Changes in hydrothermal stability of leather and parchment with deterioration: a DSC study

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Abstract

Differential scanning calorimetry (DSC) allows to study the enthalpy changes which are associated with the denaturation of collagen, of which shrinkage is the macroscopic manifestation. Damage to skin, leather or parchment may be expected to manifest as a decrease of either the temperature of denaturation and/or the enthalpy changes. These modifications are associated with those of other chemical and mechanical characteristics.

DSC is a very fine tool to follow in an easy way the reaction of leather or parchment to an ageing or a conservation treatment. While for leather ΔH seems to have less than T_d, for parchment, it must be looked at very carefully since an unchanged T_d value can be accompanied by a fall of ΔH. The case of archeological waterlogged leather is particular since the state of deterioration is apparently not correlated with the hydrothermal stability. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Collagen; Denaturation; Ageing (natural and artificial); Environment; Conservation

1. Introduction

Hydrothermal stability of collagen in skin, leather or parchment is characterized by a shrinkage of the material when heated in water at a defined temperature. This thermal behaviour can be measured by different methods: by direct observation or under a microscope, by using the isometric tension test, where the sample is mechanically restrained and the resultant internal tension measured, or by differential scanning calorimetry (DSC). The latter allows the study of enthalpy changes which are associated with the denaturation of collagen, of which shrinkage is the macroscopic manifestation, together with the temperature at which the phenomenon occurs.

DSC has been used by different authors to elucidate the native structure of collagen, and how the molecule associates to a tannin [1–10]. Denaturation is defined as a transition from the triple helix to a randomly coiled form, taking place in the domains between the cross-links. The bonds which stabilize the superhelix are hydrogen, hydrophobic, van der Waal's bonds and interactions between oppositely charged residues on side chains. The non-random distribution of ionisable and hydrophobic side chains along the repeating unit results in the occurrence of charged and hydrophobic patches that contribute to stabilization of the structures through electrostatic and hydrophobic interactions. On the other hand, hydrogen bonded water plays a big part in the stabilization of the molecule. All these non covalent bondings break down on heating, the breakdown starting at the weakest points of the helix, between the stabilizing clusters. A small region con-

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taining a few linkages of low energy will act as a favourable site to initiate denaturation. Covalent bonds increase the size of cooperating units through inter- and intramolecular cross-links and increase the temperature of denaturation (T_d). This occurs on natural ageing and the increase varies with age, nature and the degree of the cross-links. Thus the T_d of soluble collagen, close to $35°C$, reaches $66°C$ in the mature skin. Tanning, by introducing cross-links, generally increases T_d, but its influence on the shrinkage behaviour varies with its nature and the kind of reactive groups which are involved [7–9]. The enthalpy changes, ΔH, result from reactions having different thermal behaviour: rupture of intra- and interchain H-bonds (endothermic), rupture of interchain hydrophobic bonds (exothermic), formation of new hydrophobic bonds in the random coiled phase (endothermic) [2].

Different factors influence the temperature of denaturation (or shrinkage). They can be classified into biological and non-biological factors. Biological factors relate to the nature of animal species, its living conditions, environment, age of the animal, and location on the body. Furthermore, it was shown by different authors that the shrinkage temperature (T_s) is related to the imino acid content of collagen, their position in the chain and their hydroxylation. Fish skin, for example, which has a lower content of imino acids has a lower T_s than mammal skin [1,3]. Non-biological factors include the nature of the heating medium, ionic environment, salts, tanning and ageing. The content of water in the material influences strongly the value of T_d. Thus, the lower the water content, the higher the T_d. Komanowsky [5] attributes this phenomenon to the fact that on drying, heat stabilization is enhanced by formation of strong inter- and intramolecular and ionic bonds between acidic and basic groups. But the conventional shrinkage phenomenon is measured in an aqueous medium, and for this reason, the T_d measurements are carried out in excess of water. On the other hand, lowering or raising the pH away from the isoelectric point will cause swelling, consequently the distance between the reactive groups will increase, altering the strength of the bonds, and T_d will be reduced. Nevertheless we observed in our samples that the thermal characteristics are stable between pH 3 and 5 [11]. Salts affect T_d in a variety of ways, and the destabilisation of the

triple helix by the addition of neutral salts confirms that at least part of the structural stability of collagen is due to electrostatic interactions [10].

The hydrothermal stability of collagen can be used to evaluate the modifications occurring in skin manufacture process, such as beaming and tanning [12,13]. However, the determination of the T_s obtained on heating a leather sample in water and recording when it shrinks is more commonly used in tanneries to follow the degree of tannage. Damage to skin may be expected to manifest as a decrease of either the temperature of denaturation or the enthalpy changes (ΔH), and we found the method a very fine tool to follow in an easy way the reaction of leather or parchment to a conservation treatment. We usually proceed by comparing an untreated reference to a treated sample. However, we need to know the reaction of the material not only during the treatment itself, but also after the treatment, and artificial ageing is used to predict the passage of time. Therefore we studied also the behaviour of leather and parchment when aged, either artificially or naturally.

2. Experimental

2.1. Materials and method

The measurements were performed using a Perkin–Elmer DSC 7 calorimeter. It was calibrated for temperature and heat flow with indium (melting point: $156.6°C$ and latent heat of melting: 28.45 J/g). The samples are weighed (around 3 mg), immersed in water for several hours, then hermetically sealed into aluminium crucibles. Thermal changes are measured with respect to a similar water sample, while flushing the chamber with nitrogen. The two pans (reference and sample) are heated separately at the constant rate of $10°C/min$. The instrument records the rate at which heat must be applied to maintain the two pans at the same temperature. When the test sample undergoes an endothermic process, the energy input required to maintain temperature will increase and give a signal on the raising temperature curve.

Our experiments were carried out on different samples of new, naturally and artificially aged vegetable tanned calf and goat leather and parchment, and on materials submitted to different treatments. New
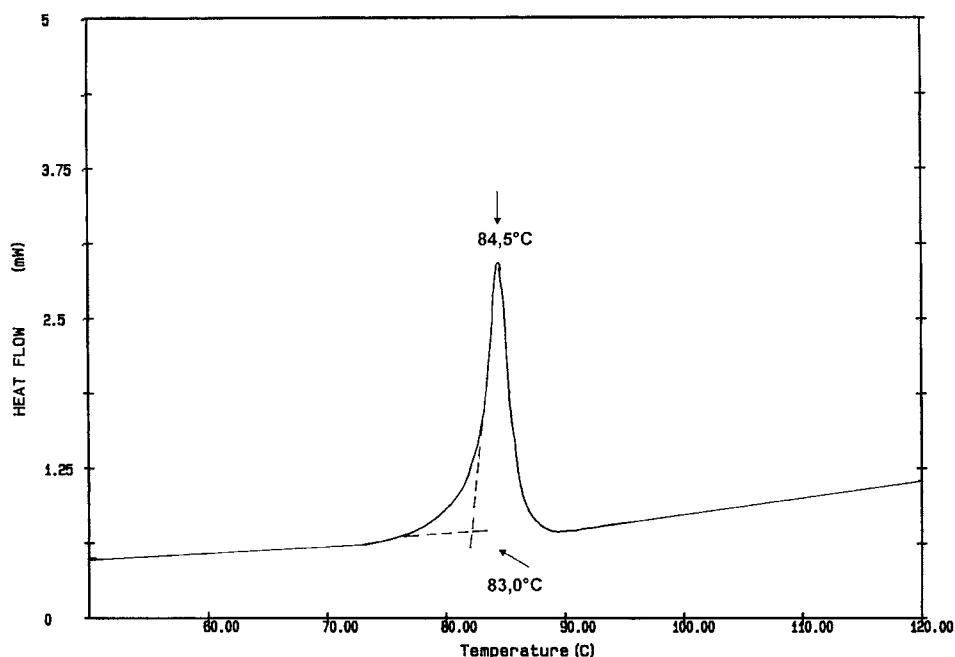
Fig. 1. Thermogram of a new vegetable tanned leather.

leathers, especially prepared at the British School of Leather Technology in Northampton, UK, for two European projects at which we participated on deterioration and conservation of vegetable tanned leather [14,15] were analysed, as well as historical and long term storage trial leathers. The historical leathers, dated from 16th to 19th century, came from different collections in Denmark, the Netherlands, UK and France, and were in different states of deterioration. The long term storage leathers were part of an investigation started at the end of 1931 by the British bookbinding industry in co-operation with leather and paper associations [16]. Some of the new leathers and parchments came from French bookbinding suppliers, and other parchments were prepared by a craftsman[1] in the Netherlands. The historical parchments were part of another European project on "Micro-analysis of parchment";[2] they came from

different libraries in Denmark and France and most of them dated from 17th and 18th century.

Fig. 1 shows a typical DSC curve of a new vegetable leather with an extrapolated onset temperature of 88.0°C, a peak at 90.0°C, a temperature range of 16.0°C, and a ΔH of 21.8 J/g (of leather). For the estimation of T_d, we use the extrapolated onset temperature (intersection of the baseline and the rising portion of the peak) which has been shown by several authors to be in the closest agreement with the conventional shrinkage data. This point is indistinguishable from the onset (temperature at which the curve begins to depart from the base line) in case of high deterioration which results in flat and large peaks. When the heating rate is lower, 5 or 2°C/min, the phenomenon is shifted to lower temperatures.

3. Results and discussion

3.1. Leather

With ageing of leather, both T_d and ΔH are decreased, but differently according to the type of deterioration. Some examples are given below.
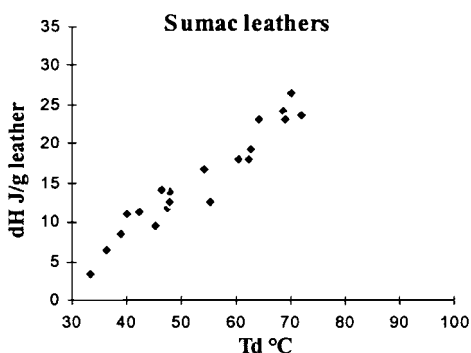
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[1] Henk de Groot, Rotterdam, the Netherlands.

Fig. 2. Relation between T_d and ΔH of artificially new sumac leather.

3.1.1. Artificial ageing

Experimental mimosa and sumac leathers, condensed and hydrolysable types, respectively, were exposed to simulated pollution in a chamber built in the laboratory. The exposure was performed according to the conditions established by the STEP project devoted to the correlation between natural and artificial ageing [14], i.e. cycles of dry heat (120°C) and pollution (25 ppm of SO_2 and 10 ppm of NO_2, at 35°C and 40% RH), during 4–16 weeks.

Plotting T_d vs ΔH as in Figs. 2 and 3 shows that the two parameters evolve rather concomitantly. This can be explained by a certain homogeneity of the samples, mostly due to the ageing, but also to the method of preparation, although different vegetable tannins were used. At a certain stage of deterioration, when temperature reaches about 29–30°C, the energy involved is so low that there is no more signal to be noticed.

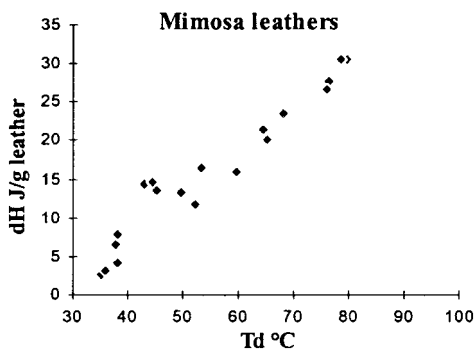This point represents the ultimate temperature at which a phenomenon can be recorded.

3.1.2. Natural ageing: historical leather

A set of 20 historical leathers vegetable tanned, dated from 16th to 19th century has been analysed during the STEP and ENVIRONMENT projects. They also exhibited lower values of T_d and ΔH, compared to a new leather, but apparently these two parameters do not seem to have decreased simultaneously; for example a T_d of 48.0°C, quite low, allied to a quite high ΔH of 24.7 J/g, (of leather) or at the opposite for another leather, 67.0°C and 13.6 J/g. Nevertheless, the most deteriorated leathers showed low values for both parameters (34.0°C/6.9 J/g and 36.5°C/9.6 J/g). As in artificial ageing, the ultimate phenomenon recordable is close to 30°C, but unlike it, the T_d and ΔH values are not at all correlated (see Fig. 4). This is probably due to the fact that the leathers, although all vegetable tanned, were prepared in different ways, and coming from different collections, they had been exposed to various environmental conditions.

During the two European projects we had access to samples which were stored in UK during 60 years. In 1931, different leathers were bound to two sets of books which were placed in two locations: at the British Library (BL) in London, a very polluted site, and at the National Library of Wales (NLW), much less polluted. Several of them have been thoroughly analysed and the results summarized in the conclusion of the STEP project [17]. Briefly, let us say that in the polluted area, the leathers deteriorated mainly through hydrolysis, while in the other location they
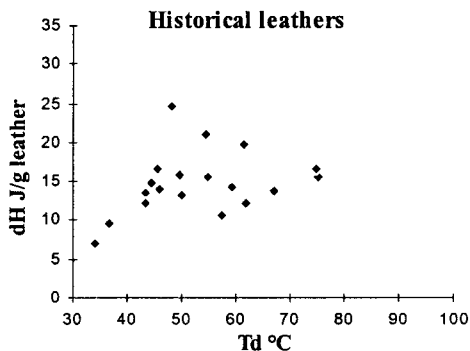

Fig. 3. Relation between T_d and ΔH of artificially new mimosa leather.

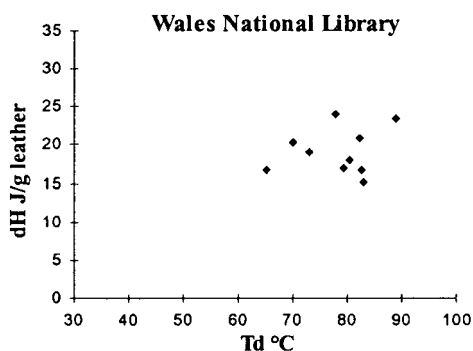Fig. 4. Relation between T_d and ΔH of historical leathers.

Fig. 5. Relation between T_d and ΔH of the Wales leathers.

deteriorated mainly under oxidation. Their physical state of conservation is very different, the polluted samples being on the whole much more deteriorated than the others.

Under DSC analysis, they showed as expected low values of T_d and ΔH, compared to new leather, but these values are not correlated as shown in Figs. 5 and 6, even inside one group, BL or NLW, where the leathers were stored in the same environmental conditions. This seems to indicate that the process of manufacture carries more weight than storage conditions. The ΔH values of all samples lied roughly between 13 and 25 J/g (of leather), with one exception for a very deteriorated leather in BL (30°C and 4 J/g). The T_d values showed a larger range of temperatures, from 30°C (BL) to 89°C (NLW). The leathers from the British Library exhibited the lowest temperatures (below 70°C), while those from the Wales National Library were above 65°C. It is then interesting to note that hydrolysis has a stronger effect on T_d than oxidation, and this corresponds also to the worst state of
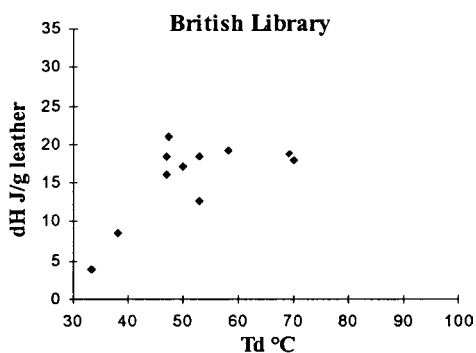

Fig. 6. Relation between T_d and ΔH of the British Library leathers.

conservation of the leathers stored in a polluted environment.

3.1.3. Natural ageing: archeological leather

Archeological waterlogged leathers show a particular hydrothermal behaviour in that the T_d's can be very high, some of them reaching values as high as 80–85°C, quite unexpected for such old and generally weakened samples. Nevertheless, the ΔH values are on the whole low, generally below 5–7 J/g, which denotes a high level of alteration.

Another characteristic worth mentioning is the behaviour of the material when left to dry in a normal environment, and measured with no water added. In the case of new or historical leathers in different states of deterioration, but stored in the atmosphere, a clear signal is observed at high temperature, generally over 120–130°C with an energy of around 15–20 J/g. In waterlogged archeological leathers, the signal obtained is much weaker (a few Joules per gram), often occurring at lower temperature (100°C and below), and in some cases of heavy deterioration, no peak is even present on the raising temperature curve (see Fig. 7). The shift of the peak to higher temperatures, as quoted before, is due to a lesser amount of water present during the measurement (overwetting in the conventional measurement of T_d versus the amount of water that the material contains at 50% RH, i.e. 14–12% when in good condition, and less, down to 9–8%, when deteriorated). Nevertheless, in any case of new, historical, or archeological samples, returning the dry material (before having heated it of course) in water restores the normal hydrothermal phenomenon.

The particular hydrothermal behaviour of the archeological leathers after drying indicates that very little water is left in the structure, and that when deterioration is severe, even part of the water participating in the stabilization of the triple helix can also be affected. During the prolonged storage in wet soils of these leathers, which have been buried in more or less good state of conservation, strong modifications of the interactions between collagen and water occur. Deterioration releases reactive polar sites where water can be fixed (phenomenon which is responsible for the swelling of such finds), but the linkage to polar groups on side chains could be different than that in main chains, resulting in weaker binding of water, and the modifications of the amino acid composition induced
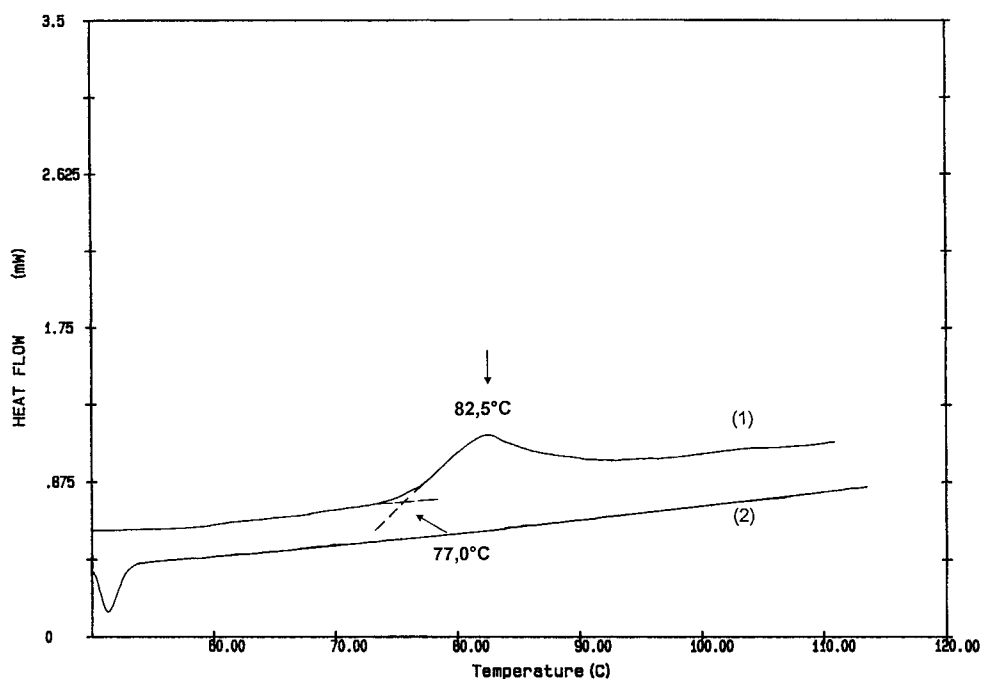
Fig. 7. Thermograms of an archeological leather: (1) measured in water; (2) measured after drying with no addition of water.

by deterioration contribute probably also to this phenomenon. Other investigations would be necessary to better understand the behaviour of these leathers, in particular to evaluate the amount of triple helix left, and to clarify what kind of water binding occurs in aqueous media. Total amino acid composition and identification of breakdown products which are under progress in the laboratory, will also contribute to determine the kind of deterioration.

The alteration of the water-retaining property of such leathers implies that after their excavation from a wet soil, they require a special treatment to be dried in good condition, because left uncured they would lose too much water, shrink, harden, become brittle and very fragile, and could then be definitely lost. One possible treatment is the impregnation before drying with a humectant such as polyethylene glycol, which helps maintaining the dimensions and the suppleness of the objects.

Even after proper drying treatment, the leathers give DSC data which apparently do not correspond to the state of conservation as shown by the examples of Table 1, with low T_d values for relatively well conserved samples and high values for deteriorated ones.

Nevertheless, when the material is very weak, powdery, there is no signal observable on the thermal analysis curve. On the other hand, the particularly high heterogeneity of the material should be mentioned.

In conclusion of the results obtained on leather, we can consider T_d as the important parameter to be used to point out a deterioration. Its variations have been closely correlated to the modifications of other chemical characteristics [17], and except for

Table 1
T_d and ΔH of some archeological leathers after drying treatment

	T_d (°C)	ΔH (J/g)
Good state of conservation	74.0	3.6
	77.0	3.5
	65.0	5.0
	50.0	3.9
	71.0	7.0
Bad state of conservation	80.5	<1.0
	63.0	3.8
	88.0	2.6
	68.0	4.8
	77.0	7.3

archeological leathers, it corresponds well also to the more or less good physical state of deterioration. In fact, we generally observe that a fall of T_d corresponds to a loss of mechanical strength. T_d can then be used to follow the behaviour of the material. For example, it showed itself to be very useful in a study carried out to understand the long term effect of polyethylene glycol left in leather after the drying treatment [18].

3.2. Parchment

According to their way of manufacture, parchments exhibit different hydrothermal stability. New parchment prepared in a quite industrial process usually has a T_d below that of fresh skin, and rather close to that of limed skin, at 55–56°C, with a ΔH around 30–35 J/g, (of parchment) a quite low value considering that parchment is mainly composed of collagen (from literature, fresh skin collagen has a T_d close to 50–55 J/g of collagen). The craftman's way of preparation results in a material presenting both higher T_d and ΔH values, respectively, above 60°C and up to 40 J/g (of parchment). Table 2 gives some values observed on some samples of new parchment.

Table 2
T_d and ΔH values of new parchments

Parchments	T_d (°C)	ΔH (J/g)
"Industrial"	56.0	33.6
	51.5	37.0
	52.0	37.5
	55.5	34.3
"Hand crafted"	64.0	31.9
	64.5	35.7
	60.5	41.7
	63.0	39.1

The thermal analysis curves of the "hand crafted" parchments exhibit a clear second peak in the high temperatures, as shown on Fig. 8, which is not so clearly seen on the "industrial" samples. Such phenomenon has been reported in literature for fresh skins of young animals. Flandin et al. [19] observed on rat skin a main peak at around 58–60°C lying between two shoulders. They calculated that the enthalpy change of the first shoulder decreases slowly with age, while the enthalpy change of the second shoulder or third peak increases, then stabilizes. All this is
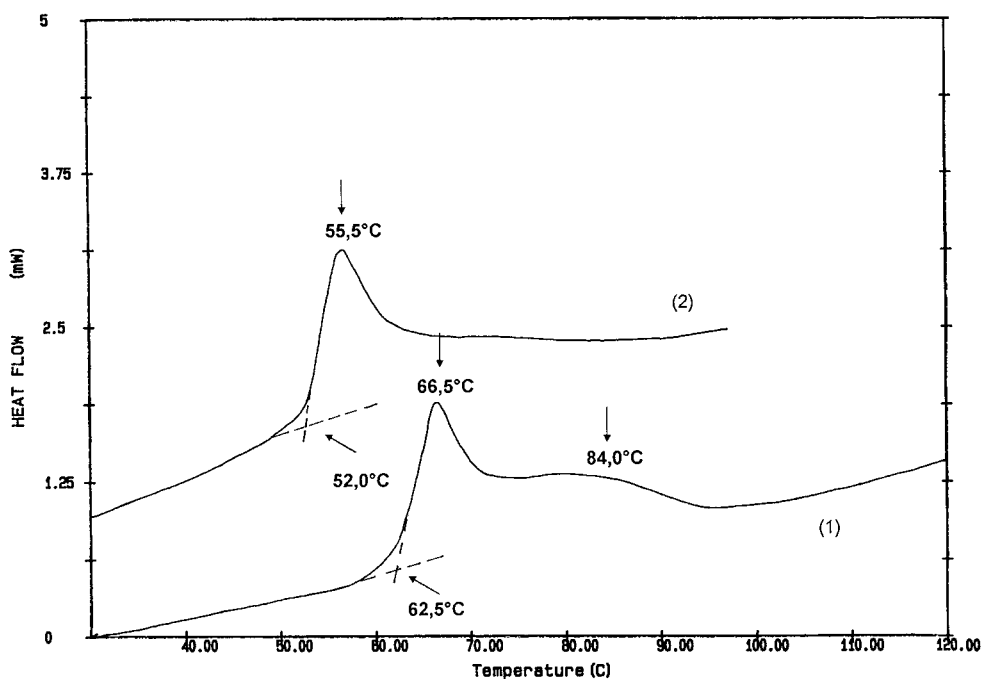
Fig. 8. Thermograms of new parchments: (1) handcrafted parchment; (2) industrial parchment.

related to heat labile and heat stabile cross-links participating in the stabilization of the collagen molecule and evolving with age. Kronick and Maleef [20] observed the same three peaks on calf skin, but quoting that the first shoulder is never present in hides of mature cattle.

The results obtained with these parchments suggest that the "industrial" manufacture is rather damaging for the skin: use of disinfectant, such as formaldehyde which partially tans the skin, excessive liming, use of bleaching such as hydrogen peroxide, etc., are responsible for the lower hydrothermal stability compared to that of the craftsman preparation which is much more respectful of the skin integrity.

3.2.1. Artificial ageing

On ageing, parchment exhibits loss of T_d and ΔH, but in the different conditions of our ageing, no clear relation can be seen in these two parameters.

Fig. 9 presents experiments performed on an "industrial" new parchment (INP) artificially aged by heat, 120°C (1 day, 2 days, 2 weeks) and 80°C (3 days, 10 days, 2 weeks), pollution (16, 48, 64 weeks), and cycles of heat (80°C) with pollution (4, 8 and 16 weeks). Under these conditions of ageing, ΔH is more affected than T_d, except for a strong deterioration such as 2 weeks at 120°C approaching an ultimate state of deterioration. Unlike vegetable tanned leather, pollution does not deteriorate strongly parchment, which even after 64 weeks of this treatment has a T_d fall of
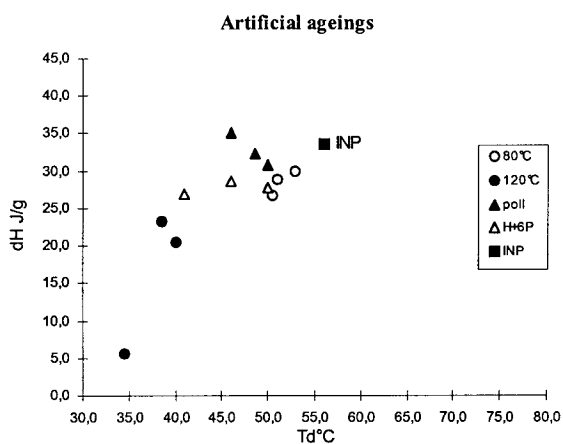
only 10°C, and an enthalpy variation quite unchanged. Heat is more damaging, especially at high temperature and when used in cycles with pollution.

We observed in another experiment on "industrial" new parchment, using xenon light[3] for the ageing in an atmosphere maintained at 30°C and 50% HR, that after 25 days of illumination, while T_d was not much modified, with a loss of 4–5°C, ΔH was considerably decreased, from 35 to 12 J/g (of parchment). In the same time, physical testing performed on this material after the same exposure showed a considerable loss of strength: −30% on the tensile test and −45 to 60% on the elongation at break. The good level maintained on T_d can partly be interpreted as the creation of new cross-links induced by light, resulting in a stiffer tissue and therefore loss of the mechanical strength. Nevertheless, the collagen molecule suffered strong modifications of structure induced by the light ageing as shown by the decrease of the energy required for the denaturation.

3.2.2. Natural ageing

We know nothing about the original characteristics of the historical parchments at the moment of their preparation, but we can assume that the methods used in the past were not too aggressive to the skin, and therefore they should have been closer to the "hand crafted" material than the "industrial" one.

Several remarks can be given on the graph of Fig. 10, showing the relation between T_d and ΔH, but what is first striking is the low energy of the historical samples, even when the corresponding T_d value is high (in one case for example we have 68°C and 14.9 J/g). Except for three samples, the ΔH values are below 16 J/g, very low compared to new parchment (30–40 J/g), while the T_d values lie between 35 and 68°C. It is quite surprising that five samples have $T_d = 60°C$, which corresponds to new parchment, but their $\Delta H < 17.9$ J/g imply important modifications of structure. These high T_d values could be attributed to cross-linking occurring during storage, under the effect of dehydration, light ageing, or whichever kind of ageing. Nevertheless, this cross-linking does not

Fig. 9. Relation between T_d and ΔH of artificially aged parchments (compared to the reference INP).

**Historical parchments
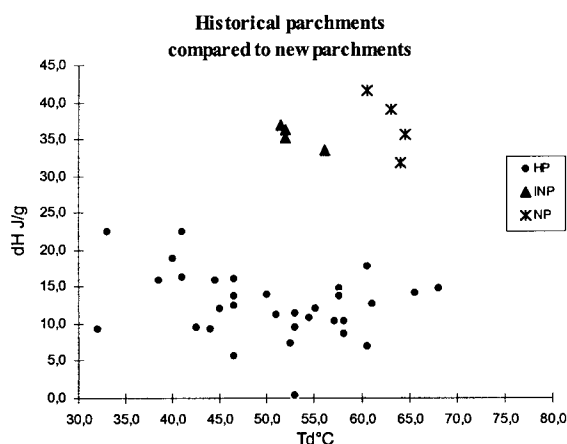compared to new parchments**

Fig. 10. Relation between T_d and ΔH of historical parchments HP (compared to new parchments, NP and INP).

prevent from breakdown and both phenomenon are responsible for re-arrangements of the molecular structure of collagen. In a study dedicated to estimate the alteration of parchment using steric exclusion chromatography to determine the molecular weight distribution of collagen, we were confronted by the problem of low solubility of these parchments [21].

We saw that for leather, when deterioration is important, ΔH value falls tremendously due to destruction of the triple helix, decreasing toward zero. In the same time T_d decreases, but never below 29–30°C: beyond that state, no signal can be seen on the raising temperature curve. As for leather, a very deteriorated parchment presents a flat thermal analysis curve, but nevertheless we observed in one case of natural ageing a faint signal happening at the relatively high temperature of 53°C.

As for leather, DSC analysis can be quite useful to follow the reaction of parchment to a conservation treatment. We used it, for example, when studying the effect of bleaching treatments, and particularly that of hydrogen peroxide which, if metallic ions are present can depolymerise collagen through the release of free radical. This reaction, not detectable at the moment of the application would amplify with time, and the following up of the modifications occurring in the hydrothermal stability was very useful to evaluate and compare the effectiveness of different protecting treatments [22].

4. Conclusion

The DSC technique provides a suitable tool for the rapid determination of changes in the collagenous structure of leather or parchment. The reproducibility of the technique is good, but the high heterogeneity of the material, especially when deteriorated, must be taken into consideration. We use it to follow the effect of treatments, by comparison with untreated reference samples. It must be noted that both parameters of denaturation, T_d and ΔH, must be considered to evaluate a modification in the material. While for leather ΔH seems to have a less interest than T_d, in the case of parchment, it must be looked at very carefully. In fact an unchanged T_d value, if considered alone, can result in a false evaluation, since in certain cases of deterioration, an accompanying fall of ΔH, denoting a re-arrangement of the molecular structure which is correlated with a loss of mechanical strength, has been recorded as pointed out in our experiment with light. Cross-linking is probably responsible for this phenomenon, as it is for the hydrothermal behaviour of certain historical parchments where high T_d values ($\geq 60°C$) are associated with low ΔH values (<18 J/g). In fact parchment can suffer both cross-linking and breakdown depending on the environmental conditions and which occur simultaneously or successively.

The case of archeological waterlogged leather is particular since the state of deterioration is apparently not correlated with the hydrothermal stability. More studies would be necessary to understand this special behaviour, and especially to clarify the kind of binding which associates water molecules to collagen in aqueous media.

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