DETERMINATION OF THE COLLAGEN THERMAL TRANSITIONS IN CHROME LEATHER BY TMA

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ABSTRACT

The collagen thermal transitions of bovine chrome leather were studied by thermomechanical analysis (TMA). The application of dynamical stresses under transverse compressive penetration and longitudinal extension modes allows us to determine the glass-transition temperature of collagen in conditioned and dried states and the denaturation temperature in dried state of collagen. The results agree with those obtained by differential scanning calorimetry (DSC) and thermogravimetry (TGA). The thermal transitions determined by TMA in transverse compressive penetration mode showed a good relationship with the origin and final application of leather.

INTRODUCTION

Skins and hides are converted into leather through the tanning process in order to prevent their putrefaction and achieve certain handle, mechanical and esthetical characteristics. The basis of these modifications is based on the collagen fibrous protein, which forms the main structure of leather.

As a semicrystalline polymer, collagen undergoes a thermal transition at low temperature *Tg*, known as the glass-transition. This event is associated with the relaxation of the polypeptidic chains. At much higher temperatures, collagen undergoes a denaturational transition *Td*, which is accompanied by a melting of the hydrated crystallites. As a result of this process, there is a rupture of hydrogen bonds and a rearrangement of the triple helix into a random configuration.

In a previous study,[1] both the glass-transition and the denaturation temperatures of a chrome bovine leather were determined by differential scanning calorimetry. The effect of the moisture content on both thermal transitions was also studied. As with other bipolymers,[2] water acted as a natural plasticiser depressing the value of *Tg* and *Td*. The results obtained led to the following conclusions:

- The denaturation temperature was shifted to lower values when the water content was increased. In a completely dehydrated sample *Td* was approx. 229°C; in a room-conditioned sample (15.2% of moisture content in accordance with the I.U.C.-5 standard method[3]) was 118°C; and in a water-soaked sample was 97°C.
- The glass-transition temperature of the room-conditioned leather, determined as the onset of the step in the DSC curve, had an approximate value of 45°C.
- A step of 15% weight loss in the TGA curve was observed from 60 to 120°C due to the evaporation of water.

The thermomechanical analysis has already been applied to the determination of collagen thermal transitions in leather[4,5] and type I collagen fibres.[6] However, all these studies were focused on the denaturation of collagen in excess of water. Being that dimensional changes can be measured by TMA,[7] this article explores the application of the thermomechanical analysis to the characterisation of room-conditioned leather. This has only been done in compressive penetration mode in the temperature range of 20-120°C.[8] On the other hand, the differential mechanical thermal analysis (DMTA) has been used to characterise vegetable and semi metal bovine leather (which had been previously conditioned at 65% RH) in the temperature range of 25-300°C.[9] All leathers examined showed a peak in the loss tangent in the 40-70°C range, which was attributed to some form of glass-

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TABLE I

Origin, Application, Characteristics and Moisture Content of the Leathers Studied

Leather	Origin	Application	Characteristics	Moisture content (%) I.U.C.-5
A	Bovine	Shoe upper - upholstery	Nappa	15.2
B	Bovine	Shoe upper - upholstery	Suances	14.1
C	Goatskin	Shoe upper	Dongola	14.2
D	Sheepskin	Shoe upper	Nubuck	13.1
E	Sheepskin	Clothing	Nubuck	12.5

transition in collagen. A further peak in the loss tangent was also observed at temperatures greater than 200°C.

In the first part of this article, the application of the thermomechanical analysis to the bovine chrome leather is discussed. The measurements were done in both transverse compressive penetration and longitudinal extension modes from room temperature up to 250°C. The transverse compressive penetration test was done through the application of a ball-point measuring probe 1.5 mm in radius onto the flesh side of the specimen. This test mode involves a progressively increase of the surface of contact between probe and sample as penetration increases and a radial distribution of stresses over the leather surface in contact with the probe, being the maximum at the top of the probe. Water was left to freely evaporate during the heating run. This allowed the determination of collagen thermal transitions in a completely dehydrated state.

In the second part, the transverse compressive penetration mode is applied to leathers of different origin and characteristics. The relationship between their final application and the thermal transitions determined by TMA in compressive penetration mode is explored.

EXPERIMENTAL

Prior to their characterisation the leather samples were conditioned for 48 hours at 20 ± 2°C and 65 ± 2% of humidity level, according to the I.U.P.-3 standard method.[10] The origin, application and characteristics of the 5 leathers studied are displayed in TABLE I. All of them were chrome tanned. Leather A had been previously characterised by differential scanning calorimetry and thermogravimetry.[1] The moisture content was calculated according to the I.U.C.-5 standard method.[3]

The thermomechanical analysis was carried out by means of a Mettler Toledo TMA/SDTA 840 apparatus. A flow of 30 mL.min^{-1} of N_2 was applied. The scanning rate was 5°C/min.

RESULTS AND DISCUSSION

1. TMA of Leather A under transverse compressive penetration mode

Figure 1 shows the TMA curve of a circular sample (4 mm diameter) of Leather A submitted to a dynamic compression

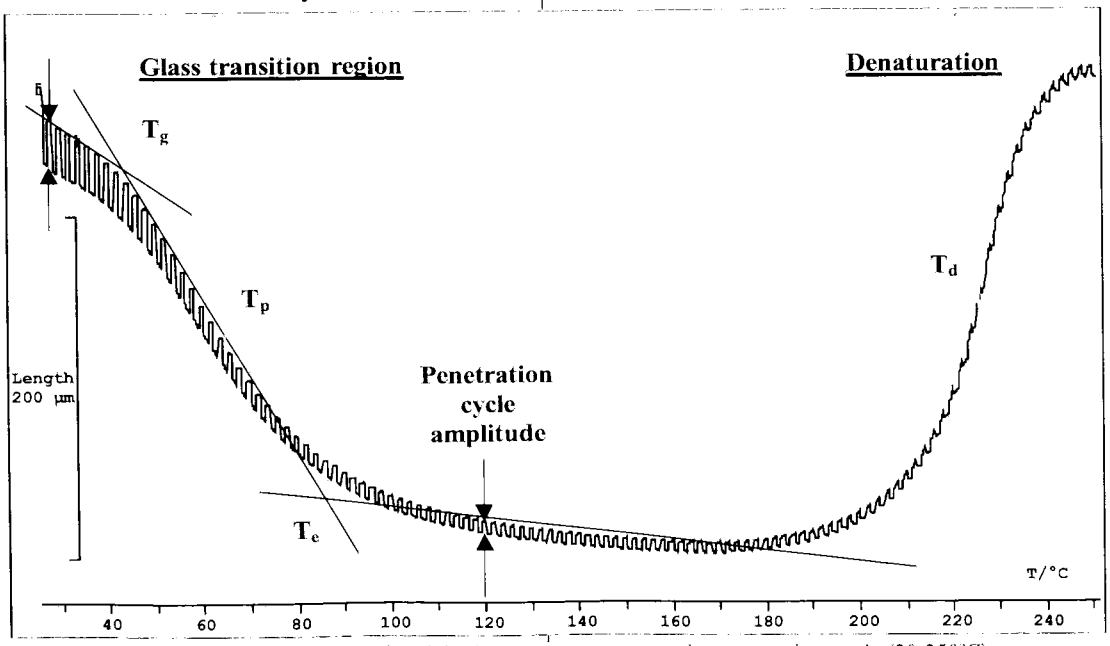

Figure 1. - TMA curve of Leather A in the transverse compressive penetration mode (20-250°C).

using a ball point measuring probe 1.5 mm in radius. Compressive loads ranged from 0.1 to 0.2 N (period = 12s) and temperature from 20 to 250°C. The temperatures at which the penetration coefficient is suddenly modified allows us to define three regions:

- *From room temperature up to 80°C*: The penetration is considerably increased. A first change in penetration rate is observed at approx. 43°C and then it remains constant up to 80°C. The amplitude of the penetration cycle induced by the periodic load levels is progressively reduced, which reveals an increase in sample stiffness. This could be due to the progressive increase of the contact surface between probe and sample, and the loss of water by heating.
- *From 80°C up to 200°C*: Neither changes in penetration coefficient nor in the amplitude of the deformation cycle (0.48%) are observed.
- *From 200°C up to 250°C*: The probe is suddenly pushed off by the sample that overcomes the initial thickness.

The penetration rate changes at 43°C (Figure 1) very close to the Tg determined by differential scanning calorimetry[1] and the glass transition determined by DMTA for vegetable and semi metal leathers.[9] Therefore this change of slope can be associated with the glass-transition phenomenon. At *Tg* the polypeptidic chains of collagen relax[4] that could make easier the probe penetration into the sample.

From 200 to 250°C the denaturation of the collagen molecule, the so-called helix-coil transition occurs. This non-reversible transition is due to the breaking of hydrogen bonds that are holding together the collagen structure.[11] As shown in Figure 1, the probe is pushed off from the sample overcoming the initial thickness. Wiederhorn and Reardorn[12] investigated the mechanical behaviour of such a material. The denatured sample behaves like an elastic solid, with a deformation amplitude due to the loads applied very narrow (0.48%) in comparison with the initial deformation amplitude under compression (2.0%). The inflexion point of the mean TMA curve in this region occurs at approx. 229°C, which agrees with the denaturation temperature previously determined by DSC.[1] The moisture content of the samples during the thermomechanical analysis was similar to that of the samples used when employing perforated lids in the differential scanning calorimetry.

2. *TMA of Leather A in longitudinal tensile mode*

Figure 2 shows the TMA curve of a cut 15x5 mm specimen of the same chrome bovine leather 1.54 mm in thickness. A dynamic stressing load from 0.1 to 0.2N (period = 12s) was applied in the tensile mode. Two samples parallel and perpendicular to the backbone were tested. As known, fibre bundles are not oriented in random directions throughout the skin or hide. The head to tail direction is the main orientation due to the need to keep together the animals body in the main automotive direction. The second major orientation is perpendicular to the backbone[13]. In this test collagen fibres were mainly oriented towards the test direction. The TMA curves of Figure 2 allow us to define three regions:

- *From room temperature up to 110°C*: The sample length remains constant till around 60°C. At this temperature a slow shrinkage begins up to 110°C reaching the 3% of its original length. Although the periodical stress applied ranged from 13 to 26 kPa, the strain amplitude remained

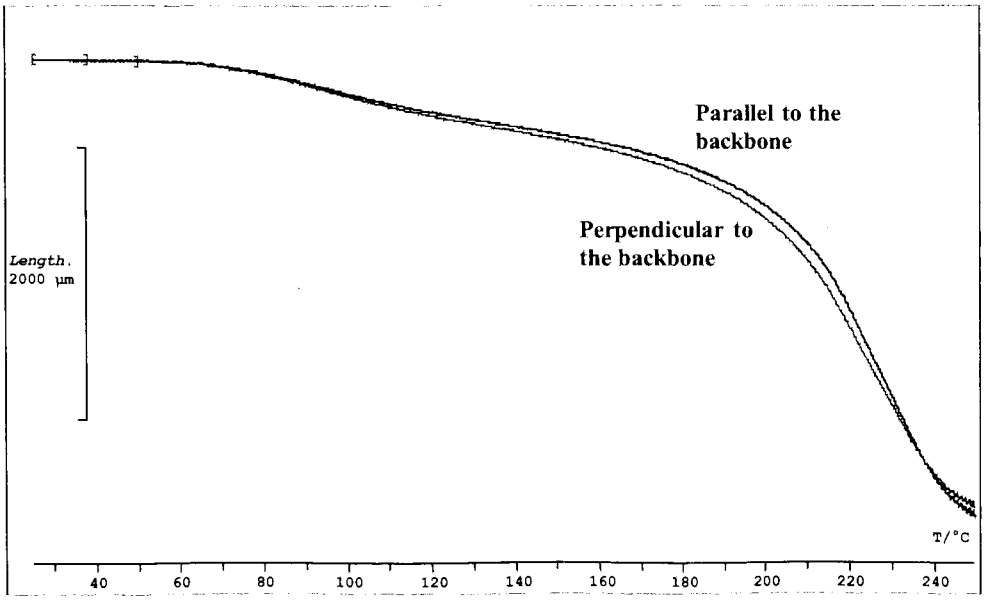

Figure 2. - TMA curve of Leather A in longitudinal tensile mode (samples cut parallel and perpendicular to the backbone)

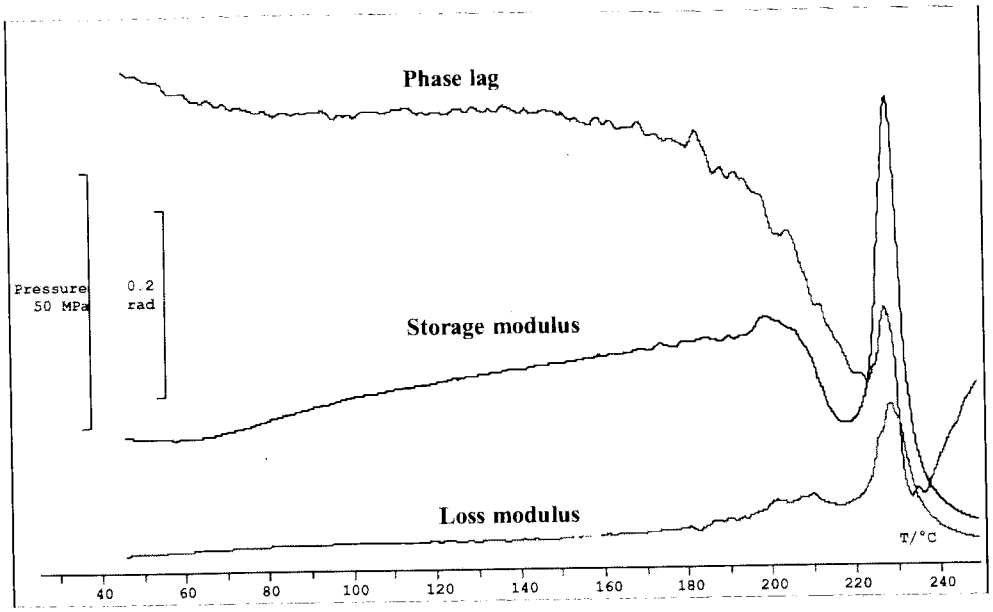

Figure 3. - Young modulus' analysis of the TMA curve of Leather A in longitudinal tensile mode (sample cut parallel to the backbone)

almost constant and was very low when compared with the amplitude of the compressive penetration mode. In the longitudinal mode, a great number of collagen fibres are strained in the test direction and therefore they contribute to balance the stress at which the specimen is submitted.

- *From 110°C up to 200°C*: The thermal contraction rate decreases in relation with the previous temperature interval and remains constant.
- *From 200°C to 250°C*: There is a sharp and sudden contraction in the sample length. The sample shrinks up to a quarter of its original length.

Figures 1 and 2 point out the differences between the transverse and the longitudinal testing modes. While the thermal transitions between 40 and 80°C in the transverse penetration curve (Figure 1) are due to the glass-transition of leather sample with a significant moisture content, the step observed from 60 to 110°C in the longitudinal tensile curve (Figure 2) can be attributed to the shrinkage due to water evaporation. This step agrees with that previously measured by thermogravimetry.[1] On the other hand, the denaturation process is now noticed by a sudden contraction of almost the 20% of its initial length. This confirms the observed transformation in the I.U.C.-16 standard test,[14] which records the collagen denaturation in excess of water. Therefore, after this first-order transition, the specimen has a higher thickness and a much lower length as shown by Figures 1 and 2 respectively.

Figure 3 shows the Young modulus' analysis of one curve in Figure 2 (no significant differences were observed between the specimens cut parallel and perpendicular to the backbone). As seen, the denaturation-melting process is noticed by a sharp maximum in the storage modulus around 227°C which agrees with that determined in the transverse compressive penetration mode. It should be also noted the presence of a minimum around 217°C in the storage modulus curve. The mechanical behaviour of the sample during this transition seems to have a different character than that observed during the denaturation process. According to Fakirov et al., gelatin (a hydrolisated form of collagen) exhibits a peculiarity as a polymeric material: the unusually close values of *Tg* and *Tm*, 217 and 230°C, respectively, in dry state. The existence of such a high value of glass-transition for completely dehydrated collagen has also been reported by other researchers[16,17] and it is now demonstrated by the minimum observed in the storage modulus curve of Figure 3. The phase lag curve shows two minimums at approx. 221 and 233°C that could be attributed to the glass-transition of the dry collagen in leather and the denaturation-melting temperature of the collagen fibres.

Although the TMA does not allow the determination of the enthalpy of denaturation, the measurement of both thermal transitions, *Tg* and *Td*, seems to be accurate and not masked by the evaporation of water, as it occurs with the differential scanning calorimetry. The collagen glass-transition of room-conditioned samples[10] is depicted in the TMA curve under transverse compressive penetration mode. The collagen glass-transition in dry state is seen in the Young modulus analysis of the TMA curve of the heating run in the longitudinal tensile mode.

On the other hand, the mechanical characteristics of leather can be also studied. Figure 1, by instance, allows the determination of the behaviour of leather when submitted to a

transverse compressive penetration test. The amplitude of the sample deformation induced by the periodical load gives information about the sample stiffness, which could be related to such an important parameter as the softness of leather.[18]

It is worth noting that thermogravimetry[1] confirms the fact that the mechanical changes detected by TMA are not only due to the evaporation of water. At 60°C, when the glass-transition in conditioned samples has already taken place in the penetration curve, only 2% of the sample weight has been lost. At the temperature range 80-160°C, when no significant changes are measured by TMA in the transverse compressive penetration mode, the evaporation process of water studied by TGA is still gradually going on. However, as previously stated, the changes detected in the tensile curve in this temperature range seem to be more directly related to the removal of moisture from the sample.

3. Glass-transition region of room-conditioned leathers by TMA in transverse compressive penetration mode

Leathers A-E were tested by TMA in the transverse compressive penetration mode from room temperature to 120°C in the conditions above described (5°C.min^{-1}; load = 0.1 - 0.2 N; period = 12 s). The experiments were performed in duplicate and a relative error lower than 5% was obtained for all the parameters determined. TABLE II shows the values of the characteristic parameters of the curve, as follows:

- *Tg* : The first extrapolated onset of the average curve in Figure 1. It corresponds to the glass-transition temperature.
- *Te*: The second extrapolated onset of the average curve in Figure 1. It targets the end of the glass-transition region.
- *Tp*: Temperature of the maximum penetration rate that corresponds to the inflexion point of the mean TMA curve.
- Cycle penetration amplitude at 20°C: Amplitude in % of leather thickness (Figure 1).
- Cycle penetration amplitude at 120°C: Amplitude in % of leather thickness (Figure 1).

TABLE II shows information about the glass-transition region of the previously conditioned samples. Leathers of bovine origin (A and B) exhibit higher values of Tg, Te and Tp and lower amplitudes of the probe penetration cycle. This agrees with the higher compactness and stiffness of bovine hides designed for shoe upper and upholstery manufacture.

The TMA of previously conditioned samples using the transverse compressive penetration mode allows us to distinguish between Leathers C and D (shoe upper goods) and E (clothing). Sample E shows higher glass-transition temperature values and significantly lower amplitude in the elastic penetration values.

CONCLUSIONS

1. Application of thermomechanical analysis to room-conditioned bovine chrome leather was explored. The technique allows the determination of the collagen glass-transition temperature (approx. 43°C), the glass-transition temperature in a completely dehydrated state (approx. 218°C) and the denaturation temperature in dry state (approx. 229°C).
2. Application of the transverse compressive penetration mode and the longitudinal extension mode has been explored. The different behaviour of the sample could be related with he relative main orientation of collagen fibres versus the testing direction.
3. The glass transition of room conditioned samples can be accurately determined through the transverse compressive penetration mode.
4. The glass transition of the completely dehydrated sample can be accurately determined through the longitudinal tensile mode.
5. Both the transverse compressive penetration and the longitudinal extension modes can accurately determine the denaturation temperature of the completely dehydrated samples.
6. The TMA curve in the longitudinal tensile mode shows

TABLE II

Parameters of the TMA curve of transverse compressive penetration mode

Leather	Tg [°C]	Te [°C]	Tp [°C]	Amplitude of the penetration cycle at 20°C [%]	at 120°C [%]
A	42.8	79.6	57.9	2.0	0.48
B	41.3	72.7	54.4	2.7	0.51
C	37.5	64.2	43.8	4.0	0.74
D	37.8	66.2	45.4	3.6	0.65
E	40.7	69.4	50.6	2.5	0.63

ıe shrinkage of the specimen linked to the free evaporation f water produced by heating between 60 and 110°C.
. The thermomechanical analysis between 20-120°C in the ransverse compressive penetration mode show different alues that depends on the origin and final application of the eather samples.

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