The protection of parchment collections in public and private libraries, archives, museums face nowadays several challenges such as environmental pollution, climate change, limited energy consumption, lack of testing/diagnosis protocols in conservation science as well as lack of standardisation of best practices in conservation. In this paper, the applications of thermal microscopy (imageMHT) and unilateral NMR to surface characterisation of parchments are presented. As parchment is a biomaterial with hierarchical structure characterised by the intimate relationship and connectivity between individual molecules and their organisation in micro-fibrils, fibrils and fibres, a micro to nano-scale investigation protocol is required for a reliable assessment.

Keywords: parchment, imageMHT, unilateral NMR.

INTRODUCTION

Parchment is a biomaterial endowed with great stability, strength and resilience, together with exceptional longevity. Studies carried out in the last decades showed that historical parchments are, however, especially sensitive to temperature and relative humidity variations in the storage environment, or elicited by the conservation and/or restoration methods (Hansen et al., 1992; Bowden and Brimblecombe, 2002). Deterioration is often more advanced on the parchment surface which may prevalently contain destabilised collagen or even gelatin. A stiff glassy surface with a flexible fibre layer below is a very risky condition for the preservation of writings and illuminations since the surface rigid layer is subjected to mechanical stress induced by even small variations of relative humidity and temperature due to its different capability of contraction and swelling compared with the underlying fibres. Characterisation of damage related to temperature and relative humidity was mainly achieved through the examination of parchments exposed to accelerated ageing procedures (Badea et al., 2012a; Della Gatta et al., 2007). Appropriate advanced physical-chemical and structural investigation techniques were developed in the compass of several EC, international and national projects, i.e. IDAP (EVK4-CT-2001-00061), OPERA (CIPE-D39) and COLLAGE (PNCDI II 224/2012) where over 300 samples were exposed to various hydrothermal ageing. In this paper, the use of thermal microscopy (imageMHT method and unilateral nuclear magnetic resonance (NMR)) to reveal whether there is any evidence for change to collagen on parchment samples’ surface after their exposure to ageing in various controlled T and RH conditions is illustrated.
EXPERIMENTAL METHODS

Micro Hot Table Method (imageMHT)

The MHT method and equipment was already described (Badea et al., 2012b). Shrinkage motion of collagen fibres was digitally recorded and evaluated by the imageMHT software developed within the research project COLLAGE (www.collage.com.ro) (Miu et al., 2014).

Unilateral Nuclear Magnetic Resonance (NMR)

NMR measurements were performed at room temperature using a bar magnet NMR-MOUSE sensor with a double-D radio-frequency coil controlled by a Bruker Minispec spectrometer working at 20.05 MHz $^{1}$H resonance frequency as described earlier (Badea et al., 2016). The NMR signal stems from a depth of about (0.5–1) mm from the surface of the sensor. The proton spin-lattice relaxation times $T_{1}$ were measured with a saturation-recovery pulse sequence using a Hahn-echo with an echo-time of about 25 µs for detection.

PARCHMENT DETERIORATION RELATED TO TEMPERATURE AND RELATIVE HUMIDITY

Standards and guidelines in force impose narrow $T$ and RH intervals for parchments storage and display implying a high consumption of energy no longer sustainable. Loosening these tight environmental standards has thus become a much debated concept. In 1994, the Smithsonian Institution’s Conservation Analytical Laboratory issued revised guidelines allowing for as much as 15 % fluctuation in RH (35 % to 65 %) and 10 °C in temperature (11 °C to 31 °C), regardless of the materials from which objects were made. Progress in materials research pushed forward the conclusion the most museum objects can tolerate, without mechanical damage, larger fluctuations than previously thought (Erhardt and Mecklenburg, 1994). In the case of parchment and leather, reaction to RH changes is more complex as they are heterogeneous materials composed of distinct collagen populations and gelatin (Badea et al., 2012a; Della Gatta et al., 2007; Badea et al., 2012b; Badea et al., 2011). The differential behaviour of these components in terms of response to RH changes is a key factor to be considered for setting up appropriate storage and exhibition RH ranges. The intrinsic heterogeneity of historical parchment and leather has therefore great implications for setting environmental standards since not all artefacts react in a similar way to the same environmental RH. Consequently, some targeted research is required in this direction. The use of imageMHT and unilateral NMR as valuable techniques for characterizing parchment is illustrated in what follows.

Thermal Stability by Shrinkage Activity

The shrinkage temperature $T_{s}$ of collagen fibres has been extensively as a gross metric of deterioration of collagen-based artefacts without considering their intrinsic structural heterogeneity. It has been reported that $T_{s}$ very well relates with denaturation temperature $T_{d}$ of fibrillar collagen measured by differential scanning calorimetry.
DSC) (Badea et al., 2012a; Badea et al., 2012b; Budrugeac et al., 2010) as illustrated in Figure 1.

![Figure 1](image1.png)

**Figure 1.** Plot showing the correlation between $T_d$ and $T_s$ for 32 parchments exposed to various hydrothermal ageing treatments.

Moreover, depending on $T_d$ value parchments were previously classified in four groups: stable/native (N) for $48 \, ^\circ\text{C} < T_d \leq 56 \, ^\circ\text{C}$; stabilized (S) for $T_d > 56 \, ^\circ\text{C}$; unstable (U) for collagen with $30 \, ^\circ\text{C} < T_d \leq 48 \, ^\circ\text{C}$ and gelatin-like (G) for $T_d \leq 40 \, ^\circ\text{C}$ (Figure 2).

![Figure 2](image2.png)

**Figure 2.** Variation of $T_d$ depending on ($T$, RH) ageing conditions. $T_d$ values correspond to parchment samples exposed to 32-day ageing.

Evaluation of both main shrinkage $C$ and total shrinkage $\Delta T$ intervals provides further information about the level of structural heterogeneity and degree of damage. Based on the study of parchment samples exposed to thermal ageing at various $T$ (e.g. 40, 60, 70, 80 and 100 °C) and RH (dry, 30%, 40%, 60% and 80%) we have found that $T$ interval increases as the structural heterogeneity increases, whereas it tends to decrease as a consequence of cross links formation (Badea et al., 2012a; Della Gatta et al., 2007; Badea et al., 2016; Budrugeac et al., 2010; Carşote et al., 2016; Della Gatta et al., 2005; Şendrea et al., 2016b). On the other hand $C$ interval generally tends to increase as a result of collagen thermal destabilization. However, in case of extended scission of
primary peptide bonds, C interval dramatically decreases or even disappears. Accordingly, by measuring the shrinkage activity in situ with portable equipment, we can obtain important information about the thermal stability, structural heterogeneity and degree of deterioration of historical parchments (Figure 3).

Figure 3. Shrinkage intervals for three parchment rolls (State Archives of Turin, XIV century), one parchment document issued by the chancery of the Stephen the Great (Library of the Romanian Academy, XV century) and one newly manufactured parchment. \( \Delta T = T_{\text{last}} - T_{\text{first}} \), where \( T_{\text{last}} \) and \( T_{\text{first}} \) are the temperatures at which the very first and very last fibre motions are observed, respectively.

Unilateral Nuclear Magnetic Resonance (NMR)

It has been shown that longitudinal (or spin-lattice) relaxation time, \( T_1 \), can be used to qualitatively assess the changes of collagen structural water environment within historic parchments (Badea et al., 2008; Masic et al., 2012). This noninvasive and nondestructive approach proved to be very sensitive in studying the effects of deterioration factors on the organization of bound water within the collagen structure (Badea et al., 2016; Ţendrea et al., 2016a; Badea et al., 2008; Masic et al., 2012; Ţendrea et al., 2015). Specific trends were observed for \( T_1 \) during hydrothermal accelerated ageing of parchments. For example, parchments subjected to 80 °C and 80% RH showed a slight shortening of \( T_1 \) values, whereas in the case of parchments aged by a combined action of \( \text{SO}_2 \), light irradiation, and heating, an increase of \( T_1 \) relaxation values was found. A complex behaviour was displayed by the parchments exposed to ageing at fix temperature (i.e. 40 and 80 °C) and daily alternate RH (i.e. 40% and 80%) (Figure 4). This behavior was related to the competition of two deterioration processes, i.e. thermal stabilization through cross-links formation (responsible for \( T_1 \) value decrease as a result of the reduced chain mobility) and peptide bonds cleavage (responsible of \( T_1 \) value due to the opening of triple helix structure, which allows random exposure of hydrophobic residues to free water). In order to better discriminate the deterioration effects on the organization and properties of water and collagen within ancient manuscripts, the decays should be analysed by fitting the experimental data to a
double or triple exponential function as reported for archival Italian documents (Badea et al., 2008) and for Dead Sea Scrolls (Masic et al., 2012).

![Variation of the proton relaxation time $T_1$ for parchments exposed to hydrothermal ageing at 40 °C/80 °C in controlled RH conditions as a function of ageing time.](image)

**IMPLICATIONS OF THIS STUDY FOR CONSERVATION PROFESSIONALS**

Analysis of the changes in collagen at the surface of parchment subjected to hydrothermal heating has shown that both thermal stability and structure of parchment are affected. Both imageMHT and unilateral NMR techniques provide valuable markers to assess such changes. Moreover, the availability of portable equipments (e.g. the imageMHT equipment developed within the project COLLAGE and NMR MOUSE from Magritek GmbH) allows for *in situ* testing and evaluation of the "health state" of historical documents and objects. For conservation professionals, the assessment protocols based on imageMHT and unilateral NMR represents a step forward in the routine conservation activity as valuable tools for diagnosis and monitoring the historical, archaeological and artistic objects made of collagen.

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