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A DSC study of deterioration caused by environmental chemical pollutants to parchment, a collagen-based material☆

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ABSTRACT

A DSC study of new parchments exposed at 25 °C for 1–16 weeks to controlled atmospheres containing 50 ppm of gaseous chemical pollutants (NO_2, SO_2, $NO_2 + SO_2$) and 50% relative humidity (RH) was performed. Samples were exposed to chemical pollutants alone, as well as after previous heating at 100 °C for 2–16 days and/or irradiating with visible light (1.7×10^5 lx) for 4–16 h. DSC measurements were performed in both sealed crucibles in static air atmosphere at 25–200 °C and open crucibles under gas flow (nitrogen, oxygen, synthetic air) at 25–280 °C. Analysis of DSC curves provided the variation induced by ageing on the thermodynamic parameters associated with both parchment denaturation and softening of collagen crystalline fraction. All the ageing procedures decreased both temperature and enthalpy of denaturation and increased broadness of DSC peak in function of ageing time. The occurrence of thermal oxidation peaks and/or lower temperature endothermic peaks was observed. The temperature of the first softening peak always indicated a general tendency to decrease as a function of ageing time. Shrinkage temperature of collagen fibres measured by thermomicroscopy also decreased as a result of accelerated ageing treatments.

1. Introduction

Parchment manuscripts are priceless records of the beliefs, ways of living and governing that form the roots of the European civilisation. It is known that parchment is a collagen-based biomaterial endowed with great stability, strength, resilience and exceptional longevity. However, it is sensitive to environmental damaging factors and especially to humidity associated with temperature variations [1–3]. Since the end of 19th century, atmospheric pollution has become typical of many urban areas and enhanced parchment degradation. Damage assessment and diagnosis, monitoring and modelling of the environment impact on parchment demand detailed investigations of the mechanisms and paths of collagen deterioration at all levels of its hierarchical structure. Thermal analysis methods such as TG/DTG, DTA, DSC, DMA and micro hot table (thermomicroscopy method for shrinkage temperature determination) have often been employed to characterise recently manufactured (new and artificially aged) and historical parchments to reveal changes in their thermal and mechanical stability induced by environmental conditions [4–21]. In this paper, DSC investigation of new and artificially aged parchments has been performed using either sealed crucibles in static air conditions or open crucibles under various gas flow. Moreover, the micro hot table (MHT) has been employed to correlate microscopic and mesoscopic deterioration of parchment.

2. Experimental

2.1. Materials

Accelerated ageing protocols established within the compass of the EU project IDAP (Improved Damage Assessment of Parchment) were applied at the Centre de Recherches sur la Conservation des Documents Graphiques, Paris, using a humidity and temperature test chamber (Servathin) and a dry oven (Memmert). The accelerated ageing procedures (Table 1) consisted in 1–8 consecutive steps of the following treatments:

(i) exposure to gaseous solutions of chemical pollutants (50 ppm of NO_2, 50 ppm of SO_2, 25 ppm $SO_2 + 25$ ppm NO_2) at 50% RH and 25 °C;

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Table 1
Symbols of parchments subjected to various accelerated ageing procedures using solutions of gaseous pollutants alone, and combined with light irradiation and/or dry heating.

No. ageing steps	NO_x	Light-NO_x	Heat-NO_x	Light-heat-NO_x
1	CR14	CR18	CR22	CR26
2	CR15	CR19	CR23	CR27
4	CR16	CR20	CR24	CR28
8	CR17	CR21	CR25	CR29
No. ageing steps	SO_2	Light-SO_2	Heat-SO_2	Light-heat-SO_2
1	CR30	CR34	CR38	CR42
2	CR31	CR35	CR39	CR43
4	CR32	CR36	CR40	CR44
8	CR33	CR37	CR41	CR45
No. ageing steps	$NO_x + SO_2$	Light-($NO_x + SO_2$)	Heat-($NO_x + SO_2$)	Light-heat-($NO_x + SO_2$)
1	CR46	CR50	CR54	CR58
2	CR47	CR51	CR55	CR59
4	CR48	CR52	CR56	CR60
8	CR49	CR53	CR57	CR61

Duration of the various ageing steps: pollutant exposure, 2 weeks; light irradiation, 4 h; dry heating, 2 days.

(ii) exposure to chemical pollutants, in the same condition as in (i), after a pre-treatment by irradiation with visible light (1.7×10^5 lx);
(iii) exposure to chemical pollutants, in the same condition as in (i), after a pre-treatment by heating at 100 °C in dry oven;
(iv) exposure to chemical pollutants, in the same condition as in (i), after a pre-treatment by both irradiation with visible light (1.7×10^5 lx) and dry heating (100 °C).

Duration of one ageing steps was 2 weeks for pollutant exposure, 4 h for visible light irradiation and 2 days for dry heating.

For sake of clarity, it should be taken into consideration that after NO_2–air mixing, a mixture of nitrogen oxides commonly indicated by NO_x is formed in the ageing chamber.

New parchment (SC70) from the School of Conservation, Royal Academy of Fine Arts, Copenhagen, was used to prepare artificially aged samples (Table 1) and served as the reference for DSC and MHT measurements. New parchments from INCDTP-ICPI, Bucharest, were used to complement DSC and MHT results.

2.2. *Differential scanning calorimetry*

DSC measurements in sealed stainless steel crucibles and static air atmosphere were made in the temperature range 25–200 °C, at 5 °C min^{-1} heating rate, with a Setaram DSC 111 microcalorimeter at the Department of Chemistry IFM, Turin. DSC measurements in gas flow (nitrogen, oxygen and synthetic air with purity higher than 99.999%, 20 ml min^{-1} flow rate) were performed in the temperature range 25–280 °C, at 10 °C min^{-1} heating rate, in open aluminium crucibles with a Netzsch DSC 204 F1 Phoenix calorimeter at INCDIE-ICPE-CA, Bucharest. At least three measurements for each reference subsample were performed, whereas two measurements were usually performed for artificially aged samples. Samples were analysed after a few days' storage in a controlled environment (ca. 20 °C and 50% RH) as described in Refs. [13,18].

2.3. *Shrinkage temperature measurement*

Shrinkage temperature T_s was determined by the micro hot table (MHT) method at INCDTP-ICPI, Bucharest. A Caloris micro hot table controlled by a temperature processor coupled with a Wild Heergbrugg stereo microscope (50×) assisted by a home-made software was used. Samples composed of a few fibres from flesh side of parchment were wetted with demineralised water and kept for 10 min on a microscope slide with concavity. After fibres separation, samples were covered with a slide, placed on the hot table and heated at 2 °C min^{-1}. T_s is the temperature at which two fibres simultaneously and continuously displayed shrinkage activity [21].

3. Results and discussion

3.1. *Differential scanning calorimetry in gas flow*

3.1.1. *New parchments*

DSC curves for new parchments measured in open crucibles and gas flow (Fig. 1) typically display a broad endotherm (peak I) ranging from room temperature to 150 °C associated with loss of moisture, which fully overlaps the denaturation endotherm occurring at about 120 °C [13], followed by a small endotherm (peak II) at temperatures higher than 200 °C [18–20]. Simultaneous TG/DTG + DSC analysis in nitrogen flow did not show weight loss within the temperature range of this second endotherm [12,18]. Peak II was first detected in collagen from the Achilles tendon by Okamoto and Saeki [22]. It has recently been observed by some of us for collagen-based materials (parchment and leather) and proposed

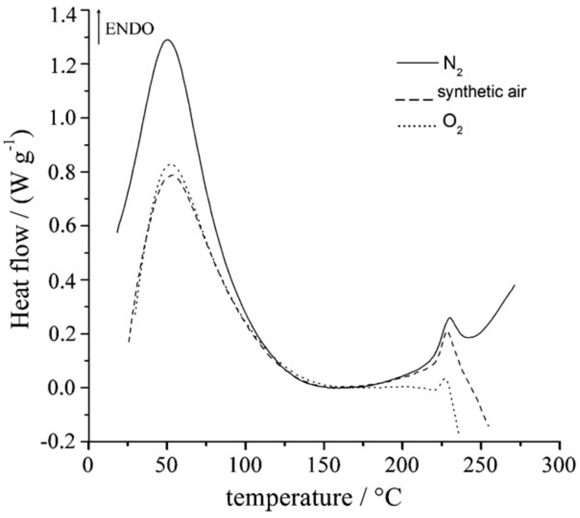

Fig. 1. DSC curves obtained in open crucibles and nitrogen/oxygen/synthetic air flow for new calf parchment displaying a broad endotherm associated with loss of moisture (peak I) and a small endotherm assigned to softening of the rigid-crystalline fraction of collagen (peak II).

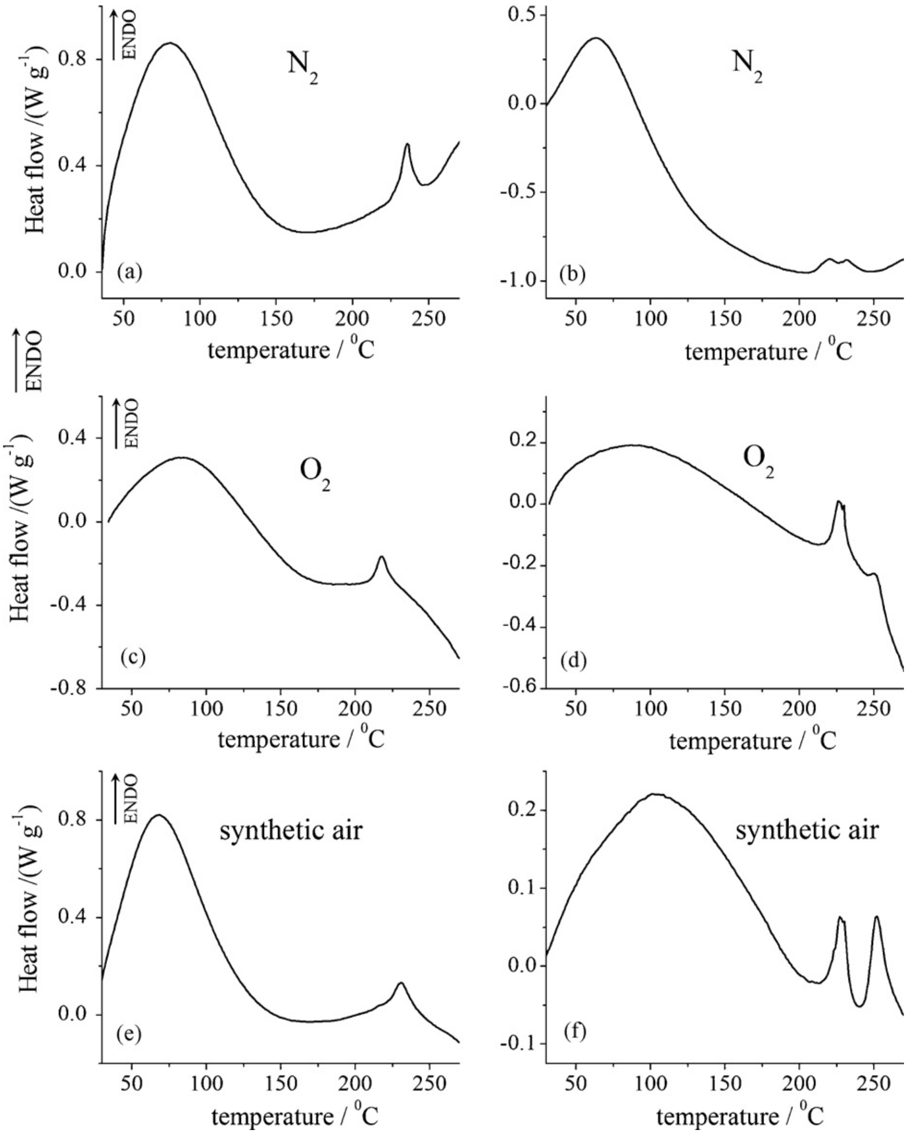

Fig. 2. DSC curves obtained in open crucibles and nitrogen/oxygen/synthetic air flow for samples exposed to SO_2: (a) CR30, (b) CR40, (c) CR37, (d) CR43, (e) CR32, and (f) CR43.

as a qualitative marker for collagen damage assessment [18–20]. It was explained by a biphasic amorphous-crystalline structure of collagen [22–24], according to which the crystalline triple-helix is embedded in an amorphous matrix and ascribed to “melting” of the crystalline fraction of collagen in parchments [18]. Very recent solid-state NMR data on pure collagen, recent manufactured parchments and leathers, historical parchments and leathers led to the elaboration of a model of collagen-based materials consisting of a rigid-crystalline phase, a mobile-amorphous phase and an interphase [20]. These phases, respectively, correspond to the crystalline collagen filaments, the amorphous collagen matrix in which they are embedded and the interface zone between the crystalline and amorphous regions. Thermal denaturation of collagen consists in the transition of ordered helix structures into random coil structures. Parchment holding its natural moisture content (about 17%) displays denaturation at 118 °C [15,25]. At this temperature, the complete denaturation of the rigid-crystalline phase is hindered by the amorphous matrix and thus thermally dehydrated (and partially denaturated) parchment usually exhibits a further helix-coil transition at higher temperature. Such a transition associated with peak II was called “melting of the collagen crystalline phase” [18,22]. In this paper, to avoid any ambiguity, we call this transition “softening” of rigid-crystalline fraction of parchment”.

Preliminary measurements on new parchments from different suppliers showed values of softening temperature, T_m, and associated enthalpy change, $\Delta_m H$, independent of flowing gas: $T_m = (231.9 \pm 3.9)\,^\circ C$, $\Delta_m H = 6.4 \pm 1.4\,J\,g^{-1}$ in nitrogen flow; $T_m = (228.3 \pm 3.2)\,^\circ C$, $\Delta_m H = (5.9 \pm 1.1)\,J\,g^{-1}$ in oxygen flow; $T_m = (229.9 \pm 3.9)\,^\circ C$, $\Delta_m H = (7.9 \pm 1.9)\,J\,g^{-1}$ in synthetic air flow. In particular, T_m seems to be a valuable indicator for undamaged parchments since its uncertainty in different flowing gases is lower than the intrinsic uncertainty of the measurements for each flowing gas.

3.1.2. Parchments subjected to accelerated ageing

All parchment samples exposed to SO_2 and SO_2 combined with light irradiation displayed one softening peak at $T > 200\,^\circ C$ (Fig. 2a, c and e), whereas for samples pre-heated at 100 °C two softening peaks (Fig. 2b, d and f) were generally observed. Those exposed to NO_x or $NO_x + SO_2$ displayed one softening peak (Figs. 3a and 4a, c, e) or one softening peak followed by an exothermic peak (Fig. 3b and e), or one/two exothermic peaks (Figs. 3c, d, f and 4b, d, f). Exother-

Table 2

Softening temperature, T_m, and enthalpy change, $\Delta_m H$ values for parchments exposed to NO_x alone and combined with light irradiation and/or dry heating measured by DSC in open crucibles under gas flow.

Sample	n	Nitrogen flow		Oxygen flow		Synthetic air flow	
		T_m (°C)	$\Delta_m H$ (J g^{-1})	T_m (°C)	$\Delta_m H$ (J g^{-1})	T_m (°C)	$\Delta_m H$ (J g^{-1})
SC70[a]	0	237.7 ± 0.9	5.3 ± 0.3	233.0 ± 1.1	5.4 ± 0.3	234.6 ± 1.2	9.9 ± 0.8
NO_x							
CR14	1	233.2	7.2	231.1	3.8	230.6	4.9
CR15	2	210.3	6.9	208.2	5.8	209.2	4.0
CR16	4	200.5	6.4	[b]	[b]	199.7	8.9
CR17	8	202.6	3.2	[b]	[b]	200.7	4.5
Light-NO_x							
CR18	1	223.5	11.6	222.0	13.7	224.6	6.1
CR19	2	212.0	3.8	209.4	4.0	210.6	4.4
CR20	4	202.6	4.5	[b]	[b]	203.0	11.4
CR21	8	195.9	[c]	[b]	[b]	194.3	8.4
Heat-NO_x							
CR22	1	228.7 229.9	3.8	225.5	7.0	228.2	8.3
CR23	2	218.9	8.2	214.8	6.6	217.7	5.9
CR24	4	205.8	2.2	[b]	[b]	197.7	8.9
CR25	8	210.3	4.2	[b]	[b]	211.5	0.9
Light-heat-NO_x							
CR26	1	225.6	3.4	221.3	4.4	224.0	5.4
CR27	2	227.3	7.6	221.4	6.1	227.7	5.1
CR28	4	211.8	4.8	210.6	2.8	212.4 228.3	2.4
CR29	8	210.8 213.0	1.9	[b]	[b]	[b]	[b]

Mean values from two measurements. n = number of ageing steps.

[a] Average values obtained from three measurements on each of the four reference subsamples. Uncertainty is the standard deviation.

[b] Melting process not observed because of its complete overlapping with exothermic oxidation.

[c] $\Delta_m H$ not evaluated since the end of endothermic process partially overlaps the onset of the exothermic oxidation.

Table 3

Softening temperature, T_m, and enthalpy change, $\Delta_m H$ values for parchments exposed to SO_2 alone and combined with light irradiation and/or dry heating measured by DSC in open crucibles under gas flow.

Sample	n	Nitrogen flow		Oxygen flow		Synthetic air flow	
		T_m (°C)	$\Delta_m H$ (J g^{-1})	T_m (°C)	$\Delta_m H$ (J g^{-1})	T_m (°C)	$\Delta_m H$ (J g^{-1})
SC70[a]	0	237.7 ± 0.9	5.3 ± 0.3	233.0 ± 1.1	5.4 ± 0.3	234.6 ± 1.2	9.9 ± 0.8
SO_2							
CR30	1	236.1	9.8	233.1	6.5	235.8	4.1
CR31	2	230.4 234.0	11.1	[b]	[b]	231.1	3.8
CR32	4	230.6	9.9	225.6	8.5	230.8	6.7
CR33	8	216.1	8.3	219.3	8.6	223.2	5.7
Light-SO_2							
CR34	1	234.8	10.3	231.9	7.5	235.6	7.1
CR35	2	232.1	9.3	226.6	6.4	229.8	10.3
CR36	4	230.4	9.0	228	5.9	227.1	10.3
CR37	8	220.3	6.6	217.5	6.3	218.0	7.9
Heat-SO_2							
CR38	1	235.6	8.7	231.4	7.2	223.9 235.6	4.1
CR39	2	223.9	9.0	226.7	7.3	224.9	7.7
CR40	4	220.0 231.2	9.7	213.1	6.3	226.8	6.3
CR41	8	206.1 211.8 228.5	1.5 0.9	217.0	6.3	211.4	0.9
Light-heat-SO_2							
CR42	1	235.9	8.9	233.9	7.4	235.8	6.3
CR43	2	226.3 229.1	8.9	226.8 229.5	8.6	226.9	5.0
CR44	4	216.3 232.1	0.3 1.8	[b]	[b]	234.0	6.0
CR45	8	202.5 226.5	0.6 2.3	[b]	[b]	205.8	1.6

Mean values from two measurements. n = number of ageing steps.

[a] Average values obtained from three measurements on each of the four reference subsamples. Uncertainty is the standard deviation.

[b] Melting process not observed because of its complete overlapping with exothermic oxidation.

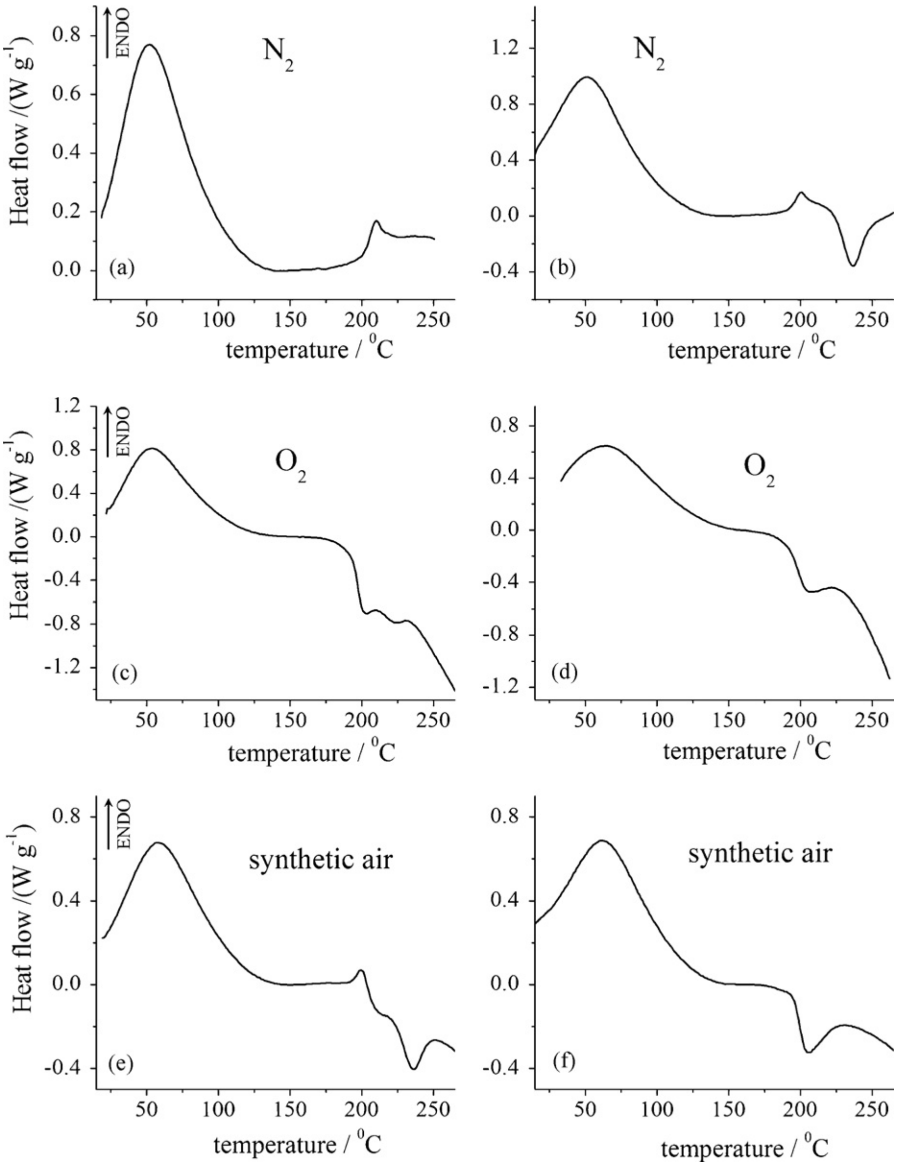

Fig. 3. DSC curves obtained in open crucibles and nitrogen/oxygen/synthetic air flow for samples exposed to NO_x: (a) CR15, (b) CR25, (c) CR21, (d) CR29, (e) CR16, and (f) CR29.

mic peaks mostly occur at temperature higher than 200 °C. As a consequence, exothemic peaks are in the first instance attributable to oxidation induced by exposure to the oxidation agent NO_x. The temperature of the first softening peak always indicated a general tendency to decrease with the number of ageing steps (Tables 2–4). We previously reported that T_m increases with cross-linking degree in collagen-based materials (e.g. parchment and leather) [19]. Results in Tables 2–4 thus indicate a decrease of cross-linking degree in parchment as a function of the number of ageing steps. Multiple softening peaks are probably due to the heterogeneity of the deteriorated parchment induced by random fragmentation of the original matrix during ageing [20]. The closeness of the T_m values in synthetic air and in nitrogen flow indicated good stability of the samples to thermal oxidation, though T_m values in oxygen flow were generally lower than those measured in nitrogen flow. Pre-heating at 100 °C weakens parchment structure and increases its susceptibility to the oxidation mediated by NO_x. No softening peaks were thus observed for longer ageing periods of pre-heated samples (e.g. CR24, CR25, CR29, CR44, CR45, CR57, CR61) as thermal oxidation compensates and even exceeds endothermic melting (Figs. 3b, d, f and 4b, f). The exothermic peaks could be due to reactive oxygenated groups formed during the exposure to NO_x/$NO_x + SO_2$ [17,25] which lead to oxidation of the collagen matrix at a relatively low temperature. High oxidation level induced by NO_x is evidenced by the presence of exothermic peaks in inert gas flow, i.e. N_2 as for CR25 (Fig. 3b). Disappearance of softening peak and exothermic peak shifting to temperature lower than 200 °C as for CR57 and CR61 (Fig. 4b and f) indicate a high degree of structure disrupting induced by extensive oxidation.

3.2. *Differential scanning calorimetry in sealed crucibles*

Sealed stainless steel crucibles do not allow any release from samples, and their moisture content remains constant over measurement temperature ranges. This means that the main endothermic peak is now related to thermal denaturation only [4,13,15].

3.2.1. *New parchments*

DSC evidence for new parchments consists of a narrow endothermic peak at 120–125 °C attributable to thermal denaturation of fibrillar collagen as showed in Fig. 5 [13]. Table 5 sets

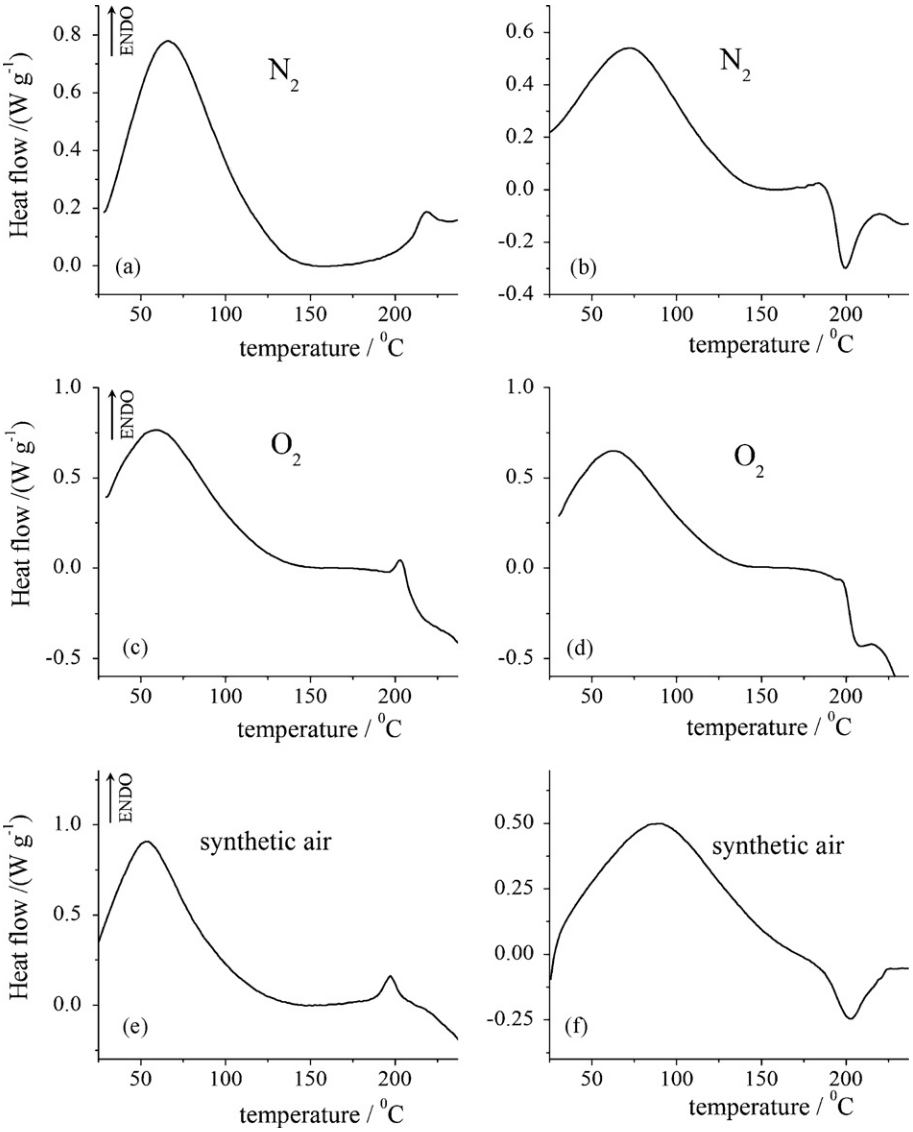

Fig. 4. DSC curves obtained in open crucibles and nitrogen/oxygen/synthetic air flow for samples exposed to ($NO_x + SO_2$): (a) CR46, (b) CR61, (c) CR51, (d) CR56, (e) CR49, and (f) CR57.

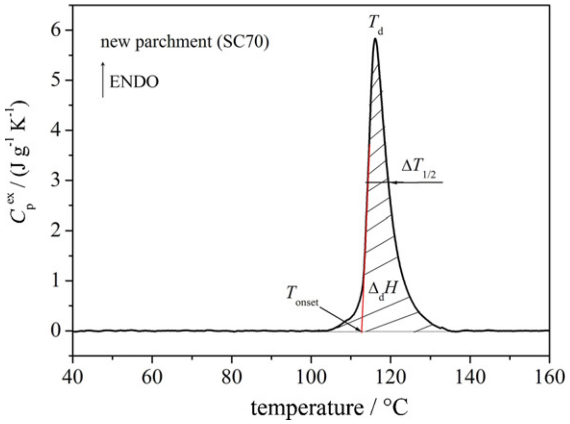

Fig. 5. Typical DSC curve in sealed crucible for thermal denaturation of fibrillar collagen in new parchments: T_d = temperature of denaturation; $\Delta_d H$ = enthalpy of denaturation (peak area); $\Delta T_{1/2}$ = peak half-width.

out average values of thermodynamic parameters, T_{onset}, T_d, $\Delta T_{1/2}$ and $\Delta_d H$, of thermal denaturation of new calf parchments from two manufacturers and their averaged values. Very low uncertainties (ca. 1–5%) show that thermodynamic parameters from DSC measurements are valuable indicators for undamaged parchments.

3.2.2. Parchments subjected to accelerated ageing

According to previous DSC studies, lower T_d and $\Delta_d H$ values reflect decreased stability of collagen caused by its progressive deterioration. A broader shape of the peak, measured by its half-width $\Delta T_{1/2}$, as well as the occurrence of small shoulders, correspond to an increased diffuse or specific heterogeneity in terms of thermal stability [2,13,15].

Changes of DSC denaturation peak parameters provoked by the chemical pollutants alone and combined with light irradiation and/or dry heating, as a function of the number of ageing steps, are reported in Tables 6–8. All the ageing procedures decreased T_d and $\Delta_d H$, and increased $\Delta T_{1/2}$ in function of exposure time. The T_d maximum decrease does not exceed 30% even in the case of longest exposure times to pollutant and dry heat (i.e. CR25, CR41, CR57) and to pollutant, dry heat and light irradiation (i.e. CR29, CR45,

Table 4
Softening temperature, T_m, and enthalpy change, $\Delta_m H$, values for parchments exposed to $NO_x + SO_2$ alone and combined with light irradiation and/or dry heating measured by DSC in open crucibles under gas flow.

Sample	n	Nitrogen flow		Oxygen flow		Synthetic air flow	
		T_m (°C)	$\Delta_m H$ (J g^{-1})	T_m (°C)	$\Delta_m H$ (J g^{-1})	T_m (°C)	$\Delta_m H$ (J g^{-1})
SC70[a]	0	237.7 ± 0.9	5.3 ± 0.3	233.0 ± 1.1	5.4 ± 0.3	234.6 ± 1.2	9.9 ± 0.8
$NO_x + SO_2$							
CR46	1	218.7	4.6	214.2	4.2	218.4	10.9
CR47	2	202.8	5.0	197.7	6.7	203.1	11.5
CR48	4	199.0	6.0	201.7	0.5	197.3 200.0	5.0
CR49	8	199.5	7.6	197.4	4.9	197.4	9.7
Light-($NO_x + SO_2$)							
CR50	1	220.6	4.4	212.9	6.0	214.6 216.1	11.1
CR51	2	205.3	7.2	202.9	9.7	205.6	8.9
CR52	4	[b]	[b]	199.8	0.2	[b]	[b]
CR53	8	196.4	8.3	194.5	9.1	196.8	5.7
Heat-($NO_x + SO_2$)							
CR54	1	220.2	4.2	214.3	7.4	215.1	3.3
CR55	2	222.9	2.9	216.2	2.9	222.5	7.7
CR56	4	201.9	6.9	[b]	[b]	200.7	7.8
CR57	8	[b]	[b]	[b]	[b]	[b]	[b]
Light-heat-($NO_x + SO_2$)							
CR58	1	218.4 225.3	3.3	215.3	[c]	220.7	8.7
CR59	2	210.5	4.7	209.4	[c]	213.7	9.0
CR60	4	204.0	7.5	199.1	6.6	200.5	7.2
CR61	8	[b]	[b]	[b]	[b]	[b]	[b]

Mean values from two measurements. n = number of ageing steps.

[a] Average values obtained from three measurements on each of the four reference subsamples. Uncertainty is the standard deviation.

[b] Melting process not observed because of its complete overlapping with exothermic oxidation.

[c] $\Delta_m H$ not evaluated since the end of endothermic process partially overlaps the onset of the exothermic oxidation.

CR61), whereas the $\Delta_d H$ decreasing is more abrupt, namely about 40% for samples exposed to pollutant and dry heat and more than 50% for those exposed to pollutant, dry heat and light irradiation. $\Delta T_{1/2}$ shows, as expected, an opposite trend and its increase reach more than 300% for samples previously subjected to dry heating or light irradiation and dry heating for more than 4 ageing steps. Percent variations in Tables 6–8 indicate that dry heating strongly influences all DSC parameters and enhances the damaging effect of chemical pollutants, whereas previous exposure to light has less influence. We have already reported that simple visible light irradiation (1.5×10^5 lx) of SC70 subsamples does not produce systematic effects after 8 ageing steps, whereas dry heating (100 °C) and combined dry heating and light irradiation result in a progressive decrease of both T_d and $\Delta_d H$, and increase of $\Delta T_{1/2}$ with ageing time [13]. On the other hand, statistical analysis (ANOVA, PCA) confirmed that dry heating induced the most significant variation of DSC parameters. The number of ageing steps showed a discriminating power similar to that of heating, whereas chemical pollutants alone showed a lower discriminating power, with SO_2 being less discriminative than NO_x.

Deterioration level of aged samples can be evaluated from the departure of T_d, $\Delta_d H$ and $\Delta T_{1/2}$ from the reference values by using the simple quantitative scheme we have proposed to grade historical parchments deterioration [17,26].

The DSC peak shifted to lower temperature with ageing time, and became shorter and broader following deterioration trends illustrated by their parameters' variation. In addition, some peaks displayed a less sharp onset or even a small shoulder on the ascending part of the curve (Fig. 6c, curves CR58 and CR59) attributable to the increasing formation of loosened collagen fractions. Lower T_d reflect a decreased stability of the residual native (not denatured during the previous accelerated damage) collagen, while the broad shape of the peak and/or the presence of shoulders correspond to an increased dispersion in terms of stability of the still intact fibrils. Further evidence appears in the form of a small, broad endothermic peak at 50–70 °C (Fig. 6a–c) which increases with ageing time, as illustrated in Fig. 7. This peak and its increase, as well as the small shoulder, were already observed for parchments solely exposed to dry heating at 100 °C (Fig. 8) [17]. Since, according to Wess and Orgel [8], dry heating alters polypeptide chains within the gap region of microfibrils, the peak at 50–70 °C could be ascribed to parchment deterioration at molecular level. The presence of this endothermic peak at low temperature correlates with the occurrence of multiple softening peaks evidenced

Table 5
Thermodynamic parameters for thermal denaturation of new parchments measured by DSC in sealed crucibles under static air atmosphere.

Sample	T_{onset} (°C)	T_d (°C)	$\Delta T_{1/2}$ (°C)	$\Delta_d H$ (J g^{-1})
New parchment SC70[a]	118.3 ± 0.5	121.1 ± 0.9	5.6 ± 0.1	34.2 ± 0.3
New parchments ICPI[b]	111.3 ± 1.9	117.2 ± 2.1	6.3 ± 0.3	34.3 ± 0.7
Average[c]	114.8 ± 2.0	119.2 ± 2.3	6.0 ± 0.3	34.3 ± 0.8

[a] Values obtained from measurements on four SC70 subsamples provided by the School of Conservation, Copenhagen [13]. Uncertainty is the standard deviation.

[b] Values obtained from measurements on four different new parchments provided by ICPI, Bucharest. Uncertainty is the standard deviation.

[c] Average values with their combined uncertainties.

Table 6
Thermodynamic parameters for thermal denaturation of parchment exposed to NO_x alone and combined with light irradiation and/or dry heating measured by DSC in sealed crucibles under static air atmosphere.

Sample	n	T_d		$\Delta_d H$		$\Delta T_{1/2}$	
		°C	%	J g^{-1}	%	°C	%
SC70[a]	0	121.1 ± 0.9	100	34.2 ± 0.3	100	5.6 ± 0.1	100
NO_x							
CR14	1	116.3	96.0	32.3	94.4	5.6	100
CR15	2	106.0	87.5	27.1	79.2	7.4	132
CR16	4	97.7	80.7	26.5	77.5	6.4	114
CR17	8	96.6	79.8	24.0	70.2	6.3	113
Light-NO_x							
CR18	1	112.3	92.7	29.1	85.1	6.1	109
CR19	2	104.8	86.5	26.5	77.5	7.3	130
CR20	4	96.2	79.4	27.6	80.7	6.6	118
CR21	8	91.3	75.4	27.1	79.2	6.4	114
Heat-NO_x							
CR22	1	106.9	88.3	31.5	92.1	7.2	129
CR23	2	102.5	84.6	28.0	81.9	10.1	180
CR24	4	93.6	77.3	23.7	69.3	15.5	277
CR25	8	88.7	73.2	19.9	58.2	18.7	334
Light-heat-NO_x							
CR26	1	104.5	86.3	27.5	80.4	8.9	159
CR27	2	103.8	85.7	25.7	75.1	8.8	157
CR28	4	91.8	75.8	25.3	74.0	12.3	220
CR29	8	88.4	73.0	17.2	50.3	20.8	371

Mean values from two measurements. n = number of ageing steps.

[a] Average values from Table 5.

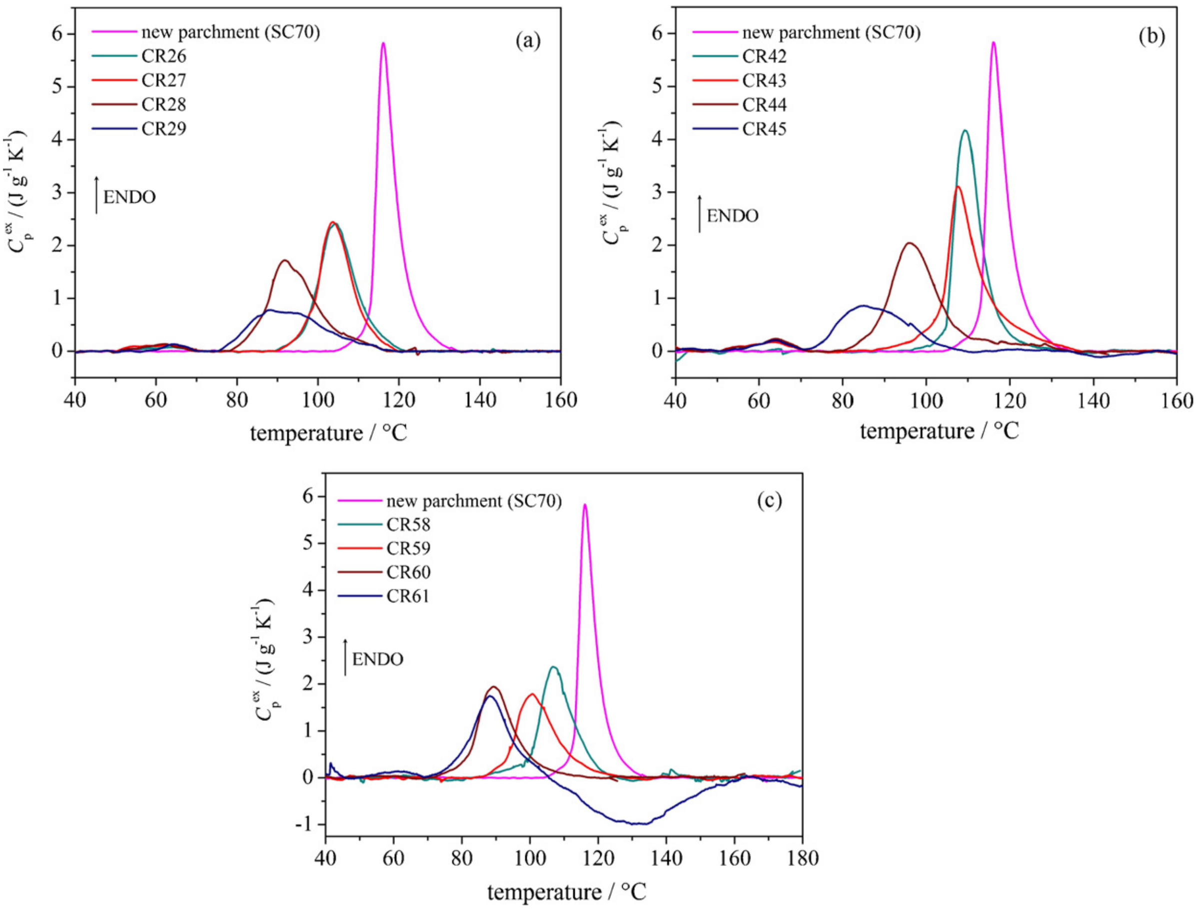

Fig. 6. DSC curves obtained in sealed crucibles for samples exposed for increasing time to: (a) light irradiation, dry heating and NO_x; (b) light irradiation, dry heating and SO_2; (c) light irradiation, dry heating and $NO_x + SO_2$.

Table 7
Thermodynamic parameters for thermal denaturation of parchment exposed to SO_2 alone and combined with light irradiation and/or heating measured by DSC in sealed crucibles under static air atmosphere.

Sample	n	T_d		$\Delta_d H$		$\Delta T_{1/2}$	
		°C	%	J g^{-1}	%	°C	%
SC70[a]	0	121.1 ± 0.9	100	34.2 ± 0.3	100	5.6 ± 0.1	100
SO_2							
CR30	1	117.5	97.0	33.8	98.8	5.7	102
CR31	2	114.5	94.5	33.2	97.1	6.0	107
CR32	4	112.0	92.5	32.3	94.4	6.5	116
CR33	8	107.7	88.9	31.9	93.2	6.8	121
Light-SO_2							
CR34	1	95.0	96.5	34.0	99.4	6.0	107
CR35	2	91.6	93.0	32.1	93.9	6.3	113
CR36	4	91.5	93.0	32.4	94.7	6.7	120
CR37	8	85.6	87.0	31.6	92.4	7.6	136
Heat-SO_2							
CR38	1	89.3	90.8	30.8	90.1	7.3	130
CR39	2	79.9	81.1	28.8	84.2	9.2	164
CR40	4	76.6	77.9	27.4	80.1	10.0	179
CR41	8	72.7	73.8	20.5	59.9	16.4	293
Light-heat-SO_2							
CR42	1	90.2	91.6	30.7	89.8	7.0	125
CR43	2	88.8	90.2	28.2	82.5	7.7	138
CR44	4	79.3	80.5	26.9	78.7	12.0	214
CR45	8	70.7	71.8	15.2	44.4	19.4	346

Mean values from two measurements. n = number of ageing steps.
[a] Average values from Table 5.

by DSC in open crucibles and gas flow (e.g. CR22, CR25, CR29, CR44, CR45 in Tables 2 and 3 and in Figs. 6a, b and 9). Moreover, because the main effect of dehydrothermal treatment is to modulate the fibrillar packing prior to alteration of the helical structure [8,27], the small shoulder in the ascending part of DSC curve for samples subjected to 2–4 ageing steps can be assigned to the presence of a fraction of collagen with only fibrillar deterioration [2,13,15]. Dry heating and NO_x exposure were strongly oxidative, whereas SO_2 is known to mainly induce hydrolysis of peptide chains [25,28]. The deterioration of samples observed in this study can thus be referred to scission of inter-fibrillar bonds followed by cleavage of covalent bonds caused by peptide chain oxidation and hydrolysis.

Samples exposed to $NO_x + SO_2$ and dry heating (CR57) or $NO_x + SO_2$, light irradiation and dry heating (CR61) for 8 ageing steps displayed an exothermic peak at about 130 °C, just following denaturation. Since new, undamaged parchment undergoes oxidative decomposition at $T > 230$ °C [25], we could infer that this peak,

Table 8
Thermodynamic parameters for thermal denaturation of parchment exposed to $NO_x + SO_2$ alone and combined with light irradiation and/or heating measured by DSC in sealed crucibles under static air atmosphere.

Sample	n	T_d		$\Delta_d H$		$\Delta T_{1/2}$	
		°C	%	J g^{-1}	%	°C	%
SC70[a]	0	121.1 ± 0.9	100	34.2 ± 0.3	100	5.6 ± 0.1	100
$NO_x + SO_2$							
CR46	1	113.6	93.8	32.5	95.0	7.9	141
CR47	2	111.2	91.8	31.7	92.7	6.9	123
CR48	4	105.4	87.0	27.2	79.5	8.6	154
CR49	8	102.1	84.3	25.9	75.7	6.5	116
Light-($NO_x + SO_2$)							
CR50	1	113.0	93.3	34.8	101.8	7.3	130
CR51	2	110.1	90.9	32.6	95.3	7.0	125
CR52	4	102.1	84.3	31.6	92.4	8.8	157
CR53	8	103.1	85.1	30.7	89.8	7.6	136
Heat-($NO_x + SO_2$)							
CR54	1	102.1	80.0	33.9	99.1	13.3	238
CR55	2	96.9	84.3	26.7	78.1	9.4	168
CR56	4	95.1	78.5	23.0	67.3	7.5	134
CR57	8	93.4	77.1	21.5	62.9	20.8	371
Light-heat-($NO_x + SO_2$)							
CR58	1	106.8	88.2	29.1	85.1	10.3	184
CR59	2	100.8	83.2	25.9	75.7	12.2	218
CR60	4	89.2	73.7	20.1	58.8	11.6	207
CR61	8	88.3	72.9	16.4	48.0	11.7	209

Mean values from two measurements. n = number of ageing steps.
[a] Average values from Table 5.

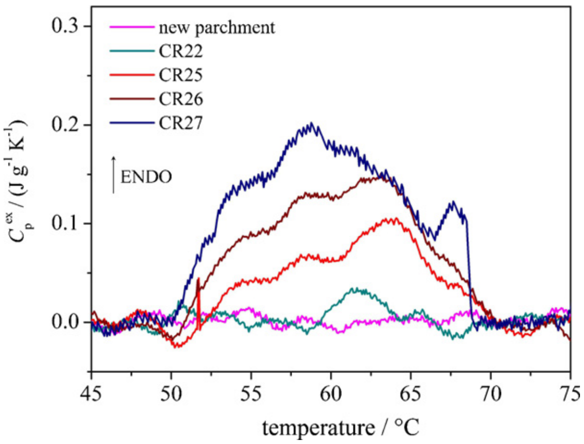

Fig. 7. Magnified view of the broad endothermic peak occuring at 50–70 °C for samples exposed for increasing time to dry heating and NO_x (CR22 and CR25) and light irradiation, dry heating, and NO_x (CR26 and CR27).

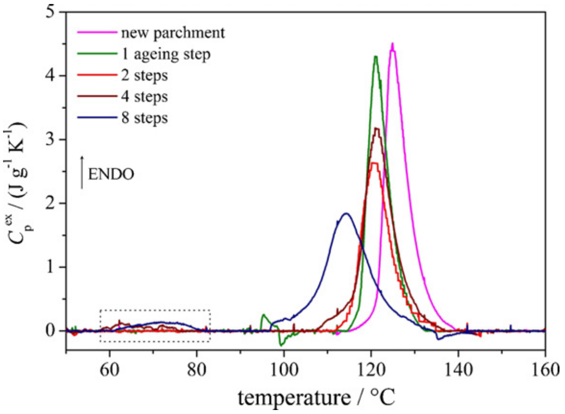

Fig. 8. DSC curves obtained in sealed crucibles for samples exposed to dry heating at increasing time (1 step = 2 days) from Ref. [17].

not yet reported in the literature, is due to the decomposition of a fraction of collagen heavily degraded by the combined action of chemical pollutants and dry heating. LC–MS analysis revealed a strong oxidative damage disrupting the parchment structure in such conditions [29]. This process can be tentatively supposed to progress during DSC measurement giving rise to exothermic events at lower temperature. The high susceptibility to oxidation of CR57 and CR61 is confirmed by the DSC measurements in open crucibles: softening peaks are no longer present and exothermic peaks occur at $T < 200$ °C in both inert and oxidising atmosphere (Fig. 10).

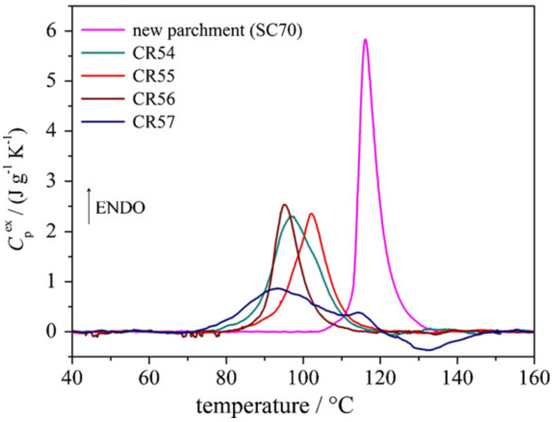

Fig. 9. DSC curves obtained in sealed crucibles for samples exposed for increasing time to dry heating and ($NO_x + SO_2$).

3.3. Shrinkage temperature measurements

Shrinkage temperature, T_s, characterises hydrothermal stability of collagen-based materials, as described in Section 2.3, and is considered a highly valuable parameter for a broad range of research and conservation applications for skin and leather materials in museums, libraries and archives. Indeed, very good correlation between T_s and the onset of DSC denaturation peak, T_{onset}, for samples analysed in wet conditions has been reported for new and artificially aged parchments [19]. For new parchments we obtained the average value $T_s = (57.8 \pm 3.8)$ °C in very good agreement with $T_s = (57.9 \pm 3.0)$ °C reported by Larsen et al. [21]. However, heterogeneity of deterioration in historical parchments as well as “local” character of sampling makes T_s a rather poor indicator for evaluation of their environmental deterioration [30]. Establishment of

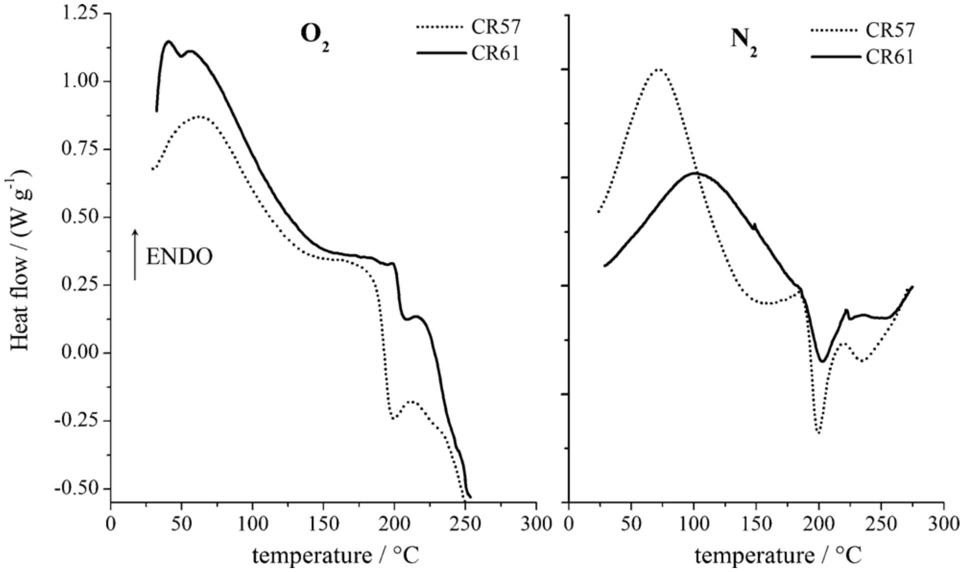

Fig. 10. DSC curves obtained in open crucibles and nitrogen/oxygen flow for samples exposed to dry heating and $NO_x + SO_2$ (CR57) and light irradiation, dry heating and $NO_x + SO_2$ (CR61).

Table 9
Shrinkage temperature, T_s, of parchments exposed to pollutants alone and combined with light irradiation and/or heating measured by MHT.

n	Sample	T_s		Sample	T_s		Sample	T_s	
		°C	%		°C	%		°C	%
0	Reference	63.0	100		63.0	100		63.0	100
	NO_x			SO_2			$NO_x + SO_2$		
1	CR14	51.2	81.3	CR30	49.9	79.2	CR46	45.1	71.6
2	CR15	48.2	76.5	CR31	49.5	78.6	CR47	47.1	74.7
4	CR16	49.4	78.4	CR32	53.0	84.1	CR48	48.7	77.39
8	CR17	49.5	78.6	CR33	46.7	74.1	CR49	42.1	66.8
	Light-NO_x			Light-SO_2			Light-($NO_x + SO_2$)		
1	CR18	56.5	89.7	CR34	50.2	79.7	CR50	43.7	69.4
2	CR19	47.0	74.6	CR35	50.0	79.4	CR51	40.8	64.8
4	CR20	44.7	71.0	CR36	46.7	74.1	CR52	46.4	73.7
8	CR21	34.7	55.1	CR37	43.7	69.4	CR53	39.2	62.2
	Heat-NO_x			Heat-SO_2			Heat-($NO_x + SO_2$)		
1	CR22	43.7	69.4	CR38	38.0	60.3	CR54	45.8	72.7
2	CR23	38.7	61.4	CR39	32.5	51.6	CR55	40.7	64.6
4	CR24	37.7	59.8	CR40			CR56	47.5	75.4
8	CR25			CR41			CR57	46.3	73.5
	Light-heat-NO_x			Light-heat-SO_2			Light-heat-($NO_x + SO_2$)		
1	CR26	38.9	61.7	CR42	39.7	63.0	CR58	46.8	74.3
2	CR27	36.0	57.1	CR43	33.2	52.7	CR59	39.2	62.2
4	CR28	35.5	56.3	CR44			CR60	35.8	56.8
8	CR29			CR45			CR61		

n = number of ageing steps. Mean values from at least three measurements. Maximum standard deviation of MHT determinations was lower than 5%.

a correlation between T_s and other DSC data would improve the content of the information provided by MHT measurements.

Our results (Table 9) indicate a sharper decreasing of T_s by comparison with T_d, especially for samples subjected to 4–8 ageing steps. The T_s values for 1–2 steps confirm this pattern, though some contradictory results were obtained for relatively low damage, since sampling uncertainty plays a not negligible role. Sampling of surface fibres emphasizes the heterogeneity of low and medium levels of damage. For damaged samples, heterogeneity can no longer influence the average value of T_s. Heavily damaged samples showed substantial decrease of T_s until the shrinkage activity ceased as in case of combination of dry heating with SO_2 (i.e. CR40, CR41, CR44, CR45) or NO_x/$NO_x + SO_2$ (CR25, CR29, CR57, CR61) (Table 9). This behaviour would suggest complete disruption of collagen fibrillar structure. In fact, these samples display molecular damage indicated by low temperature endothermic peaks (Figs. 6 and 9), high oxidation level illustrated by data in Tables 2–4 and DSC curves in Fig. 10, and a 50–60% denaturation of native collagen (Tables 6–8). However, in spite of their heavy and complex deterioration, collagen structures are still present in these samples. Their weakened structures become highly instable in the aqueous milieu, which enhanced deterioration level as revealed by our DSC measurements in excess water conditions [17], resulting in a complete loss of shrinkage activity. As a consequence, the use of T_s data, even though provides an overall indication of deterioration, can easily become misleading if not correlated with other data.

4. Conclusions

DSC measurements enabled us to evaluate accelerated deterioration of parchment due to ageing by exposure to gaseous chemical pollutants (SO_2, NO_x) at 25 °C and 50% RH. The synergic effect of ageing pre-treatments, i.e. dry heating and/or visible light irradiation, was also assessed.

(A) Experimental evidences of deterioration were extensively analysed and DSC indicators associated with collagen denaturation were employed to assess the damage level of parchment.
  (i) Deterioration of fibrillar collagen in parchment holding its natural moisture content was evaluated from the departure of denaturation parameters T_d (denaturation temperature), $\Delta_d H$ (associated enthalpy) and $\Delta T_{1/2}$ (peak half-width) from the reference values. A general ageing trend characterised by progressive shift of T_d to temperatures lower than 120 °C, DSC peak shape broadening and lowering, i.e. increase of $\Delta T_{1/2}$ and decrease of $\Delta_d H$, was observed. The small shoulders in the ascending part of the DSC denaturation curve were assigned to a fraction of collagen with high fibrillar deterioration.
  (ii) The softening peak at $T > 200$ °C attributed to denaturation of a small intact fraction of rigid-crystalline collagen still present in almost completely denaturated parchment provides further information on parchment's deterioration. Generally, its temperature, T_m, decreases with deterioration until the complete disappearance of the peak.

(B) Correlation between the DSC results obtained with open and sealed crucibles provided some further deterioration markers for molecular level of parchment. The endothermic peak detectable at 50–70 °C using sealed crucibles was ascribed to parchment deterioration at molecular level, i.e. loss of triple-helical structure of collagen. This peak correlates with the occurrence of multiple softening peaks revealed by DSC in gas flow.

(C) Strong oxidative damage induced by NO_x generates exothermic peaks even in inert gas flow in open crucibles, whereas in sealed crucibles, it is revealed by the occurrence of exothermic peaks following that of denaturation.

(D) Weakened structures of heavily damaged parchments highly instable in the aqueous milieu are characterised by much lower shrinkage temperature T_s and even lack of shrinkage temperature.

(E) Generally, pollutants' attack is significantly enhanced by preheating treatments, whereas light irradiation shown a slight power to potentiate pollutant-induced damage.

Acknowledgements

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