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ASSESSMENT OF DAMAGE IN OLD PARCHMENTS BY DSC AND SEM

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Environmental impact on parchment was investigated by differential scanning calorimetry (DSC) and scanning electron microscopy (SEM). Parchments subjected to accelerated ageing and old parchments were compared to evaluate quality and extent of deterioration. Stability of fibrillar collagen within parchment was determined from the changes in thermodynamic parameters associated with thermal denaturation. Parchment surface was characterised, and specific morphological criteria were selected for damage assessment. The thermodynamic and morphological changes of collagen induced by deterioration are discussed, and their correlations are proposed as a means of ranking damage in old parchments.

Keywords: *accelerated and environmental damage, collagen stability, DSC, parchment, SEM*

Introduction

Parchment was used as the common writing material from the 2nd century BC to the end of the Middle Ages, when it was joined by paper. However, it has continued to be used for special purposes, such as official documents and bookbindings. Since it is manufactured from animal hide after strong alkaline removal of the epidermis and subcutaneous tissues, fibrous collagen is by far its main component. Deeper understanding of the physical and chemical changes of parchment by ageing would enable conservators and restorers to provide specific answers to the problems raised by the environmental deterioration. The standard model for the structure of collagen is an ascending bundling hierarchy. Collagen type I is a triple helix about 285 nm long and 1.5 nm in diameter resulting from the right-handed super-coiling around a common axis of three left-handed helices (α-chains) (Fig. 1a) [1–3]. Lateral and longitudinal packing results in the assembly of almost cylindrical fibrils of 40 to 100 nm in diameter. Native collagen fibrils exhibit 65.5 nm banding following the quarter-stagger arrangement of 4–5 molecules (Fig. 1b) observable by electron microscopy [4]. Fibres with diameters ranging from 50 to 300 μm are then built up through the tight packing of fibrils by means of intermolecular interactions [5]. The mesoscopic arrangement of collagen fibres in skin is a three-dimensional network of thin, wavy and loosely intertwined bundles (Fig. 1c) [6, 7]. The great stability and strength conferred to collagen by this supramolecular structure account for its functional efficiency in a variety of tissues and explain the exceptional longevity of documents and other artefacts made from parchment [8].

Some damage may be caused in the production of parchment, e.g. liming has an effect on the axial spacing, lateral packing and lipid bilayer thickness, while drying affects the lateral intermolecular packing [9]. Humidity, heat, irradiation, pollutants and micro-organisms are mainly responsible for the ageing and deterioration of parchment, while floods, fires, wars, etc. are obvious sources of damage. Deterioration proceeds from a molecular to a mesoscopic level, resulting in impairment of the hierarchical organisation of collagen and hence the stability of parchment. In chemical terms, deterioration is primarily due to oxidation and hydrolysis [10]. Oxidation is generally caused by free radicals generated by heating, UV light [11], and SO_2 [12] and NO_x pollution [13]. The side chains of some amino acids are initially involved, but oxidation can also occur in the backbone of collagen through the rupture of N–C covalent bonds [14]. Hydrolysis is catalysed by both hydroxyl and hydrogen ions [15], especially when atmospheric pollutants such as SO_2 [16] and NO_x act in conjunction with air humidity, and cleavage of peptide bonds disrupts the hierarchical structure of collagen. Gelatinisation, consisting in further denaturation and aggregation, leads the irreversible formation of a heavily hydrated gel matrix [17]. Partially degraded collagen is especially susceptible to gelatinisation in damp and warm environments, since H-bonds are exposed to the action of water [18].

Ageing of ancient parchments results from the combined action of deterioration mechanisms, and

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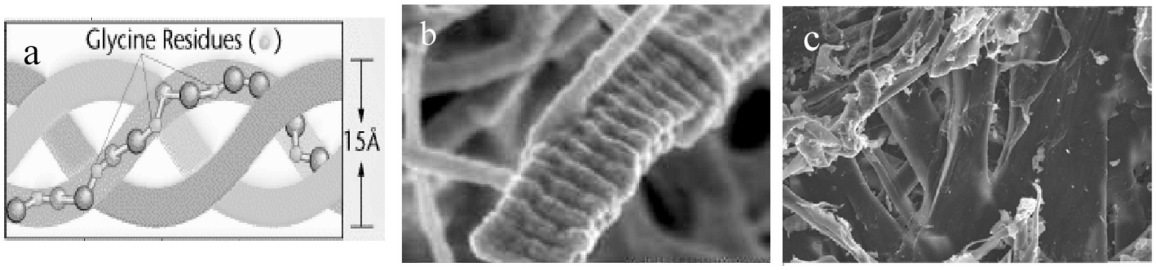

Fig. 1 Structural organisation levels of collagen type I in parchment: a – model for molecular collagen: right-handed triple helix formed by three left-handed chains based on the amino acid sequence (Gly-X-Y)*n* where X, Y are often Pro and Hyp (molecular level); b – single fibril (nanoscopic level); c – fibre network (mesoscopic level)

their complexity renders the suffered damage rather heterogeneous [19]. As a consequence, the pathways of collagen deterioration in parchment have not yet been mapped and appropriate damage assessment protocols have still to be readily elaborated.

The present study is concerned with the relationships between the deterioration of parchment and the thermodynamic parameter values provided by DSC. In addition, the morphological alterations induced by the loss of collagen hierarchical structure have been monitored by SEM. DSC illustrates changes in the thermal stability of collagen by measuring the energy associated with thermal denaturation. A wealth of thermodynamic information has been gathered on molecular collagen from different tissues in dilute solution, whereas only a few studies of collagen in parchment have been performed [20–23]. Our measurements on dry samples can however give valuable information on different intermolecular interactions (e.g. hydrophobic, van der Waals, etc) which are only minor in wetted skin or absent in very dilute solution of collagen. Results obtained would be useful for comparison with those already obtained on dehydrated collagen [24], collagen with different hydration level [25, 26] and soluble collagen [27, 28].

Experimental

Materials

The parchment samples examined are listed in Table 1. Accelerated ageing procedures were used to assess the deterioration induced in days or weeks in the search for parallel criteria for the evaluation of environmental damage sustained over a number of centuries. Accelerated ageing protocols were set up and performed at Centre de Recherches sur la Conservation des Documents Graphiques, Paris, and School of Conservation, Royal Danish Academy of Fine Arts, Copenhagen.

Four groups were compared:

- New parchments prepared from calf, sheep, goat and pig hides supplied by different manufacturers. These samples were also used as the reference for both aged and old parchments.
- Calf parchment ageing for increasing time by light irradiation was made with an ATLAS MTT BV Solarconstant 2500/4000 lamp ($1.7\ 10^5$ lx illuminance). Dry heating treatments were performed inside an oven maintained at 100°C.
- Calf parchment ageing by humid heating (60°C and 60% RH, 60°C and 80% RH, 80°C and 60% RH, and 80°C and 80% RH) for 1, 2, 4, 8, 16 and 32 days was performed in appropriate chambers.
- Old parchments from the National Archives of Scotland, Stirling, UK, the School of Conservation, Royal Danish Academy of Fine Arts and The Royal Library of Copenhagen. The Scottish parchments are documents from the 18th and 19th centuries originally stored in boxes in an office at Perth and one not dated bookbinding. The Danish samples are undated bookbinding whose prior history is not known. All samples are included among those circulating within the IDAP EU project (http://www.idap-parchment.dk).

Methods

Differential Scanning Calorimetry

DSC measurements were made with a SETARAM 111 microcalorimeter at 5°C min^{-1} heating rate in the temperature range (30 to 190)°C. Several melting runs with certified reference materials (naphthalene, benzoic acid, indium) performed under the adopted experimental conditions showed agreement with the recent IUPAC recommended values [29] within 0.07% for temperature and 0.25% for enthalpy.

All types of samples (new parchments, parchments subjected to accelerated ageing and old parchments) were analysed in dry condition after few days storage in a controlled environment (approximately 20°C and 50% RH). All runs were performed in static air conditions by carrying out 2–3 measurements per sample. Denaturation of parchment is irreversible and no heat effect was observed on rescanning. Not more

Table 1 List of the parchments

New parchments		
Origin	Animal	Symbol
De Groot, NL	calf	SC69:1
De Groot, NL	calf	SC69:2
De Groot, NL	calf	SC69:3
De Groot, NL	calf	SC69:4
SC*, Copenhagen, DK	calf	SC70:B10
SC*, Copenhagen, DK	calf	SC70:E9
SC*, Copenhagen, DK	calf	SC70:E1
SC*, Copenhagen, DK	calf	SC70:C5
SC*, Copenhagen, DK	calf	NP10
Supplier, Romania	calf	SC84
Supplier, Romania	goat	SC87
Supplier, Romania	sheep	SC88
Supplier, Romania	pig	SC83

*SC: School of Conservation, Royal Danish Academy of Fine Arts

Artificially aged parchments		
Ageing	Exposure time	Symbol
Humid heating (60°C, 60% RH)	1 day	SC108
	2 days	SC109
	4 days	SC110
	8 days	SC111
	16 days	SC112
	32 days	SC113
Humid heating (60°C, 80% RH)	1 day	SC90
	2 days	SC91
	4 days	SC92
	8 days	SC93
	16 days	SC94
	32 days	SC95
Humid heating (80°C, 60% RH)	1 day	SC96
	2 days	SC97
	4 days	SC98
	8 days	SC99
	16 days	SC100
	32 days	SC101
Humid heating (80°C, 80% RH)	1 day	SC102
	2 days	SC103
	4 days	SC104
	8 days	SC105
	16 days	SC106
	32 days	SC107
Visible light irradiation ($1 \cdot 10^{-5}$ lx)	4 hours	SC70:F3
	8 hours	SC70:C8
	16 hours	SC70:C3
	32 hours	SC70:A7
Visible light irradiation ($1 \cdot 10^{-5}$ lx)+dry heating (100°C)	4 hours+2 days	SC70:42
	8 hours+4 days	SC70:84
	16 hours+8 days	SC70:168
	32 hours+16 days	SC70:3216

Old parchments			
Origin	Type	Date	Symbol
Natl. Archives of Scotland, Stirling, UK	document	1765	USH1
	document	1769	USH2
	document	1775	USH3
	document	1827	USH4
	document	1824	USH5
	document	1832	USH6
	document	1828	USH7
	document	1817	USH8
	bookbinding	not dated	USH9
	document	1765	USH10
	document	1792	USH11
	document	1740	USH12
School of Conservation, Copenhagen, DK	bookbinding	not dated	HP17:1
	bookbinding	not dated	HP17.2
	bookbinding	not dated	HP31:1
Royal Library, Copenhagen, DK	uncertain	18thcentury	SC72:1
	uncertain	18thcentury	SC72:2
	bookbinding	not dated	SC73:1
	bookbinding	not dated	SC73:2
	bookbinding	not dated	SC75:1
	bookbinding	not dated	SC75:2
	bookbinding	not dated	SC76:1
	bookbinding	not dated	SC77:1

than 2 measurements were made on the old samples because very few were available. Sealed stainless steel crucibles were employed. Sample mass $(5 \text{ to } 10) \cdot 10^{-3}$ g was determined with a Mettler microelectrobalance AE-163 with $1 \cdot 10^{-5}$ g resolution.

The calorimetric output was acquired both with an 8210 Sefram recorder and digitally with a home-made software before processing with Microcal Origin 6.1 and converted to apparent specific heat (C_p/J K^{-1} g^{-1}) by dividing measured heat-flow rate by

scan rate and sample mass. Onset temperature, T_{onset}/°C, maximum peak temperature, T_d/°C (denaturation temperature), enthalpy associated with endothermic effect, ΔH/J g^{-1} (denaturation enthalpy) and peak half-width, $\Delta T_{1/2}$, were determined.

Scanning Electron Microscopy

SEM observations were made at (5 to 30) kV accelerating voltage with a Leica Stereoscan 420 apparatus equipped with a tungsten filament. Samples were short-pulse coated with graphite to avoid damage due to overheating. Observations by Environmental Scanning Electron Microscopy (ESEM), which does not require coating, were also carried out on some samples for comparison. The absence of morphological differences between the SEM and ESEM observations showed that the coating had not caused any damage. Increasing magnifications were used to inspect: (*i*) the general features of the surface of samples, (*ii*) the shape of the fibre patches, and (*iii*) the structure of individual fibres. Electron Dispersion Spectroscopy (EDS) was also employed in *a* very few cases to look for residues of inorganic compounds.

Results and discussion

Differential scanning calorimetry

New parchments

The typical DSC endotherms for the calf, goat, sheep and pig parchment samples are shown in Fig. 2, and generally consist of a narrow peak in the range (120–125)°C ascribed to the thermal denaturation of fibrillar collagen. Decreasing of parchment moisture content makes denaturation of collagen to occur at progressively higher temperatures [30]. Present results compare favourably with previous literature data [31–34]. Table 2 sets out the T_{onset}, T_d, $\Delta T_{1/2}$ and ΔH values for three series of new parchments from different suppliers. For samples obtained from the same parchment with a standard sampling procedure, T_d and ΔH variations were small, less than ±1% and ±2% respectively (Fig. 2a, Table 2, sections a and b). Somewhat greater variations were found for the average values of T_d (±2%) and ΔH (±5%) in parchments from different suppliers (Fig. 2b and Table 2, all sections). On the other hand, dispersion for calf, goat, sheep and pig parchments manufactured by the Romanian supplier is still low: about ±2% for both T_d

Table 2 Thermodynamic parameter values for thermal denaturation of new parchments manufactured by different suppliers from various animal hides

Sample	T_{onset}/°C	T_d/°C	$\Delta T_{1/2}$/°C	ΔH/J g^{-1}
a) Reference samples from the same calf parchment (De Groot, NL)				
SC69:1	123.1	126.1	6	30.8
SC69:2	122.1	125.4	5.5	31.2
SC69:3	121.1	124.8	6	32.1
SC69:4	122.1	126.1	6	32.3
SC69 (average)[a]	122.1±0.7	125.6±0.5	5.9±0.2	31.6±0.6
b) Reference samples from the same calf parchment (SC, DK)				
SC70:B10	118.1	121.2	5.5	34.0
SC70:E9	118.5	121.5	5.8	34.3
SC70:E1	119.0	122.1	5.5	33.7
SC70:C5	117.6	119.6	5.5	34.6
SC70 (average)[a]	118.3±0.5	121.1±0.9	5.6±0.1	34.2±0.3
c) Reference samples from parchments made by the SC, DK and Romanian suppliers from various animal hides				
NP10	115.3	120.1	6.0	36.3
SC84	115.8	120.1	6.0	35.3
SC87	111.7	115.1	6.5	34.1
SC88	112.6	116.6	6.0	33.6
SC83	112.1	117.1	6.5	34.2
Average[a]	113.5±1.7	117.8±2.0	6.2±0.2	34.7±1.0
d) Total average[a]	117.6±3.8	121.2±3.5	5.9±0.3	33.6±1.5

Mean values from two measurements.
[a]Uncertainties are standard deviations of the mean.

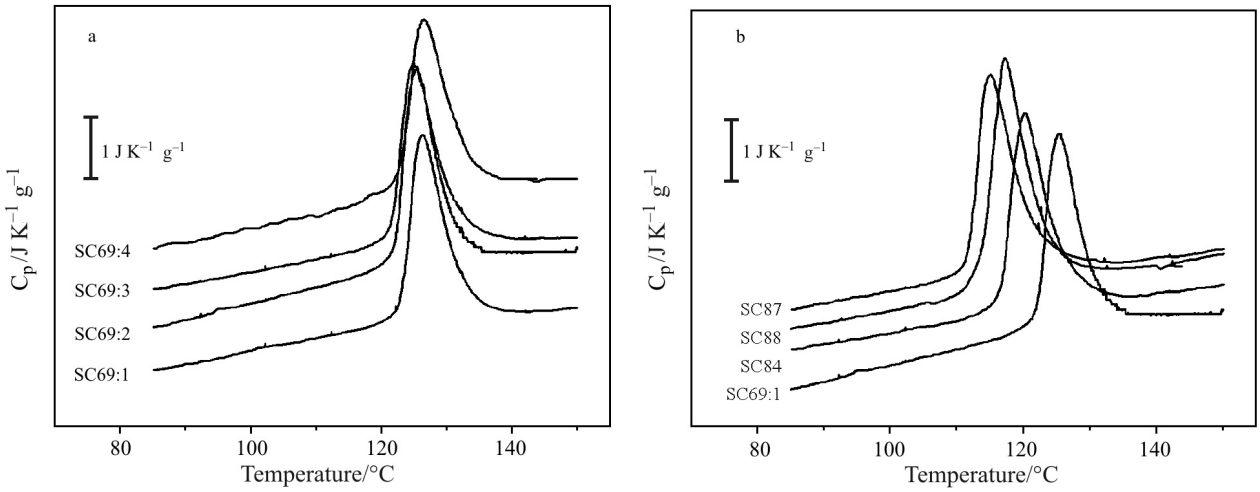

Fig. 2 DSC curves of new parchments taken as reference for measurements: a – manufactured by a single supplier; b – from different animal species and manufactured by two suppliers

and ΔH (Table 2, section c). Animal origin has some effect on T_d values (Table 2, section c), though the differences are clearly offset by variations induced by manufacturing. It may thus be suggested: (*i*) the manufacturing procedure can result in not negligible alterations in the thermal stability of collagen; (*ii*) T_{onset} and T_d values can be indicative of the animal species, whereas $\Delta T_{1/2}$ and ΔH values are inconclusive in this respect; (*iii*) variations higher than those mentioned should thus be ascribed to the accelerated / environmental damage suffered by parchments. Larger variation in both T_d and ΔH values were reported for parchments measured in wet condition [20], probably due to a non-uniform distribution of water in the samples [32]. Low standard deviations (Table 2) support our choice for DSC measurements on parchments 'in dry condition'.

Parchments subjected to accelerated ageing

New calf parchments were aged by heating in dry or humid conditions and exposed to light irradiation to simulate damage suffered by old parchments. Samples from only two new parchments were used for dry and humid ageing procedures, respectively, to restrict intrinsic dispersion of results. Damage induced by accelerated ageing is expected to appear as a decrease of both T_d and ΔH [26, 35]. The more deteriorated a parchment is, the less energy is needed for its thermal denaturation. Since all samples within a series were from the same parchment, changes in the area, height and width of the DSC peaks should only be related to deterioration induced by accelerated ageing. Tables 3 and 4 compare the thermodynamic parameter values for the aged samples and the corresponding reference values. Figure 3 shows the DSC curves for a group of

Table 3 Thermodynamic parameter values for thermal denaturation of parchments subjected to dry ageing

Sample	T_{onset}/°C	T_d/°C	$\Delta T_{1/2}$/°C	ΔH/J g^{-1}
SC70 (average)[a]	118.3±0.5	121.1±0.9	5.6±0.1	34.2±0.3
a) Exposure to visible light irradiation ($1.7\cdot10^5$ lx)				
SC70:F3	117.8	122.1	6.0	35.1
SC70:C8	117.6	120.8	5.5	32.6
SC70:C3	117.1	121.1	6.0	36.5
SC70:A7	115.4	119.1	6.0	32.0
b) Exposure to visible light irradiation ($1.7\cdot10^5$ lx) and dry heating (100°C)				
SC70:42	115.1	119.6	6.3	28.8
SC70:84	114.4	119.4	7.5	25.0
SC70:168	111.6	116.9	7.3	21.8
SC70:3216	103.5	112.1	10.6	20.2

Mean values from two measurements.
[a] Reference values from Table 2

Table 4 Thermodynamic parameter values for thermal denaturation of parchments subjected to humid ageing

Sample	T_{onset}/°C	T_d/°C	$\Delta T_{1/2}$/°C	ΔH/J g^{-1}
SC69 (average)[a]	122.1±0.7	125.6±0.5	5.9±0.2	31.6±0.6
a) Heating at 60°C in humid environment (60% RH)				
SC108	122.1	126.1	6.0	33.0
SC109	123.1	127.1	6.0	34.2
SC110	122.1	125.1	6.0	33.3
SC111	121.1	124.6	6.0	33.8
SC112	120.6	124.1	6.0	31.2
SC113	121.1	124.6	6.0	32.1
b) Heating at 60°C in humid environment (80% RH)				
SC90	123.1	126.1	6.0	30.8
SC91	124.6	127.1	6.0	29.1
SC92	124.6	127.5	6.0	29.4
SC93	124.1	127.1	6.0	31.7
SC94	124.6	126.0	5.5	31.8
SC95	123.8	127.0	6.0	28.7
c) Heating at 80°C in humid environment (60% RH)				
SC96	122.1	125.4	5.5	31.2
SC97	126.9	128.6	5.0	30.8
SC98	125.8	130.2	5.0	30.4
SC99	128.0	131.3	6.0	32.0
SC100	126.7	129.3	5.0	31.9
SC101	126.5	129.8	6.0	33.0
d) Heating at 80°C in humid environment (80% RH)				
SC102	121.1	124.8	6.0	30.0
SC103	125.6	128.1	6.0	31.1
SC104	126.1	129.1	5.5	29.5
SC105	129.1	132.0	5.5	30.0
SC106	127.1	130.5	5.5	31.8
SC107	128.1	131.0	6.0	29.3

Mean values from two measurements. [a]Uncertainties are standard deviations of the mean

selected aged samples. Combined dry heating at 100°C and light irradiation resulted in a progressive decrease of both T_d and ΔH, and increased the $\Delta T_{1/2}$ on ageing time, and are indicative of a tendency to heavy deterioration (Table 3, section b). DSC peaks became broader and smaller, and shifted to lower temperatures (Fig. 3a). This trend can also be interpreted by considering collagen as formed of structural blocks that break down cooperatively during denaturation [36]. Deterioration due to dry heating at 100°C may thus be supposed to result in a progressively lower and broader energy distribution of these blocks. They will collapse on heating, at lower temperature and in wider ranges giving rise to smaller DSC peak areas. After 16 days at 100°C, baseline diffuse disturbances manifested in a wide range of temperature from 50°C to the lower limit of the region of denaturation. This finding could be tentatively assigned to the transition of a disordered fraction of collagen originated by partial denaturation induced by ageing. By contrast, simple irradiation does not produce any effect detectable by calorimetry (Table 3, section a).

Smaller changes were observed for parchments heated in a humid environment (Table 4). Samples heated at 60°C displayed almost no changes in the considered parameters except slightly higher ΔH values at 60% RH, whereas somewhat higher T_d, almost constant ΔH and partially lower $\Delta T_{1/2}$ were found for samples heated at 80°C at both 60 and 80% RH. The shape of the DSC peaks was much the same for all the humid-heated samples (Fig. 3b). It can thus be deduced that parchment can withstand moderate temperatures in a humid environment for up to 32 days with no evident signs of deterioration. Our slightly

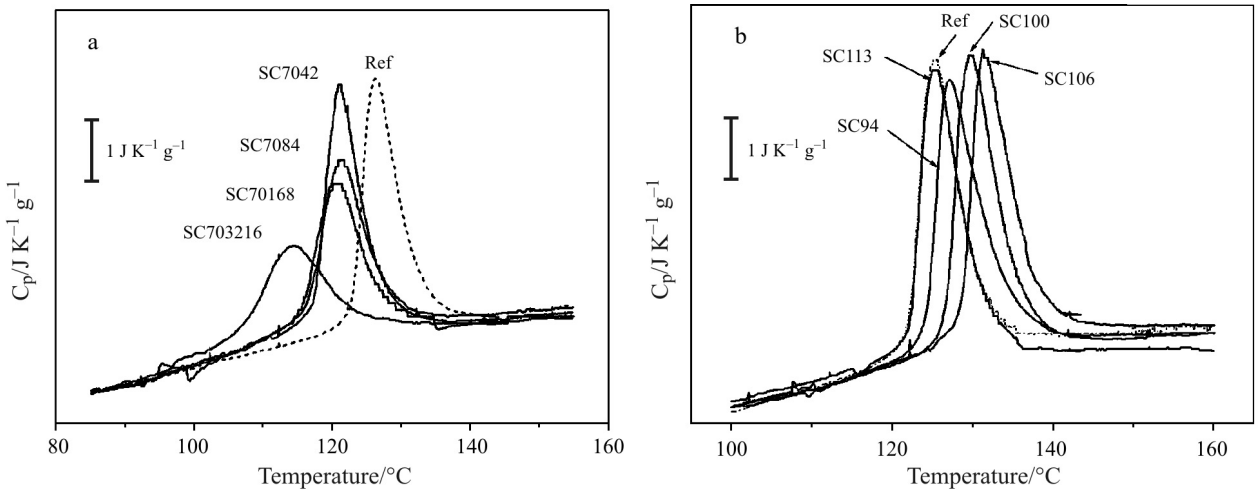

Fig. 3 DSC curves of artificially aged parchments: a – subjected to dry heating and exposed to light irradiation for increasing times (ref. SC70E1); b – heated at 80°C and 80% RH for increasing times (ref. SC69:2)

higher T_d associated to lower $\Delta T_{1/2}$ values (Fig. 3b and Table 4, section c and d) may be related to crosslink formation [37] and regarded as an early sign of deterioration [25].

ΔH *vs.* T_d and $\Delta T_{1/2}$ *vs.* T_d plots (Figs 4 and 5) were set up to provide a comprehensive picture of thermal stability changes for use in the comparison of aged and old parchments. Points in these plots represent correlations between the per cent values of the considered couples of parameters. Percent values were obtained by relating the experimental results to the corresponding reference average, namely SC70 for samples aged by irradiation and/or dry heating (Table 3) and SC69 for samples aged by humid heating (Table 4). Per cent values for reference samples (points not reported) are thus comprised in the marked rectangular areas, whose height and width represent their maximum average uncertainties (Table 2). Dotted lines extend this subdivision to the entire plot area and reveal the (ΔH, T_d) and ($\Delta T_{1/2}$, T_d) per cent deviations from the reference values for both the aged and old samples.

The effects of accelerated ageing are illustrated in Figs 4a and 5a: (*i*) heating at 60°C and both 60% and 80% RH had practically no effect on structural thermal stability, since the relevant points are prevalently located in the rectangular areas; (*ii*) heating at 80°C and both 60 and 80% RH displaced the points to higher T_d values accompanied by a slight $\Delta T_{1/2}$ decrease, whereas the ΔH values remained within the limits of the reference samples; (*iii*) exposure to visi-

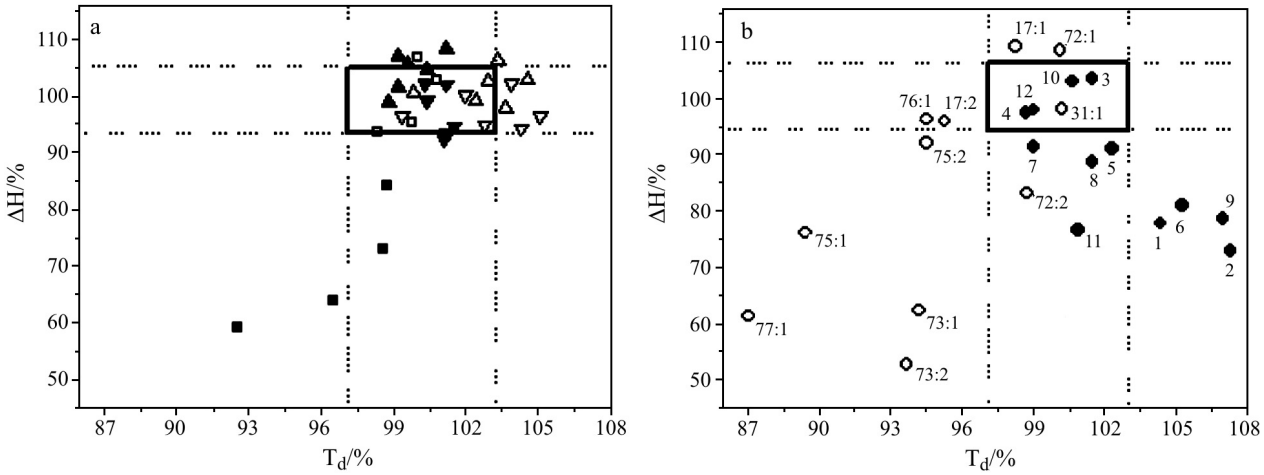

Fig. 4 ΔH *vs.* T_d interplay. a – artificially aged parchments subjected to: □, irradiation by visible light; ■, irradiation and dry heating at 100°C; ▼, heating at 80°C and 60%RH; ▲, heating at 60°C and 60%RH; ▽, heating at 80°C and 80%RH; △, humid heating at 80°C and 80%R; b – historical parchments ● from National Archives of Scotland and, ○, from School of Conservation and The Royal Library, Copenhagen. Codes of old parchments were shortened using only their numeric part. Reference data are comprised in the rectangular area

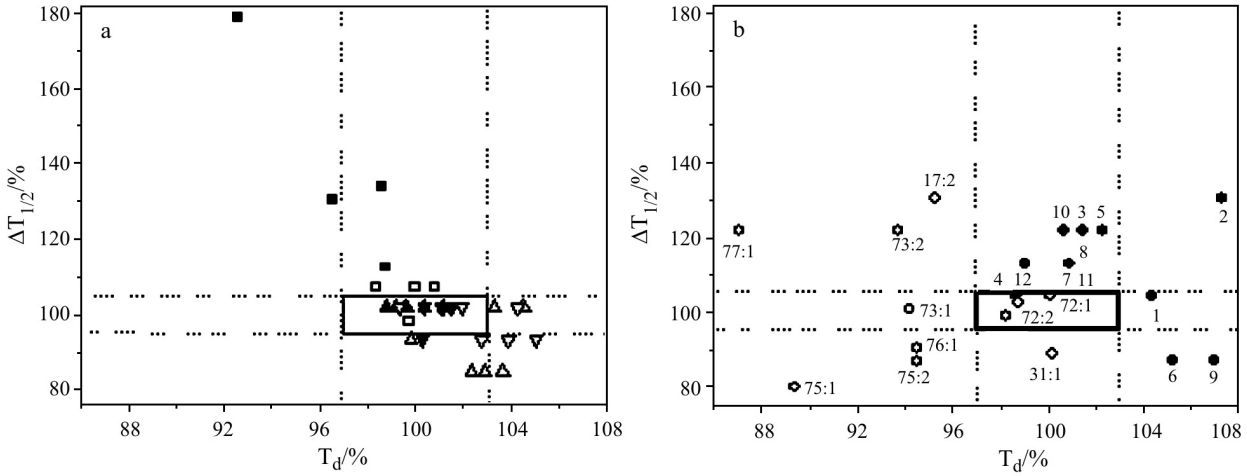

Fig. 5 $\Delta T_{1/2}$ *vs.* T_d interplay. a – artificially aged parchments subjected to: □, irradiation by visible light; ■, irradiation and dry heating at 100°C; △, heating at 80°C and 60%RH; ▲, heating at 60°C and 60%RH;▽, heating at 80°C and 80%RH; ▼, humid heating at 80°C and 80%RH; b – historical parchments ● from National Archives of Scotland and, ○, from School of Conservation and The Royal Library, Copenhagen. Codes of old parchments were shortened using only their numeric part. Reference data are comprised in the rectangular area

ble light only had no significant effect; (*iv*) combined dry heating at 100°C and visible light exposure led to a progressive decrease of T_d values, accompanied by an up to 60% decrease of ΔH and $\Delta T_{1/2}$ increase to almost twice the reference value. Dry heated samples are strongly influenced by the treatment time, since the points indicate progressive impairment of all the thermodynamic parameter values instead of the narrowly spread distribution observed for all the other samples subjected to accelerated ageing.

Old parchments

The results for the old parchments are presented in Table 5 and Figs 4b and 5b. The distribution and clustering of points in the two plots and their correlation with the results for aged samples were used to rank the 23 old parchments in four classes according to their collagen denaturation level (Table 6), which can be grouped as follows. Samples with no significant deviation of their (ΔH, T_d) and ($\Delta T_{1/2}$, T_d) points from the references thus falling in the marked areas. This group (undamaged class) consists of seven dated documents (USH3, USH4, USH5, USH7, USH8, USH10, USH12) and one dateless sample (HP31:1) that are well preserved and stable. A further group, corresponding to both slightly and medium damaged classes, is composed of six Danish bookbindings (HP17:1, HP17:2, SC72:1, SC72:2, SC75:2, SC76:1) and four dated Scottish documents (USH1, USH6, USH9, USH11) with a negative ΔH deviation and small negative or positive T_d and $\Delta T_{1/2}$ deviations. The last group (heavily damaged class) composed of one document and four bookbindings displaying substantial T_d and ΔH negative deviations (USH2, SC 73:1, SC73:2, SC75:1, SC77:1). The higher the T_d and ΔH negative deviations, the greater the risk of irreversible deterioration when parchments are kept in inappropriate environment.

The overall picture illustrated in Figs 4 and 5 shows that deterioration variously affects T_d, ΔH and $\Delta T_{1/2}$ values. Thus, DSC peak shape is an early indicator of the degree of deterioration. The relationship between deterioration and peak shape of old parchments is illustrated in Fig. 6. The peaks for undamaged or slightly damaged samples almost overlap the reference peak, sometimes at somewhat lower or higher

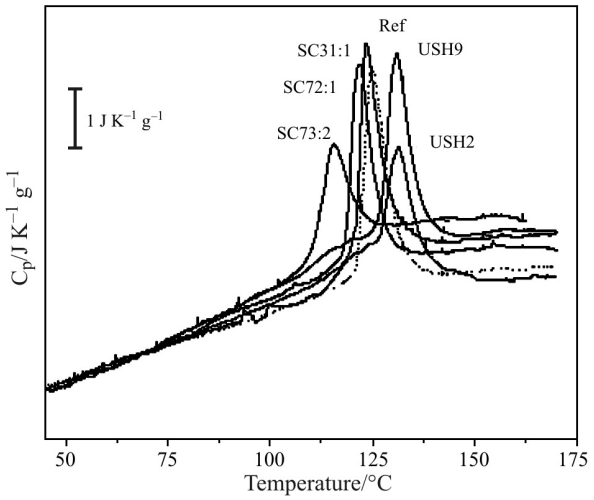

Fig. 6 DSC curves for some historical parchments showing thermal stability variation induced by environmental ageing: almost no changes (SC31:1); minor changes (SC72:2); major changes (USH9); heavy changes (USH2, SC73:2) (ref. SC69:3)

Table 5 Thermodynamic parameter values for thermal denaturation of old parchments from three archives

Sample	T_{onset}/°C	T_d/°C	$\Delta T_{1/2}$/°C	ΔH/J g^{-1}
National Archives of Scotland, Stirling, UK				
USH1	124.6	127.6	6.0	26.0
USH2	109.6	115.6	7.5	24.4
USH3	119.6	124.1	7.0	34.6
USH4	116.9	120.7	6.0	32.6
USH5	121.6	125.1	7.0	30.5
USH6	126.1	128.7	5.0	27.1
USH7	117.1	121.1	6.5	30.6
USH8	120.6	124.1	7.0	29.7
USH9	127.6	130.8	5.0	26.3
USH10	118.6	123.1	7.0	34.5
USH11	119.1	123.4	6.5	25.6
USH12	117.2	121.1	6.5	32.8
School of Conservation, Copenhagen, DK				
HP17:1	117.9	121.2	5.7	35.6
HP17:2	111.2	117.5	7.5	31.3
HP31:1	121.1	123.6	5.1	32.0
The Royal Library, Copenhagen, DK				
SC72:1	120.1	123.5	6.0	35.4
SC72:2	118.2	121.8	5.9	27.1
SC73:1	112.6	116.2	5.8	20.3
SC73:2	110.3	115.6	7.0	17.2
SC75:1	107.2	110.3	4.6	24.8
SC75:2	113.7	116.6	5.0	30.0
SC76:1	113.4	116.6	5.2	31.4
SC77:1	102.6	107.4	7.0	20.0

Mean values from two measurements

Table 6 Estimation of the degree of deterioration of old parchments from thermal stability and morphological criteria

Deterioration level	Investigation technique	Parchment
undamaged	DSC	USH3, USH4, USH5, USH7, USH8, USH10, USH12, HP31
	SEM	USH1, USH4, USH5, USH8, USH10
slightly damaged	DSC	USH1, USH11, HP17.1, HP17:2, SC72:1, SC76:1
	SEM	USH3, USH7, USH11, USH12, HP31:1, SC17:1, SC76:1
medium damaged	DSC	USH6, USH9, SC72:2, SC75:2
	SEM	USH9, HP17:2, SC75:2
severely damaged	DSC	USH2, SC73:1, SC73:2, SC75:1, SC77:1
	SEM	USH2, USH6, USH9, SC72:2, SC73:1, SC73:2, SC75:1, SC77:1

temperatures (Figs 4b and 5b). Moreover, baselines show to be rather dispersed, especially after denaturation. These experimental evidences cannot univocally be interpreted since they could have different origins: humps underlying peaks and/or specific heat variations (positive or negative). However, they can substantially be ascribed to the heterogeneity of samples not belonging to single series as for artificially aged parchments. The wide-range slight deviation from reference baseline before denaturation peak may be attributable to transitions of collagen partially denaturated as a consequence of ageing. [38–39].

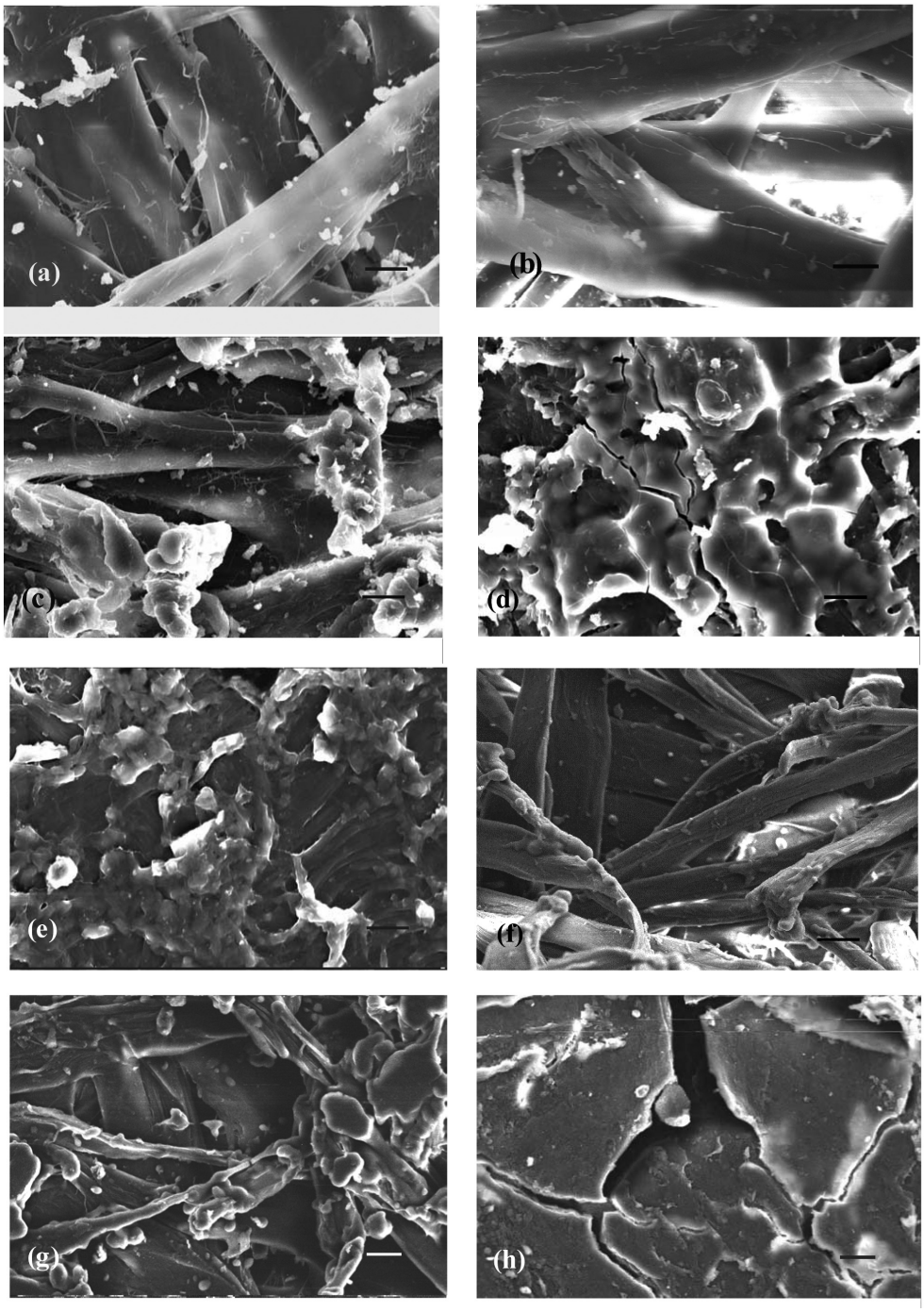

Fig. 7 High magnification SEM showing typical morphology evolution for artificially aged parchments: a – intact fibre network (ref sample); b – slight swelling and spacing of fibres after exposure to light irradiation (32 h); c – some shrunk fibres (4 h light irradiation+2 days dry heating at 100°C); d – swollen and melt-like fibres (16 h light irradiation+8 days dry heating at 100°C); e – extended melt-like surfaces and deep cracks (32 h light irradiation+16 days dry heating at 100°C); f – spaced out and shrunk fibres (16 days at 60°C and 60% RH); g – swollen, detached and weakened fibres (16 days at 80°C and 60% RH) ; h – extended glassy surface with cracks (32 days at 80°C and 80% RH).
Magnification bar: 6 μm for a to e and 10 μm for f to h

Generally, as the degree of damage increases, the peak becomes lower and wider, and a small shoulder appears on their ascending side for peaks shifted to higher temperatures, or on their descending side for those shifted to lower temperatures. The presence of a shoulder before denaturation peak can be assigned to a collagen fraction with lower thermal stability that may be outset by ageing [23, 40, 41].

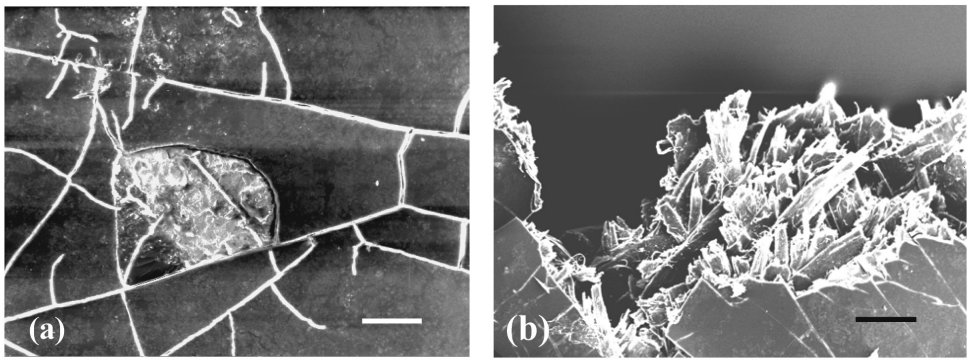

Fig. 8 SEM pictures showing bulk morphology for the SC107 sample (32 days at 80°C and 80% RH) with extended glassy surfaces: a – almost intact fibre network beneath glassy layer, b – bulk fibres at the cut edge of the sample Magnification bar: 50 μm

Scanning Electron Microscopy

New and aged parchments

SEM is a high-resolution imaging technique. Its 3D images of the network of collagen fibres were used to assess their surface state and follow progress of deterioration [42]. The two experimental techniques employed in this study are complementary: DSC provides an integral response concerning the sample mass whereas SEM furnishes a large collection of local images.

The new parchments (Table 1) used as reference displayed a network of integral collagen fibres with clear contours and sharp edges (Fig. 7a), whereas accelerated ageing resulted in changes of their shape. Samples irradiated by visible light alone (Table 2a) displayed unchanged thermodynamic parameter values, but their fibres were slightly swollen and rounded (Fig. 7b), indicating incipient deterioration detectable solely by SEM. Ageing by light irradiation and dry heating led to changes in thermal stability (Table 3, section b) together with a progressively deteriorated morphology: (*i*) random shrinkage of fibres (Fig. 7c), (*ii*) formation of melt-like zones (Fig. 7d), (*iii*) loss of fibre network and appearance of deep cracks (Fig. 7e). Deterioration following humid heating also started with slight increase of spacing between fibres and beginning of fibres shrinking (Fig. 7f), followed by swelling and apparent melting of fibres that gave rise to thin, glass-like cross-fissuring surfaces. For samples aged by humid heating at 60°C the melted zones and glass-like surfaces only appeared after 32 days, but the underlying fibrous network was still visible (Fig. 7g). Heating at 80°C produced extensive melted zones and cracks after only 8 days treatment and the samples were completely glassy after 16 days at 80% RH (Fig. 7h). These alterations correspond to small changes in the thermodynamic parameter values, compared to those measured for the dry-heated samples (Tables 3 and 4). Ageing by humid heating may thus be supposed to mainly affect the surface of a parchment. Observation of the bulk morphology of parchment at both edge fractures and internal layers showed an almost intact fibre network even in case of extended glassy-like damaged surfaces (Fig. 8).

Old parchments

The main morphological features used to draw up a scheme for ranking damage in old parchments are: a) persistence of the fibre network and b) a glassy surface. Parchments with a persistent network are divided into four classes displaying: (*i*) fibres with clear contours; (*ii*) swollen and rounded fibres; (*iii*) shrunk fibres with incipient globular aspect; (*iv*) fragmented fibres. Parchments with a glassy surface were divided into three classes displaying: (*i*) incipient melt-like surfaces; (*ii*) an extensive glassy surface; (*iii*) a glassy surface with cracks and detached layers [43].

The surface morphology of old parchments was much more varied than that of those subjected to accelerated ageing (Fig. 9). However, assignment of intensity scores to persistence of the network and to the presence of a glassy surface enabled the average level of deterioration to be determined. Damage ranking based on the DSC results was in good agreement with the SEM criteria even though the thermodynamic data essentially refer to bulk properties, whereas our SEM observations are solely concerned with the surface morphology (Table 6).

Conclusions

DSC measurement of the thermal stability of collagen in parchments and SEM investigation of their surface morphology have provided a preliminary set of criteria for the assessment of deterioration at both the mesoscopic and nanoscopic level.

The results obtained with the aged samples were used to investigate aspects of the deterioration of a parchment structure, a process whose complexity in-

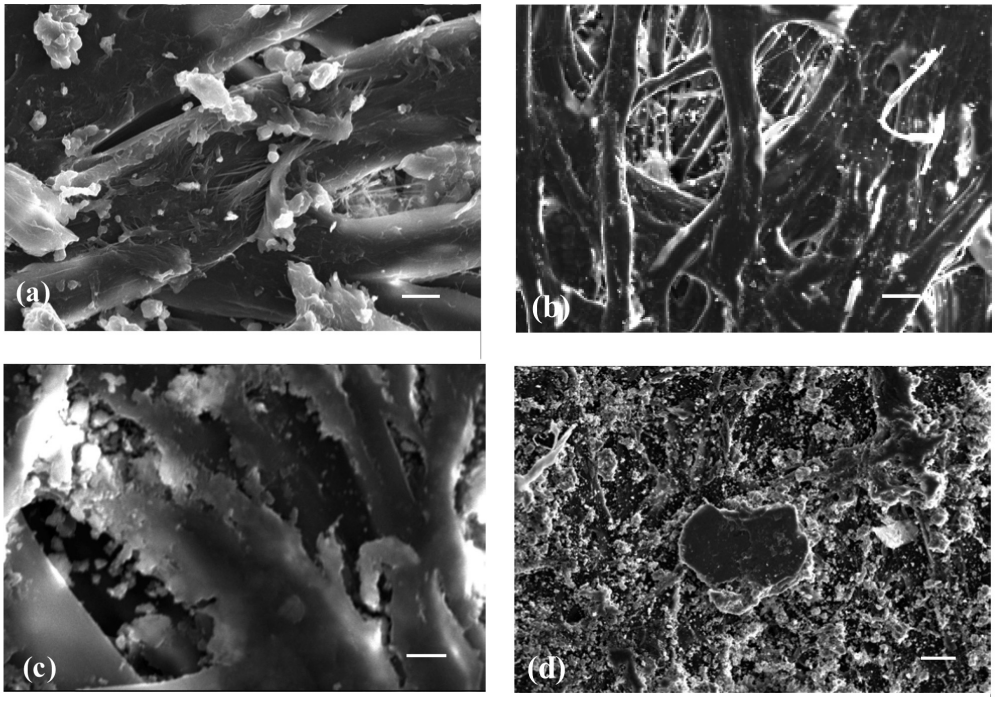

Fig. 9 High magnification SEM images illustrating typical morphology of historical parchments: a– undamaged parchment with almost intact fibre network (USH8); b– swollen and detached fibres in a slightly damaged parchment (USH3); c– initial fragmentation of fibres in a damaged parchment (HP17:2); d– severely damaged parchment with multilayer deterioration and fibre extensive fragmentation (SC75:1). Magnification bar: 6 μm for a and c, and 20 μm for b and d

volves all the hierarchical structure of parchments thus ranging from the microscopic to the mesoscopic and molecular levels.

Samples subjected to dry heating and visible light displayed appreciable deterioration. Reduction of thermal stability was reflected in decreasing of both ΔH and T_d, however the latter showed a less sharp decrease, possibly due to crosslink formation prior to deterioration through polypeptide chain cleavage. This behaviour could be explained by sample dehydration during ageing treatment, which produces crosslink formation. However, bond water removal can induce an irreversible destabilization of collagen and disrupts its structure as it has been proved by a progressive decrease of entropy when collagen is ultimately dried to 2.2% moisture content [44].

The lower effect of humid heating on thermal stability was evident in the slight changes observed in thermodynamic parameter values. Presence of humidity during ageing promotes the formation of water bridges and increases intra- and intermolecular interactions. As a consequence, collagen can withstand moderate temperatures for short exposure periods showing slightly higher T_d (Table 4).

SEM disclosed appreciable surface changes. Rounded and swollen collagen fibres appeared first, followed by thin glassy surfaces as the ageing proceeded. Glassing may be due to slight gelatinisation, since the underlying fibre network is still visible through cracks in the glassy layer.

The damage caused by accelerated ageing afforded only a partial explanation of the complex experimental evidence of deterioration displayed by the old parchments. However, it pointed to correlations between the degree of deterioration level and changes in thermal stability and surface morphology that could be used to rank damage in old parchments.

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