Melting of gelatin crystals below glass transition temperature:

a direct crystal - glass transition as revealed by microhardness.

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Abstract

In the present paper the effect of crystallization conditions on the melting temperature (T_m) of chemically cross-linked gelatin is studied by means of differential scanning calorimetry (DSC) and microhardness (H) measurements as extension of our recent findings (Ref. 11). A rather unusual situation when polymer crystals melt below the glass transition temperature (T_g) is confirmed by DSC. This is highlighted on dry gelatin which is characterized by relatively high, and close to each other, T_g and T_m values (217 and 230°C, respectively). By depressing T_g, using water as plasticizer, rather imperfect crystallites are obtained which melt well below the T_m of dry gelatin. It is shown that H increases with temperature mostly due to the drying out of the room-conditioned gelatin. In the 160 - 180°C range H reaches values of about 390 MPa. In this temperature range the imperfect crystallites melt (according to DSC), however without formation of a typical liquid phase (since H remains constant). It is expected that this direct crystal - glass transition can be used to obtain highly ordered polymer glasses. The unusual high H values as well as the low coefficient of thermal softening of gelatin, in contrast to those common for synthetic polymers, makes this material attractive for practical applications.

Introduction

Gelatin is a high molecular weight polypeptide derived from collagen, the primary protein component of animal connective tissues, such as bone, skin and tendon [1]. The collagen molecule itself consists of a three helical peptide chains (α chains) held in close, parallel association, in the form of a rigid rod. Thermal denaturation occurs near 40°C for solutions of most mammal collagens and is marked by abrupt changes in intrinsic viscosity, specific optical rotation, sedimentation, and light scattering intensity [2]. Because of intramolecular cross-links near the ends of the α chains, three distinct gelatin species can be formed: α, β and γ gelatin. Frequently, they are referred to as protogelatins, and correspond approximately to monomer, dimer, and trimer, respectively. The conversion of collagen into gelatin by thermal denaturation involves the disruption of only non-covalent bonds, such as coulombic, hydrophobic or hydrogen bonds, and van der Waals forces.

The cross-link centers in gelatin are crystallites of helical chains which melt at high temperatures [1]. Owing to the large number of side functional groups of the molecule, gelatin readily undergoes chemical reactions including chemical cross-linking when a multifunctional reagent is used [3]. Aqueous gelatin dries to a semicrystalline solid, which is clear, tough and brittle, swellable in cold water, and completely soluble in hot water in the absence of cross-linking [1]. From calorimetric measurements [4-6] it is known that well aged, high quality gels are

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~20% crystalline and that dried films may reach up to 40% crystallinity.

Subsequent annealing can affect gel and solid-state properties through the

increase of the thermal stability and the degree of crystallinity [4-8].

Solid-state properties are dominated by the thermal history and the

presence of diluents, notably water. If dried below the melting temperature T_m,

the degree of crystallinity is proportional to the gelatin concentration,

the magnitude T_m-T (over the course of drying), and the drying time. If gelatin is

dried above T_m (so-called sol drying), an amorphous solid is formed which

undergoes a glass transition T_g [4-7]. For anhydrous, solid gelatin, $T_g = 217°C$ and

$T_m = 230°C$, but both values are reduced by moisture [4].

These two contrasting peculiarities of gelatin, namely (i) the very close

values of T_g and T_m in the dried state and (ii) the very strong plasticizing effect

of water (T_g drops to 25°C when the water content reaches 20% [1]) allows one to

observe a rather unusual situation when the polymer crystals melt below T_g.

In order to observe this effect, one has to reduce T_g by means of a plasticizer to a

value $T'_g << T_g$ and perform a crystallization at high undercooling ΔT (but above

T'_g of the plasticized polymer!) to obtain crystallites of rather low perfection ,

having a melting point T'_m much lower than T_m. Furthermore, the plasticizer can

be removed by drying at low temperatures and as a result T'_g will increase,

approaching the T_g- value typical of the neat polymer, i.e., without plasticizer.

The drying temperature should be low enough in order to avoid recrystallization,

i.e., formation of more perfect crystals with a higher melting temperature T_m. At this stage, a rather unusual structure of the material could be obtained: imperfect crystallites embedded in a solid polymer glass with T_g possibly higher than the melting temperature of the crystallites T'_m.

The described possibility of melting below T_g has another peculiarity. Since the imperfect crystallites are surrounded by a glassy matrix (after removing the water), the melting process results in the formation of a solid amorphous (glassy) phase rather than of a liquid phase as in the common cases, i.e., a direct "crystalline solid - amorphous solid" transition is observed.

In a recent systematic study on mechanical properties and transition temperatures of cross-linked drawn and undrawn oriented gelatin it was suggested [9-11] that by using the above approach it is possible to observe the melting of polymer crystals below the T_g of the polymer. The transition temperatures were followed by dynamic-mechanical thermal analysis (DMTA) and differential scanning calorimetry (DSC).

During the last two decades it was shown [12-14] that the microhardness technique, owing to its simplicity and high sensitivity, allows to derive a detailed information about the microstructure of polymers. Relationships between microhardness and crystallinity, crystallite perfection, chain conformation and other structural parameters have been derived [15-18].

The aim of the present study is to conclusively demonstrate, by using the microhardness technique, that melting of gelatin below T_g represents a direct

transition of crystal to glass, i.e., without formation of a liquid phase after melting as in the common cases. The microhardness technique is particuliarly suitable for such a test, because of the drastic difference in the H-values for the liquid and the glassy state [14], in contrast for example with the information which can be obtained from X-ray diffraction.

Experimental

Materials

Food grade gelatin powder (Merck) and a formaldehyde solution of at least 37 wt% (Merck) were used. The gelatin was soaked overnight at 5°C, then dispersed at 50°C (water bath) in the presence of a phosphate buffer. A buffered solution of the cross-linking agent was added at 50°C, with thorough mixing. In this way, the concentration of formaldehyde in the final solution varied between 0.02 and 0.1 M, and the pH of the solution between 5.5 and 6.5. Gelatin concentrations varied between 2 and 14 %. All of these ranges are optimum for the effective cross-linking of gelatin [19]. Portions of the resulting gelatin solutions were dried in order to obtain isotropic, chemically cross-linked films. Before testing the samples they were completely dried, the water content at the room-conditioned samples being typically between 15 and 18 wt%. Thereafter they were moistured in order to achieve the desired depression in T_g (typically T'_g around 60°C), and were let to crystallize at temperatures T_c between 60 and 90°C

in order to produce low-temperature melting crystallites. Crystallization was performed in an oven in presence of water in order to prevent that the sample would dry during this process. The next step was to dry the sample in a vacuum oven at temperatures below T_c in order to exclude recrystallization leading to higher melting crystallites. The complete removal of water was checked by wheighing before and after the DSC tests.

Techniques

DSC measurements were performed in a DuPont 9900 instrument. The sample weight was 5-15 mg, and the heating rate was 10°C/min.

Microhardness (H) was measured at room and elevated temperature using a Leitz Tester equipped with a square based diamond indenter and a hot stage. The H-value was derived from the residual projected area of indentation according to the expression [12]:

$$H = kP / d^2 \ (MPa)$$

where d is the length of the impression diagonal in meters, P is the contact load applied in N and k is a geometrical factor equal to 1.854. A loading cycle of 0.1 min and a load of 2 N were used. Ten microindentations to determine the hardness for each point were averaged. Since differences in the T_g and T_m values calculated by DSC and H measurements were found, the hot stage temperature was calibrated using the melting points of organic crystals in the range of interest (70-210°C). In the temperature range 25 - 160°C indentations were carried out

every 5-10°C step. In the 160-220°C range each measurement was performed

every 2°C. In addition, the ten indentations at a given temperature were

performed within 10-15 min. The selection of such relatively small increasing

temperature steps offers enough time (around 3 hours) for complete drying of

gelatin before attaining a temperature of 150-160°C. When the same temperature

was reached in a shorter time the H-values obtained were lower, due to

incomplete drying.

Results

Calorimetry

In contrast to previous reports [10,11], where the influence of orientation and

water content on the values of T_m and T_g was followed, in the present study the

T_m value was investigated as a function of crystallization temperature T_c.

Fig. 1 illustrates the DSC curves of room-conditioned cross-linked gelatin

subjected to different treatments.

The thermogram of the Fig. 1a was taken from a sample immediately after

drying at 140°C for 5 h. One can see that below 200°C there is no evidence for

any transition - a rather straight line is observed. The first transition takes place

near 215°C which should be assigned to the glass transition T_g followed by a

slight indication for crystallization, and finally by melting at T_m = 239°C. These

results are in agreement with our preceeding studies [9-11].

If, after drying at 140°C for 5 h (Fig. 1a), the sample is moistured up to 10 wt% of water content, followed by crystallization at T_c = 90°C for 5 h and a final drying at 80°C for 5 h in vacuum, the shape of the thermogram substantially changes (Fig. 1b). In this case no transitions are seen up to 170°C, where melting starts. The next conspicuous peak (near 225°C, Fig. 1b) cannot be assigned to melting. In this temperature interval the glass transition takes place (T_g = 215°C [1,10,11]). The softened gelatin crystallizes thereafter (T_c= 240°C) and melts again at T_m = 253°C. Of particular interest is the narrow interval between the first melting T'_m and the glass transition T_g (200-215°C). In this range the gelatin has to be in the glassy state since T_g = 217°C for the dry material [4] and as found in the present case T_g amounts to 215°C (Fig. 1a).

In order to examine the nature of this endothermic interval under different conditions, an attempt was done to broaden it by producing even less perfect crystallites with a lower T'_m value. This was done by applying lower treatment temperatures (T_c= 80°C), and vacuum drying at 70°C for 5 h. In addition, the starting sample contained more water than the other ones - 18 wt%. Consequently the depression in T_g should be larger [1,11]. The corresponding thermogram is displayed in Fig. 1c. Again, in accordance with the previous sample (Fig. 1b) no transition is observed up to 160°C when the first melting starts (T'_m= 170°C), followed by the glass transition step, the crystallization process and the neat melting above 250°C. The glassy interval after the first melting T'_m is in this case

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wider (180-220°C) due to the depression of T'_m as compared to the former case (200-215°C, Fig. 1b).

Microhardness Study

Fig. 2 shows the variation of microhardness for a room-conditioned gelatin and for a dried one as a function of temperature. Most interesting is the fact that the room-conditioned gelatin (containing about 15 wt% of water) is characterized by relatively high H-values - above 200 MPa (Fig. 2a). With rising temperature H gradually increases, reaching the value of 390 MPa in the range 135 - 160°C. One can assume that this doubling in the H-value is correlated, both, to the crystallization process and to the dry state of the sample. At 160°C the gelatin is completely dried, as found earlier by DSC and gravimetric measurements [11]. Therefore H remains constant up to ~180°C where it drops some 40 MPa reaching a minimum value of 350 MPa at 204°C followed by a new increase up to roughly 400 MPa at about 210°C. Finally, a decrease to 350 MPa at 220°C (Fig. 2a) is again observed. In agreement with the literature [1,10,11] and DSC observations (Fig.1), the observed changes in H-values above 180°C can be related to the softening in the glass transition interval (around 180-204°C) followed by crystallization (the peak of H is at T_c ~ 210°C) and melting of the formed crystals (Fig. 2a). The observed difference in the T_g values of about 15-20°C evaluated by means of the two techniques (Figs. 1 and 2) originates from

the well known fact that various methods lead to different T_g values, differences being up to 30°C [20].

The suggested interpretation of the experimentally observed transitions (Fig. 2a) is supported by the results obtained in the dried sample (Fig. 2b). In this case the increase of H with the temperature increase in the 20-160°C range is missing. The predried sample (Fig. 2b) shows H-values typical for the high temperature interval (160-180°C) of the originally undried sample (Fig. 2a). This indicates that the increase of H during heating up to 160°C in the room conditioned sample (Fig. 2a) is mostly related to the removal of water, resulting in a T'_g increase [11]. Furthermore, the slight H decrease displayed in the 110 - 190°C range (Fig. 2b) allows one to calculate the coefficient of thermal softening β of dry gelatin from the hardness variation:

$$H = H_0 \, e^{-\beta T}$$

where H is the hardness value at temperature T and H_0 is a constant. For the temperature range below T_g one obtains $\beta = 0.52 \times 10^{-3} \ K^{-1}$.

It is worth to highlight the common features of the two curves (Figs. 2a and 2b):

(i) the dried sample (Fig. 2b) displays the same transitions in the same temperature interval as the room-conditioned one (Fig. 2a).

(ii) in both cases the microhardness values after softening (at $T_g \sim 196°C$) and after melting ($T_m = 220°C$) are nearly the same.

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The data of Fig. 2 reveal two types of softening leading to a significant drop in H: the first softening is related to the T_g transition while the second one is assigned to the melting T_m of the recrystallized material. In addition, it is noteworthy that no drop in H-values is observed during the melting T'_m of the crystallites created in the system gelatin - water (Fig. 1, b and c). In other words, we are not observing the common crystal melting process with formation of a soft liquid.

Discussion

The present DSC measurements (Fig. 1) support our former findings [10,11] that a depression of the T_g values of gelatin, using water as a plasticizer, is compatible with a crystallization at large undercoolings and yet above the T'_g value of the gelatin - water system. This is done after removing the water at low temperature, to observe the melting of imperfect crystals (T'_m around 160-180°C) below the glass transition temperature of the dried gelatin ($T_g \sim 215$°C). This rather unusual situation is distinguished by another peculiarity - melting of polymer crystals without formation of a liquid state typical for the common cases, as melting takes place below T_g. This means that one deals with a direct "crystalline solid - amorphous solid" transition, i.e., the phase transition is not accompanied by a change in the aggregation state.

The last assumption derived originally from the observation that melting occurs below the T_g of the dry gelatin [10,11], is clearly supported by the temperature dependance of microhardness illustrated in Fig. 2. If a liquid state were formed as a result of the melting of the imperfect crystallites (in the region of 160-180°C, Fig. 1 and Ref. 11), a drop in the microhardness should be observed (as actually it takes place at higher temperatures during softening, T_g = 196°C, and melting, T_m =220°C, Fig. 2). However, below T_g this is not the case for even a large temperature range such as 160-180°C. This indicates that the glassy state of the dried gelatin is preserved, on the whole, within this temperature interval. The melting process in this particular case should be related with slight displacements of chain segments from their equilibrium positions typical for the crystalline state. Such displacements are obviously sufficient for the loss of the crystalline order but insufficient to account for a solid - liquid transition since the polymer is below its T_g. Thus, one can conclude that starting from a gelatin - water system, through crystallization at large undercooling and removal of water at temperatures below T_c it is possible to observe a direct "crystal - glass" transition. This approach offers another interesting opportunity of obtaining ordered polymer glasses. Furthermore, the results displayed in Fig. 2 allow one to conclude that: (i) the microhardness value of gelatin in the crystalline or in the glassy state are rather close to each other, (ii) the increase of H with increasing temperature is mainly due to the T_g increase as a result of water removal, and that the coefficient of thermal softening of dry gelatin is, for

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example, twice smaller than that for poly(ethylene terephtalate) (β = 1.2 x 10^{-3} K^{-1})[21], the lowest one for a number of typical thermoplastics.

Conclusions

In conclusion, the data presented in Fig. 2 show that gelatine is characterized by unusual high microhardness values surpassing those of commonly used synthetic polymers, soft metals and their alloys [22]. Even in a plasticized state (room-conditioned material) gelatin has a microhardness value comparable with those of the majority semicrystalline synthetic polymers. What is more important - the extremely high H-values (of 380-400 MPa) are preserved at high temperature (up to 180°C). For this reason gelatin seems to be a good candidate for surface protection for many technical purposes, including aerospace applications. The clarification of these potentials is in progress.
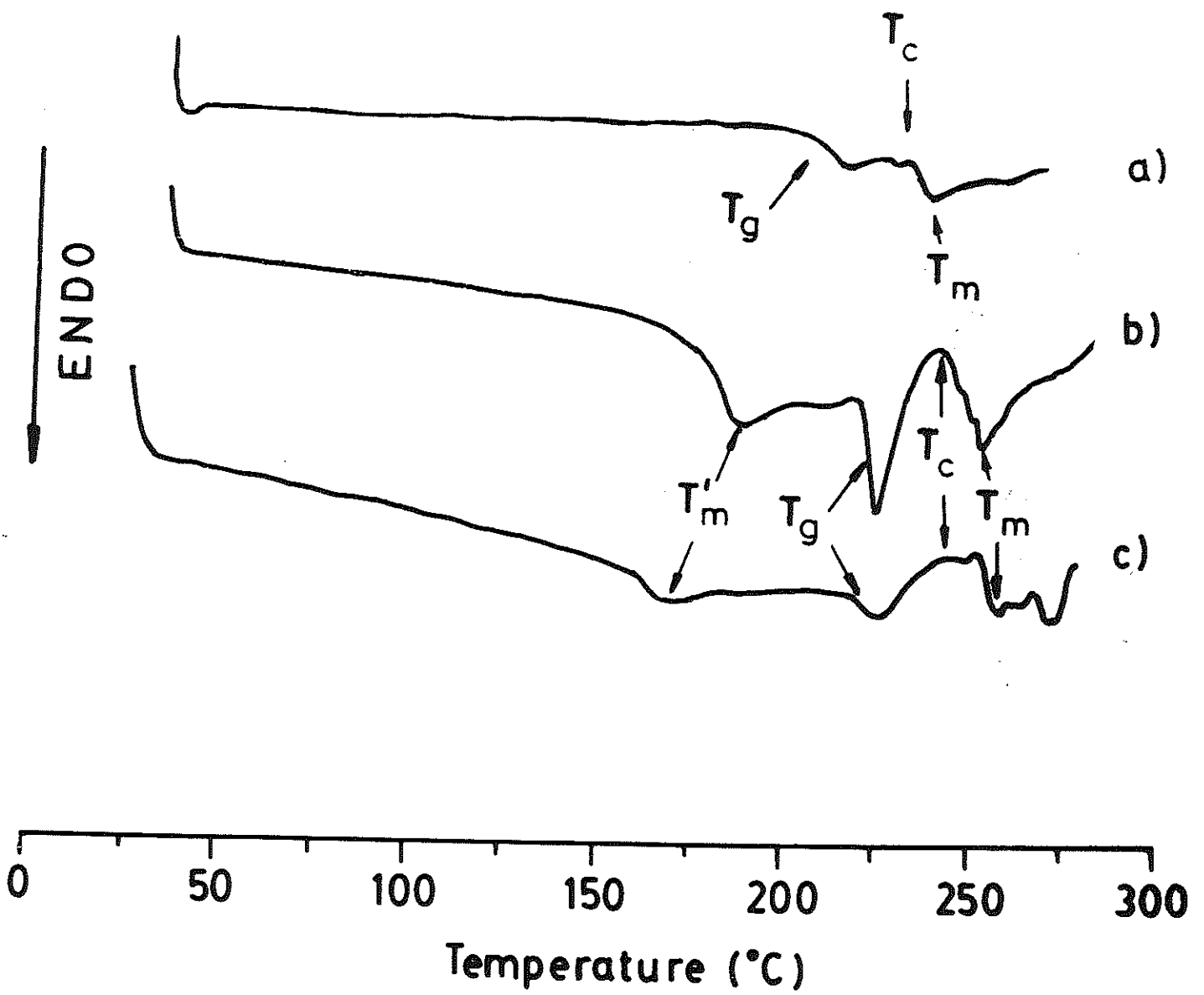
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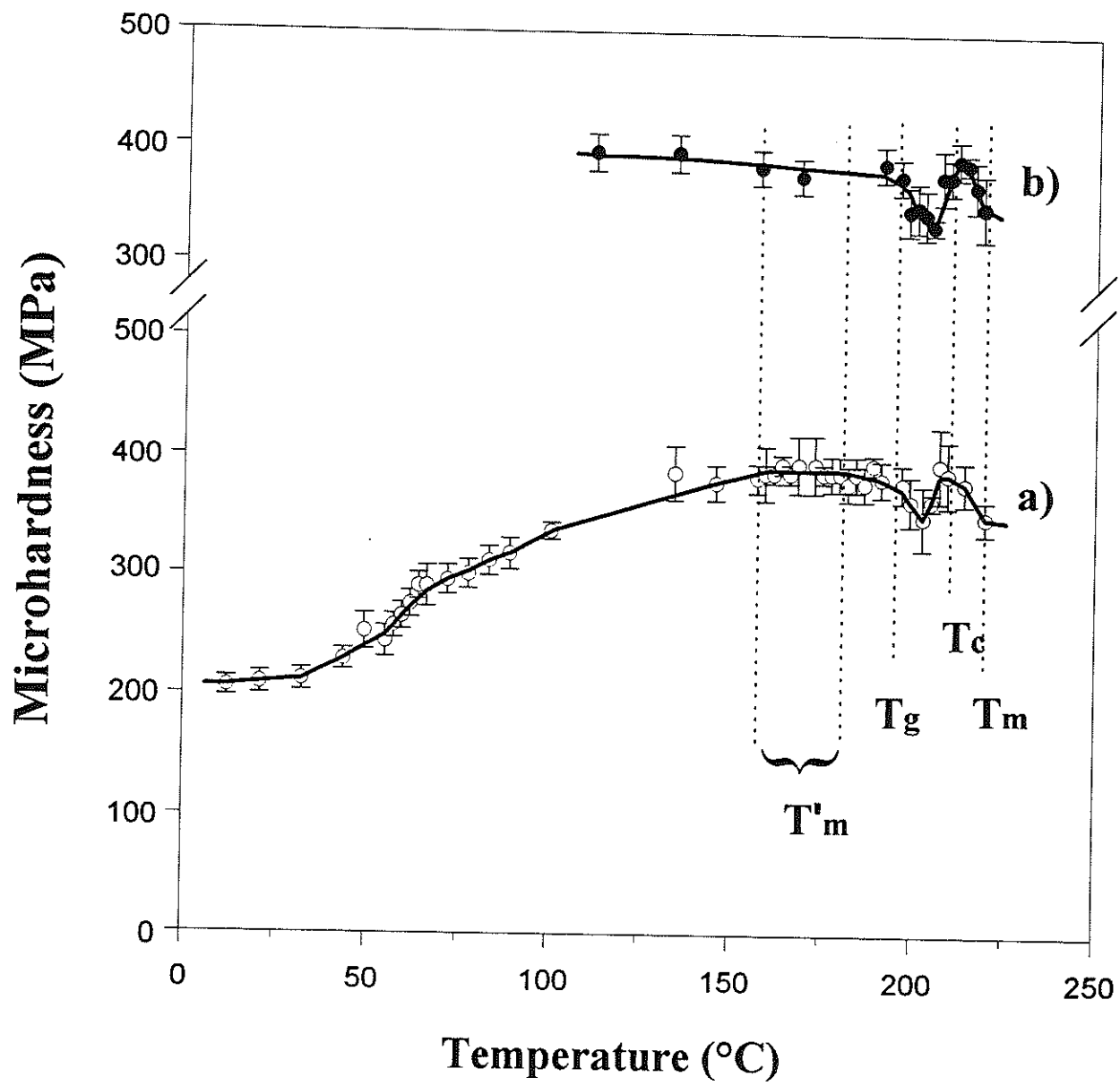[10]. Z. Sarac, S. Fakirov and T. Anbar: *Polymer Networks'94*, Prague,

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Figure captions

Fig. 1 DSC curves of cross-linked isotropic gelatin: a) - dried at 140°C for 5 h.; b) - dried as in a) and thereafter moistured up to 10 wt% water content followed by crystallization at $T_c = 90°C$ for 5 h and finally dried at 80°C for 5 h in vacuum; c) - dried as a) and moistured up to 18 wt% water content, crystallized at $T_c = 80°C$ for 5 h and finally dried at 70°C for 5 h in vacuum.

Fig. 2 Temperature dependence of microhardness H for: a) - room-conditioned cross-linked isotropic gelatin film; b) - gelatin dried at 140°C   for 5 h.
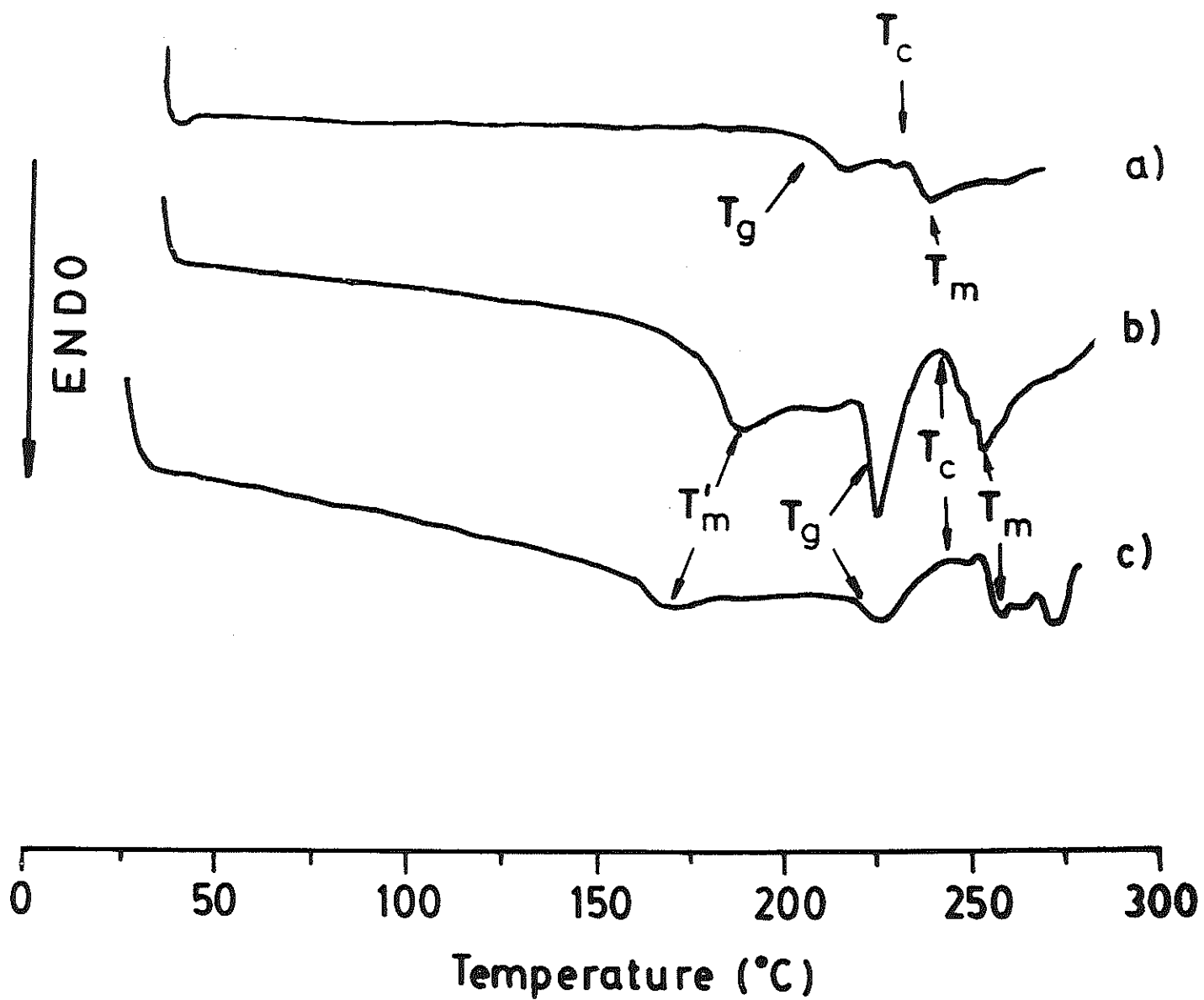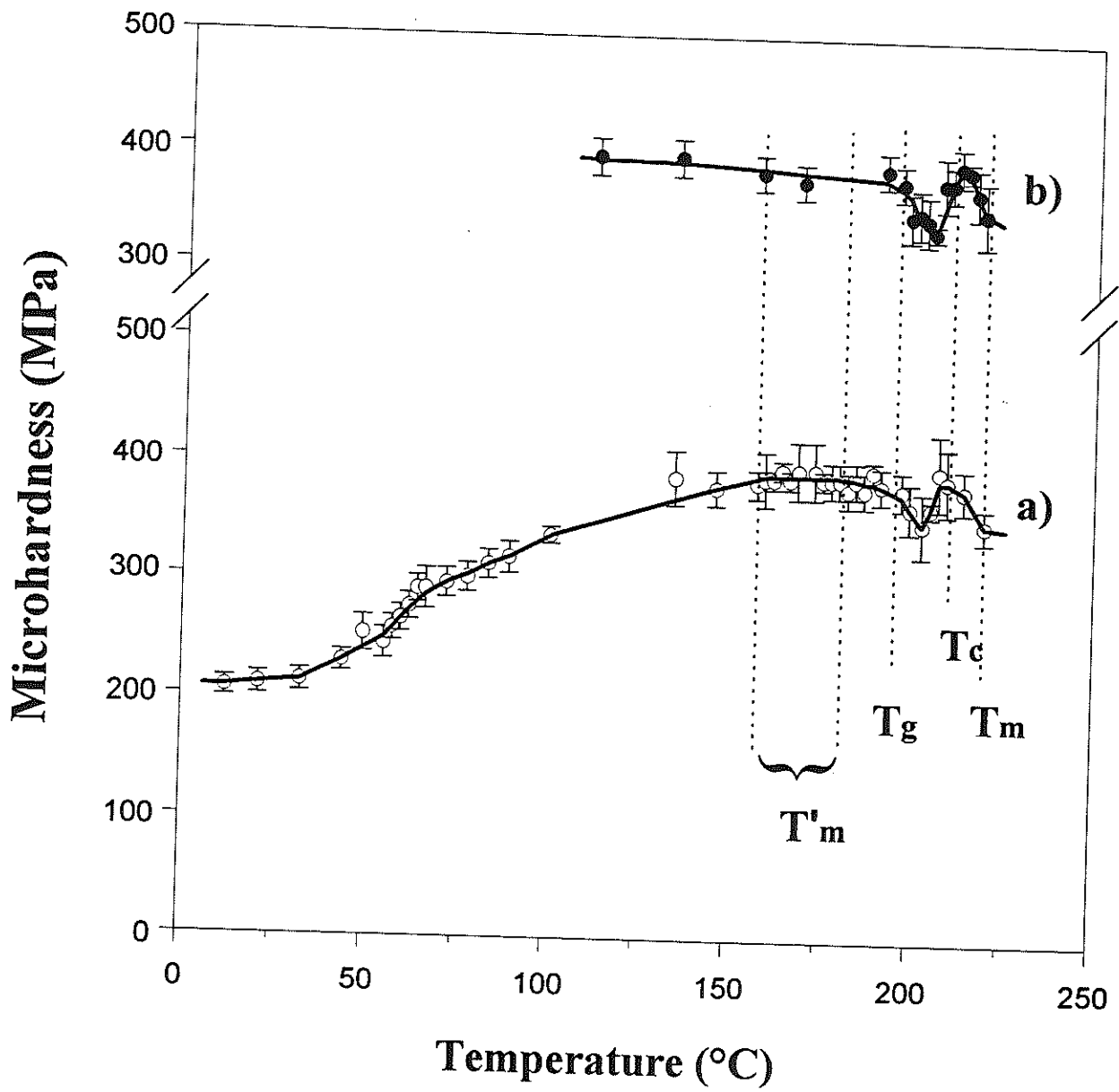
Fig. 1

Fig. 2