\alpha, T_\mathrm{i}(t_\alpha)]}{J[E_\alpha, T_\mathrm{j}(t_\alpha)]} \tag{1}$$

where

$$J[E_\alpha, T_\mathrm{i}(t_\alpha)] \equiv \int_{t_{\alpha-\Delta\alpha}}^{t_\alpha} \exp\left[\frac{-E_\alpha}{RT_\mathrm{i}(t)}\right] \mathrm{d}t \tag{2}$$

Numerical integration over small time segments eq. (2) provides the second advantage which is elimination of a systematic error [25] found in the simpler integral isoconversional methods when E_α varies significantly with α. In eq. (2), α changes from $\Delta\alpha$ to $1-\Delta\alpha$ with a step $\Delta\alpha = m^{-1}$, where m is the number of intervals selected for computation. The integral, J in eq (2), is evaluated via the trapezoid rule. The minimization routine is carried out for each value of α to reveal a variation of E_α with α.

4. Results and discussion

4.1. Selecting the range of annealing temperatures

Because the gel melting is associated with rather small thermal effects, it has been important to identify the range of temperatures, annealing at which yields the largest amount of gel. This can be accomplished by using annealing temperatures from the vicinity of the temperature of the maximum gelation rate. The latter is known [28] to pass through a maximum in a fashion similar to the rate of isothermal crystallization of solutions [29] and melts [30,31]. By quickly cooling 17 wt.% gelatin solution to various annealing temperatures and using modulated DSC to measure the heat release, it has been previously found [19] that the maximum gelation rate lies between 14 and 19 °C. Following the same experimental procedure the present study has yielded the maximum temperatures of the gelation rate respectively around 15 and 20 °C for 10 and 40 wt.% solutions. As expected, the more concentrated solution has a somewhat higher temperature of the maximum gelation rate that means that it gels more readily, i.e., at smaller undercoolings. The temperatures evaluated appear consistent with the results of similar measurements by Tseretely and Smirnova [32] who find the maximum between 17 and 27 °C for 20 wt.% solution, and by Cuppo et al. [33] who report the maximum around 14 °C for 4.5 wt.% solution. Note that in the original paper by Goddard et al. [28] the maximum rate temperatures are reported to be markedly lower, e.g., for 20 wt.% solution the value is found to be between 5 and 10 °C. At any rate the present measurements suggest that the use of annealing temperatures around 15–20 °C should yield the larger amounts of gelatin gel when gelling the solution in the range of concentrations 10–40 wt.%.

4.2. *Effect of the annealing time*

Once the range of the temperatures for the gelation rate maximum has been estimated, a series of experiments has been carried out to probe the effect of the annealing time on the activation energy of the gelatin gel melting. The effect has been systematically studied on the 40 wt.% gels whose melting yields the best DSC signal to noise ratio. Other concentrations have not been explored because, as shown later (Section 4.3), the effect of the concentration on the thermal stability of gels is very insignificant. For this reason, the results obtained for the 40 wt.% gels are considered representative of the all gels studied in this work. The 40 wt.% gels studied here have been obtained by annealing the gelatin sols at 20 °C for periods of time 15, 30, 60, and 120 min. While arbitrarily chosen, this range has been found sufficient to identify the general trend. The use of the times shorter than 15 min would be associated with the problem of measuring of very small heats of melting. On the other hand, further doubling the longest annealing time (120 min) would make it impossible to run all annealing experiments followed by heating at the five heating rates within one week, which was found to be the useful life time of a single sample. The same sample had to be used within a single series of runs to exclude the undesirable sample-to-sample variation.

The melting curves presented in Fig. 1 demonstrate that the heat of melting increases markedly with increasing the annealing time. That is, a gel annealed for longer time forms more crosslinks. However, this does not necessarily mean that a gel becomes more thermally stable, e.g., more resistant to melting. An increase in the annealing time from 15 to 120 min has raised the melting peak temperature only by a little over 1 °C (Fig. 1). It means that while the total number of crosslinks increases, the stability of the network junctions has not increased much. It should be noted that a small increase of the melting peak temperature is still detectable calorimetrically over a period of several days [28,32]. In gelatin gels the network junctions are frequently purported to be refolded triple helix segments. By assuming that a relationship [30] between the melting temperature and lamellar thickness of polymer crystals holds for the melting of gelatin gels, Guo et al. [34] have proposed that an increase in the melting peak temperature of the gel should reflect an increase in the length of refolded segments.

Isoconversional analysis of melting of the gels respectively formed after different annealing times have produced virtually identical E_α dependencies for all annealing times. To avoid overcrowding Fig. 2 shows two E_α dependencies that correspond to the shortest and longest annealing times. As mentioned earlier, the experimental values of E_α are expected to reflect the energy barriers to destruction of the network junctions in gel. Therefore, the similarity of the obtained E_α dependencies suggests that these energy barriers are practically unaffected by the annealing time, at least when the latter is raised from 15 to 120 min.

Another important observation (Fig. 2) is that the effective activation energy decreases significantly (from ~170 to 95 kJ mol^{-1}) throughout the process of melting. This behavior is rather unexpected in a view of a previous work [22] in which the activation energies for melting of isothermally prepared polystyrene–CS_2 gels have been found practically constant throughout the process. The difference in the E_α dependencies for the two types of gels hints at a difference in their melting mechanisms. A discovery that E_α varies with α is typically an indication that the overall process includes multiple steps having different activation energies [16,17]. One could perhaps assume that the gel formed may consist of the network junctions having different strength and, thus, a different energy barrier to melting. However in such a case the weaker network junctions would disintegrate first followed by disintegration of the stronger ones so

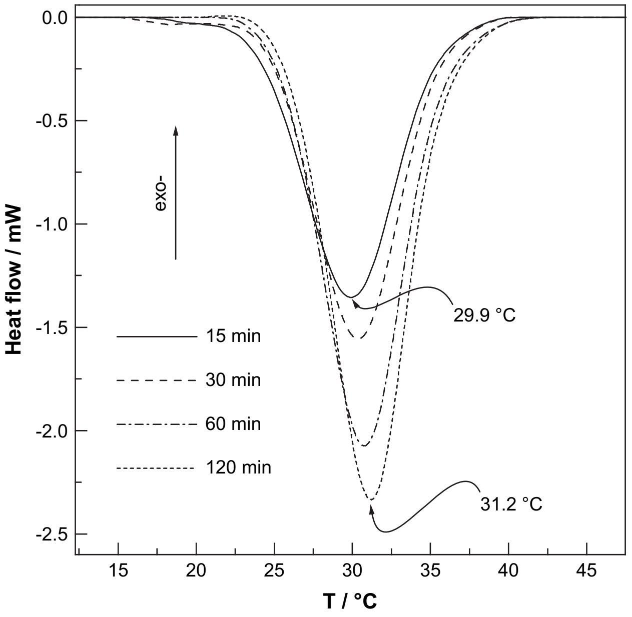

Fig. 1. Melting of 40 wt.% gels formed on annealing at 20 °C for periods of time from 15 to 120 min. Curved arrows mark the peak positions. Heating rate is 2.5 °C min^{-1}, sample mass 70.8 mg.

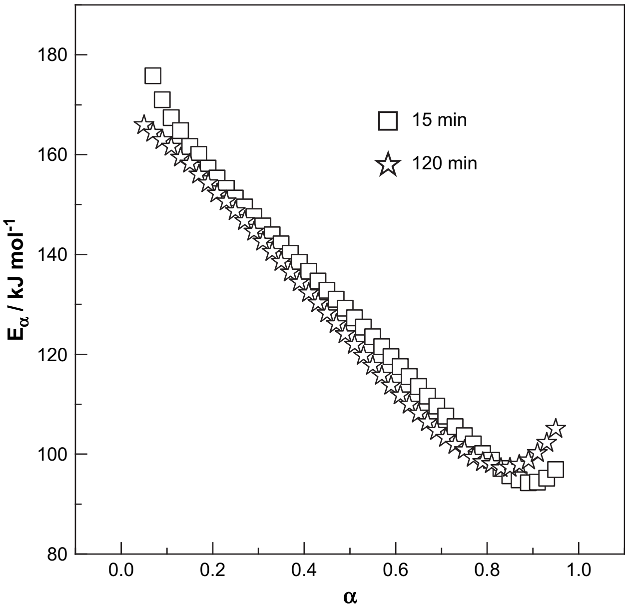

Fig. 2. E_α dependencies obtained for melting of 40 wt.% gels annealed at 20 °C for 15 and 120 min.

that the E_α value would rather have to increase with increasing α. A decrease observed in Fig. 2 shows a remarkable similarity to a decrease in E_α reported [16,17,35] for the glass transition in various polymeric and nonpolymeric glasses. In glasses, a large energy barrier for the initial stages of the glass transition reflects the strongly cooperative character of the molecular motion in the conditions of constricted free volume. As the temperature rises, the free volume increases relieving energetic constrains and causing the E_α values to decreases. It is proposed that a somewhat similar situation may occur in melting of gelatin gels. That is, disintegration of a typical network junction requires cooperative (i.e., simultaneous) breaking of multiple hydrogen bonds that crosslink the polypeptide chains. Since a typical range of hydrogen bond energies is 10–40 kJ mol^{-1} [36], the number of bonds to be broken cooperatively is in the range 4–17 as can be estimated from the E_α values for the initial stages of melting (Fig. 2). Once this initial large energy barrier is overcome and multiple crosslinks become broken, the polypeptide chains of the network junction acquire new conformational degrees of freedom. The resulting intensification of the chain motion destabilizes the network junction, therefore, lowering the energy barrier to gel melting.

4.3. Effect of the concentration

The next series of runs have been carried out to see whether the concentration of a gelatin solution has an effect on the activation energy of the gel melting. The gels have been prepared from 10, 20, and 40 wt.% solutions by annealing them for 1 h at 20 °C. As explained in the previous section (Section 4.2), the reasonable annealing times are limited by the range 15–120 min. Since the annealing time has been found not to practically affect the energy barrier, one could select any annealing time from that range. The annealing time of 1 h has been chosen as an optimum period that allows one to keep the total time of all annealing runs well within 1 week and at the same time yields a significant amount of gel whose melting results in a good DSC signal to noise ratio even for the system of the lowest concentration (10 wt.%).

Fig. 3 displays DSC melting curves of the respective gels. It is seen that the heat of melting increases with increasing the concentration that obviously indicates the presence of proportionally larger number of crosslinks in the gels of higher gelatin concentration. However, the melting peak temperature appears to be independent of the concentration. This suggests that the concentration of the gel does not affect the stability of the network junctions. In other words, the resistance toward melting or thermal stability of the isothermally prepared gels is practically unaffected by their concentration.

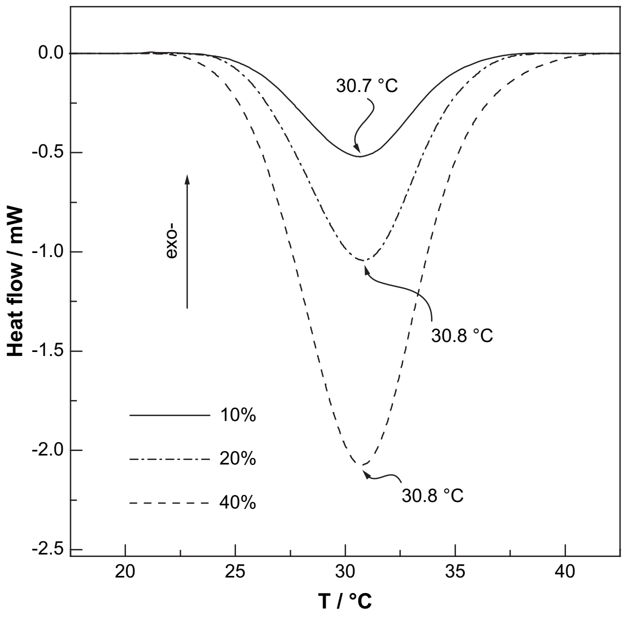

Fig. 3. Melting of gels of different concentration formed on annealing for 1 h at 20 °C. Curved arrows mark peak positions. Heating rate is 2.5 °C min^{-1}, sample mass is 63.9 mg (10 wt.%), 61.4 mg (20 wt.%), and 70.8 mg (40 wt.%).

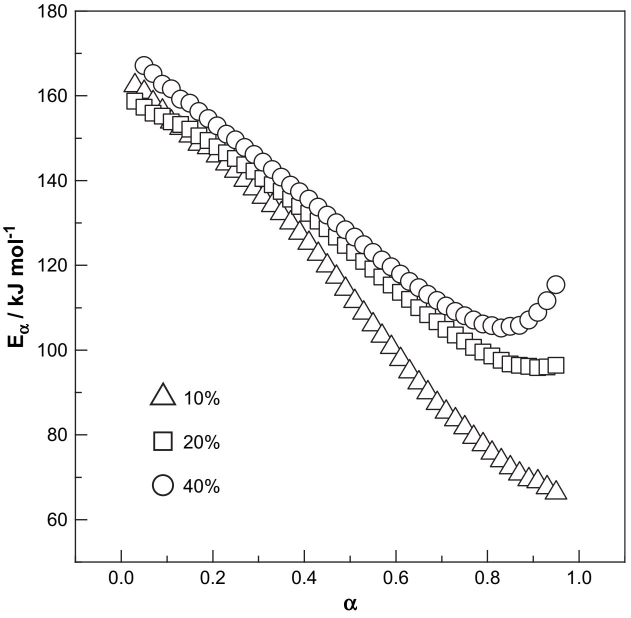

Fig. 4. E_α dependencies obtained for melting of gels of different concentration annealed for 1 h at 20 °C.

The DSC data on melting of the gels of the three different concentrations have been subjected to isoconversional analysis. The resulting E_α dependencies are presented in Fig. 4. As seen from this figure, the E_α dependencies for melting of the gels of different concentration are very similar up to the conversions values of about 0.4. Note that at higher conversions a significant fraction of the original gel structures has melted producing uncrosslinked polypeptide chain segments that can now participate in the growth of more thermally stable network junctions, therefore, causing structural reorganization of the original gel. A possibility of such process finds its confirmation in temperature modulated DSC measurements that, as discussed later (Section 4.6), demonstrate local reversibility of the gel melting. In the mean time, it should be stressed that the differences observed at higher conversions do not have to be directly linked to thermal stability of the original gels. The energy barriers to melting of the original gels are represented by the low conversion values of E_α that are about 160–170 kJ mol^{-1} for all three concentrations. These values are similar to the respective values obtained earlier for the 40 wt.% gels formed after different periods of annealing. It can again be concluded that disintegration of the network junctions requires cooperative breaking of multiple hydrogen bonds. It should also be noted that all three E_α dependencies in Fig. 4 demonstrate analogous decreasing trends, which are also similar to the trends observed for melting of the 40 wt.% gels obtained after different annealing times (Fig. 2). The similarity of the trends hints at the occurrence of a common melting mechanism.

4.4. Effect of the annealing temperature

The effect of the annealing temperature on the activation energy of melting has been examined on two sets of the 40 wt.% gels prepared by annealing for 30 min at 15 and 25 °C respectively. As shown in the previous Sections 4.2 and 4.3 both annealing time and concentration have practically no effect on the energy barrier of melting. Therefore, one can, in principle, select any of the three concentrations and five annealing times studied. The selection of the 40 wt.% gel has been motivated by the fact that, compared to 10 and 20 wt.% systems, the melting of this gel absorbs the largest amount of heat, which secures the best DSC signal to noise ratio. Furthermore, the heat of melting is large enough to obtain a good signal to noise ratio after only 30 min of annealing that has been selected as the annealing time for this series of the runs.

DSC curves for melting of the resulting gels are shown in Fig. 5. Melting of the gel obtained at 15 °C demonstrates a larger heat of melting indicating that it has a larger number of crosslinks. On the other hand, the melting peak temperature for the gel obtained at 25 °C has been reproducibly 5–6 °C greater than that for the gel obtained at 15 °C. Clearly, the gel produced at higher annealing temperature is more resistant to melting and, thus, is more thermally stable. The increase in stability must be linked to stabilization of the network junctions as a result of forming longer refolded triple helix segments [34] or, more generally, forming more crosslinks within the individual junctions.

Isoconversional analysis of the DSC data on melting of the gels prepared at 15 and 25 °C has yielded the E_α dependencies presented in Fig. 6. Both dependencies demonstrate a decreasing trend analogous to that observed for the dependencies estimated from melting of the gels obtained under other conditions (cf. Figs. 2 and 4). This again is a clue about the occurrence of a possible common melting mechanism for gelatin gels prepared under different conditions. Compared to the E_α values for melting of the gel obtained at 15 °C the respective E_α values for the gel produced at 25 °C are consistently larger by 10–30 kJ mol^{-1} which is an equivalent of 1–3 hydrogen bonds. The initial E_α values that are directly related to thermal stability of the gels are 170 and 190 kJ mol^{-1} for the gels respectively produced on annealing at 15 and 25 °C. The values are close to the initial E_α values for melting of the gels obtained under other conditions (Figs. 3 and 5) and once more point at the cooperative nature of the energy barrier, overcoming which requires simultaneous breaking of multiple hydrogen bonds.

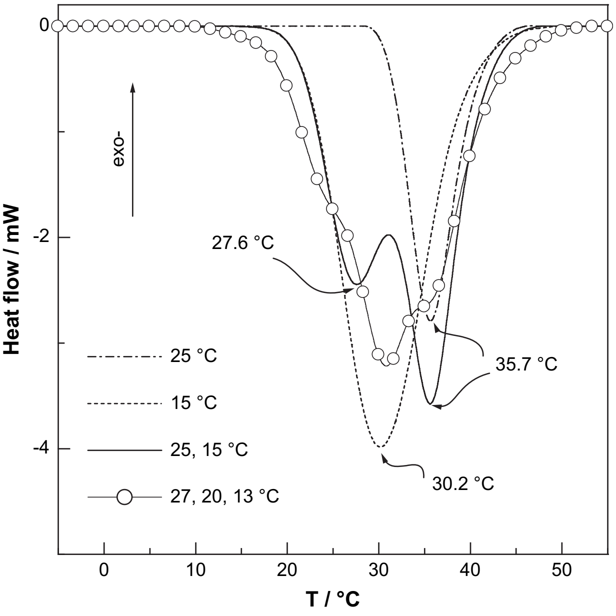

Fig. 5. Melting of 40 wt.% gel obtained in one-, two-, and three-step annealing. Temperature of the annealing steps is shown by the curve types. Duration of each step is 30 min. Curved arrows mark the peak positions. Sample mass is 65.8 mg (one- and two-step annealing) and 68.1 mg (three-step annealing). Heating rate is 5 °C min^{-1}.

The study of the effect of annealing temperature has been complemented by an additional set of experiments involving two successive 30 min steps of annealing at 25 and 15 °C. DSC data for melting of the resulting gel is shown in Fig. 5. The DSC curve demonstrates two distinct melting peaks that apparently reveal the formation of two gel structures, which differ in their thermal stabilities. The higher temperature melting peak appears at the same temperature as the melting peak for the gel obtained by single-step annealing at 25 °C. This is consistent with a rheological study by te Nijenhuis [37] who concluded that the structures formed at higher annealing temperatures persist at annealing at lower temperatures. On the other hand, the lower temperature peak is found at a temperature about 2–3 °C lower than the melting peak temperature for the gel obtained by single-step annealing at 15 °C. The peak also demonstrates a noticeable decrease in the heat of melting compared to that associated with melting of the gel obtained at 15 °C. The decrease in the melting heat simply indicates that in the two-step annealing procedure the less stable gel structure is formed in a smaller amount. This seems to be a trivial consequence of the fact that by the time the system reaches 15 °C it does not have that many uncrosslinked polypeptide chains because most of them have crosslinked in the first step at 25 °C. A more interesting observation is that the gel structure formed in the second annealing step at 15 °C melts at a lower temperature than the gel formed in a single-step annealing at 15 °C. This suggests that the presence of the gel structures formed at 25 °C limits the growth of the network junctions being formed at 15 °C. Apparently the limitation arises from the fact that in partially crosslinked gel the conformational degrees of freedom of the polypeptide chains are largely restricted. This makes it more difficult for new crosslinks to form and, therefore, for the network junctions to grow.

Also, several three-step annealing procedures have been tried. Unfortunately, all of them resulted in gels whose melting yields strongly overlapped DSC peaks. An example of melting of one such gel obtained by consecutive 30 min annealings at 27, 20, and 13 °C is shown in Fig. 5. Because of the strong overlap of the DSC peaks no further analysis has been pursued.

Isoconversional analysis has been applied to the DSC data on melting of the gels annealed consecutively at 25 and 15 °C. Fig. 6 shows the resulting E_α dependence that can be easily understood by comparison with the individual dependencies determined for melting of the gels prepared in the respective single-step annealings at 15 and 25 °C. The initial descending portion of this dependence is quite similar to the initial part of the E_α dependence for melting of the gel prepared at 15 °C. At the lowest conversions both dependencies demonstrate E_α around 170 kJ mol^{-1} that suggests the existence of similar energy barriers to melting. From DSC data (Fig. 5) it is clear that melting of the gel structures formed on annealing at 15 °C provide smaller contribution to the overall heat of melting than melting of the gel structures formed on annealing at 25 °C. Therefore, a transition from melting of the lower temperature structures to melting of the higher temperature structures should take place below $\alpha = 0.5$. Indeed, the descending E_α dependence breaks at around $\alpha = 0.3$ and climbs sharply to $E_\alpha \cong 195$ kJ mol^{-1} at $\alpha \cong 0.5$. Although melting of the lower temperature structures appear to persist past this region, the overall process becomes dominated by melting of the gel structures formed on annealing at 25 °C. In agreement with this, the second descending portion of the E_α dependence demonstrates the initial values similar to the E_α values found at lowest conversion in the E_α dependence for melting of the gel prepared at 25 °C. Remarkably, the initial portions of both descending dependencies demonstrate the presence of two different energy barriers whose values are

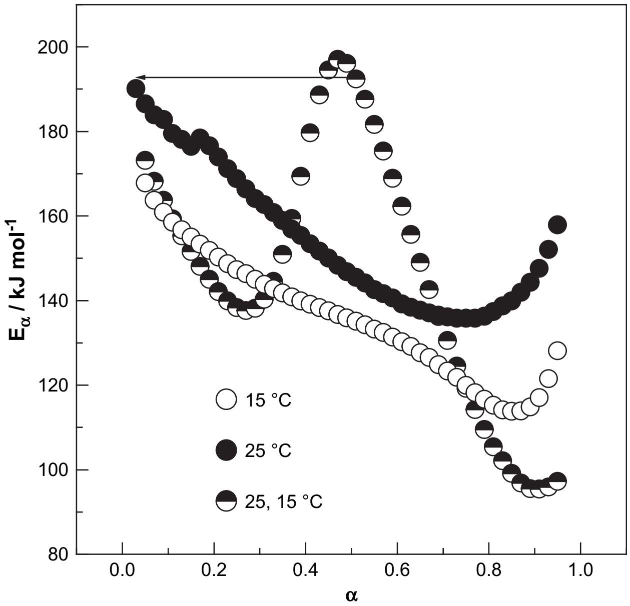

Fig. 6. E_α dependencies obtained for melting of 40 wt.% gels obtained in one- and two-30 min annealing steps performed at temperatures shown by the point types. Arrow shows respectively comparable parts of two- and one-step annealing processes.

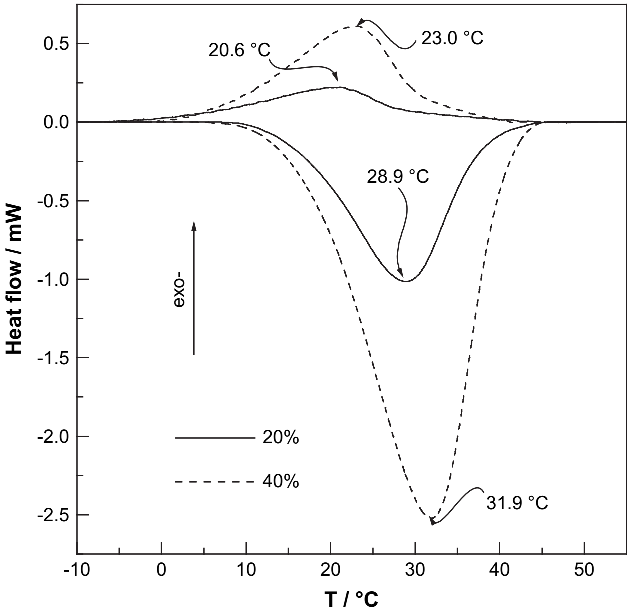

Fig. 7. Formation and melting of the gels obtained from 20 and 40 wt.% solutions. The respective sample masses are 61.4 and 66.2 mg. Exothermic gelation is measured on cooling at 1 °C min^{-1}, endothermic melting at 5 °C min^{-1}.

consistent with the values determined for melting of the individual gels prepared by annealing at 15 and 25 °C respectively.

The final stages of the melting process ($\alpha > 0.8$), demonstrate some unexpectedly low E_α values. However, as mentioned earlier (Section 4.3), the behavior of the E_α dependencies at high conversions should not be linked directly to the stability of the original gel structures that dominate the early stages of the overall melting process. The original structures disintegrate releasing uncrosslinked polypeptide chain segments that give rise to the formation of new gel structures. Note that the local reversibility of the gel melting is confirmed by modulated DSC (Section 4.6). As a result, the rate and, thus, the E_α values observed for the late stages of melting are most likely to be determined by the process of either formation of these new gel structures or their melting.

4.5. Nonisothermally prepared gels

Fig. 7 presents DSC data on melting of the gels prepared by continuous cooling at 1 °C min^{-1}. The major difference in melting isothermally and nonisothermally prepared gels is that in the latter melting starts at lower temperatures and proceeds over a wider temperature range. This behavior indicates the presence of a wide distribution of the gel structures having differing thermal stabilities. It is seen from Fig. 7 that the exothermic process of crosslinking (i.e., gelation) starts at ~40 °C and continues till ~0 °C. The maximum heat release rate takes place at ~23.0 and 20.6 °C in 40 and 20 wt.% solutions respectively. The gelation process occurring under these conditions can be thought of as a large number of very short annealing steps performed consecutively in the temperature range from 40 to 0 °C. An annealing program of such type should obviously produce a variety of gel structures whose melting peak temperatures decrease with decreasing the annealing temperature in manner similar to that observed in Fig. 5. The mass fractions of the structures formed should be proportional to the rate of crosslinking (Fig. 7). Since crosslinking on cooling at 1 °C min^{-1} occurs at its maximum rate around 20 ± 5 °C, the structures formed in largest fractions should have thermal stability comparable to that of the structures formed on isothermal annealing at 15, 20 and 25 °C. However, the DSC data also indicate that crosslinking continues to occur at a significant rate at temperature below 15 °C. The process in this range should give rise to the gel structures whose melting peak temperatures are lower than that of the gel obtained isothermally at 15 °C. These structures should have lower resistance to melting and should, thus, be associated with lower energy barriers.

Fig. 8 presents the results of isoconversional analysis as applied to the DSC on melting of nonisothermally prepared gels of the concentration 40 and 20 wt.%. Both E_α dependencies demonstrate a similar shape that differs markedly from that of the E_α dependencies found (Figs. 2, 4, and 6) for melting of isothermally prepared gels. Both dependencies in Fig. 8 demonstrate an increase in E_α in

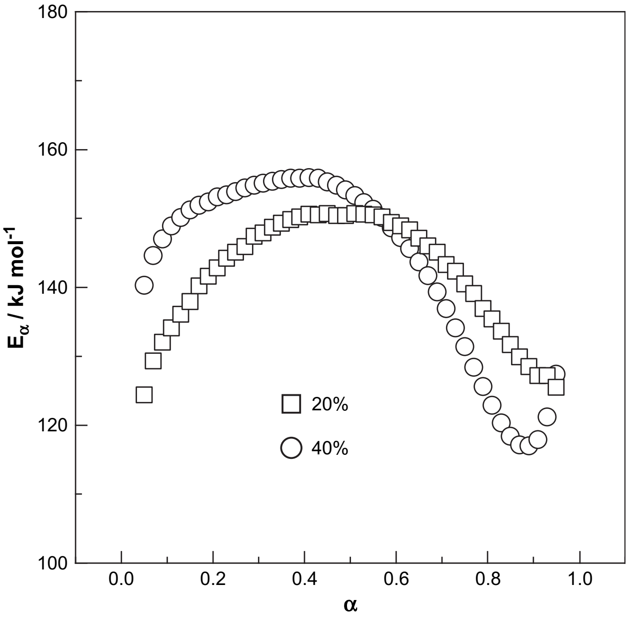

Fig. 8. E_α dependencies obtained for melting of 20 and 40 wt.% gels obtained on cooling of the respective solutions at 1 °C min^{-1}.

the range of $\alpha < 0.4$. The initial E_α values are around 125 and 140 kJ mol^{-1} for melting of the 20 and 40 wt.% gels respectively. Either of these values is markedly smaller than any of the initial values found for melting of the isothermally prepared gels. This indicates the presence of the gel structures whose disintegration faces a lower energy barrier or, in other words, the structures, in which network junctions involve fewer crosslinks. As temperature rises melting proceeds through disintegration of the increasingly more thermally stable structures, i.e., the structures having network junctions held together by more crosslinks. This process is reflected in the increasing E_α dependence. A decrease in E_α observed at $\alpha > 0.4$ appears to be an effect of a nonuniform mass distribution of the gel fractions. As mentioned above, the gel structures formed at 20 ± 5 °C should make the largest mass fraction of the total gel formed on cooling at 1° min^{-1}. More stable structures whose disintegration is associated with larger energy barriers are present in the overall gel in increasingly smaller mass fractions. Although they melt at higher temperatures, the overall melting process is dominated by the final stages of melting of overwhelmingly present less stable structures. As a result, the effective activation energy of the overall process demonstrates a decreasing dependence similar to that observed in the isothermally obtained gels.

Another noteworthy fact is that the obtained E_α dependencies demonstrate an effect of the concentration on the energy barrier to melting. Recall that no such effect is detected in isothermally prepared gels (Fig. 4). The effect seems rather straightforward for the initial E_α values that are directly associated with the melting and stability of the originally formed gel. As seen from Fig. 7, crosslinking occurs faster in the more concentrated 40 wt.% solution and reaches its maximum rate at 23.0 °C. In the 20 wt.% solution, the maximum is reached at a lower temperature, 20.6 °C. Since gelation of the 40 wt.% solution takes place at higher temperatures than gelation of the 20 wt.% solution, a gel formed from the former should be composed from more thermally stable structures than a gel formed from the latter. For this reason, melting of the originally formed 40 wt.% gel should encounter larger energy barriers than melting of the originally formed 20 wt.% gel.

Unexpectedly, the effect of concentration reverses for the later stages ($\alpha > 0.4$) so that melting of the 40 wt.% gel appears to encounter a smaller energy barrier than melting of the 20 wt.% gel. However, as mentioned earlier, the behavior of the E_α dependencies at high conversions should not be linked directly to the stability of the original gel structures. Since about a half of the original structures have already disintegrated at this point, the E_α values observed at $\alpha > 0.4$ are likely to be determined by an intricate combination of the energy barriers for the formation and melting of new gel structures as well as for possibly continuing melting of the survived original structures.

4.6. Stochastically modulated DSC

Stochastically modulated multi-frequency DSC is a recently introduced [38] technique that combines isothermal or continuous heating or cooling temperature program with stochastic temperature perturbations. It splits the total heat flow, Φ_{tot}, into the reversing, Φ_{rev}, and non-reversing, Φ_{non}, parts that at small perturbations and slow rates of temperature change become respectively similar to the sensible, Φ_{sen}, and latent, Φ_{lat}, heat flows [38]

$$\Phi_{\mathrm{tot}} = \Phi_{\mathrm{rev}} + \Phi_{\mathrm{non}} = \Phi_{\mathrm{sen}} + \Phi_{\mathrm{lat}} \tag{3}$$

Since Φ_{sen} originates from temperature variations and is proportional to $\beta = \mathrm{d}T/\mathrm{d}t$, it can be estimated as Φ_{rev}, which is a part of Φ_{tot} correlated with perturbations in β that arise from temperature perturbations:

$$\Phi_{\mathrm{sen}} \cong \Phi_{\mathrm{rev}} = mc_{\mathrm{p}}\beta \tag{4}$$

where m is the mass and c_{p} is the heat capacity. The correlated part of the heat flow permits evaluating the complex (i.e., frequency, ω dependent) heat capacity that can be represented as the sum of two parts:

$$c_{\mathrm{p}}^{*}(T,\omega) = c_{\mathrm{p,fast}}(T) + c_{\mathrm{p,slow}}^{*}(T,\omega) \tag{5}$$

The first part, $c_{\mathrm{p,fast}}$ is associated with fast molecular motions, such as vibrations, whose characteristic time is significantly shorter than the characteristic time of temperature perturbations. This part is frequency independent. The second part is frequency dependent because it represents relaxation processes associated with slow molecular motions, whose characteristic time is comparable to that of temperature perturbations. The complex heat capacity evaluated at zero frequency yields the quasi-static heat capacity, $c_{\mathrm{p,0}}$, which depends only on temperature and at sufficiently low rates of temperature change and small temperature perturbations becomes identical with regular equilibrium heat capacity. When Φ_{lat} originates from fast irreversible transformations it cannot be reversed by small temperature perturbations. However, in the case of reversible transformations temperature perturbations may partially reverse Φ_{lat} therefore providing an excess contribution to the measured values of Φ_{rev} and $c_{\mathrm{p,0}}$. The latter then acquires a meaning of time dependent apparent heat capacity.

$$c_{\mathrm{p,app}}(T,t) = c_{\mathrm{p,0}}(T) + c_{\mathrm{p,exc}}(T,t) \tag{6}$$

where $c_{\mathrm{p,exc}}$ represents the excess contribution. The excess contributions have been widely observed for melting of various polymer crystals [23]. A recent application of stochastically modulated multi-frequency DSC has revealed [19] the excess contribution in gelation of gelatin solutions. Here, the technique is applied for the first time to the process of gel melting. The runs have been performed on 40 wt.% gels prepared by using isothermal and nonisothermal procedures. Isothermally prepared gels have been obtained by cooling 40 wt.% solution from 60 to 20 °C at 20 °C min^{-1} and then annealing it at this temperature for 1 h. Nonisothermally prepared gels have been produced by cooling 40 wt.% solution from 60 to −10 °C at 1 °C min^{-1}.

Fig. 9 presents the total and reversing heat flows measured by stochastically modulated DSC on isothermally and nonisothermally prepared gels. It is seen that melting in both systems demonstrates the presence of the reversing component. The presence of such component has been previously detected by applying the same technique to the thermal denaturation collagen (i.e., collagen to gelatin conversion) [18] as well as to gelation of gelatin (i.e., sol to gel transition) [19]. However, in both cases the reversing component has amounted to only a few percent of the total heat flow. For melting of gelatin gels, the reversing component amounts to ~40% of the total heat flow. The reversing component represents the fraction of the latent heat flow that is correlated with the stochastic temperature perturbations and is associated with the part of the gel melting that proceeds reversibly on the time scale of the perturbations. In other words, a part of the gel melted during the positive temperature perturbations can convert to gel during the negative perturbations. The active reversibility observed in a wide temperature range suggests that the global disintegration of the gel structures that occurs on melting may partially be accompanied by local gelation formation. This can occur when disintegration of less thermally stable network junctions cuts loose several polypeptide chains fragments that become capable of readily producing new crosslinks, the formation of which can contribute to the growth of

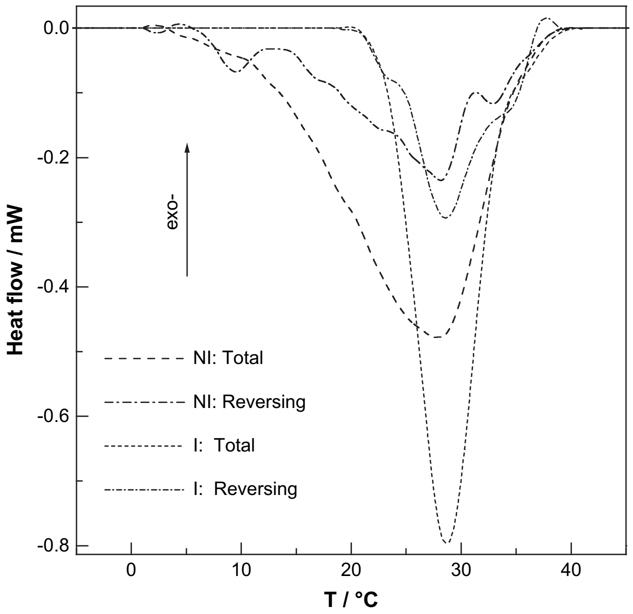

Fig. 9. Reversing and total heat flow measured by modulated DSC for melting of isothermally (I) and nonisothermally (NI) prepared gels. Heating rate is 1 °C min^{-1}, sample mass is 66.0 mg.

the existing more thermally stable junctions. Note that the process of the gel formation remains possible as long as the temperature is below the gelation temperature, which according to the DSC data (Fig. 7) is around 40 °C for 40 wt.% gel.

Fig. 10 displays the results of quasi-isothermal measurements of the heat capacity performed by applying stochastically modulated DSC to isothermally and nonisothermally prepared gels. The steady state heat capacity has been measured over 15 min periods at multiple temperatures. The obtained values have been averaged over the respective periods and assigned to the respective temperatures. It is seen that the resulting values represent an apparent heat capacity that contains a noticeable excess contribution that reveals itself in the form of the peaks. Such peaks have been widely observed [23] in quasi-isothermal heat capacity measurements on reversible melting of crystalline polymers. The observed excess contribution (Fig. 10) again suggests that melting of isothermally and nonisothermally prepared gels is partially reversible so that the process of disintegration of less thermally stable gel structures can contribute to their reorganization into the more thermally stable structures.

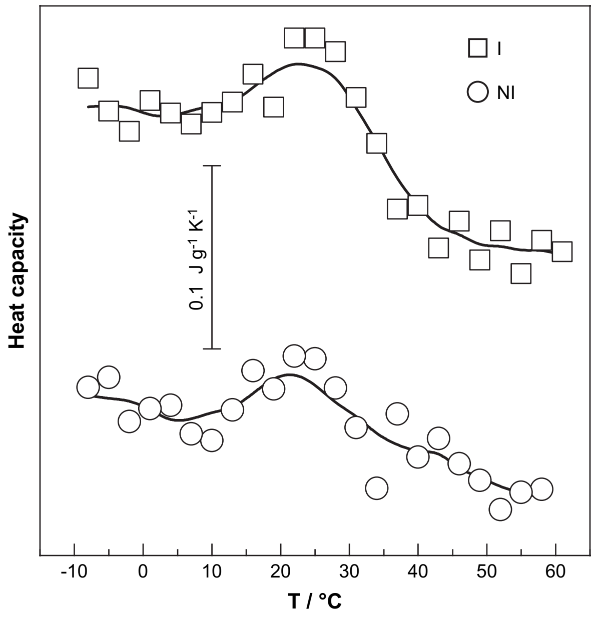

Fig. 10. Apparent heat capacity for melting of isothermally (I) and nonisothermally (NI) prepared gels measured by modulated DSC in quasi-isothermal mode. Solid line is a smoothed trend. Sample mass is 66.0 mg.

5. Conclusions

The study has comprehensively explored the effect of the conditions of preparation of gelatin gels on the activation energy barriers to the gel melting. Isoconversional analysis of melting of isothermally prepared gels has revealed a significant decrease in activation energy with increasing conversion. The large values estimated at low conversions suggest that the energy barrier originates from cooperative breaking of multiple hydrogen bonds. Of explored preparation conditions, the time of annealing and the concentration of gelatin solution have been found to have no significant effect on the energy barriers to melting. In contrast, an increase in the annealing temperature has been demonstrated to cause an increase in the energy barrier to the gel melting. Isoconversional analysis of melting of nonisothermally prepared gels has yielded effective activation energy that passes through a maximum with increasing conversion. The activation energies estimated at low conversions have been markedly smaller than the respective values for isothermally prepared gels. It has been discovered that an increase in the concentration of gelatin solutions causes an increase in the energy barrier to melting of nonisothermally prepared gels. Temperature modulated DSC has revealed that melting of both isothermally and nonisothermally prepared gels is a partially reversible process that may lead to reorganization of the original gel structures. The results suggest that an experimentally derived dependence of the activation energy on conversion can serve as an effective means of describing the thermal stability and melting of polymer gels.

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