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# Characterisation and evaluation of the environmental impact on historical parchments by differential scanning calorimetry

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Cultural Heritage Special Chapter


**Abstract** Our recent developments concerning the assessment of parchments deterioration using DSC are reported. Measurements performed on samples in excess water conditions, in static air and gas flow provided qualitative and quantitative information on parchment ageing and deterioration at microscopic and mesoscopic level, when assembly of fibres/fibrils is weakened, partially and eventually completely lost, and at molecular level, when triple helix uncoiling occurs. A damage ranking scale based on a large collection of DSC parameters obtained by investigating artificially aged samples was set up. Deconvolution of the DSC thermal denaturation peaks in excess water enabled evaluating and discriminating stability of parchments with similar damage levels. Further experimental evidences such as softening of the crystalline fraction of collagen, thermal-oxidation and collagen gelatinisation were detected by DSC measurements in gas flow and static air, and related to specific deterioration patterns. DSC measurement of wet samples provided an objective and reliable method for evaluating parchment shrinkage temperature overcoming the limitations of conventional methods.

**Keywords** Parchment · Collagen · DSC · Ageing · Cultural heritage

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## Introduction

Parchment conservation practice has long time been regarded and executed as a craft governed by secrecies and empiricism, while parchment conservation science has started to develop only in the second half of twentieth century. One of the first scientific approaches to the restoration of parchment was the pioneering work of the Italian chemist Icilio Guareschi, *Della pergamena, con osservazioni ed esperienze sul ricupero e sul restauro di codici danneggiati negli incendi e notizie storiche* [1], following the fire ravage of the manuscripts of the National University Library of Turin in 1904. In the last decades, the holistic approach of cultural heritage has produced a deep attention towards aspects previously considered as merely secondary, such as materials properties and their deterioration mechanisms. Parchment heritage, despite its exceptional longevity, is subjected to physical, biological and chemical deterioration induced by manipulation, consultation, exhibition, storage in unsuitable conditions, previous restoration treatments. Its safe and long-term conservation requires a comprehensive understanding of chemical and physical deterioration mechanisms and of how they can be successfully slowed down. Most progress in the assessment and diagnosis of parchment as material was achieved in the European research projects: *Methods in the Microanalysis of Parchment* (MAP, SMT4-96-2101) and *Improved Damage Assessment of Parchment* (IDAP, EVK-CT-2001-00061). MAP concerned with the analysis of historical parchments and pointed towards the necessity of accelerated ageing studies. This was then developed in IDAP and damage assessment techniques for macro-, micro-, meso-, nanoscopic and molecular levels of parchment were devised for the first time. IDAP also described in detail the damage types and causes and established for the first time

standard methods based on physical chemical advanced techniques for diagnosis and assessment of damage. The recent Italian project *Old Parchment: Evaluating Restoration and Analysis* (OPERA, CIPE 04, Section Nanotechnology and Nanosciences, D39) has deepen the knowledge and refined the tools developed in IDAP by studying archival and library parchment collections. The Romanian project *Multidisciplinary research for establishing the deterioration mechanisms of historical and cultural parchment documents* (PERGAMO, CEEX 165) also dealt with deterioration aspects of historical parchments. It is worth to be mentioned that projects like *Domesday Book* (National Archives, UK), *Codex Sinaiticus* (UK, Germany, Russia, Egypt, USA) and *Systematic description of the deterioration of leather and parchment fibers at microscopic level* (Denmark) are currently using the diagnostic methods and damage ranking protocols developed within IDAP.

This work concerns with the advantages offered by DSC in such a complex area of investigation and its capability to answer the questions raised by conservation practice: how can we evaluate deterioration both qualitatively and qualitatively, what are causes and mechanisms of deterioration, when parchment state becomes unstable and turns to irreversible damage.

Pioneering of DSC application to cultural heritage study is due to Hans G. Wiedemann. He demonstrated that DSC offers the possibility of distinguishing between ancient silk fabrics and showed that the shape of DSC curves was dependent on the age of papyrus sheets and different treatment to which they were subjected [2–4]. In the last two decades, DSC has extensively been applied to the study of parchment and leather deterioration [5–7]. Present study reports the results of a research programme based on a large number of parchments (new, subjected to various accelerated ageing treatments and historical) aiming at describing thermal and hydrothermal properties of collagen in different deterioration conditions and finding out suitable DSC parameters that links them with the overall response of parchment on deterioration [8–10]. Different DSC techniques enabled collagen macroscopic and microscopic (fibrous network), mesoscopic (fibrils) and molecular organisations to be investigated. A damage scale based on DSC quantitative parameters was built up and employed to classify deterioration level in historical parchments.

## Parchment structure and deterioration

The key of success of parchment as biomaterial rests on its complex structure characterised by an intimate relationship and connectivity between individual collagen molecules and their hierarchical supramolecular organisation (Fig. 1). Type I collagen molecules are composed of three

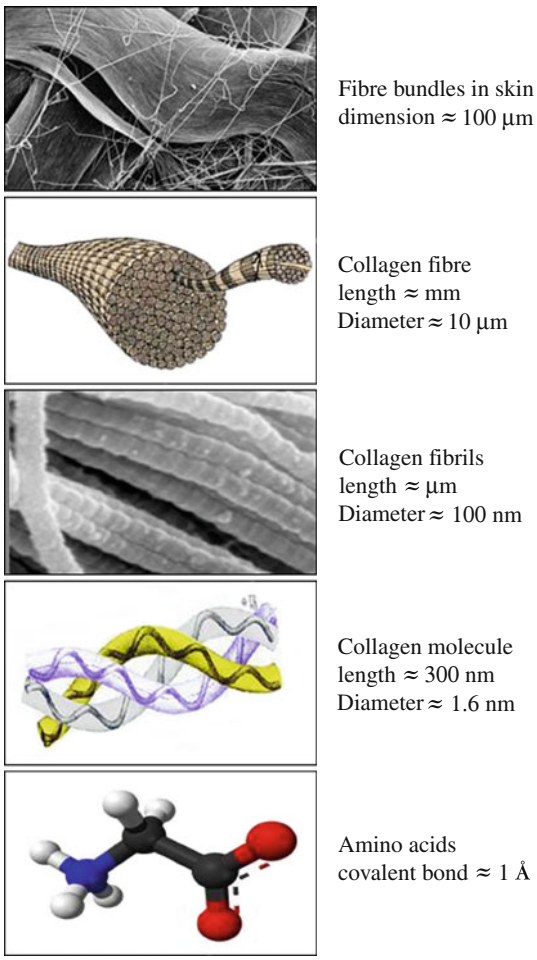


**Fig. 1** Hierarchical structure of collagen-based materials. Each collagen molecule is a right-handed triple helix made of three left-handed α-chains. Collagen molecules aggregate both in lateral and longitudinal directions to form fibrils. Multiple fibrils make up collagen fibres mainly through crosslinking

polypeptide chains, consisting of 1300–1700 amino acid residues each, organised into a central triple helix configuration. This triple helix of 285 nm length and 1.4 nm diameter consisting of a repeating triplet (G-X-Y)*n*, where G is glycine and X and Y are often proline or hydroxyproline, is stabilised by a network of hydrogen bonds, mainly associated with hydroxyproline. Within microfibrils, collagen molecules are arranged head-to-tail, with a 67 nm gap between each (*D*-band), in a quarter-stagger arrangement. Collagen fibrils with a diameter of 100–500 nm and a length up to millimetres range are formed through the bundling of several microfibrils, each of them containing clusters of five collagen molecules. At the next level of the hierarchy, multiple fibrils make up collagen fibres. Fibre bundles intertwine in the three-dimensional collagen fibre network. This supramolecular hierarchical structure stabilised by covalent links between terminal ends of collagen triple-helices, carbonyl-water hydrogen bonds, hydrophobic and

van der Waals interactions [11, 12] confers great stability and strength to parchment and makes it an extremely long-lived material.

Parchment deterioration occurs as a result of alterations induced in both its molecular structure and the hierarchy of its multi-level organisation by residual chemicals (e.g. cations catalysing oxidation processes) and fat, interaction between ink or binders and collagen, environmental factors (humidity, temperature, light, pollutants), biological agents, improper handling and wrong conservation and/or restoration operations as well as extreme events (fires, floods, earthquakes, wars). Manufacturing process itself was shown to induce the first structural alterations of collagen, i.e. liming has an effect on the axial spacing and on the lipid bilayer and drying has an effect on intermolecular lateral packing [13]. Parchment main deterioration mechanisms are oxidation and hydrolysis which often act simultaneously, reinforcing one another and finally causing the irreversible helix-coil transition (denaturation) of collagen at molecular level.

## Experimental

### Materials

More than 30 new parchments manufactured by different suppliers from calf, goat, sheep, baby goat, baby sheep, pig and deer hides were investigated. Some calf parchments were subjected to various accelerated ageing treatments by exposure to single and combined actions of heat, humidity, light irradiation and chemical pollutants. In total, more than 150 new and artificially aged parchments were investigated.

A number of historical parchments in form of single sheets, documents and bindings from the National Archives of Stirling (UK), Royal Library (Denmark), State Archives of Turin, Genoa and Florence, Historical Archives of the City of Turin, Historical Archives of the University of Turin (Italy), Municipal Museum of Bucharest, National Museum of History, National Military Museum and Moldova Museum Complex (Romania) were investigated.

### Differential scanning calorimetry

DSC measurements were performed on as received, wet and in excess water parchment samples to obtain a comprehensive spectrum of thermal responses.

(i) DSC measurements of as received samples were performed in static air atmosphere using sealed stainless steel crucibles in the temperature range 25–200 °C, at 5 K $min^{-1}$ heating rate, with a SETARAM DSC 111 microcalorimeter at the Department of Chemistry IFM, Turin. The measurements in open crucibles under gas flow (nitrogen, oxygen, and synthetic air, purity better than 99.999%, 20 mL $min^{-1}$ flow rate) were made in the temperature range 25–280 °C, at 10 K $min^{-1}$ heating rate, with a NETZSCH DSC 204 F1 Phoenix calorimeter at the INCDIE ICPE-CA, Bucharest. In both cases, samples ($\approx$5 mg) were analysed after a few days storage at about 20 °C and RH 50%. Since in sealed crucibles any release of matter is prevented, moisture content of the sample remains constant over the entire measurement temperature range and DSC results are thus related to thermal denaturation only [8]. Denaturation peaks are sharp, narrow and reach their maximum at about 120 °C [8, 15]. In open crucibles and gas flow, a broad endotherm ranging from room temperature to 150 °C is the experimental evidence of two overlapping processes, moisture loss and collagen thermal denaturation. Thermal denaturation cannot thus be evaluated, but transitions related to softening/melting of the collagen crystalline fraction as well thermal oxidation can be detected at higher temperatures [14, 15].

(ii) DSC measurements in excess water were performed with a SETARAM Micro DSC III calorimeter at the Department of Chemistry IFM, Turin, in the temperature range 5–95 °C, at 0.5 K $min^{-1}$ heating rate and using a 0.5 M acetate buffer solution (pH = 5.0). Before measurement, samples ($\approx$2 mg) were kept in their calorimetric cell containing buffer solution for 2 h at 5 °C to assure reproducible hydration conditions.

(iii) DSC measurements of wet samples in sealed crucibles were made in the temperature range 25–110 °C, at 10 K $min^{-1}$ heating rate with a NETZSCH 204 F1 Phoenix calorimeter at the INCDIE ICPE-CA, Bucharest. Samples ($\approx$5 mg) were wetted with 35 μL deionised water, hermetically sealed in aluminium crucibles and kept at room temperature for 24 h before measurement.

Measurements in excess water condition were selected for quantitative evaluation of deterioration since at high hydration level, i.e. more 30 molecules of water per three residue (Gly-X-Y) unit, both temperature and enthalpy of thermal denaturation were shown to be constant [16] and allowed to obtain very reproducible results. In this condition, collagen fibrils are swollen to their maximum extent in water which is distributed in two phases, intrafibrillar and external water. Further water addition merely increases its presence in the external pool without affecting intrafibrillar water or interaxial molecular spacing of collagen molecules in the fibre lattice. By contrast, at intermediate hydration levels, as for so-called wet samples, results may be less reliable, since DSC parameters can vary with the collagen hydration level.

Certified reference materials such as naphthalene, benzoic acid and gallium were used to check the calibration constants supplied by SETARAM in the working temperature regions for DSC111 and micro DSC III calorimeters.

Several melting runs performed under same experimental conditions as parchment measurements showed an agreement with the recent IUPAC recommended values [17] of 0.05% for temperature and 0.25% for enthalpy. As a check, DSC scans of calf skin pure collagen were performed and both denaturation temperature and associated enthalpy were in excellent agreement with values obtained by other authors [18–20]. NETZSCH 204 F1 Phoenix calorimeter was calibrated by using high-purity standards (In, Bi, Hg, Zn, CsCl, $KNO_3$) supplied by the producer.

Two to five measurements per sample were carried out depending on sample availability.

## Results and discussion

### DSC measurement in excess water and static air

DSC measurements provide $C_p^{ex} = f(T)$ curves whose integration gives the energy exchanged during the thermal events. Highly cooperative structures such as proteins, stabilised by the cooperation of numerous weak forces (e.g. hydrogen bonding, electrostatic and hydrophobic interactions, etc.), undergo thermal transitions resulting from changes in conformation, hydration/dehydration, denaturation, aggregation, de-aggregation, oxidation. Thermal denaturation is a time-dependent irreversible transformation of the native triple helical structure into uncoiled structure [21–24]. Regardless of the underlying molecular mechanisms, collagen heating gives rise to sharp endothermic peaks at defined temperatures, $T_{max}$, depending on the hydration level as shown in Fig. 2. DSC peak temperature has been assumed as denaturation temperature of collagen within parchment. The higher denaturation temperature for the same sample measured in static air is mainly due to the higher crosslink content and fibrillar cohesion. Tables 1 and 2 set out average values of the calorimetric parameters of thermal denaturation of new undamaged parchments measured in excess water and static air conditions. For sub-samples selected using the IDAP standard sampling procedure, $T_{max}$ and $\Delta H$ variations were very small, lower than ±2% and ±3%, respectively. Somewhat larger variations for average values of $T_{max}$ (±3%) and $\Delta H$ (±9%) in new parchments from different suppliers were found. It can thus be assumed that manufacturing procedures result in not negligible alterations in thermal stability of collagen and variations higher than those mentioned (3%, 9%, respectively) have to be ascribed to ageing/deterioration suffered by parchments.

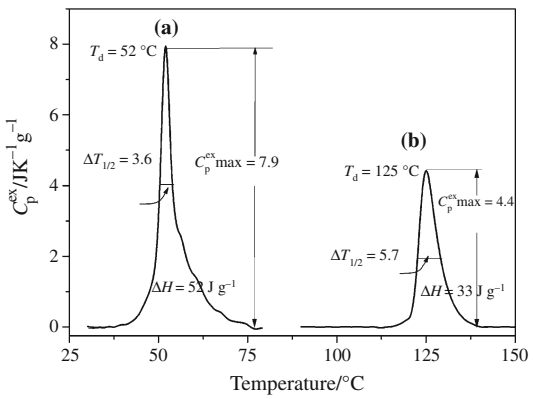


**Fig. 2** Typical thermal denaturation DSC curves of a calf new parchment obtained in sealed crucibles in (*a*) excess water and (*b*) static air conditions and associated thermodynamic parameters: peak temperature, $T_{max}$, enthalpy change, $\Delta H$, peak half-width $\Delta T_{1/2}$ and maximum height $C_p^{ex}$max

**Table 1** DSC parameters of thermal denaturation for new parchments measured in excess water

| Parchment | *n* | $T_{max}$/°C | $\Delta H$/J g$^{-1}$ | $\Delta T_{1/2}$/°C | $C_p^{ex}$max/J K$^{-1}$ g$^{-1}$ |
|---|---|---|---|---|---|
| Calf (SC69)[a] | 4 | 51.9 ± 0.6 | 54.0 ± 1.4 | 5.4 ± 0.3 | 6.8 ± 0.3 |
| Calf (SC81)[a] | 3 | 56.7 ± 0.3 | 53.3 ± 1.5 | 4.6 ± 0.4 | 7.4 ± 0.4 |
| Calf (SC82)[a] | 2 | 54.6 ± 0.5 | 49.3 ± 1.4 | 3.8 ± 0.2 | 7.7 ± 0.2 |
| Calf (SC70)[b] | 2 | 53.1 ± 0.4 | 45.8 ± 1.2 | 4.1 ± 0.3 | 5.6 ± 0.3 |
| Calf average | | 53.9 ± 1.6 | 51.5 ± 4.6 | 4.7 ± 1.0 | 6.9 ± 1.1 |
| Goat[c] | 16 | 49.1 ± 0.9 | 52.0 ± 1.1 | 4.7 ± 0.3 | 7.5 ± 0.4 |

Uncertainties are twice standard deviation. Average values are given as weighted mean with combined uncertainties

*n* = number of subsamples; for each subsample at least three measurements were performed

[a] Origin: de Groot (NL)

[b] Origin: School of Conservation (DK)

[c] Average values for 16 subsamples from 8 parchments manufactured at INCDTP-ICPI (RO)

**Table 2** DSC parameters of thermal denaturation for new parchments measured in static air

| Parchment | *n* | $T_{max}$/°C | $\Delta H$/J g$^{-1}$ | $\Delta T_{1/2}$/°C | $C_p^{ex}$max/J K$^{-1}$ g$^{-1}$ |
|---|---|---|---|---|---|
| Calf (SC69)[a] | 4 | 125.6 ± 0.5 | 32.6 ± 0.6 | 5.7 ± 0.5 | 4.6 ± 0.4 |
| Calf (SC81)[a] | 3 | 126.4 ± 0.8 | 31.2± 0.3 | 5.4 ± 0.2 | 4.9 ± 0.3 |
| Calf (SC82)[a] | 2 | 126.0 ± 0.5 | 30.2 ± 0.4 | 5.5 ± 0.3 | 4.7 ± 0.2 |
| Calf (SC70)[b] | 2 | 123.2 ± 0.4 | 34.2± 0.3 | 5.6 ± 0.3 | 4.9 ± 0.3 |
| Calf average | | 125.5 ± 1.9 | 32.1 ± 1.5 | 5.6 ± 1.2 | 4.8 ± 1.1 |
| Goat[c] | 4 | 116.3 ± 0.9 | 34.0 ± 0.3 | 5.2 ± 0.4 | 5.1 ± 0.4 |

Uncertainties are twice standard deviation. Average values are given as weighted mean with combined uncertainties

*n* = number of subsamples; for each subsample at least three measurements were performed

[a] Origin: de Groot (NL)

[b] Origin: School of Conservation (DK)

[c] Average values for 4 subsamples from 2 parchments manufactured at INCDTP-ICPI (RO)

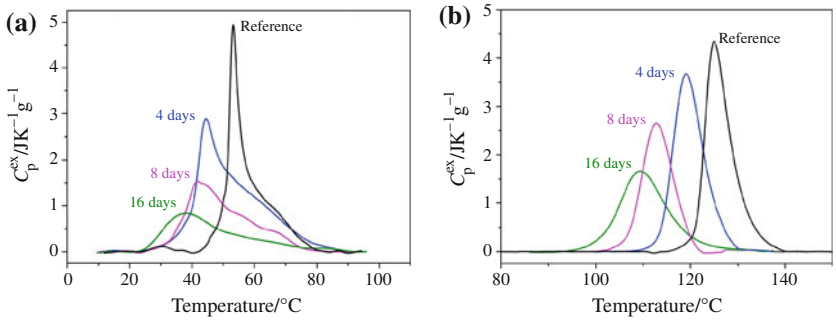


**Fig. 3** DSC curves for a series of parchments subjected to heating at 100 °C for increasing time measured in **a** excess water and **b** static air conditions

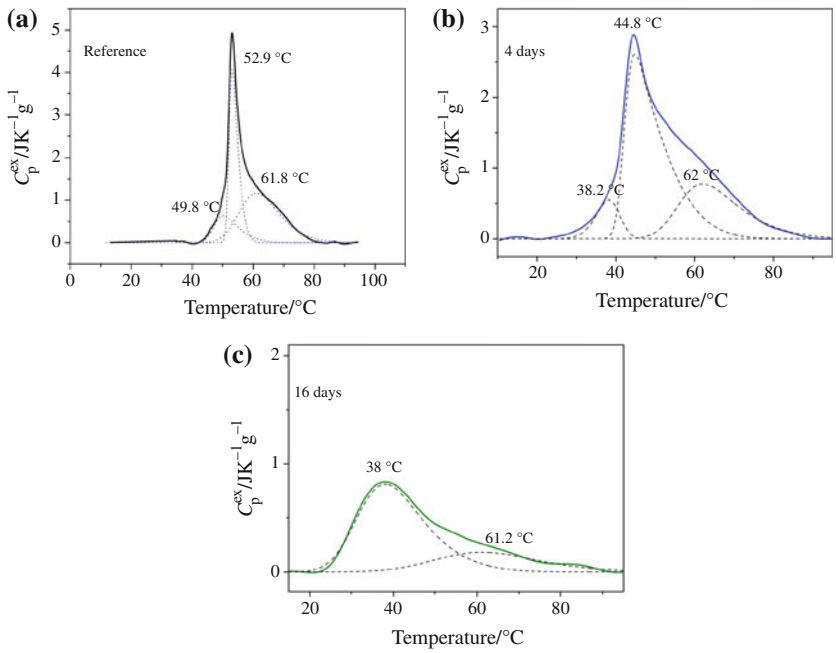


**Fig. 4** Deconvolution of DSC curves in excess water from Fig. 3a: **a** reference (new parchment); **b** parchment heated at 100 °C for 4 days and **c** parchment heated at 100 °C for 16 days

Accelerated ageing treatments resulted in distinct variation patterns for DSC parameters depending on the ageing factors and hence on the induced deterioration mechanisms [8–10]. At high hydration level the multiple character of DSC curves is resolved (Fig. 3a) and multiple peaks can be singled out by appropriate deconvolution (Fig. 4). As a consequence, besides their excellent reproducibility, measurements in excess water offer a more comprehensive evaluation of thermal denaturation of parchments [25]. DSC parameters of thermal denaturation in excess water were therefore selected for the quantitative evaluation of damage and stability of parchment. The large collection of DSC parameters obtained for parchments exposed to various accelerated ageing treatments enabled us to set up a deterioration scale ranking parchments in four damage classes (no damage, minor, medium and major damage) [10]. To this purpose, scores from 1 to 4 were attributed to the DSC full peak parameters, $T_{max}$, $\Delta H$ and peak shape index $I_S = \Delta T_{1/2}/C_p^{ex}$max, in function of their departure from reference values (i.e. those of the new, untreated parchment): score 1 = no damage, score 2 = minor damage, score 3 = medium damage and score 4 = major damage) [10, 26]. For the temperature of DSC full peak, $T_{max}$, scores were attributed as follows: score 1 for 50 °C $< T_{max} <$ 55 °C; score 2 for 45 °C $< T_{max} <$ 50 °C and $T_{max} >$ 55 °C; score 3 for 40 °C $< T_{max} <$ 45 °C and score 4 for $T_{max} <$ 40 °C. For $\Delta H$, scores were calculated as percent variation from reference value: score 1, less than 10%; score 2, 10–20%; score 3, 20–35% and score 4, >35%. For $I_S$, scores were attributed as follows: score 1 for $I_S < 1$; score 2 for $1 < I_S < 5$; score 3 for $5 < I_S < 15$ and score 4 for $I_S > 15$. The overall damage score, $S_{tot}$, is calculated by assigning empirical weight coefficients to each DSC parameter:

**Table 3** Percent fraction of distinct collagen populations within parchments exposed to heating at 100 °C for increasing time obtained by deconvolution of the full DSC peak

| Sample | $\Delta H^a$/ J g$^{-1}$ | Population 1 | | Population 2 | | Population 3 | |
|---|---|---|---|---|---|---|---|
| | | $T_1$/°C | % $\Delta H_1$ | $T_2$/°C | %$\Delta H_2$ | $T_3$/°C | % $\Delta H_3$ |
| Reference | 54 | 50.0 | 11 | 52.9 | 41 | 61.8 | 48 |
| 4-day | 44 | 38.2 | 8 | 44.8 | 64 | 62.0 | 28 |
| 8-day | 33 | 38.5 | 8 | 43.1 | 79 | 61.9 | 13 |
| 16-day | 22 | 38.0 | 75 | – | – | 61.1 | 25 |

[a] $\Delta H$ = overall thermal denaturation enthalpy

$T_i$, $\Delta H_i$ = peak temperatures and enthalpies of DSC peak components obtained by deconvolution

$$S_{\text{tot}} = 0.2S(T_{\max}) + 0.5S(\Delta H) + 0.3S(I_S) \quad (1)$$

Damage ranking based on the above scheme concerns bulk mesoscopic properties of parchment and by correlation with other rankings, e.g. those based on Scanning Electron Microscopy, mainly related to deterioration of superficial structures, e.g. fibres and fibre bundles, and on FTIR spectroscopy, related to chemical alterations at molecular level, a detailed picture of parchment damage from molecular to macroscopic level bas been obtained [25–28].

Alteration of DSC full peak on ageing/deterioration determined from its shifting, broadening and shortening by respect to reference is an indication of increasing heterogeneity of distribution of collagen populations with different thermal stability. Their separation is obtained by deconvolution of full DSC peak as illustrated in Fig. 4. New parchments displays up to three components: (i) a minor peak at $T < T_{\max}$, (ii) the main transition peak at $T_{\max}$ and (iii) a shoulder at $T > T_{\max}$ (Fig. 4a). These peaks were associated to labile, stable and crosslinked collagen populations, respectively [10, 27]. Quantification of the collagen populations with distinct thermal stability is given by the enthalpy associated to the corresponding fractions. Figure 4b and c illustrates deconvolution of DSC curves for parchments subjected to heating at 100 °C for 4 and 16 days, respectively, and Table 3 reports thermal stability (i.e. peak temperature) and percent distribution (i.e. percent enthalpy) of distinct collagen populations, as well as the overall thermal denaturation enthalpies. Partial denaturation and conversion of collagen stable fraction with $T = 52.9$ °C into less stable collagen showing thermal transition at $T = 44.8$ °C occurred after 4-day heating (Fig. 4b). Longer time heating induced further denaturation and progressive conversion of the latter into highly unstable collagen ($T = 38$ °C). A parallel decrease of the most stable collagen population occurred whereas its thermal stability remained practically unmodified ($T \approx 62$ °C) (Fig. 4c). This collagen population stabilised by high concentration of crosslinking also showed a clear tendency to withstand deterioration induced by heating in humid atmosphere, as well as by combined heating at 100 °C and exposure to $NO_x$ atmosphere (50 ppm) (unpublished data). By contrast, in parchments subjected to $SO_2$ atmosphere (50 ppm) after preliminary heating at 100 °C, this stabilised collagen fraction completely disappeared as a result

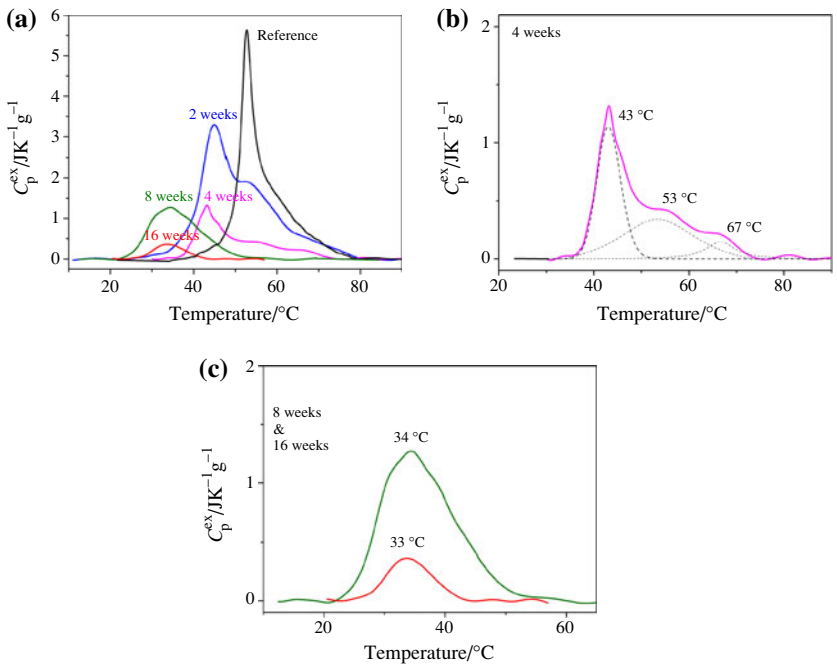


**Fig. 5** **a** DSC curves in excess water for a series of parchments subjected to accelerated ageing in $SO_2$ atmosphere (50 ppm) for 2,4, 8 and 16 weeks, after previous exposure to visible light and heating at 100 °C. **b** Deconvolution of 4-week peak; **c** 8- and 16-week peaks showing only highly unstable collagen fractions with $T_{\max} < 35$ °C

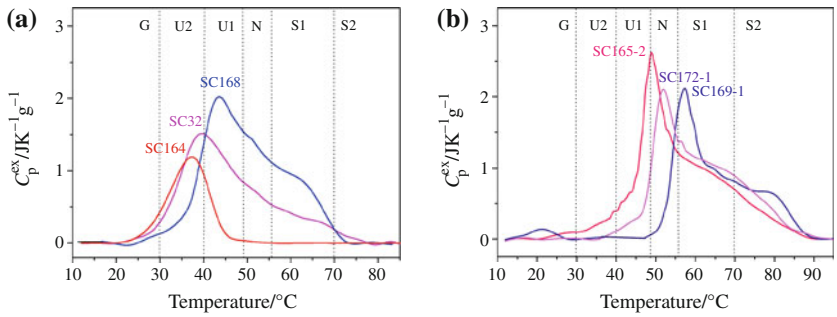


**Fig. 6** DSC peaks in excess water of historical parchments showing two opposite deterioration patterns characterised by: **a** prevailing presence of unstable collagen with $T_{max} < 48$ °C and **b** prevailing presence of stable collagen with $T_{max} > 48$ °C. N (native) interval: 48 °C $\leq T \leq$ 56 °C; S (stable) interval: $T > 56$ °C; U (unstable) interval: 30 °C $\leq T \leq$ 48 °C; G (gelatine) interval: $T < 30$ °C

of the hydrolysis promoted by $HSO_3^-$ (Fig. 5a). In fact, both collagen fractions with $T > 52$ °C directly transform into less stable structures (peak at $T = 43$ °C) (Fig. 5b) and after 8-week ageing the remaining uncoiled, almost gelatinised collagen structures collapsed at $T < 35$ °C (Fig. 5c). Longer ageing induced further hydrolysis of this highly unstable collagen, as indicated by the decrease of both width and height of the 16-week peak (Fig. 5c).

Thermal stability and quantitative distribution of collagen populations within parchment has been successfully used to evaluate the stability of historical parchments and discriminate between parchments with comparable levels of deterioration. In Fig. 6, DSC curves in excess water of some historical parchments from the State Archives of Florence are reported. Parameters of their full denaturation endotherms, shrinkage temperature, $T_s$, measured by thermal microscopy and damage scores are listed in Table 4. Damage scores indicate a net ranking of parchments in three damage classes: minor damage for SC165-2 and SC172-1, medium damage for SC169-1 and SC168 and major damage for SC32 and SC164.

To better point out the energetic features of DSC peaks, temperature range of measurement was divided in six intervals in function of the main thermal events occurring in each of them (Fig. 6). N interval (48 °C $\leq T \leq$ 56 °C) corresponds to thermal denaturation of native fibril collagen. More stable collagen populations display thermal transition in S1 (56 °C $\leq T \leq$ 70 °C) and S2 ($T > 70$ °C) intervals, whereas unstable and highly unstable collagen show transitions in U1 (48 °C $\leq T \leq$ 40 °C) and U2 (30 °C $\leq T \leq$ 40 °C) intervals. Interval G ($T < 30$ °C) corresponds to the temperature range of reversible gelatine thermal transition. DSC peak enthalpy distribution revealed two distinct deterioration patterns illustrated by parchments showing thermal transition principally in U (Fig. 6a) or S (Fig. 6b) intervals. Parchments in S interval (SC165-2, SC172-1 and SC169-1) are characterised by an enthalpy contribution of the more stable collagen fraction higher than 50% whereas that of the remaining "healthy" collagen (i.e. denaturing in the N interval) ranges between 25 and 35%. Parchments in U interval (SC168, SC32 and SC164) show a much lower contribution of the more stable collagen ($T \geq 56$ °C) accompanied by a very high contribution (about 80%) of the unstable collagen ($T < 48$ °C). As a consequence, it can be inferred that age-related crosslinking stabilised SC165-2, SC172-1 and SC169-1 parchments and hence protected collagen from transforming into less organised, unstable structures, whereas disruptive deterioration mechanisms in SC168, SC32 and SC164 promoted the conversion of collagen to unstable structures. Samples in U interval are significantly more susceptible to rapid ageing and deterioration as the largest fraction of their collagen lost the native supercoiled organisation, allowing thermal collapse to occur at lower temperatures. Enthalpy distribution of DSC peaks is therefore a valuable stability parameter for discriminating between parchments with comparable damage scores. For example, SC169-1 and SC168 samples were both ranked as $S_{tot} = 3$. The distribution between "healthy" collagen (31%) and most stable collagen (69%) displayed by SC169-1 indicate this parchment can better and longer stand the progress of natural

**Table 4** Shrinkage temperature, $T_s$, denaturation full DSC peak parameters and relevant damage scores, $S_{tot}$, from Eq. 1 for historical parchments from the State Archives of Florence

| Parchment | Type | Treatment | $T_s$/°C[b] | $T_{max}$/°C | $\Delta H$/J g$^{-1}$ | $I_S$ | $S_{tot}$ |
|---|---|---|---|---|---|---|---|
| SC165-2[a] | Binding | Flattening | 46 | 49 | 44 | 3.2 | 2.0 |
| SC172-1[a] | Binding | Unknown | 47 | 52 | 40 | 7.3 | 2.1 |
| SC169-1 | Binding | Unknown | 49 | 57 | 33 | 4.0 | 3.0 |
| SC168 | Binding | Flattening | 36 | 44 | 41 | 9.7 | 3.0 |
| SC32[a] | Binding | Flattening | 33 | 40 | 31 | 10.7 | 3.6 |
| SC164[a] | Sheet | Drying | 33 | 37 | 19 | 8.8 | 3.8 |

[a] Water damaged

[b] $T_s$ = shrinkage temperature determined by Micro Hot Table method (MHT)

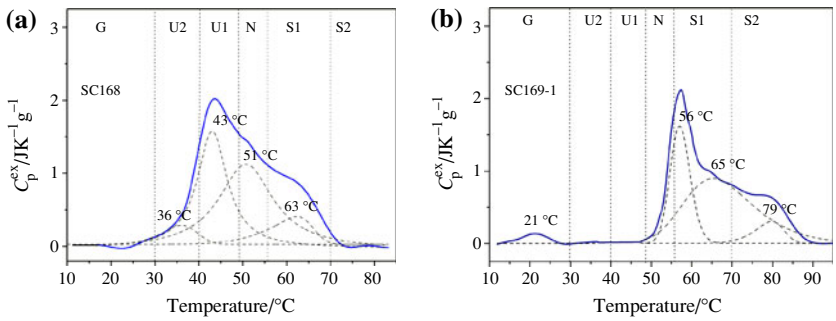


**Fig. 7** Deconvolution of DSC peaks for **a** SC168 and **b** SC169-1 historical parchments from Fig. 6, providing thermal stability and quantitative distribution of collagen fractions

ageing by comparison with SC168, whose denaturation enthalpy is mainly attributable to unstable collagen fraction (59%) and only 29 and 12% comes from the "healthy" and most stable fractions, respectively (Fig. 7). This is also in agreement with $T_s$ values determined by thermal microscopy. Following the enthalpy distribution criterion, SC32 ($S_{tot} = 3.6$) results more stable than SC164 ($S_{tot} = 3.8$) in spite of their similar damage scores and shrinkage temperatures. It is known that both parchments suffered water damage, but SC164 was dried by heating and thus lost its collagen fraction with $T > 40$ °C which was fully denatured. At this point, samples in U interval may easily pass towards higher deterioration and instability levels if improper conservation treatment or storage is applied. It should be stressed that $T_s$ alone cannot reliably evaluate either damage, or stability level of historical parchments (Table 4). This is, however, possible by the overall analysis of DSC data. Correlation of denaturation and shrinkage parameters is a further step of our study that could improve the capability of thermal microscopy to warn against instability threshold closeness in historical parchments.

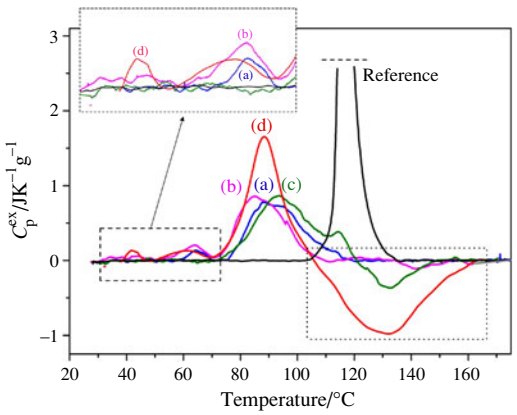


**Fig. 8** Endothermic (30 – 80 °C) and exothermic (110–160 °C) DSC peaks occurring before and after thermal denaturation (80–100 °C), in static air condition, for parchments exposed to chemical pollutants (50 ppm) for 16 weeks: (*a*) $NO_x$; (*b*) $SO_2$; (*c*, *d*) ($NO_x + SO_2$). (*a*), (*b*) and (*d*) after previous visible light irradiation and heating at 100 °C; (*c*) after previous heating at 100 °C

Measurements in static air can provide additional experimental evidences and information related to deterioration (Fig. 8). First evidence is represented by the exothermic peak at about 130 °C, observed in parchments subjected to accelerated ageing in strong oxidative conditions, such as combined exposure to chemical pollutants ($NO_x + SO_2$) and dry heating at 100 °C. This behaviour is a consequence of the increased disruption susceptibility of parchment whose structure was strongly weakened by the oxidative damage [15]. Second evidence is represented by small and broad endothermic peaks in the range 30–80 °C detected in parchments exposed to dry heating at 100 °C as well as to combined action of chemical pollutants and dry heating for long times (samples a, b and d) [10, 15]. Such a transition was ascribed to alterations at the molecular level of parchment structure [15]. DSC curves of the same parchments in excess water displayed a small peak at $T < 35$ °C, confirming the presence of uncoiled collagen and gelatine (Fig. 5c). Moreover, due to their highly unstable collagen these samples did not longer displayed shrinkage activity [15]. Third evidence is the short and broad denaturation peak occurring at 80–110 °C in the closeness of the small endotherms mentioned above indicating severe damage and high level of heterogeneity for the remaining collagen.

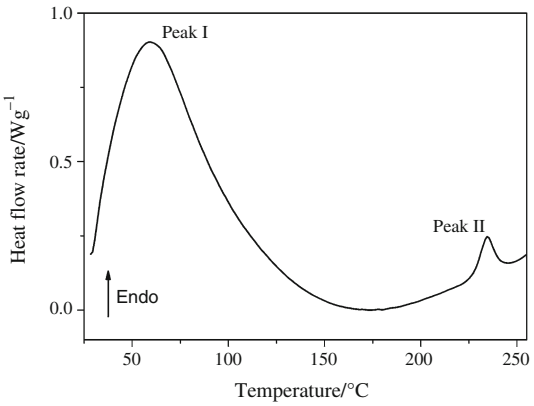


**Fig. 9** Typical DSC curve obtained for a new parchment in open crucible and nitrogen flow. *Peak I* results from dehydration and thermal denaturation overlapping; *peak II* is related to softening of collagen crystalline fraction

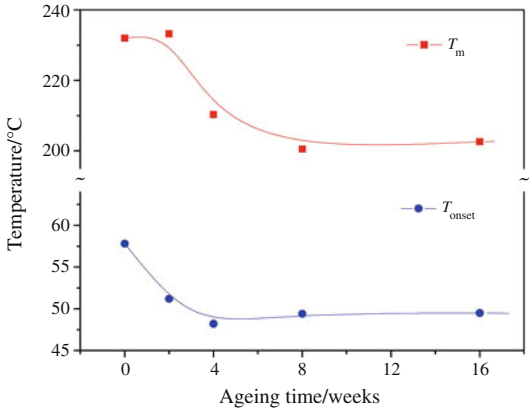


**Fig. 10** Variation of softening, $T_m$, and onset, $T_{onset}$, temperatures for parchments subjected to accelerated ageing in $NO_x$ (50 ppm) atmosphere at increasing time. $T_m$ was measured in open crucibles under nitrogen flow. $T_{onset}$ was measured on wet samples

## DSC measurement in open crucibles and gas flow

DSC curve of a new parchment in open crucible under nitrogen flow (Fig. 9) typically displays two main transitions in the range 25–250 °C: a major endotherm (peak I) between room temperature and 150 °C and a minor endotherm (peak II) around 235 °C. Peak I results from two overlapping processes, i.e. loss of moisture and thermal denaturation, whereas peak II is associated with softening/melting of the collagen crystalline fraction [7, 14, 15, 29–31]. As a consequence, peak II temperature, $T_m$, is related to crystalline network strength, and the associated enthalpy change, $\Delta H$, can be assumed as representative of the parchment crystalline degree.

Investigation of 21 new parchments from different animals hide (calf, goat, sheep, deer) showed somewhat spread out values for both softening temperature (226 °C $\leq T_m \leq$ 236 °C) and associated enthalpy change (1.1 J $g^{-1} \leq \Delta H \leq$ 8.0 J $g^{-1}$). $T_m$ decreases as a result of exposure to chemical pollutants (e.g. $NO_x$) for increasing times [14]. Weakening of crystalline network is likely due to molecular cleavage and disruption of the hierarchical organisation of collagen caused by oxidation and/or hydrolysis promoted by chemical pollutants. It is interesting to note that $T_m$ trend as a function of ageing time is similar to that showed by $T_{onset}$ (Fig. 10), even though these two temperatures are in connection to thermal stability of crystalline phase and amorphous matrix of collagen, respectively. It was already reported that samples exposed to $NO_x$ showed a sharp decrease of $T_m$, whereas those exposed to $SO_2$ displayed multiple softening peaks, even though $T_m$ decrease was less sharp. Moreover, the combined action of $NO_x$ and $SO_2$ determined a sharper decrease of $T_m$ for short and medium ageing time, whereas samples treated for longer times (8 and 16 weeks) did no longer show any softening peak [15]. The occurrence of multiple softening peaks was related to a random fragmentation of the original collagen matrix and subsequent heterogeneous deterioration of the embedded crystalline fractions. By contrast, samples heated at 195 °C for short times (1–2 h) showed almost unchanged $T_m$ values by comparison with the reference sample in both nitrogen and oxygen gas flow as reported in Table 5. It can thus be inferred that softening behaviour of a parchment can indicate specific deterioration patterns, e.g. disrupting oxidation for samples with low $T_m$ values, crosslinking formation for those with high $T_m$ values, hydrolytic deterioration in the case of multiple softening peaks. In addition, it is worth to be mentioned that for most parchments showing $T_m < 210$ °C or multiple peaks in nitrogen flow, softening was no longer observed when the same sample was measured in oxygen flow, indicating their high susceptibility of related fractions to oxidative deterioration.

**Table 5** Temperature, $T_m$, and associated enthalpy change, $\Delta H$, of softening DSC peaks measured in open crucibles under gas flow for goat parchments artificially aged by heating at 195 °C

| Parchment[a] | Ageing time/h | Nitrogen flow | | Oxigen flow | |
|---|---|---|---|---|---|
| | | $T_m$/°C | $\Delta H$/J $g^{-1}$ | $T_m$/°C | $\Delta H$/J $g^{-1}$ |
| E7 | Reference | 232.9 | 6.22 | 227.6 | 6.61 |
| | 0.5 | 230.4 | 4.78 | 226.3 | 5.05 |
| | 1 | 230.4 | 4.85 | 228.4 | 5.95 |
| | 2 | 230.1 | 5.18 | 229.6 | 5.66 |

[a] Origin: INCDTP-ICPI (RO)

Historical parchments, however, display even more spread out $T_m$ values than those of new ones. For example, a series of old Romanian documents (fifteenth and sixteenth centuries) gave only one softening peak at 214 °C $\leq T_m \leq$ 239 °C and enthalpy change of 1.4 J $g^{-1} \leq \Delta H \leq$ 13.0 J $g^{-1}$. The same parameters measured in oxygen or synthetic air flow were close to those obtained in nitrogen flow suggesting these parchments were relatively stable to thermal oxidation in the temperature range 25–240 °C [7]. Their high crystalline degree suggested by high $\Delta H$ values can be ascribed to formation of either crosslinks within the collagen structure or specific collagen-lipid interactions [28, 32]. High crosslinking concentration is confirmed by their high shrinkage temperatures (see Fig. 11b, samples 16–24).

A different softening behaviour was observed for a series of old bookbindings (fourteenth to sixteenth century) from the Historical Archives of the City of Turin which displayed one melting peak at 228 °C $\leq T_m \leq$ 237 °C and enthalpy change of 5.8 J $g^{-1} \leq \Delta H \leq$ 8.0 J $g^{-1}$ in nitrogen flow. In static air condition most of these samples showed a second endothermic peak at about 160 °C which

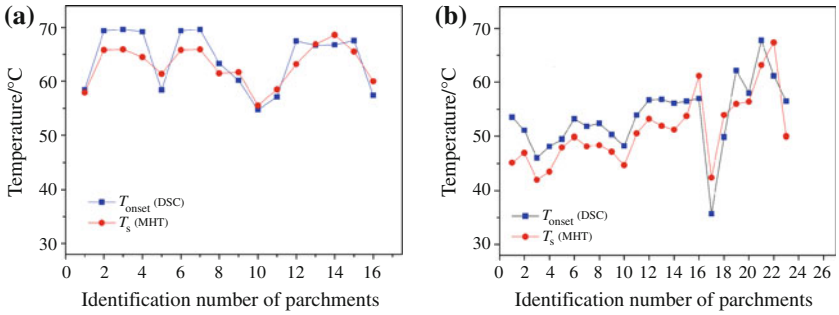


**Fig. 11** Shrinkage temperatures determined by DSC and MHT methods for: **a** new parchments from different animal hides: calf (1 to 7), deer (8 and 9), goat (10 to 15), sheep (16); **b** historical parchment bookbindings from Historical Archives of the University of Turin (1 to 10), Historical Archives of the City of Turin (11 to 15), and various Romanian parchment documents (16 to 24)

could be assigned to a crystalline fraction weakened by the oxidative deterioration, as also suggested by FTIR oxidation markers (e.g. band intensities of hydroxyl and carbonyl groups) [28]. Their shrinkage temperatures of about 56 °C (see Fig. 11b, samples 11–15) indicates slightly reduced hydrothermal stability. Such behaviour has to be attributed to various oxidation mechanisms promoted by direct exposure of bookbindings to visible light, gaseous pollutants and variations of temperature and relative humidity.

### Shrinkage temperature measurement by DSC measurement and thermal microscopy

Most common methods used for determining $T_s$ are (i) standardised method described by TEST IUP 16 of the International Union of Leather Technologists and Chemical Societies [32]; (ii) thermal microscopy [33] and (iii) DTA or DSC techniques. The first method, recommended for leather industry, requires a sample of (50 × 3) mm, and cannot be applied to historical artefacts, whereas thermal microscopy and DTA/DSC techniques, needing decisively smaller samples (a few fibres and a few mg, respectively), are suitable for the historical objects assessment. Witnauer and Wisnewski [34] firstly used DTA for $T_s$ determination. More recently, Chahine et al. [5, 35, 36] and Budrugeac et al. [7, 37–39] have used the DSC technique to evaluate shrinkage temperature of new and aged parchments and leathers. The extrapolated onset temperature of wet parchment DSC peak, $T_{onset}$, showed to be very close to $T_s$ determined by micro hot table (MHT), a thermal microscopy method [33, 37–39]. Small differences between $T_{onset}$ and $T_s$ values could be attributed both to difficulty of sampling such a heterogeneous material as parchment and errors inherent in evaluation of the extrapolated onset temperature. It should be stressed that $T_{onset}$ is a bulk material property, whereas $T_s$ generally reflects the hydrothermal stability of few surface fibres. As a consequence, $T_{onset}$ offers a more objective and reliable evaluation of shrinkage temperature.

Investigation of a number of new and artificially aged parchments enabled a rough ranking of old parchments in two main classes: (i) parchments with low hydrothermal stability displaying $T_{onset} < 58$ °C and (ii) parchments in good conservation state with $T_{onset}$ close to those of new parchments. For a group of 16 new parchments, $T_{onset}$ ranged between 58 and 69 °C (Fig. 11a) whereas for a group of 24 historical parchments the corresponding range was 36–67 °C (Fig. 11b). $T_s$ values determined by MHT method are also reported. Generally, $T_{onset}$ is slightly higher than $T_s$ for both new and old parchments.

## Conclusions

DSC is a sophisticated and versatile technique that allows to measure heat flow associated with collagen thermal transitions as a function of temperature using 1–5 mg microsamples. Its employment as advanced tool for the assessment of historical parchment offers damage diagnostics and ranking, as well as warning indices for damage progress prediction.

(i) Comprehensive characterisation of parchments thermal stability at both fibril level, by measurements in excess water, and fibre level, by measurements in static/dynamic atmosphere;
(ii) Assessment of deterioration by classifying historical parchments in four damage classes (no damage, minor, medium and major damage) based on DSC quantitative denaturation parameters obtained in excess water;
(iii) Evaluation of stability of deteriorated parchments and their capability to further stand ageing through mapping thermal stability and transition enthalpy of distinct collagen populations (stable–unstable) within samples;
(iv) Quantitative or qualitative characterisation of various processes, i.e. crosslinking, hydrolysis, oxidative

breakdown, gelatinisation, revealed by the parameters and features of thermal transitions;

(v) Reliable evaluation of the shrinkage temperature of bulk samples through the measurement of $T_{onset}$ of thermal transition of wet samples;

(vi) Determination of softening temperature of parchment crystalline fraction and associated enthalpy change related to crystalline network strength and parchment crystalline degree, respectively.

Synthetically, we can say that DSC assesses the present deterioration state of a historical parchment and can provide an estimation of its future damage progress.

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