Thermal properties of gelatin films

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Thermal properties of dehydrated 'hot-cast' gelatin films, obtained from hake skin, were studied using differential scanning calorimetry (d.s.c.) and thermal mechanical analysis (t.m.a.). Two glass transition temperatures, at 120°C and at 180°–190°C, were obtained. The low-temperature transition is assigned to the devitrification of blocks rich in α-amino acids, while the high-temperature transition is attributed to the devitrification of blocks rich in imino acids. For hydrated gelatins both transitions are shifted to lower temperatures. Differences in the behaviour of fish and mammalian gelatins were found. The influence of crosslinking with formaldehyde upon the thermal properties is analysed. The crosslinked fish gelatin devitrifies progressively in the 100°–200°C temperature range.

(Keywords: gelatin; collagen; hake skin; glass transition; glassy state relaxations; crosslinked gelatin)

INTRODUCTION

Collagen, a major protein constituent of connective tissues, is characterized by four different levels of order[1]. Gelatin includes some semicrystalline or amorphous state collagen (lacking partially or completely in tertiary (triple helical) and quaternary structural order).

Gelatin is obtained from collagen by heating above the helix–coil transition temperature. When such heating is carried out in solution, it causes a collapse of the rodlike, three stranded collagen unit (tropocollagen molecule) into a random coil. Moreover, there is a partial disaggregation of individual chains leading to α (one chain), β (a covalently bonded pair) and γ (three covalently bonded chains) coiled polymeric units.

Gelatin films cast from solution at temperatures below the helix–coil transition temperature ('cold-cast' gelatin) partially rebuild the tertiary structure, whereas films cast above the critical temperature level ('hot-cast' gelatin) are completely amorphous.

The thermal properties of gelatin samples have been extensively studied in the past[1-8]. Both Hirai[2] and Flory and Garrett[3] reported a glass transition temperature, (T_g), at 95°C for dry gelatin samples. A dilatometer with a very high sensitivity was used to show the very weak second-order transition[2]. Okamoto and Saeki[4] reported a T_g at 120°C by viscoelastic measurements. However, Yannas and Tobolsky[5] observed a T_g close to 190°C, associated with a sharp drop in the shear modulus of dry gelatin. Koleske and Faucher[6] studied the mechanical loss properties of gelatin samples containing 9.6 wt% moisture. They reported a low temperature transition at $-85°C$, a large loss peak at 130°C and another at 180°C, showing the complex nature of this material. Later, Gillham[7] reported a T_g at 200°C by torsional braid analysis for a carefully dehydrated gelatin sample. At the same time, Yannas and Tobolsky[8] reported values of T_g at 175°±10°C for uncrosslinked gelatin samples and

196°±3°C for crosslinked samples using differential thermal analysis and viscoelastic methods. Moreover, Yannas[1] stated the possibility that reported T_g values for gelatin (which were lower than 150°C), had been obtained on imperfectly dehydrated specimens. However, it is difficult to reconcile this statement with the experimental evidence reported in the literature. In any case, the T_g of gelatin samples still seems to be an open question.

The aim of this study is to present and discuss results related to the thermal transitions of 'hot-cast' gelatin films. The influence of the gelatin primary structure, the moisture content and the introduction of crosslinks will be analysed.

EXPERIMENTAL

Materials

The gelatin source was the skin of the patagonian hake (*Merluccius hubbsi*). The hake is broadly distributed in the Atlantic and Pacific Oceans (*Merluccius bilinearis*: USA, east coast; *M. productus*: USA, west coast; *M. gayi*: Chile and Perú; *M. albidus*: Gulf of Mexico; *M. carpensis* and *M. paradoxus*: South Africa; *M. merluccius*: Europe; *M. senegalensis*: Africa, etc.). It is one of the most important sources of white fillet, giving skins as an important by-product. In general, fish collagens have a lower imino acid content than mammalian collagens[9]. Its influence upon the observed thermal properties will be discussed in the following section.

The procedure used for the leaching of collagen from hake skin has been described previously[10,11]. Briefly, it consists of the following operations: (a) washing of the skins in running water to remove foreign particles, and rinsing with demineralized water to remove salts; (b) cutting of the skins in several pieces to obtain a good contact with the solution in the following step; (c) leaching of the skins with an acetic acid solution (3% by volume of
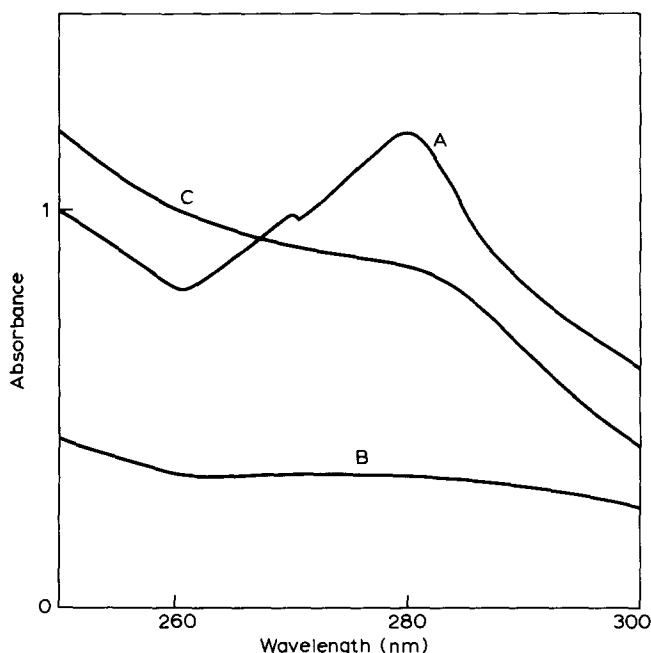
Figure 1 U.v. absorption spectra of collagen/gelatin solutions; curve A, beef-tendon collagen after a single extraction with Na_2HPO_4 (after ref. 12); curve B, rat-tail collagen highly purified (after ref. 12); curve C, gelatin leached from hake skin

glacial acetic acid in water), in a 1:1 ratio (weight of skins, g:volume of solution, ml), at 80°C, during 3 h, followed by filtration, to give a liquor containing 8–10 g gelatin/dl. The purity of the resulting solution was analysed with u.v. spectra in the region 250–290 mμ[1].

Films were cast from the gelatin solution by solvent evaporation and annealing at 60°C, during 48 h at atmospheric pressure. Different annealing periods were used when studying their influence upon the thermal properties of the films.

Some samples were also prepared following the procedure described by Yannas[1], to obtain films with an extremely low level of hydration. It consisted in a two-step dehydration of the films cast at 60°C, starting with a 24 h treatment at 105°C under atmospheric pressure, followed by a 24 h step at 105°C under a vacuum of 5×10^{-3} Torr.

Also, gelatin films containing formaldehyde were prepared by adding 5% by volume of a technical formalin solution (37 wt% formaldehyde) to the gelatin liquor, and casting at 60°C as described above.

Due to the hydrophilic character of the gelatin films, they were tested immediately after preparation.

Equilibrium moisture

Films were exposed to atmospheres of different relative humidities, in equilibrium with sulphuric acid solutions of different concentrations, at 18°C. The absorption was followed until a constant weight was obtained. A period of two months proved to be adequate. The weight increase was referred to a zero level arbitrarily assigned to the sample prepared at 105°C.

Thermal properties

The thermal properties of gelatin films were measured using differential scanning calorimetry (d.s.c.) and thermal mechanical analysis (t.m.a.). A Du Pont 990 thermal analyser provided with a d.s.c. 910 pressure cell, and a t.m.a. 943, calibrated with usual procedures, were used.

The d.s.c. runs were carried out at a 10°C min^{-1} scanning rate, under nitrogen at 4.5×10^6 Pa (650 psi). The t.m.a. runs were performed using an expansion probe with an added weight (5–10 g), at a 5°C min^{-1} heating rate. The use of the liquid nitrogen accessory permitted to make measurements below room temperature.

RESULTS AND DISCUSSION

Purity of the gelatin liquor

U.v. spectroscopy may be used to detect the presence of impurities in collagen/gelatin solutions[1,12]. The method is based on the observation that a highly purified sample shows no absorption peaks in the region 250–290 mμ. *Figure 1* shows u.v. spectra for an impurified sample (curve A), the same sample highly purified (curve B) and our own gelatin solution as resulting from the leaching process (curve C). It may be stated that impurities absorbing in the 250–290 mμ range are not present in significant concentrations in our gelatin sample. Moreover, precipitating the gelatin with $(NH_4)_2SO_4$, filtering and redissolving did not change the reported u.v. spectrum.

Equilibrium moisture

The moisture content of gelatin films cast at 60°C was found to be less than 1 wt%, with respect to films prepared at 105°C under vacuum (taken as the zero humidity level). Thus, results reported in this paper correspond to highly dehydrated gelatin specimens (unless otherwise stated).

Figure 2 shows the equilibrium moisture content (mass of water/mass of dry solid × 100) of hake skin gelatin for different relative humidities in the atmosphere. The original films behaved like brittle glasses, while samples equilibrated with air of relative humidity close to or higher than 60% showed a rubbery behaviour at room temperature. Thus, a moisture content close to 13% (g water/g dry solids × 100) leads to a glass–rubber transition at room temperature. This is a reversible transition
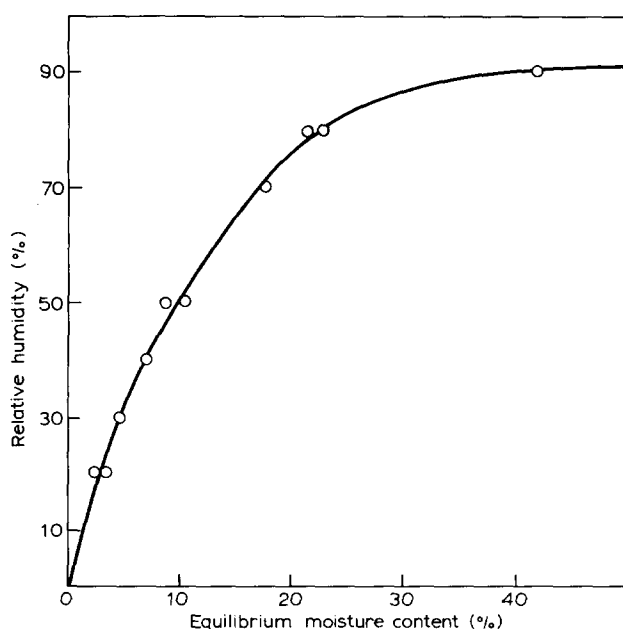
Figure 2 Equilibrium moisture content (mass of water/mass of dry solids × 100) of hake skin gelatin equilibrated at 18°C with air of different relative humidities
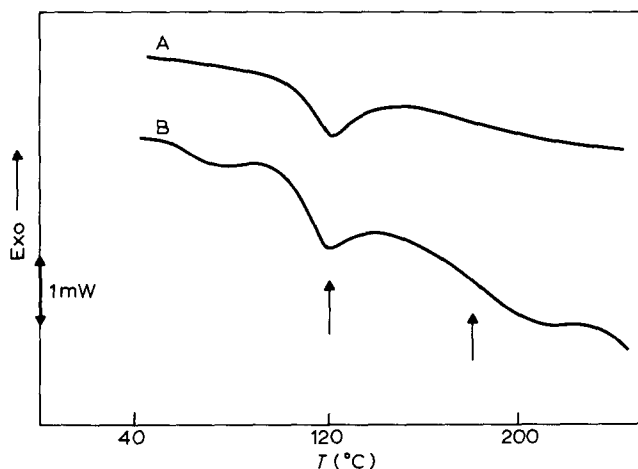
Figure 3 D.s.c. curves of gelatin samples; Curve A, sample obtained by heating 2 days at 60°C; Curve B, sample obtained by heating 2 days at 60°C, 1 day at 105°C and 1 day at 105°C under vacuum (5×10^{-3} Torr)

as qualitatively shown by the fact that films stored at ambient conditions changed the behaviour from rubber to glass and *vice versa*, depending on the prevailing relative humidity.

According to Finch and Jobling[13], mammalian gelatin films are plasticized with a 27% absorbed water (rubber-like behaviour), while a 15% was found to be insufficient to modify the glass-like behaviour of dry gelatin. It follows that fish gelatins are plasticized more easily than their mammalian counterparts. This may be attributed to the low content of the imino acids proline and hydroxy-proline possessed by fishes (particularly by cold water species). This is, in turn, probably related to the body temperature of the species. The bearing of this fact on the thermal properties of gelatin films will be further discussed in next section.

Thermal properties of uncrosslinked gelatin films

Figure 3 shows d.s.c. runs for gelatin films cast at 60°C and 105°C. There is no significant difference between both samples, giving extra evidence that they are both highly dehydrated specimens. Two glass transitions are shown in the Figure: one at 120°C, characterized by an energy absorption overlapped with the change in the specific heat, and the other, broad and less pronounced, at a temperature close to 180°C. At temperatures in the range 230°–260°C an endothermal runaway which may be associated with thermal degradation[1], takes place.

The t.m.a. behaviour, shown in *Figure 4*, agrees with the d.s.c. results. A dehydrated sample (curve A) shows two glass transitions at 120°C and 190°C; the last one is partially superimposed with the rubbery flow region starting at 200°C approximately. Moisture absorption shifts both glass transitions to lower temperatures. For a sample with 20% moisture on a dry basis (curve B), the corresponding temperatures are $-30°C$ and 80°C, while the rubbery flow region still starts at 200°C. This sample behaves as a plasticized film at room temperature. So, it may be stated that the first glass transition is associated with the overall behaviour, i.e. the one of the matrix or continuous phase.

In order to give an interpretation to the observed thermal properties it is necessary to describe the known aspects about the primary structure of gelatin. It is

accepted that, to a rough approximation, gelatin (or collagen) is a block copolymer composed of sequences in which mainly α-amino acids are present, including glycine at every third position (soft blocks), and sequences mainly made up of the imino acids proline and hydroxyproline, including glycine at every third position (rigid blocks)[1]. Thus, the rough model may be represented by

$$-(Gly-A-B)_x-(Gly-Pro-Hypro)_y-$$

where A and B denote any of the α-amino acids present in gelatin.

The restrictions on rotation imposed by the imino acids in the peptide linkage lead to the rigidity of blocks containing proline and hydroxyproline (pyrrolidone rings). Moreover, two pyrrolidine rings in sequence determine the positions of nine bonds which is one complete turn of the polyproline helix[9] (secondary structure). Thus, the imino acid content determines the extension of the protein secondary structure. Mammalian gelatins have a high imino acid content leading to a well developed secondary structure (the picture may be one of a matrix of rigid blocks with a low content of dispersed soft blocks). On the other hand, gelatin obtained from cold water fish has the lowest imino acid content[9,14]. This produces a weak secondary structure (a matrix of soft blocks containing dispersed rigid blocks).

The reported experimental results may be now explained on the basis of the proposed model. The first glass transition (at a temperature close to 120°C) is attributed
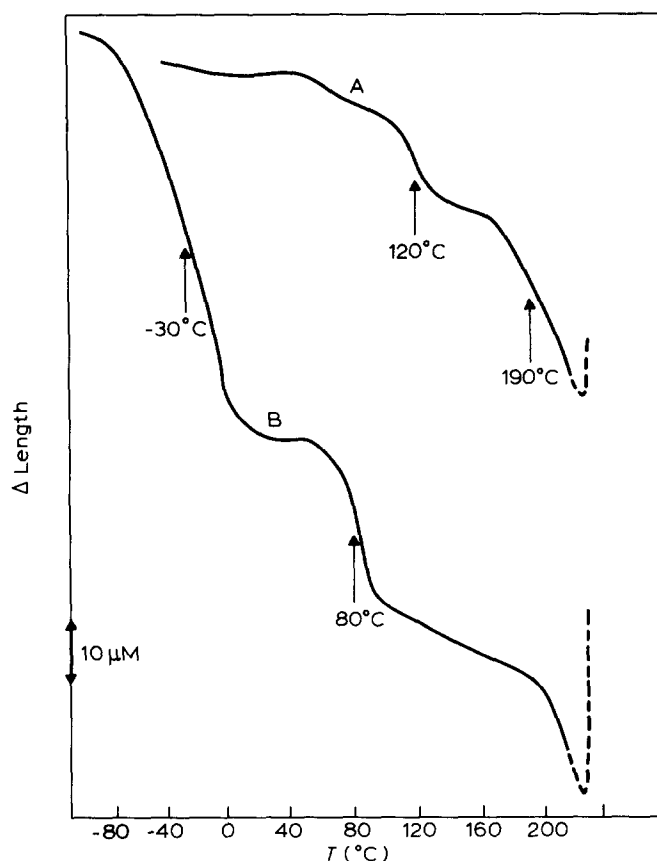
Figure 4 T.m.a. curves of gelatin samples; curve A, dehydrated sample (2 days at 60°C), weight on the probe = 7 g; curve B, sample with 20% moisture on a dry basis, weight on the probe = 5 g
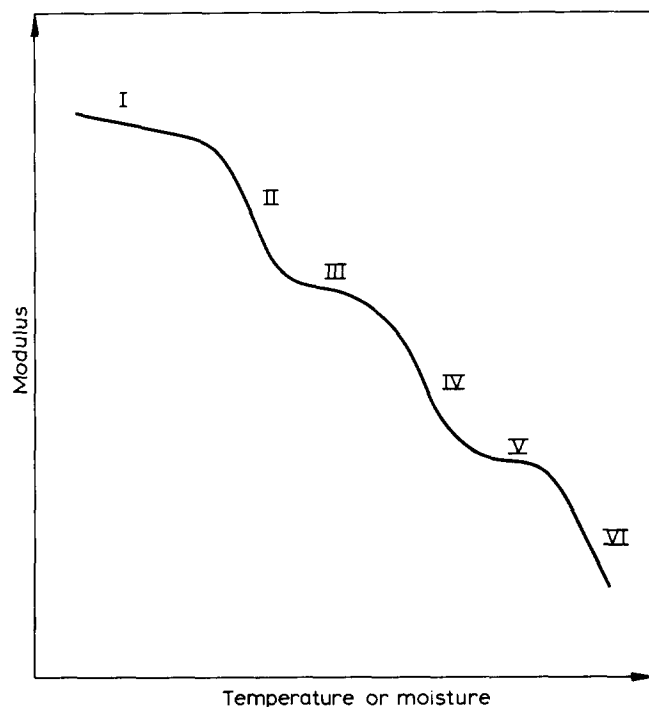
Figure 5 Schematic diagram of the mechanical modulus of fish gelatin as a function of temperature or moisture content; I, glass; II, glass transition of soft blocks; III, reinforced rubber; IV, glass transition of rigid blocks; V, rubber; VI, rubbery flow region

to the devitrification of soft blocks while the second glass transition (at a temperature in the region 180°–190°C) is assigned to the devitrification of rigid blocks. The first one is more intense in fish gelatins while the reverse is true for mammalian gelatins, according to the nature of the matrix in each case. In order to plasticize a gelatin film at room temperature, it is necessary to add a diluent (i.e. water) to shift the first glass transition in the case of fish gelatins, or in the case of mammalian gelatin, the second glass transition. This explains the low diluent levels which are necessary in the first case.

Results reported in the literature [1-8] regarding thermal properties of gelatin samples derived from mammals, may be also explained by the block copolymer model. In this case, the more intense glass transition takes place in the range 180°–200°C. Also it is this glass transition which is responsible for the overall behaviour of the material (glass or rubber appearance). This explains why several authors reported this transition as the only transition occurring[1,5,7,8]. However, some authors reported the low-temperature glass transition using techniques of adequate sensitivity[2-4]. Koleske and Faucher[6] found both transitions, as well as a third one at −85°C, but did not offer any explanation.

A schematic diagram of the mechanical modulus of fish gelatins as a function of temperature or moisture, is depicted in *Figure 5*. The first glass transition transforms the material from a glass to a reinforced rubber (rubbery matrix reinforced with glassy segments). The second glass transition leads to an ordinary rubber which eventually may begin to flow. In the case of mammalian gelatins, regions I (glass) and III (toughened glass) have almost the same mechanical modulus[1,5] (the impact resistance may, however, be different). Region IV represents a true overall glass–rubber transition.

The influence of different annealing periods upon the

first glass transition, for gelatin films cast at 60°C, is shown in *Figure 6*. Films with a short annealing period show an ordinary glass transition at 90°C (curve A). The baseline shift is due to the change in the specific heat from that of the glass to that of the rubber (over the transition temperature interval). Increasing the annealing time at 60°C leads to a significant heat absorption during the glass transition, as well as an increase in the temperature range (minimum at 120°C). When the sample is annealed, relaxation processes associated with the nonequilibrium nature of the glassy state take place. Both specific volume and enthalpy decrease with annealing time, approaching the equilibrium glassy state. When the annealed glass is reheated, the new glass transition is overlapped with an energy absorption related to the enthalpy relaxation which took place in the annealing process. The increase in the temperature range is due to the necessity of producing molecular mobility in a more dense glass.

Thermal properties of crosslinked gelatin films

In this section, the influence of crosslinking the gelatin chains upon the resulting thermal properties will be dealt with. Yannas[1] pointed out that severe dehydration, which occurs when gelatin films are cast at 105°C under a vacuum of at least 10^{-2} Torr, leads to a certain level of crosslinking. However, this treatment did not introduce significant modifications in the thermal properties (*Figure 3*). Here, crosslinks are introduced by addition of formaldehyde prior to casting, followed by a heating of the resulting film to produce the chemical reaction.

Figure 7 shows the d.s.c. curves of gelatin samples containing formaldehyde. A film cast at 60°C with the usual procedure (curve A) shows the first glass transition and a broad exothermic band, peaking at 200°C, characteristic of the reaction between formaldehyde and the protein (amino groups of lysine, arginine, hydroxylysine and histidine[15]). The heat of reaction was estimated by measuring the area under the peak, defined by drawing an
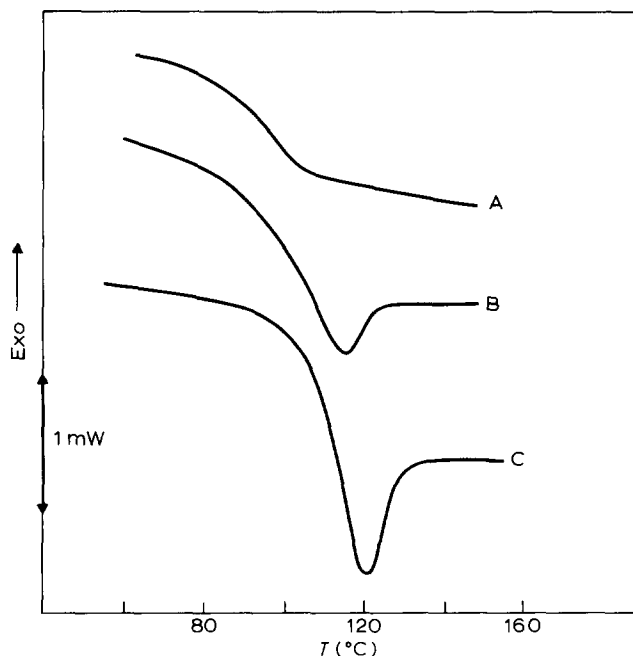
Figure 6 D.s.c. curves of gelatin samples with different annealing periods at 60°C; curve A, few hours; curve B, two days; curve C, three days
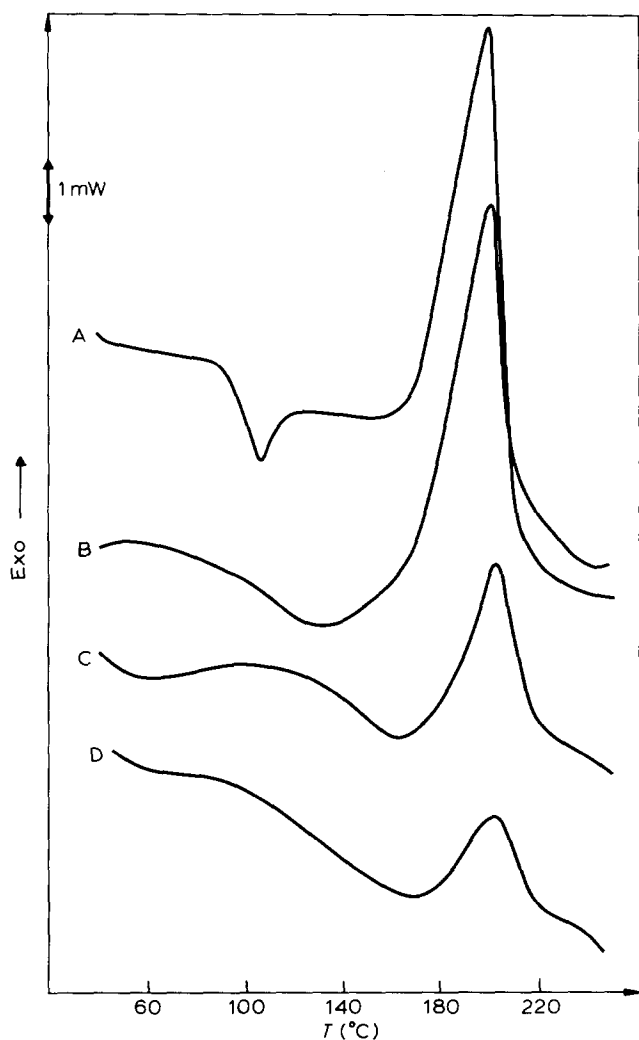
Figure 7 D.s.c. curves of gelatin samples containing formaldehyde; curve A, initial sample (2 days at 60°C); curves B, C and D, samples heated at 110°C, 130°C and 140°C, respectively, in the isothermal mode until arrest of reaction, cooled and scanned at 10°C min^{-1}. Masses of different samples are: curve A = 15.7 mg; curve B = 13.0 mg; curve C = 12.5 mg; curve D = 12.3 mg

arbitrary straight baseline between both extremes. As the baseline for reactants is different from that of products (due to the crosslinking reaction), it is not easy to place it accurately. Instead, several runs were carried out to minimize errors. The average of 8 runs was $Q_T = (6.5 \pm 1.3) \times 10^4$ J kg^{-1} (15.5 ± 3.1 cal g^{-1}).

The crosslinking reaction was carried out in the isothermal mode, at temperatures lying in the 100°–180°C range, until no further reaction could be observed. Then, the samples were cooled and run in the scanning mode of the d.s.c. Curves B, C and D of *Figure 7* represent the results obtained for crosslinking temperatures of 110°, 130° and 140°C, respectively. It may be seen that an increase in the crosslinking temperature decreases the residual reaction heat, Q_R, and expands the temperature range of the glass transition.

As formaldehyde acts crosslinking gelatin chains through α-amino acids present in the soft blocks, it is reasonable to expect an increase in the rigidity of these blocks. This leads to the expansion of the temperature range associated with the glass transition. When the crosslinking reaction is carried out at a certain tempera-

ture, a final conversion is reached at which the soft blocks are sufficiently immobilized to permit further reaction to occur. The effect is the same as the arrest of reaction due to vitrification in thermosetting systems[16].

The final conversion of the crosslinking reaction attained at a particular temperature is given by $(1 - Q_R/Q_T)$. *Figure 8* shows this conversion as a function of the reaction temperature. The reaction threshold is close to 100°C while at 160°C an almost complete conversion is attained.

The broadening of the glass transition range corresponding to the crosslinked soft blocks is illustrated in *Figure 9*, for a gelatin film reacted with formaldehyde at 160°C. The overlapping of the glass transitions for both kinds of blocks implies that the crosslinked gelatin sample behaves as a material which devitrifies progressively in the 100°–200°C temperature range.

In the scanning mode of the thermal analyser the system evolves through the rubbery region, avoiding the vitrification zone[16]. The kinetics of the overall crosslinking reaction may be obtained from the shape of the exothermic peak of initial samples (i.e. curve A of *Figure 7*), using the method developed by Barrett[17].

The reaction rate may be written in terms of the conversion $x = Q/Q_T$ (ratio of the heat evolved up to a certain temperature over total reaction heat), as

$$dx/dt = Af(x)\exp(-E/RT)$$

where $f(x)$ represents a functionality of conversion, E is the activation energy and A is the specific rate constant.

As $dx/dt = (dQ/dt)/Q_T$, where (dQ/dt) is the height of the peak over the baseline, in power units, the d.s.c. signal is a direct measure of the reaction rate along an arbitrary trajectory in a conversion–temperature plane. By taking logarithms, we get

$$\ln[(dx/dt)/f(x)] = \ln k = \ln A - E/RT$$
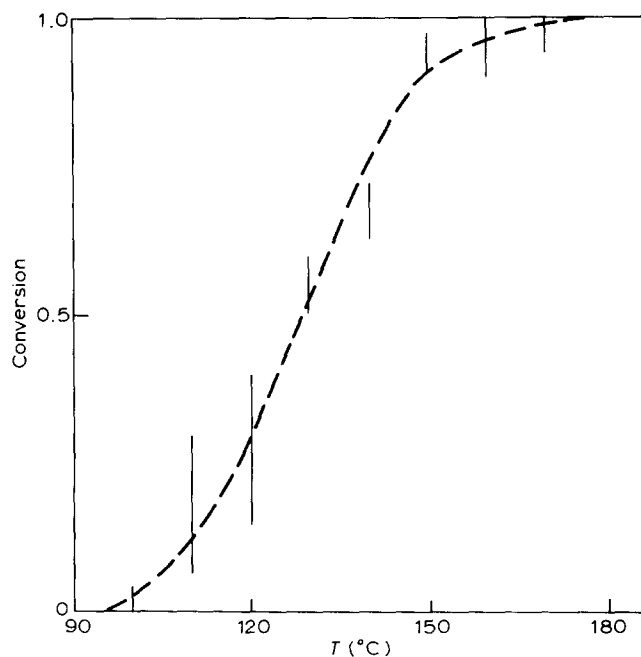
Figure 8 Final conversion of the gelatin – formaldehyde reaction as a function of the constant temperature at which the system was heated in the isothermal mode (bars represent the range covered by several runs)
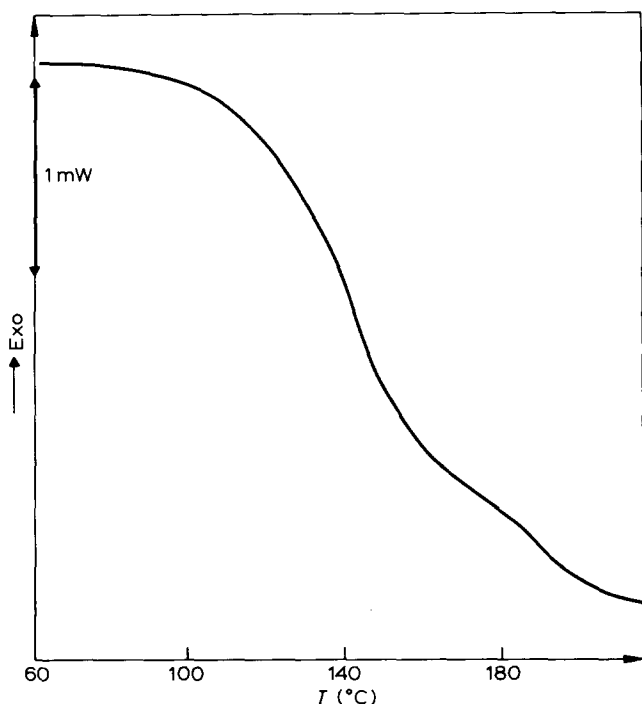
Figure 9 D.s.c. curve showing the broad glass transition of a gelatin sample crosslinked with formaldehyde at 160°C

Figure 10 Linear regression of the overall specific rate constant for the gelatin – formaldehyde reaction (four different runs)

A plot of $\ln k$ vs. $1/T$ will be linear if $f(x)$ is correctly chosen. *Figure 10* shows such a plot for $f(x)=(1-x)^2$ (second order reaction) and four different runs. In the conversion range $0 \leqslant x \leqslant 0.9$, the best linear regression is

$$dx/dt(s^{-1}) = 4.93 \times 10^{27}(1-x)^2 \exp[-31350/T(\mathrm{K})]$$

The correlation coefficient is 0.982. If first and third order reactions are fitted, the corresponding correlation coefficients are, respectively, 0.980 and 0.971. The slight difference between first and second order prevents us from extracting any mechanistic conclusion from the proposed phenomenological equation.

CONCLUSIONS

1. Dehydrated gelatin samples ('hot-cast' films) show two glass transition temperatures, one at 120°C and the other in the 180°–190°C temperature range. The low-temperature transition is assigned to the devitrification of the soft blocks (α-amino acids with glycine at every third position), while the high-temperature transition is attributed to the devitrification of the rigid blocks (imino acids with glycine at every third position), of the copolymer chain. Hydrated gelatins show both transitions shifted to lower temperatures.

2. In fish gelatins the soft blocks prevail over the rigid ones (weak secondary structure). Then, the devitrification of soft blocks leads from a glass to a rubber reinforced with dispersed glassy segments. In mammalian gelatins rigid blocks prevail (a strong secondary structure). Now, the first glass transition does not represent an overall glass–rubber transition but a change from a glass to a
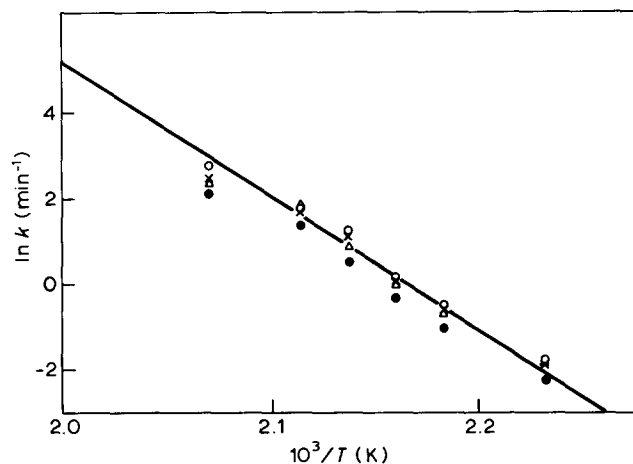
toughened glass (dispersed rubbery segments). It is the second glass transition that leads to a rubbery material.

3. The annealing of glassy gelatins results in an enthalpy relaxation approaching the equilibrium value, similar to other polymeric materials.

4. The crosslinking of fish gelatin chains with formaldehyde has a threshold temperature at 100°C, but complete conversion is attained only at temperatures higher than 160°C. As the crosslinking occurs through the soft blocks of the polymeric chains, there is a broadening of the temperature range associated with the first glass transition. The crosslinked fish gelatin behaves as a material which devitrifies progressively in the 100°–200°C temperature range. The crosslinking reaction follows a second-order kinetic law with a very high activation energy ($E = 261$ kJ mol^{-1}).

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