e-PS, 2012, **9**, 97-109
ISSN: 1581-9280 web edition
ISSN: 1854-3928 print edition

www.Morana-rtd.com

e-PRESERVATIONScience

published by MORANA RTD d.o.o.

SCIENTIFIC PAPER

DAMAGE RANKING OF HISTORIC PARCHMENT: FROM MICROSCOPIC STUDIES OF FIBRE STRUCTURE TO COLLAGEN DENATURATION ASSESSMENT BY MICRO DSC

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received: 31.12.2012
accepted: 23.01.2013

key words:
historic parchment, deterioration assessment, collagen denaturation, fibre shrinkage, micro DSC

Microscopic assessment of collagen fibre structure and their shrinkage activity was used to quantify the deterioration at a microscopic level for two collections of historic parchments, from the Royal Library of Copenhagen and the State Archives of Florence. The micro Differential Scanning Calorimetry (micro DSC) was used to quantitatively assess the deterioration at the mesoscopic level, through the analysis of fibrillar collagen thermal denaturation. The results enabled us to classify the historic parchments in four categories, i.e. non-damaged, and displaying minor, medium and major damage. The measurement protocols, deterioration parameters and criteria for assigning a parchment to one of the four categories are reported. Such categorisation of deterioration can support improved preventive care and conservation interventions in collections.

1 Introduction

Historic parchments have an extraordinary importance as they preserve, in the form of scrolls, manuscripts, codices, notary documents, records, documents of the secretaries of State, archives of ecclesiastical institutions and ancient families, almost the entire intellectual heritage of the Western World from the classical age to Renaissance. Passing this inheritance on to the future generations relies on an in-depth understanding of parchment as a material as well as of its deterioration. It is therefore important to accurately evaluate material state of deterioration in order to design the most appropriate strategies for conservation interventions, display and storage. Parchment is a hierarchically arranged collagen-based tissue consisting mainly of type I collagen fibres, randomly orientated and distributed as a feltwork.[1-4] The type I collagen molecule is a triple helix resulting from right-handed coiling around the common axis of three α-chains, each forming a left-handed helix of about 1,000 amino acid residues, with an increase of 0.29 nm per residue in the direction of the axis. These chains are formed of repeating Gly-X-Y sequences, where X is often proline or hydroxyproline.[5] The triple helix is about 300 nm long and has a diameter ranging from 1.2 to 1.5 nm depending on its hydration level. Throughout the structure, from the triple helices to fibrils, there is an

alternating left and right-handed symmetry, which gives rise to the outstanding mechanical strength of this biomaterial. Coiling throughout the whole molecular structure is stabilised by a network of intra- and intermolecular hydrogen bonds including hydrogen bonds with water, whereas covalent links between terminal ends of collagen triple-helices, carbonyl-water hydrogen bonds, van der Waals interactions and electrostatic forces hold the collagen hierarchical structure together.[6-8] Other factors, i.e. methods of production, unknown environmental and conservation histories of objects as well as the intrinsic heterogeneity of animal skin, add to this structural complexity increasing the difficulty of investigating parchment deterioration.

This paper is based on some of the outcomes of the EC project "Improved Damage Assessment of Parchment" (IDAP, EVK4-CT-2001-00061) which have subsequently been applied and improved in the projects OPERA,[a] MuSA-System,[b] MEMORI,[c] and COLLAGE.[d] The IDAP project aimed to answer the following questions: (a) what are the causes and mechanisms of deterioration of parchment, and (b) how can we detect, describe and quantify this deterioration. Due to the supramolecular and hierachical structure of collagen, the strategy of the IDAP project was complex, encompassing visual analysis, various microscopic and spectroscopic techniques, unilateral-NMR, X-ray scattering and various thermal analysis methods to either directly or indirectly investigate structural changes of collagen, induced by ageing.[9,10]

The main purpose was to develop standardised methods for early detection of deterioration, its characterisation and quantification. To this end, more than 100 artificially degraded parchments and 450 historic parchments from European archives and libraries were investigated. For the first time, the IDAP project represents an in-depth study of temperature and relative humidity effects as well as of pollutants and/or light on parchment, providing a useful basis for the assessment of the type and level of deterioration.[11-15]

Previous research on parchment deterioration was based on Differential Scanning Calorimetry (DSC) and shrinkage measurements by the Micro Hot Table (MHT) method.[16-18] DSC provides a measure of the energy of thermal denaturation, whereas the MHT method determines microscopic deformation induced by thermal denaturation. In particular, DSC measurements were recently used to validate the restoration protocols in use at the National University Library of Turin to restore manuscripts heavily damaged by a fire in 1904.[19] When micro-sampling of 1-2 mg from historic parchments was possible, micro DSC provided quantitative parameters for evaluating the level of deterioration as well as establishing threshold levels for irreversible damage.[20-22] However, within one collection there may be parchments with similar levels of damage but different stability and resistance against ageing. Micro DSC has thus revealed to be a suitable tool to evaluate the stability of a parchment, i.e. its sensitivity to environmental conditions and capacity to withstand further degradation without risking irreversible deterioration.[22,23]

On the other hand, the MHT method has been widely used to characterise the hydrothermal stability and shrinkage activity of heritage collagen-based materials.[24-28] Recent studies showed that similar structural deformation of collagen fibres takes place during natural ageing as well as during heating in water,[29] suggesting that microscopic deformations induced by denaturation are not influenced by the type of the denaturing agent (e.g. heat, acidity, oxidation, photo-oxidation).

In this paper, historic parchments from the Royal Library of Copenhagen and State Archives of Florence were investigated by micro DSC in excess water, MHT method and fibre assessment protocol. This is the first time that an in-depth correlation of results obtained by micro DSC and MHT was performed, providing a comprehensive and useful basis for the evaluation of level of deterioration of historic parchments at microscopic and mesoscopic levels.

2 Materials and Methods

2.1 Samples

A group of 17 subsamples from 14 different historic parchments (4 single sheets and 10 bookbindings) were used in this study: 9 parchments were from the Royal Library of Copenhagen, Denmark, and 5 parchments from the State Archives of Florence, Italy (Table 1). New parchments used as references were manufactured by Henk de Groot, The Netherlands (4 calf parchments) and at the National Institute of Research and Development for Textile and Leather (INCDTP-ICPI), Bucharest, Romania (2 sheep parchments and 2 goat parchments) on the basis of medieval recipes.[30] Since IDAP samples were used in further projects and papers, their original acronyms have been maintained to make it possible to track their analytical history. When possible, the origin of the animal was identified from the hair follicle pattern. The presence of glass-like layers, i.e. transparent fibres on the surface, transparent pools on grain side located around groups of hair holes, glass-like layers on one or both sides of parchment, areas transparent throughout the parchment structure, was detected by the microscopic observation of both sides, grain and flesh, of the parchments.

Sample	Type	Animal origin	Previous conservation treatment	Glass-like layer
The Royal Library, Copenhagen				
SC16	bookbinding	ND	flattened	yes
SC17-1	bookbinding	goat	untreated	no
SC17-2	bookbinding	goat	untreated	no
SC18	bookbinding	sheep	unknown	yes
SC24	bookbinding	sheep	cleaned	yes
SC31	bookbinding	calf	unknown	no
SC32	bookbinding	ND	flattened	yes
SC35	bookbinding	calf	unknown	no
SC38-1	single sheet	calf	restored with paper fillings	yes
SC38-2	single sheet	calf	restored with paper fillings	yes
SC77	bookbinding	ND	untreated	yes
The State Archives, Florence				
SC163	single sheet	ND	dried after flooding	no
SC164	single sheet	ND	dried after flooding	yes
SC166	single sheet	ND	dried after flooding	yes
SC173-1	bookbinding	sheep	unknown	no
SC173-2	bookbinding	sheep	unknown	no
SC175-1	bookbinding	ND	untreated	no

Table 1: Historic parchments. ND = not determined.

2.2 Damage Assessment Methods

Assessment of damage in historic parchment is a complex task as bulk chemical and physical properties as well as surface characteristics can vary from one area to another across the parchment. Frequently, historic parchments show zones with different degree and type of damage even within a limited area where deterioration may be superficial or partially/totally penetrated the inner structure.[31-32] A non-uniform damage picture reflects variations in conditions caused by a different exposure to environment, or conservation treatment, e.g. the differences in damage among the first and last quires of a bound parchment as compared to the edges or centre of parchment leaves in the middle of the manuscript,[33] as well as the differences between the front cover, back cover, spine and flaps of a binding.[34-35] Deterioration can have a dynamic character as is the case with environmental exposure, or can be isolated, e.g. in the case of handling, fire, floods, insects, etc. It is not uncommon that a seemingly well-preserved and macroscopically intact parchment has substantial damage at the fibrillar and molecular levels.[36] For a reliable assessment of deterioration, sampling was performed to assure that the degree of damage in the specific region from which the samples were taken was as homogeneous as possible. One or two specific areas were selected for the assessment of each parchment depending of their heterogeneity. The classification of parchments according to the IDAP damage categorisation[37] was undertaken separately for microscopy, MHT and micro DSC results. The measurement protocols, deterioration parameters and criteria for assigning a parchment to one of the four damage categories are detailed below.

2.2.1 Microscopic Assessment of Collagen Fibres

In intact fibres, the helical structure with the twisting of micro fibrils and minor fibres is visible in a micrograph. During ageing, deterioration usually manifests through flattening, splitting and/or fraying of the fibres leading to fragmentation and ending with a gel-like substance that can dissolve in water or easily hydrate in a very moist environment. In some cases the end product is represented by small hard fragments that do not react any longer in contact with water even on heating.[31]

Comparing the morphologies of historic parchments, with those produced by heating new parchments in an aqueous environment, nine major features were identified as typical and representative for damage characterisation of collagen fibres: frayed, unwound, flat, cracked, fragmented, shrunk (i.e. curled, deformed and swollen into a 'pearls-on-a-string'-like structure), bundles (i.e. fibres where separation is impossible), gel-like and dissolved (non-fibrous material that dissolves immediately when adding water).[21,25,31] In most cases, more of these features are present in various degrees in the same sample or even along a single fibre.

Microscopic assessment was performed on fibres immersed in water at room temperature. Water relaxes and swells the fibres making their morphological features more apparent. A sample consisting of a few fibres from the corium part (flesh side) of the parchment was soaked in excess demineralised water for 10 min on a microscope slide with a concavity. The

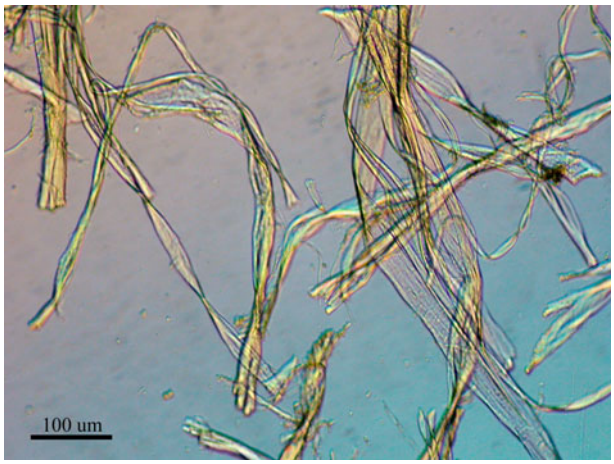

Figure 1: Microscopic image (100x magnification) of collagen fibres in water (SC17-2): unwound, flat, "pearls on a string" and frayed fibres are present.

thoroughly wetted fibres were carefully separated with fine preparation needles, well dispersed in water, covered with a microscope glass and examined under a light microscope at 100x magnification (Figure 1). Higher magnifications were used for specific details. Between 10 and 15 well-separated fibres from at least three different areas of each sample were documented using digital photography.

2.2.2 Shrinkage Activity by Micro Hot Table Method

When progressively heated in water, the collagen triple-helical structure converts to random coil disordered structures over a defined temperature interval. The macroscopic manifestation of this process called thermal denaturation can be observed through a stereomicroscope as a shrinkage motion of the collagen fibres. Collagen shrinkage activity associated with thermal denaturation is described by a sequence of temperature intervals: no activity – A1 – B1 – C – B2 – A2 – complete shrinkage[24] (Figure 2). In the first two intervals, A1 and B1, shrinkage discretely occurs in individual fibres and displays higher activity (namely higher extent of shrinkage per unit of time) in the B1 interval. Then, the majority of the fibre mass shrinks in the main interval C. The starting temperature of this interval is the shrinkage temperature, T_s. Generally, the shrinkage activity levels off through B2 and A2 intervals. T_f is the temperature at which the very first motion is observed and T_i the temperature of the very last observed motion. While shrinkage of collagen fibres from new parchments runs through all these intervals, for some historic parchments neither of these last two intervals are observed. In some cases, even the main shrinkage interval C was absent (e.g. Figure 2, sample SC 175:1).

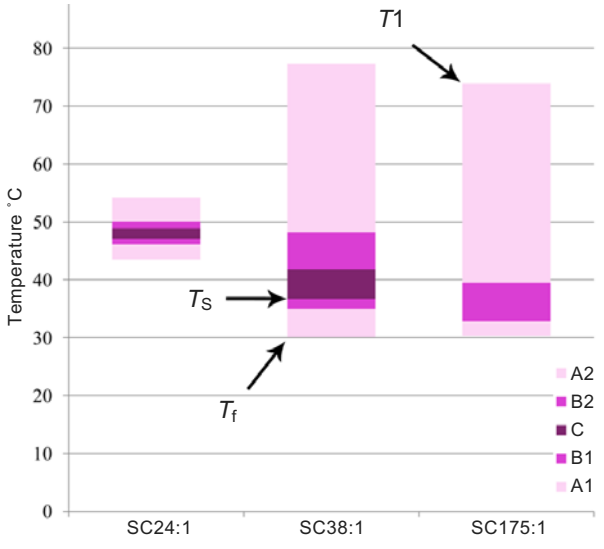

Figure 2: Shrinkage intervals for three historic parchments. The main temperatures, T_f, T_s and T_i, are indicated. SC175-1 does not show the main shrinkage interval C, and thus has no defined shrinkage temperature, T_s.

The shrinkage activity was measured using the MHT method, a thermal microscopy method which, unlike the standardised method of determining shrinkage temperature (i.e. TEST IUP 16 of the International Union of Leather Technologists and Chemical Societies[38]) and the classical DTA[39] technique, offers detailed information on the hydrothermal stability using only a few collagen fibres (0.1 – 0.2 mg)[24] and rather simple equipment.[26]

The measurements were carried out using a FP82HT Hot Table equipped with a FP90 Central Processer both from Mettler Toledo.[24,25] The fibres were wetted and separated in demineralised water as described above, placed on a microscope slide with a concavity, completely immersed in demineralised water and covered with a cover glass, then placed on the hot table and heated at a rate of 2 °C min^{-1}. The shrinkage process was digitally recorded with a camera connected to the microscope. At least three measurements per sample were carried out.

2.2.3 Thermal Denaturation of Fibrillar Collagen Using Micro DSC

Micro DSC is a sensitive technique of measurement of the energy absorbed or emitted by a sample as a function of temperature. It represents the most direct and sensitive approach for characterising the thermodynamic parameters controlling non-covalent bond formation (and therefore stability) in proteins. Collagen thermal unfolding, i.e. denaturation, is thermodynamically significant at the mesoscopic level, where the assembly of fibrils is weakened and finally lost, and at molecular level, where the triple helix uncoiling occurs. As a consequence, in a micro DSC experiment, which only requires a few milligrams of material, the collagen subjected to thermal unfolding generates an endothermic peak in a characteristic temperature range, allowing for the measurement of the associated enthalpy change ΔH through the integration of the heat capacity curve vs. T (peak area). In this experiment, the denaturation temperature T_{max} (peak maximum temperature), peak half-width $\Delta T_{1/2}$ and peak maximum height $C_{p\,max}^{ex}$ are also determined (Figure 3a). Generally, as deterioration increases, the DSC denaturation peak becomes lower and wider, and shifts to lower T (Figure 3b).[11,14,15] The lower the ΔH and $C_{p\,max}^{ex}$, the less stable the parchment and more susceptible to further deterioration and ageing due to scission of hydrogen bonds and cross-links, and disruption of hydrophobic interactions. The sharpness of the denaturation peak measured as $\Delta T_{1/2}$ is indicative of the relative cooperativity of thermal denaturation: if denaturation produces a narrow, relatively symmetric DSC peak such as the new parchment peaks in Figure 3b, the process is likely to

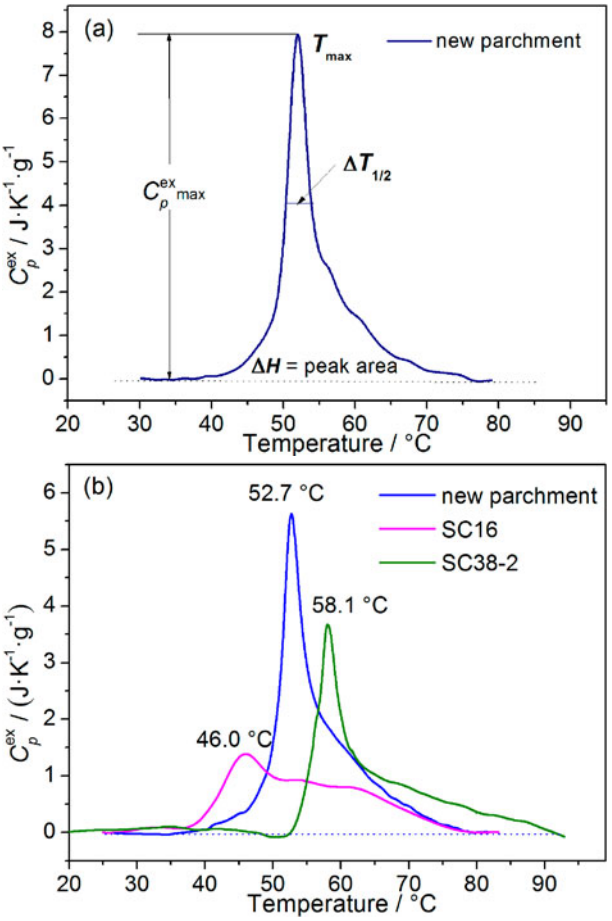

Figure 3.(a) A DSC thermal denaturation curve for a new parchment illustrating the derived parameters. (b) DSC thermal denaturation curves for a new calf parchment and two historic parchments, SC16 and SC38-2. The measurements were performed in excess water.

be highly cooperative. Broad peaks as those displayed by parchment SC16 (Figure 3b) indicate low cooperativity and high structural heterogeneity. T_{max} is the temperature where about 50% of the collagen is in its native conformation, and the other 50% is denatured.

T_{max} is an indicator of thermal stability. Generally, the higher is the T_{max}, the more thermodynamically stable is collagen. Parchments with higher T_{max} are less susceptible to unfolding and denaturation at lower temperatures.

The intensive (T_{max}, $\Delta T_{1/2}$) and extensive (ΔH, $C_p^{ex}{}_{max}$) parameters are relevant to the bulk sample and either used alone or in conjunction with the MHT method and microscopic fibre assessment data enable a quantitative and comprehensive assessment of deterioration in parchment.[22,34]

The DSC measurements were carried out with a high-sensitivity SETARAM micro differential scanning calorimeter (micro DSC III) in excess water as previously described.[15] Parchment samples were first suspended in sodium acetate buffer (pH = 5.0) in a sealed calorimetric cell and kept for 2 h at 5 °C before measurement, to assure uniform pre-hydration conditions and avoid fluctuations of the calorimetric parameters due to different hydration levels.[15] Experimental DSC data acquired with the SETARAM SetSoft2000 software were analysed using PeakFit 4.2 (Jandel Scientific) to obtain the experimental heat capacity change of the sample $C_p^{ex}{}_{max}$ in the scanned temperature interval and derive the above mentioned parchment denaturation parameters. DSC parameters of thermal denaturation for new parchments manufactured from various animal hides are reported in Table 2.

Reference	T_{max} (°C)	ΔH (J·g^{-1})	$\Delta T_{1/2}$ (°C)	$C_p^{ex}{}_{max}$ (J·K^{-1}·g^{-1})
calf parchment (average values of 11 sub-samples from 4 parchments)	53.9 ± 1.6	51.5 ± 4.6	4.7 ± 1.0	6.9 ± 1.1
sheep parchment (average values of 6 sub-samples from 2 parchments)	54.8 ± 1.9	44.0 ± 4.1	5.0 ± 1.3	4.0 ± 0.5
goat parchment (average values of 6 sub-samples from 2 parchments)	54.8 ± 1.8	46.9 ± 4.6	5.5 ± 0.9	3.2 ± 0.5

Table 2: DSC parameters of thermal denaturation for new parchments from various animal hides. DSC measurements were performed in excess water. The intervals represent the standard deviation.

3 Results and Discussion

3.1 Damage Ranking by Microscopic Assessment of Collagen Fibres

The total extent of damage was measured by summing the estimated ratio of damage of each fibre in the sample. Partial ratios (e.g. 0.25, 0.50 and 0.75) were given to partially damaged fibres.[33] The result reported as the percentage of damaged fibres was used to assign the parchment to one of the four damage categories: (i) no or very small damage: ≤30%

Sample	Damaged fibres %	Damage category	Sample	Damaged fibres %	Damage category
The Royal Library, Copenhagen			The State Archives, Florence		
SC16	76	4	SC163	100	4
SC17-1	68	3	SC164	100	4
SC17-2	79	4	SC166	96	4
SC18	72	3	SC173-1	69	3
SC24	82	4	SC173-2	85	4
SC31	35	2	SC175-1	93	4
SC32	92	4	SC175-2	92	4
SC35	95	4	SC163	100	4
SC38-1	92	4			
SC77	88	4			

Table 3: Ranking of damage in historic parchments according to the percentage of damaged fibres.

damaged fibres; (ii) minor damage: 30-50% damaged fibres; (iii) medium damage: 50-75% damaged fibres; (iv) major damage: >75% damaged fibres.[21,33]

The results presented in Table 3 indicate heavily damaged fibres for the majority of the historic samples. The best preserved fibres categorised as "medium damaged" were found in SC31, which displayed 35% damaged fibres only, whereas SC163 and SC164 were fully damaged. Samples SC17-1, SC18 and SC173-1 showed 68-72% damaged fibres. All the remaining parchments had more than 75% damaged fibres.

It should be noticed that in some cases new parchments can also show a relatively high percentage of damaged fibres due to both chemicals and methods used in modern parchment production. Given their age, all the investigated parchments were believed to have been produced according to traditional methods. Moreover, most of these parchments have or are presumed to have undergone a conservation treatment (Table 1), only 5 having been judged to be untreated. In all cases except one (SC163) conservation treatments led to gelatinisation of the grain layer with the subsequent formation of glass-like areas on the surface (Table 1) confirming that conservation treatments often had a negative impact on the stability of the objects. Apart from the conservation treatments, other factors like the original quality and storage history should be considered to explain why the majority of samples are heavily damaged.

3.2 Damage Ranking using the MHT Method

The shrinkage temperature T_s for mammalian raw hides is around 65 °C, but chemical and physical processes involved in the traditional manufacture of parchment decreases the T_s values of new parchments to about 60 °C.[14,23] However, T_s values as low as 50 °C have been also measured for newly made parchments. During accelerated dergadation, when parchment deterioration progressively increases, T_s decreases and heavily damaged samples show a substantial decrease of T_s until the shrinkage activity fully ceases as observed in the case of dry heat in the presence of pollutants (SO_2 and/or NO_x).[9] The lack of shrinkage due to complete disruption of collagen fibrillar structure may also be found in the case of historic parchments and in the worst cases leads to dissolution of parchment when put in contact with water.

New parchment characterised by uniform fibres showed a total shrinkage interval length, $\Delta T_{total} = T_l - T_f$, of about 10 °C, whereas the presence of non-uniform fibres due to either improper manufacture or deterioration resulted in larger ΔT_{total} intervals.

Damage categories	T_f	T_s
1	>45 °C	>50 °C
2	>40 °C to ≤45 °C	>45 °C to ≤50 °C
3	>35 °C to ≤40 °C	>40 °C to ≤45 °C
4	≤35 °C	≤40 °C

Table 4: MHT criteria for damage ranking in historic parchments.

Among all the shrinkage parameters defined above, T_f and T_s correlated with the level of deterioration well, as revealed by *k*-means cluster analysis, and can be thus used for grouping historic parchments into the four damage categories (Table 4).[17] Historic samples can show shrinkage of a few fibres at room temperature even if still categorised as slightly damaged according to the criteria listed in Table 4. Partial gelatinisation can occur at room temperature in samples with medium damage, whereas partial or total dissolution of fibres at room temperature indicates severe damage.[32] Moreover, the coexistence of cross-linked structures with high T_s and heavily damaged structures which no longer shrink is rather frequent.[22,40]

The results of the MHT measurements presented in Figure 4 and Table 5 show very high values of ΔT_{total} and A2 intervals for the majority of samples and rather low T_s values. This experimental evidence can be attributed to the coexistence of fibres with both low and high thermal stability. For SC 175-1 sample, the heat-induced fibre motion was recorded along a temperature interval of 43 °C without displaying the main shrinkage interval C. On the other hand, shrinkage activity occurred in a 10 °C interval for SC24-1, SC163 and SC164, as for new parchments. Their fibre mass can be therefore regarded as being homogenously deteriorated.

Two sub-samples have been selected from parchments SC17, SC38 and SC175, from areas showing apparent different conservation states. The different damage categories identified using the IDAP visual categorisation protocol,[37] which relies very much on a conservation assessment, were confirmed by the shrinkage parameters (Table 5). For example, the sub-sample SC38-1, taken from the upper right corner of the sheet subjected to a lot of dirt and grease transferred from fingers during handling, displayed a low T_s value and very large ΔT_{total} and A2 intervals and was categorised as heavily damaged according to the MHT criteria. By contrast, the sub-sample SC38-2, taken from a central and thus more protected position of the parchment sheet, characterised by T_s = 51.3 °C and ΔT_{total} = 28.9 °C, was categorised as slightly damaged.

All parchments from the State Archives of Florence have suffered damage during the flood of the Arno in

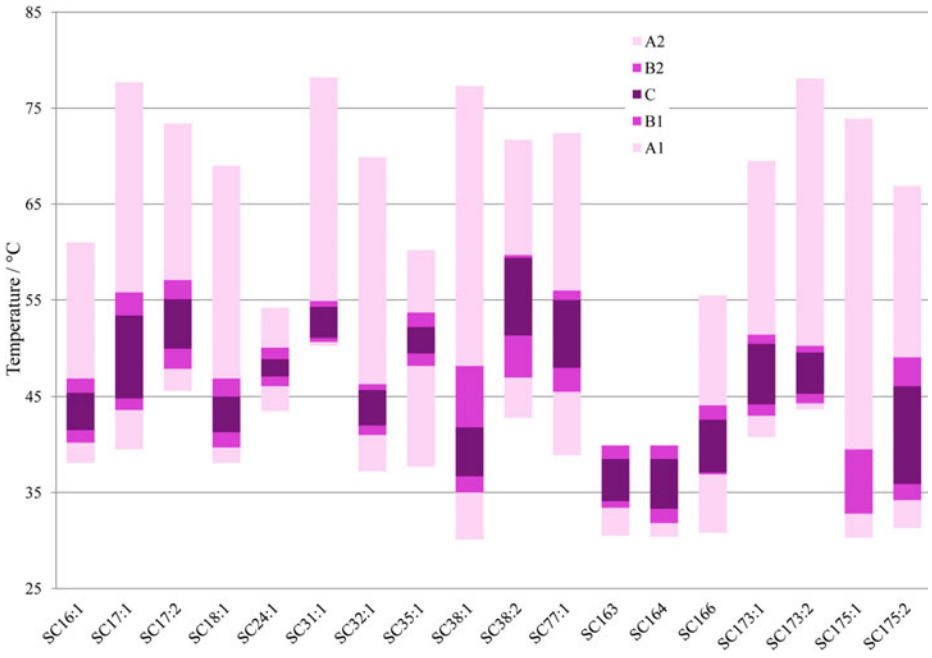

Figure 4: Shrinkage intervals for historic samples from the Royal Library, Copenhagen, and State Archive, Florence.

Sample	T_f (°C)	T_s (°C)	T_l (°C)	ΔT_{total} (°C)	Damage categories	
					S-T_f	S-T_s
The Royal Library, Copenhagen						
SC16	38.1	41.5	61.0	22.9	3	3
SC17-1	39.5	44.8	77.7	38.2	3	3
SC17-2	45.6	50.0	73.4	27.8	1	2
SC18	38.1	41.3	69.0	30.9	3	3
SC24	43.5	47.1	54.2	10.7	2	2
SC31	50.3	51.1	78.2	27.9	1	1
SC32	37.2	42.0	69.9	32.7	3	3
SC35	37.7	49.5	60.2	22.5	3	2
SC38-1	30.1	36.7	77.3	47.2	4	4
SC38-2	42.8	51.3	71.7	28.9	2	1
SC77	38.9	48.0	72.4	33.5	3	2
The State Archives, Florence						
SC163	30.5	34.1	39.9	9.4	4	4
SC164	30.4	33.3	39.9	9.5	4	4
SC166	30.8	37.1	55.5	24.7	4	4
SC173-1	40.8	44.2	69.5	28.7	2	3
SC173-2	43.7	45.3	78.1	34.4	2	2
SC175-1	30.3	–	73.9	43.6	4	4
SC175-2	31.3	35.9	66.9	35.6	4	4

Table 5: MHT parameters, T_f, T_s T_l and ΔT_{total}, together with damage categories based on T_f and T_s values.

1966. Consequently, they have been subject to conservation interventions. Water, either during the flood, or as part of the conservation treatment, has been shown to cause harm.[33] Collagen, being a hydrophilic biomaterial, interacts with water and undergoes changes of physical-mechanical properties depending on its moisture content. This explains why parchments from the flooded collections generally exhibited heavier damage.

Parchments from the Royal Library were in most cases classified into the lower damage categories, with SC31-1 even appearing undamaged. Interestingly, all single sheets were placed in the fourth damage category, while the book bindings showed less damage except SC175 which had been subjected to a conservation intervention.

By comparing damage ranking based on MHT (Table 5) and microscopic fibre assessment (Table 3), discrepancies were observed for SC17-2, SC24, SC35, SC77 and SC 175-2. Sometimes, MHT damage categories do not match the higher fibre damage categories. This can be explained either by formation of intermolecular cross-links, or by severe dehydration. Both may maintain the overall tissue fabric in spite of deterioration and increased the shrinkage temperature. In fact, according to calorimetric studies of collagen denaturation by Luescher *et al.*,[41] when water concentration is decreased below a critical value, the T_{max} increases, but ΔH decreases.

On the other hand, if the MHT damage category indicates a heavily damaged condition, this is always mirrored by the high damage category in microscopic fibre assessment.

3.3 Damage Ranking by Micro DSC

According to Badea *et al.*,[22] ageing induces distinct variations of micro DSC parameters depending on the ageing factors. The most frequent patterns are illustrated by two DSC curves in Figure 3b: the green curve similar in shape to that of a new parchment, shifted towards higher temperatures (SC 38-2) and magenta curve displaying a wide shoulder, shifted to lower temperatures (SC16).[22,35] In case of SC 38-2 both fibrillar and molecular structure of collagen were well preserved and even reinforced by intermolecular cross-links, while collagen in SC 16 underwent partial structural deterioration at both levels.

Deterioration of parchments was evaluated on the basis of DSC values (Table 6) and taking the corresponding values determined for new parchments as a reference (Table 2).[11,14] Categories from 1 to 4 were assigned to each DSC parameter on the basis of the

lower and upper limit of its variation induced by accelerated degradation. For historic parchments we observed higher variations of the extensive parameters (i.e. those parameters depending on the mass of the system, e.g. amount of collagen) compared to that of T_{max}, an intensive parameter (i.e. not depending on the collagen amount of the sample).[11,15,22,34,35] This is also evident in Table 6. Based on this general behaviour of historic parchments, the decision to give higher weight to categories assigned to extensive parameters when calculating the overall damage category S_{DSC} was taken:[20,21]

$S_{DSC} = 0.2\ S(T_{max}) + 0.5\ S(\Delta H) + 0.3\ S(I_S)$,
where:
I_S is the ratio between $\Delta T_{1/2}$ and $C_{p\,max}^{ex}$.
T_{max} categories are:

1 for 50 °C < T_{max} < 55 °C;
2 for 45 °C< T_{max} < 50 °C and T_{max} > 55 °C;
3 for 40 °C < T_{max} < 45 °C and
4 for T_{max} < 40 °C.

ΔH categories are based on its % variation from the reference:

1 less than 10%;
2 10-20%;
3 20-35% and
4 >35%.

I_s categories are:

1 for I_s < 1;
2 for 1< I_s < 5;
3 for 5 < I_s < 15 and
4 for I_s > 15.

Accordingly, historic parchments were assigned to the four damage categories defined above (Table 6, last column).

The DSC peaks were deconvoluted using the PeakFit Gaussian algorithm and the DSC parameters of each endotherm were derived. The separation of collagen populations with distinct thermal stability provided a better understanding of degradation dynamics when studying the effect of accelerated degradation[9,10,15] and indicate specific patterns of deterioration in historic parchments.[22,34,35] Figure 5 illustrates the deconvolution of the SC16 DSC curve into four components corresponding to four populations of collagen with distinct thermal and structural stability. The peaks were allocated to four intervals depending on their T_{max} values: native (N), stabilised (S1) and unstable (U1 and U2).[15,22] The temperature intervals were previously defined: native, N (48 °C ≤ T_{max} ≤ 56 °C), in accordance with the average denaturation temperatures for new undamaged parchments; stabile, S1 (56 °C ≤ T_{max} ≤ 70 °C) and S2 (T_{max} > 70 °C), unstable, U1 (40 °C ≤ T_{max} ≤ 48 °C) and U2 (30 °C ≤ T_{max} < 40 °C), and gelatine, G (T_{max} < 30 °C), in

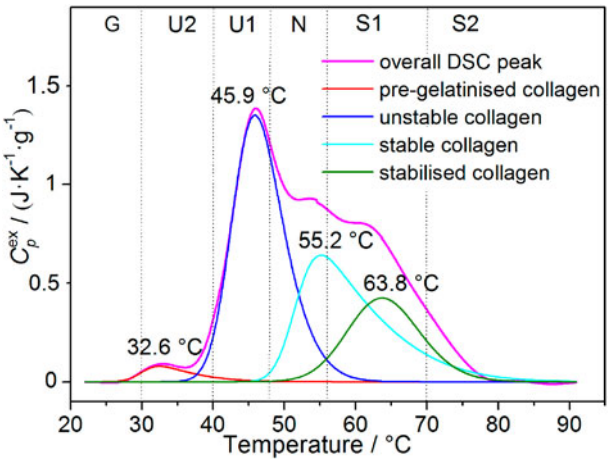

Figure 5. Deconvolution of the DSC curve of the historic parchment SC16 revealing four collagen populations: native, N (Tmax = 55 °C); stabile, S1 (Tmax = 64 °C); unstable, U1 (Tmax = 46 °C) and U2 (Tmax = 32 °C).

Sample	T_{max} (°C)	ΔH (J·g^{-1})	$\Delta T_{1/2}$ (°C)	$C_{p\,max}^{ex}$ (J·K^{-1}·g^{-1})	S_{DSC}
The Royal Library, Copenhagen					
SC16	46.0	27.9	22.3	1.4	3.6
SC17-1	54.2	24.5	7.1	1.7	2.8
SC17-2	53.7	12.7	3.6	1.5	2.8
SC18	46.5	30.7	15.4	1.7	2.8
SC24	48.9	28.3	8.8	2.0	2.8
SC31	51.2	12.6	5.6	1.5	2.8
SC32	39.6	31.6	17.3	1.5	3.5
SC35	51.8	21.5	12.7	1.4	2.9
SC38-1	45.6	33.3	16.3	1.6	3.3
SC38-2	58.1	25.4	4.2	3.7	2.5
SC77	50.7	15.3	11.9	1.0	3.2
The State Archives, Florence					
SC163	37.8	40.3	11.2	3.2	2.9
SC164	37.3	37.5	10.5	3.3	2.9
SC166	43.1	38.0	17.5	2.1	3.0
SC173-1	51.6	17.5	12.3	1.1	3.1
SC173-2	50.0	19.8	9.9	1.4	3.1
SC175-1	48.1	28.6	15.1	1.5	3.3
SC175-2	49.9	32.2	10.4	2.0	3.1

Table 6: DSC parameters of thermal denaturation for historic parchments and the overall damage categories S_{DSC}.

accordance with the thermal behaviour of artificially aged parchment.[22] It should be noted that the various populations of collagen are rarely simultaneously present in a historic parchment.

The percent contribution to the overall denaturation enthalpy of each endotherm was assumed as approximately proportional to the percentage of the corresponding collagen population.[15,22] It should be stressed that the collagen having undergone irreversible denaturation during ageing is no longer revealed by micro DSC, with the exception of gelatine

which displays thermally reversible behaviour. This is reflected by the relative amount of collagen which was calculated as the percent ratio %ΔH between the overall denaturation enthalpy of each sample and that of the new parchment (Table 7). As at $T_{max} \approx 50$ °C about 50% of the collagen is in its native state (cf. T_{max} definition above), T_{max} can be considered as a criterion to classify historic parchments in two groups independently of their damage level, which mostly depends on ΔH value (see the above S_{DSC} equation): parchments with more that 50% of their collagen fractions displaying $T_{max} \geq 50$ °C, and those with more than 50% of their collagen fractions with $T_{max} < 50$ °C.[23] The high stability of parchments in the first group suggests the formation of cross-links ensuring their high resistance even in the presence of partially collapsed collagen, as already mentioned at the end of Section 3.2. The low stability of parchments in the second group indicates they had mainly suffered from degradation related to hydrolytic bond scission and oxidation. As a consequence, the R_S ratio between the enthalpy sum of native and stabilised collagen fractions and that of unstable fractions was used to categorise historic parchments in two groups: stable ($R_S > 1$) and unstable ($R_S < 1$).

The results in Table 6 indicate that parchments from both the Royal Library and State Archives showed medium damage (S_{DSC} from 2.8 to 3.1), except SC16 (S_{DSC} = 3.6), SC32 (S_{DSC} = 3.5), SC38-1 (S_{DSC} = 3.3) and SC175-1 (S_{DSC} = 3.3). Three of these more extensively damaged samples from the Royal Library had undergone previous conservation treatments: SC16 and SC32 were flattened in the presence of moisture, while SC38 was restored using paper fillings. They also displayed the highest values of $\Delta T_{1/2}$ in the group, indicating higher heterogeneity due to a mixture of collagen populations with increasing structural disorder and decreasing thermal stability. We have already reported that parchments exposed to heating in a humid atmosphere for long periods of time showed a broad distribution of less stable collagen populations reflected in the progressive broadening of their DSC peaks, including shifts towards lower temperatures.[15,22,42] The behaviour of SC16, SC32 and SC38-1 appears very similar to that of the samples exposed to heating in humid conditions, and induced us to hypothesize the use of relatively high temperatures during conservation interventions. In fact, the reason for their high S_{DSC} values is not a significant enthalpy decrease, equivalent to a high percentage of irreversibly deteriorated collagen, but their low thermal stability and high heterogeneity, as was the case of parchments subjected to accelerated degradation by heating in a humid atmosphere. It should be pointed out that the MHT method also revealed very large shrinkage intervals ΔT_{total} (Table 5) indicating high fibre heterogeneity.

Sample	Collagen	Population U2		Population U1		Population N		Population S1	
	%[a]	T_{max} / °C	%	T_{max} / °C	%	T_{max} / °C	%	T_{max} / °C	%
The Royal Library, Copenhagen									
SC16	54.3	32.5	1.4	45.9	24.1	55.2	17.7	63.7	11.1
SC17-1	52.2	-	-	-	-	54.2	28.4	66.3	23.8
SC17-2	27.1	-	-	-	-	53.7	14.5	58.8	12.6
SC18	69.8	-	-	41.8 46.7	6.2 24.0	54.2	23.4	68.8	16.2
SC24	64.3	33.8	2.3	44.7	9.1	48.9	34.0	57.9	18.9
SC31	24.5	36.1	2.8	-	-	51.1	17.8	59.8	3.9
SC32	61.4	-	-	39.8	53.3	-	-	65.7	8.1
SC35	41.7	-	-	47.8	2.4	51.8	14.2	58.7 66.7	10.7 14.4
SC38-1	64.7	35.8	2.4	45.8	8.0	-	-	58.7 68.3	34.1 20.2
SC38-2[b]	49.4	35.8	2.3	-	-	52.1	15.2	58.5 68.3	16.5 15.4
SC77[c]	29.8			46.7	3.3	50.6	10.9	57.0 65.9	11.5 4.1
The State Archives, Florence									
SC163	78.3	-	-	37.8	77.6	53.8	0.3	67.1	0.4
SC164	72.8	-	-	37.3	72.8	-	-	-	-
SC166	73.8	37.6	4.3	43.1	56.3			57.6	13.2
SC173-1	39.8	-	-	45.8	4.5	51.7	21.1	59.2 64.3	6.6 7.6
SC173-2	45.0	29.5	0.4	42.8	2.7	50.0	29.1	59.7 66.8	5.2 7.6
SC175-1	55.3	33.1	1.4	-	-	48.1	29.9	58.0 65.4	16.5 7.5
SC175-2	62.5	33.1	0.6	-	-	49.9	39.1	58.7 67.0	10.8 12.0

Table 7: Denaturation temperatures (T_{max}) and proportion of collagen populations in historic parchments obtained by deconvolution of the DSC peaks. [a]Relative amount of collagen calculated as percent ratio between the enthalpies of sample and reference. [b]Highly stabilised collagen (7.6%) with T_{max} = 76.6 °C was detected. [c]Gelatine (0.7 %) at $T_{max} \approx 24$ °C was detected.

Parchments form the State Archives of Florence have similar S_{DSC} categories. However, for a proper interpretation of their damage, the enthalpy distribution among the collagen fractions with distinct thermal stabilities obtained by deconvolution of DSC peaks, should be considered (Table 7). In particular, SC163 and SC164, both consisting of a single population of collagen located in the U1 interval, as well as SC166, mainly made of unstable collagen distributed in U1 and U2 intervals, were classified as unstable (R_s < 1). It is known that SC163, SC164 and SC166 parchments have been dried after the flood. The extensive conversion of their collagen in unstable and disordered structures led us to assume that they were subjected to drying at high temperatures. The deterioration features are different for SC173 and SC175, where collagen content is lower but mainly consists of native and stabilized structures (R_s > 1). Moreover, the presence of pre-gelatinous structures U2 (Table 7) decreases the T_s value for SC173-2 and SC175-2 or even hides the main shrinkage interval, as for SC175-1. The presence of pre-gelatinised collagen should be considered as an additional risk factor for the stability of historic parchment as it easily converts to gelatine in a humid and warm environment at room temperature. [42]

3.4 Correlations Between Microscopy, MHT and Micro DSC Damage Ranking

Table 8 summarizes the damage categories according to the microscopic and micro DSC protocols. The microscopy category S_{micro} was calculated as the average of the categories obtained by microscopic evaluation of fibres (Table 3) and the MHT method (Table 5) since both protocols refer to fibre damage. In most cases, microscopy and MHT protocols led to an average damage category rather close to that obtained by micro DSC. This indicates homogeneous damage at both the microscopic (e.g. fibres) and the mesoscopic levels (e.g. fibrils). The differences between the two groups of results can be explained by considering both the methodological differences (micro DSC provides an integral result pertaining to the whole mass of the sample, while both the microscopic visual assessment and the MHT methods are concerned with a few fibres generally located at the corium core and sample surface) and the structures targeted (fibres and fibrils).

The Royal Library, Copenhagen			The State Archives, Florence		
Sample	S_{micro}	S_{DSC}	Sample	S_{micro}	S_{DSC}
SC16	3.3	3.6	SC163	4.0	2.9
SC17-1	3.0	2.8	SC164	4.0	2.9
SC17-2	2.3	2.8	SC166	4.0	3.0
SC18	3.0	2.8	SC173-1	2.7	3.1
SC24	2.7	2.8	SC173-2	2.3	3.1
SC31	1.3	2.8	SC175-1	4.0	3.3
SC32	3.3	3.5	SC175-2	4.0	3.1
SC35	3.0	3.2			
SC38-1	4.0	3.3			
SC38-2	1.5	2.5			
SC77	3.0	3.1			

Table 8: List of damage categories S_{micro} and S_{DSC} assigned using the microscopic method (average value obtained from microscopic evaluation and shrinkage activity measured by MHT) and micro DSC analysis, respectively.

On the other hand, historic parchments can also display highly heterogeneous deterioration. For example, for samples containing only U (e.g. SC164, SC164) collagen fractions, or both U and S collagen fractions (e.g. SC38-1, SC164, SC175-1 and SC175-2), S_{micro} was higher than the S_{DSC} damage category by 1 point. This could be ascribed to the fact that the C interval revealed by MHT method is mainly related to the mass shrinkage of fibres with lower thermal stability (U collagen), while the shrinkage of the stabilised fractions (S collagen), characterised by strong inter-molecular cross-links and consequently less cooperative, is mainly detected in the A2 interval. Moreover, since deterioration is often more advanced on the surface, the fibres taken for microscopic evaluation may predominantly contain pre-gelatinised collagen or even gelatin. As in many cases collagen fibres are still intact below the glass-like/molten layers, the damage of the bulk as characterised by micro DSC is less extensive. A stiff glass-like surface with a flexible fibre layer beneath represents a condition at risk in terms of preservation of inscriptions and illuminations since the rigid surface layer is subjected to mechanical stress induced by even small variations in relative humidity due to its different expansion and contraction characteristics compared to the underlying fibers.

Historic parchments displaying S_{DSC} values significantly higher than S_{micro} values, e.g. for SC31 and SC38-2 represent a further case of concern. Both of these parchments contain pre-gelatinised (U2), cross-linked (S) and native (N) collagen fractions (Table 7). For SC31 contains 24.5% of collagen in comparison with new parchment. However, most of this collagen, ~77% is native collagen, which is in a very good agreement with 35% of damaged fibres, as revealed by microscopic assessment, and T_s = 51.1 °C measured by MHT method. SC31 is a typical example of overestimation of the condition, which may occur if only microscopic assessment of fibres and/or shrinkage activity measurements are performed. In this case, the extensive parameters obtained with micro DSC, such as the overall amount of collagen and the proportion of collagen populations with distinct ther-

mal stability are very valuable for correct evaluation of deterioration.

4 Conclusions

Deterioration of historic parchments was studied using microscopic techniques at both room temperature (visual observation) and during heating (MHT method), and by micro DSC. The assessment of deterioration was performed using intensive and extensive physical parameters, and ranking criteria based on the extent of their variation in comparison to new parchment as reference. The upper and lower limits for each damage category were defined by using progressively deteriorated samples obtained by exposing new parchments to various types of accelerated degradation. A comparison of damage categories obtained using the different methods was carried out.

Starting from the premise that ageing of parchments proceeds progressively from the outside towards the inside of a parchment sheet and from fibre surface to the molecular level, and considering that the surface layers carry inscriptions and illuminations, the assessment of both the surface and the underlying layers is a prerequisite for a reliable ranking of damage. Microscopic evaluation can be carried out at the surface and in the bulk, offering a comprehensive picture of fibre damage.

In this paper, fibres taken for both MHT and microscopic assessment methods represent bulk corium fibres, enabling the comparison of results with those provided by micro DSC. This comparison showed that the microscopic methods are suitable for damage ranking of historic parchments. Some limitations were found for parchments consisting of either mixtures of pre-gelatinised and strongly cross-linked collagen, or only native collagen in a very low content, with respect to new parchment. Even though the actual damage level appears to be evaluated in more detail using micro DSC, which accounts for both intensive (T_{max}, $\Delta T_{1/2}$) and extensive (ΔH) physical parameters, the microscopic procedures could be improved and thus reduce the above mentioned limitations.

The fibre assessment method can become more precise through quantification of various morphological features (e.g. “pearls on a string” and “butterflies”) and measures of their dimensional variation (e.g. length and width of the so-called pearls).[29] Optimisation of the quantitative and qualitative information related to fibre morphology is ongoing and will result in an improved damage assessment procedure.[43,] On the other hand, the analysis of correlations between the various temperatures as measured using MHT and micro DSC is envisaged within the mentioned COLLAGE project and may enhance the content of information provided by the MHT method.[44]

Averaging damage categories obtained by microscopic methods and micro DSC is not recommended even when they are not contradictory because they refer to different structural levels (i.e. fibres and fibrils). In addition, MHT assessment is based on intensive parameters (i.e. T_f and T_s) only, whereas micro DSC assessment is based on both intensive (i.e. T_{max}, $\Delta T_{1/2}$) and extensive (i.e. ΔH, $C_p^{ex}{}_{max}$) parameters.

Since deterioration of collagen within historic parchment can be variously distributed throughout its structure, the use of both microscopic methods and micro DSC offers the advantage to provide complementary information on specific alterations at different structural levels and prevent partial, improper evaluation.

The microscopic assessment and the MHT method could be easily used in a non-laboratory environment and the knowledge gained so far allows for a partly quantitative assessment of deterioration at the fibre level and ranking of parchments in four damage categories.

Micro DSC unambiguously quantifies the deterioration, assessing the thermal and structural stability of collagen populations and discriminates between stable and unstable parchments. It is therefore a valuable tool for research purposes and, when possible (micro-sampling allowed, equipment and expertise available), can provide high-quality support to conservation decisions.

5 Acknowledgements

The participation of Dr Elena Badea in the EC project IDAP (EVK-CT-2001-00061) and Piedmont Region project OPERA (CIPE 2004-D39) was made possible by the “General Accord for Cooperation and Scholar Exchange in Chemistry and Physics” between the University of Craiova (Romania) and the University of Turin (Italy) through research contracts. The participation of Dr Alexandra Kurysheva in this work was made possible through a research grant funded by the Italian Ministry of Foreign Affairs (MAE) in the framework of the Bilateral Programme of Scientific and Technological Cooperation between Italy and Russia. The participation of Dr Lucreția Miu in this work was made possible through a Bilateral Agreement between the INCDTP-ICPI, Bucharest (Romania), and the Department of Chemistry, University of Turin (Italy).

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7 Endnotes

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