UNIVERSITY OF BUCHAREST
FACULTY OF CHEMISTRY
DOCTORAL SCHOOL IN CHEMISTRY

PhD THESIS
ABSTRACT

DAMAGE ASSESSMENT OF HISTORICAL LEATHERS AND PARCHMENTS

PhD student:

Cristina Carșote

PhD Supervisor:

C.S.I dr. Petru Budrugeac

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(According to the PhD thesis)

INTRODUCTION

Museums, archives, libraries and religious institutions all over the world preserve a large number of heritage objects made from leather and parchment, which are unique and irreplaceable testimonies of history and inexhaustible information sources of our culture and civilization.

Due to their organic structure, based mostly on collagen - the main structural protein of the skin, leather and parchment deteriorate over time due to the synergetic action of environmental factors (light, heat, humidity, atmospheric pollutants), biological agents (fungus, bacteria, rodents, insects) and chemicals used in conservation and/or restoration treatments. The time over which the historical objects made from leather and parchment maintain their functionality and ability to completely convey the intangible and tangible values decisively depends on the conditions in which they are stored or displayed and how they are preserved or restored.

In the cultural heritage field, establishing of the optimal conditions for storage and/or exhibition of objects, as well as the decision to apply a conservation treatment, should always start from the material properties and knowledge of it's deterioration. An accurate damage assessment of historical leathers and parchments requires knowledge of the complex hierarchical structure of collagen and it's characterisation at all structural levels by using specific physico-chemical techniques.

This paper aims an optimised use of some analytical techniques used for heritage objects investigation, such as thermal microscopy (Micro Hot Table – MHT), differential scanning calorimetry (DSC) and infrared spectrometry (FTIR), in order to obtain new scientific results which could allow significant progress in the assessment and diagnostic of historical leather and parchment objects degradation methodology, both in laboratory and *in situ*.

The thesis is structured in two parts: *literature data* and *original contributions*, combined into seven chapters.

Literature data includes two chapters:

- ✓ In the first chapter are given: (i) a brief history on the use of leathers and parchments; (ii) a brief description on the histological structure and chemical composition of the animal skin; (iii) data on the complex hierarchically-organised structure of the collagen – the main structural protein of the skin; (iv) a description of the stages of transforming animal skin into parchment or vegetable tanned leather, including the changes that occur in the collagen structure.
- ✓ In the second chapter are given: (i) the description of the main degradation factors (humidity, heat, visible and UV radiations, atmospheric pollutants, biological factors) and the deterioration mechanisms of collagen-based materials (hydrolysis, oxidation, gelatinisation) and (ii) aspects regarding non-destructive and micro-destructive analytical techniques (MHT, DSC and FTIR) which are proper for studying historical objects made from leather and/or parchment.

Original part is structured in five chapters:

- ✓ In the third chapter are described the materials (new and artificially-aged parchments and vegetable tanned leathers, as well as the historical leather and parchment objects) and the selected methods (MHT, DSC – analysis in water and in nitrogen flow, micro DSC and FTIR-ATR).
- ✓ Chapter IV presents the obtained results for the new collagen-based materials, based on the indicators provided by each of the applied analytical methods.

- ✓ Chapter V presents the obtained results on the synergistic effect of heat, humidity and visible light on parchments and vegetable tanned leathers based on the collagen degradation indicators, provided by the applied analytical methods.
- ✓ Chapter VI includes the description of the damage assessment protocol for historical leathers and parchments, based on the obtained results for the new and artificial aged collagen based materials.
- ✓ Chapter VII presents the conservation state of some historical objects made from leather and/or parchment (documents issued by the Moldavian Chancery during the reign of Stephen the Great – XV century; Marco Polo's Testament – XIV century; a Byzantine palimpsest from IX century, rewritten in XIII century; a military coat dated XVI – XVII centuries etc.), based on the damage assessment protocol.

The thesis ends with a chapter of conclusions which are comprised from the synthesis of the obtained results.

ORIGINAL CONTRIBUTIONS

The aim and the main objectives of the thesis

The aim of the thesis was the development of a damage assessment protocol for historical leathers and parchments, destined for specialists from museums in Romania and abroad for determining the conservation state of heritage objects made from leather and/ or parchment. The conservation state is considered the scientific underlie of the decisions on the optimal conditions for storage, use and exposure, as well as of the application or validation of conservation/ restoration treatments.

The main objectives of the thesis were the following:

- ✓ The characterisation of new collagen-based materials by using non-destructive and micro-destructive methods, such as thermal microscopy (MHT), differential scanning calorimetry (DSC in water, DSC in nitrogen flow, micro DSC) and infrared spectroscopy (FTIR-ATR), in order to explore all the information they can provide;
- ✓ The characterisation of artificially-aged collagen-based materials in order: (i) to establish monitoring indicators, provided by the applied analytical methods, for the collagen structure degradation in parchment and vegetable tanned leather, and (ii) to assess the effects induced by the main degradation factors (heat, humidity and visible light) on parchment and vegetable tanned leather;
- ✓ The development of a damage assessment protocol for historical leathers and parchments based on the obtained results for new and artificially-aged collagen-based materials;
- ✓ The application of the damage assessment protocol on historical materials provided by museums and archives in Romania and abroad.

CHAPTER III
MATERIALS AND METHODS

III.1 Materials

III.1.1 New parchments and vegetable tanned leathers

New parchments from sheep, goat and calf skins, as well as new vegetable tanned leather prepared from sheep and calf skins tanned with mimosa, quebracho and chestnut extracts were used.

III.1.2 Artificially-aged parchments and vegetable tanned leathers

In order to simulate the naturally ageing processes of the parchments and vegetable tanned leathers, as well as to assess the effects induced by the main degradation factors, the new collagen-based materials were exposed to the following artificial ageing treatments: (i) 70 °C and 30 % RH, for 8, 16, 32 and 64 days and (ii) 70 °C, 30 % RH and polychromatic visible light (4000 lx), for 8, 16, 32 and 64 days.

III.1.3 Heritage objects made from parchment and/ or leather

The investigated heritage objects made from parchment and/or vegetable tanned leather are as follows: 33 documents issued by the Moldavian Chancery during the reign of Stephen the Great – XV century, Marco Polo's Testament – XIV century and his Uncles' Testaments, a Byzantine palimpsest from IX century, rewritten in XIII century, a military coat dated XVI – XVII centuries and 10 leather objects, dated XVI – XIX centuries, belonging to national museums.

III.2 Methods

III.2.1 Thermal microscopy (Micro Hot Table method – MHT)

Thermal microscopy (Micro Hot Table method – MHT) allows hydrothermal stability determination (the evaluation of the shrinkage activity) of the collagen-based material fibres. The MHT measurements were made by using an equipment composed of a micro hot plate, equipped with an automatic system for temperature control, a digital microscope Dino-Lite® AD7013MZT and a computer for programming and data acquisition. The sample fibres of parchment or leather (0,1 mg), taken from the *corium* (flesh side) of the material, were thoroughly wetted in demineralised water on a microscope slide and separated using a pair of fine needles. The sample was covered with a cover glass, placed on the micro hot plate, under the microscope and heated at 2 °C·min^{-1}, in the temperature range (25 – 100) ^{o}C.

III.2.2 Differential scanning calorimetry (DSC)

The DSC measurements were carried out using DSC 204 F1 Phoenix equipment, produced by Netzsch, Germany. The used methods were as follows:

(a) DSC analysis in water. Each leather or parchment sample of about 3 mg was immersed in water for 24 hours using a hermetically sealed aluminium crucible. The thermal effect was measured in comparison to a sealed reference crucible containing an equivalent amount of water. Measurements were made with a 10 K·min^{-1} scanning rate, in the temperature range (25 – 110) ^{o}C.

(b) DSC analysis in nitrogen flow. Each leather or parchment sample of about 3 mg was placed into an open aluminium crucible, the thermal effect being measured in

comparison to an empty reference crucible. Measurements were made with a 10 $K \cdot min^{-1}$ scanning rate in the temperature range (25 – 280) ^{o}C. In the DSC oven was purged nitrogen (99.999% purity) with a flow rate of 20 $ml.min^{-1}$.

The micro DSC measurements were carried out with a high-sensitivity SETARAM Micro DSC III micro calorimeter by using 850 µl stainless steel (Hastelloy C) cells. Each leather or parchment sample of about 2 mg were suspended in 0.5 M acetate buffer with pH = 5.0 directly in the measurement cell and left for 2 h to secure fully hydrated conditions and thus reproducible calorimetric values for both temperature and enthalpy of denaturation. The thermal effect was measured in comparison to a reference crucible that contained an equivalent amount of buffer. The weights of the reference and sample cells were carefully matched. Measurements were made with a 0.5 $K \cdot min^{-1}$ scanning rate, in the temperature range (20 – 100) ^{o}C. Experimental data acquired with the SETARAM Set-Soft2000 software and deconvolution of the curves was carried out with PeakFit 4.1 (Jandel Scientific), using a Gaussian algorithm.

III.2.3 Infrared spectroscopy - Attenuated total reflection (FTIR-ATR)

FTIR-ATR measurements were carried out with a portable spectrometer Alpha Bruker Optics equipped with a Platinum ATR module, with wave number ranging from 4000 to 400 cm^{-1}, at a resolution of 4 cm^{-1} and 32 scans. Minimum three spectra were recorded for each surface of the vegetable tanned leather and parchment samples (*corium* - flesh side of the skin and *grain* – hair follicles side of the skin). For recording and evaluation of the spectra Opus 7.0 software was used.

III.2.4 Visual assessment

Visual assessment, at macroscopic and microscopic levels, was performed only on historical objects made from parchment, according to the visual assessment protocol developed within European project IDAP - *Improved Damage Assessment of Parchment* (2002 – 2005). Dino-Lite digital handheld microscope AD7013MZT has been used for microscopic assessment, observation being made at 20X and 50X magnifications.

CHAPTER IV
CHARACTERISATION OF THE NEW COLLAGEN-BASED MATERIALS

MHT method allowed the evaluation of the shrinkage activity at microscopic level [1] of new collagen-based material fibres. The hydrothermal stability indicators, namely: temperature at which the first shrinkage motion is observed (T_f), shrinkage temperature (T_s), temperature at which the last shrinkage motion is observed (T_l), main shrinkage interval (C) and total shrinkage interval (ΔT) were determined. In Figure 1 are graphically presented the shrinkage intervals (A1, B1, C, B2, A2) and the hydrothermal stability indicators for: (a) new sheep (S), goat (G) and calf (C) parchments; (b) new quebracho tanned (Sq), mimosa tanned (Sm) and chestnut tanned (Sc) sheep leathers and (c) new quebracho tanned (Cq), mimosa tanned (Cm) and chestnut tanned (Cc) calf leathers.

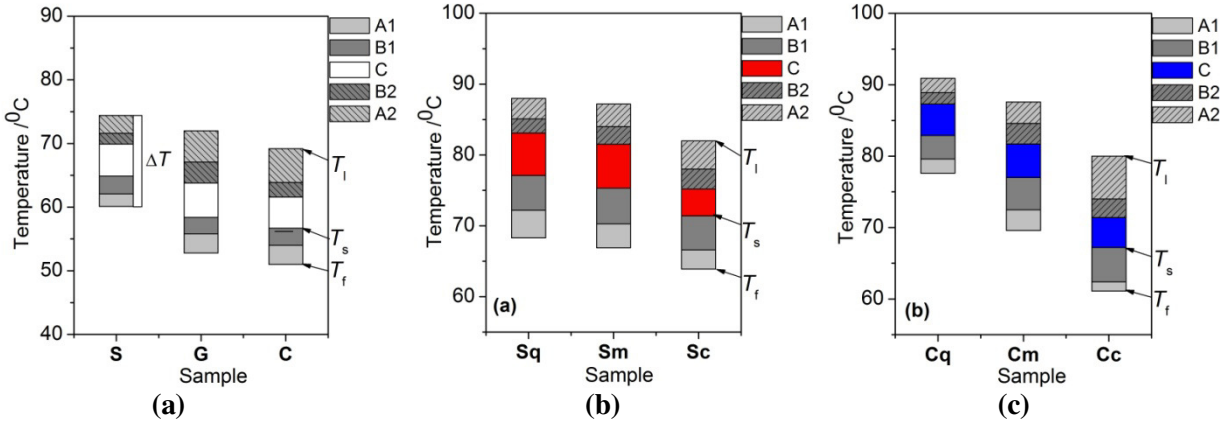


Figure 1. *Shrinkage intervals (A1, B1, C, B2, A2) for: (a) new sheep (S), goat (G) and calf (C) parchments; (b) new quebracho tanned (Sq), mimosa tanned (Sm) and chestnut tanned (Sc) sheep leathers and (c) new quebracho tanned (Cq), mimosa tanned (Cm) and chestnut tanned (Cc) calf leathers.*

MHT results showed that sheep parchment have greater hydrothermal stability in comparision with the calf and goat parchments. Vegetable tanned leathers have higher hydrothermal stability than the parchments, which depends both on tannin type and animal species: calf leather tanned with condensed tannins is more thermally stable than sheep leather tanned with condensed tannins, while chestnut tanned sheep leather is more thermally stable than chestnut tanned calf leather.

The thermal denaturation process of the new collagen-based materials, namely progressive alteration of well-organized collagen structure until its transformation into gelatine was revealed by DSC method in water [2]. The indicators assigned to the denaturation of collagen from parchment or leather, in water: the onset temperature (T_i), denaturation temperature (T_{max}) and denaturation enthalpy (ΔH) were determined. DSC results confirmed MHT results on the hydrothermal stability of new collagen-based materials. The onset temperatures (T_i) measured by DSC and the shrinkage temperatures (T_s) measured by MHT are well correlated [3-4]. The differences between these two indicators were assigned to the different experimental conditions (different scanning rate: 10 $K \cdot min^{-1}$ in case of DSC method and 2 $K \cdot min^{-1}$ in case of MHT method), the heterogeneity of collagen-based materials and the non-uniformity in tannage, in case of vegetable tanned leathers.

Until now, micro DSC method was used to determine the hydrothermal stability of collagen in parchment [3, 5-9]. In this thesis, the thermal denaturation of collagen in vegetable

tanned leather was discussed in comparison with that of collagen in calf parchment (P). Due to the sensitivity of the micro DSC method, in addition to the onset (T_i) and denaturation (T_{max}) temperatures, the other indicators of thermal denaturation of vegetable tanned leather, namely: denaturation enthalpy (ΔH), curve half-width ($\Delta T_{1/2}$) and curve height (C_p^{ex} max) were accurately measured. Micro DSC denaturation curves for the new vegetable tanned leathers are shown in Figure 2 together with the micro DSC curve for the new calf parchment.

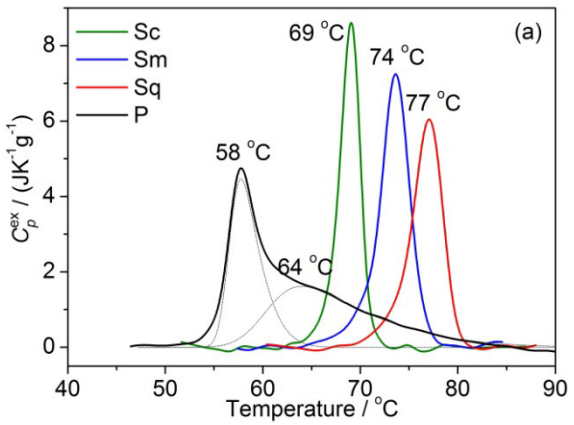


Figure 2a. *Micro DSC curves associated with thermal denaturation of collagen from quebracho tanned (Sq), mimosa tanned (Sm) and chestnut tanned (Sc) new sheep leathers and from new calf parchment (P).*

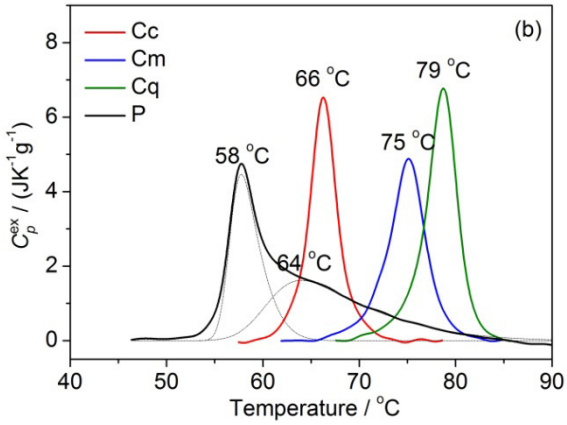


Figure 2b. *Micro DSC curves associated with thermal denaturation of collagen from quebracho tanned (Cq), mimosa tanned (Cm) and chestnut tanned (Cc) new calf leathers and from new calf parchment (P).*

The micro DSC results confirmed those obtained by DSC and MHT methods on the hydrothermal stability of vegetable tanned leather. Moreover, the comparative analysis of the micro DSC curves of leathers and parchment, as well as the constant value of the denaturation enthalpy (ΔH = 28.5 ± 1.1 J·g^{1}) for leathers allowed us to infer the full denaturation of the stabilised collagen population during pickling - the hide preparatory stage consisting in lowering of the pH value to 2.8 - 3.2 to help the penetration of tanning agents. Also, the constant value of the enthalpy of collagen denaturation in leather confirms the assumption that the denaturation process is controlled only by the breaking of intramolecular hydrogen bonds [10-13].

The denaturation temperature of the collagen crystalline phase in new parchments and leathers was determined by DSC analysis in nitrogen flow. The DSC curves obtained for the new quebracho tanned calf leather (Cq) are shown in Figure 3. Similar curves were obtained for all investigated collagen-based materials. DSC curves measured in nitrogen flow display two endothermic processes: a broad peak in the temperature range (50 – 120) °C associated with thermal dehydration of the sample and a narrow peak at about 200 °C, having smaller denaturation enthalpy in comparision with the dehydration process. For the first time, the second peak was observed for Achilles' tendon and explained by biphasic crystalline - amorphous structure of collagen according to, the triple helix of collagen is embedded in an amorphous matrix [14]. Thus, the second process was attributed to the denaturation of the collagen crystalline phase. It has been found that the denaturation of the collagen crystalline phase is actually independent of the animal species or the tannin type. The denaturation temperature of the collagen crystalline phase (T_d) in vegetable tanned leather was found to be higher than that of parchments, which is due to the strong cross-links between collagen and tannin matrix, formed during tanning.

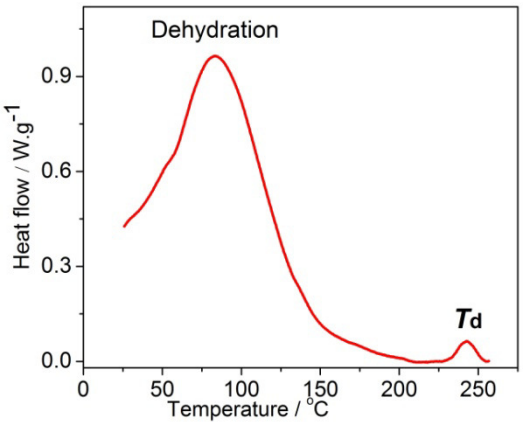


Figure 3. *DSC curve measured in nitrogen flow for new quebracho tanned calf leather (Cq).*

The spectral characteristics of new collagen-based materials were determined by FTIR-ATR method. All recorded FTIR-ATR spectra present the absorption bands of the functional groups of collagen (A_A, A_B, A_I, A_{II} and A_{III}). Apart from the collagen characteristic bands, calcium carbonate (1410 cm^{-1} and 875 cm^{-1}), originating from the manufacturing process, and fatty acids salts (1575 cm^{-1}) which are formed as a result of the slow reaction between fats residues from skin and the residues of metal oxides from various stages of the manufacturing process due to improper degreasing, were identified in the FTIR-ATR spectra (Figure 4), collected from the grain side of the sheep and goat parchments.

In case of vegetable tanned leathers, due to the overlapping of the absorption bands of the tanning agents, the positions and intensities of the collagen amide bands are disturbed. The FTIR-ATR spectra of mimosa tanned sheep leather (Sm) as compared to that of native collagen and mimosa extract are presented in Figure 5. Similar spectra were obtained for all investigated vegetable tanned leathers.

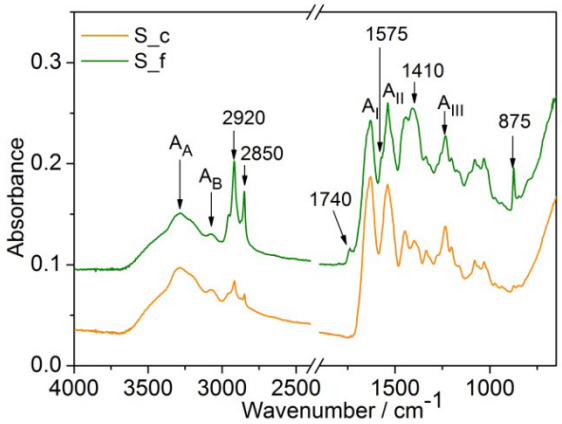


Figure 4. *FTIR-ATR spectra collected from the both sides of the sheep parchment (corium - c and grain – f). The main absorption bands of collagen (A_A, A_B, A_I, A_{II} and A_{III}), calcium carbonate (1410 cm^{-1} and 875 cm^{-1}) and fatty acids salts (1575 cm^{-1}) are indicated.*

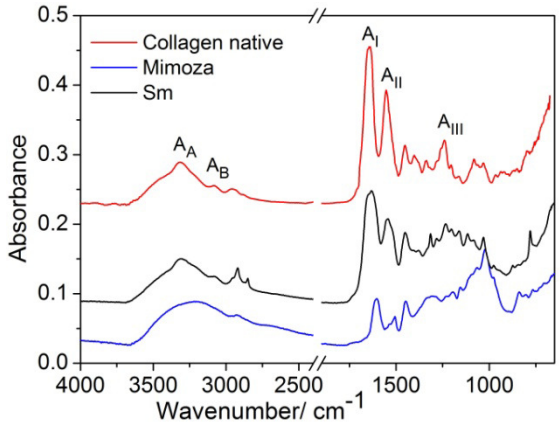


Figure 5. *FTIR-ATR spectra for mimosa tanned sheep leather (Sm) in comparision with that of native collagen and mimosa extract. The main absorption bands of collagen (A_A, A_B, A_I, A_{II} and A_{III}) are indicated.*

CHAPTER V
CHARACTERISATION OF THE ARTIFICIALLY-AGED COLLAGEN-BASED MATERIALS

The effects induced by the main degradation factors (heat, humidity and visible light) on collagen-based materials were assessed according to the indicators provided by the applied analytical methods (MHT, DSC analysis in water, DSC analysis in nitrogen flow, micro DSC, FTIR-ATR). MHT results showed that the hydrothermal stability of collagen-based materials progressively decreases with the increasing time exposure at artificial ageing treatments. Parchments present similar behaviour at heat and humidity action, independently on animal species, while the hydrothermal stability of vegetable tanned leathers depends on both the animal species and tannin type. Visible light irradiation induced a stabilised effect on collagen-based materials. The structural heterogeneity of parchments progressively increases with increasing time exposure at 70 ^{0}C and 30 % RH. Only quebracho tanned leather presented a high degree of structural heterogeneity after 64 days exposure.

DSC analysis in water confirmed MHT results. In all cases, T_i (DSC) and T_s (MHT) values are well correlated.

Micro DSC method was used to obtain detailed information on the deterioration patterns of collagen in vegetable tanned leather. The influence of both the tannin type, i.e. hydrolysable or condensed, and collagen animal species, i.e. calf and sheep, were investigated. Comparison with the behaviour of unmodified collagen in parchment was made to explain the thermal destabilisation of the chemically modified collagen in leather, induced by the main degradation factors (heat, humidity and visible light) action. The micro DSC parameters progressively varied with the increasing time (Figures 6-9). The results proved that the tannin type as well as the animal species influences the ageing behaviour of collagen in vegetable tanned leather.

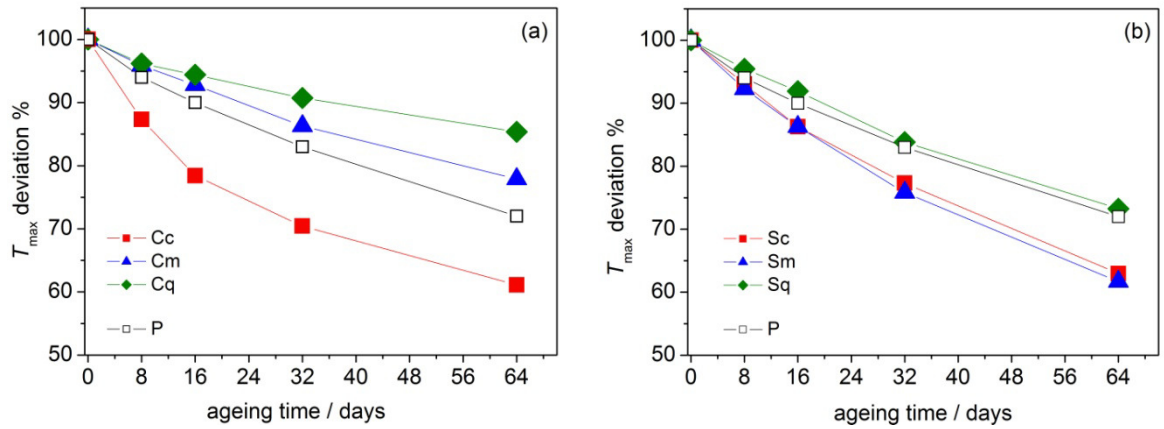


Figure 6. *Percent deviations from reference values (not treated sample) for denaturation temperature (T_{max}) for calf (a) and sheep (b) leathers subjected to accelerate ageing at 70 °C and 30% RH for 8 to 64 days. Comparison with T_{max} percent deviations displayed by parchment subjected to a similar ageing treatment.*

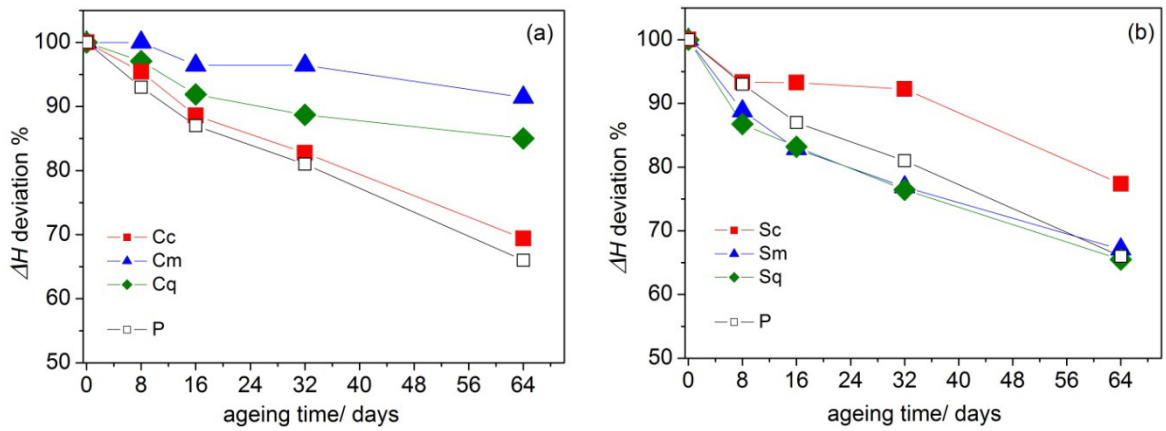


Figure 7. *Percent deviations from reference values (not treated sample) for denaturation enthalpy (ΔH) for calf (a) and sheep (b) leathers subjected to accelerate ageing at 70 °C and 30% RH for 8 to 64 days. Comparison with ΔH percent deviations displayed by parchment subjected to a similar ageing treatment.*

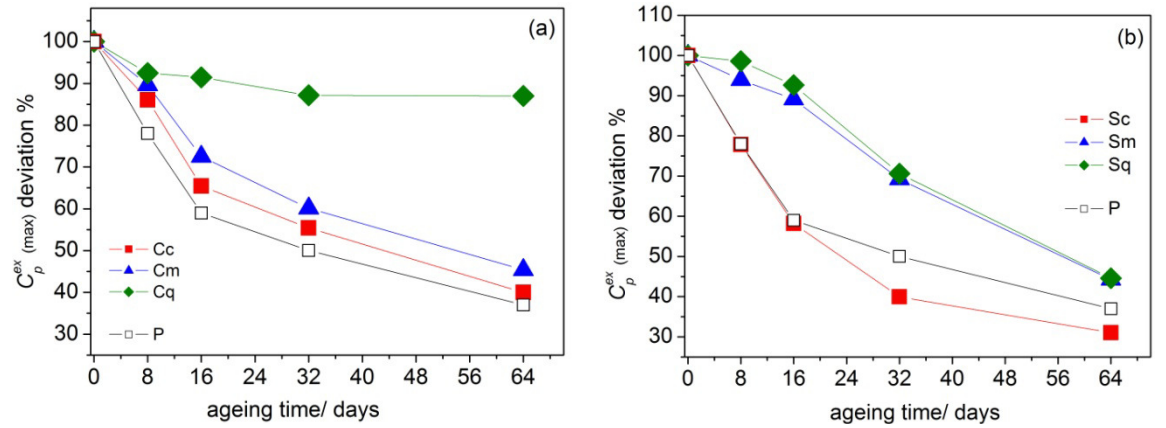


Figure 8. *Percent deviations from reference values (not treated sample) for curve maximum height (C_p^{ex} max) for calf (a) and sheep (b) leathers subjected to accelerate ageing at 70 °C and 30% RH for 8 to 64 days. Comparison with C_p^{ex}max percent deviations displayed by parchment subjected to a similar ageing treatment.*

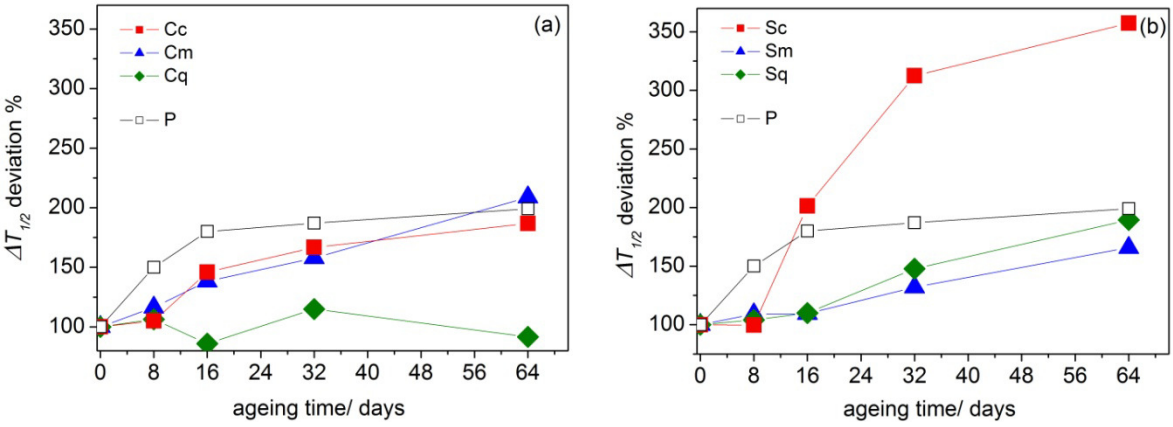


Figure 9. *Percent deviations from reference values (not treated sample) for curve half-width ($\Delta T_{1/2}$) for calf (a) and sheep (b) leathers subjected to accelerate ageing at 70 °C and 30% RH for 8 to 64 days. Comparison with $\Delta T_{1/2}$ percent deviations displayed by parchment subjected to a similar ageing treatment.*

A gradual thermal destabilisation of leathers was observed up to 16-day ageing: micro DSC curves flips to lower temperatures but maintain their sharp shape. After 32-day ageing, the curves' shape starts to broaden (Figure 10a) and display a multiple transition feature (Figure 10b). The progressive conversion of the native collagen molecules in intermediate states with lower thermal stability was observed earlier for parchments exposed to artificial ageing [9]. More than one population of collagen molecules with distinct thermal stability were identified for chestnut sheep leather only (Figure 11). Parchment-like behaviour of the chestnut sheep leather after a 32-day ageing was attributed to the collagen - chestnut complex decomposition, namely the *de-tanning* process. Thermal analysis studies [15] revealed that the tannin content of leather is more significantly affected by both natural and artificial ageing than the collagen structure, which confirms that the *de-tanning* is due to the tannin's decomposition.

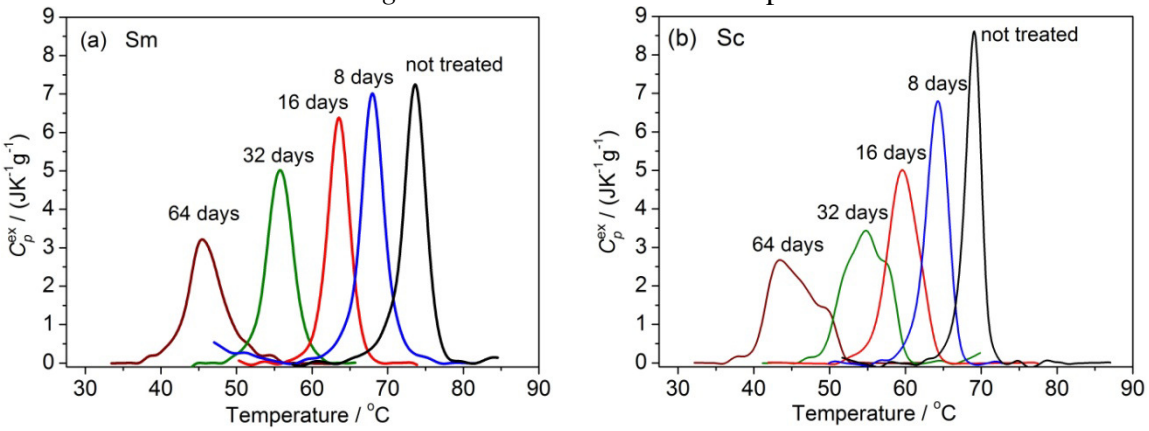


Figure 10. *DSC denaturation curves for mimosa (a) and chestnut (b) sheep leathers exposed to accelerate ageing at 70 °C and 30% RH for 8 to 64 days illustrating the influence of the tannin type on the deterioration pattern of leather.*

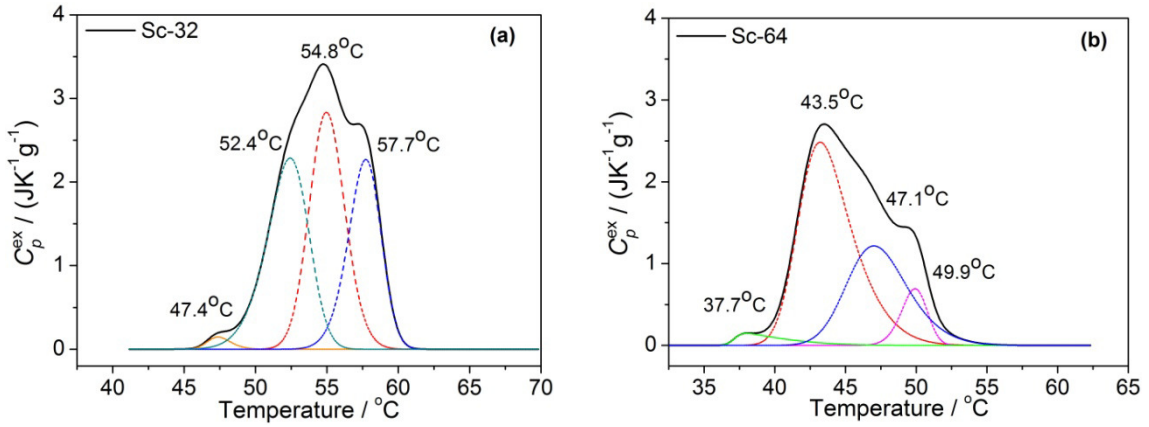


Figure 11. *Deconvolution of the DSC denaturation curve of the chestnut tanned sheep leather samples exposed to accelerate ageing at 70 °C and 30% RH for 32 days (a) and 64 days (b) revealing the populations of collagen molecules with distinct thermal stability.*

According to the obtained results, we can assert that the deterioration pattern in artificially aged vegetable leather goes through the following steps: collagen–tannin complex destabilisation, *de-tanning*, and collagen denaturation. After *de-tanning*, leather behaves like a parchment as has also been proven by the Py-GS/MS studies which revealed similar main decomposition products of aged parchment and leather [15].

Visible light irradiation induces a stabilized effect on vegetable tanned leathers. For chestnut-tanned leather (Figure 12), this effect reaches a maximum after a 16-day ageing period and then gradually declines. On the contrary, for either mimosa- (Figure 13) or quebracho-tanned leather, the stabilisation effect increases with the exposure time. The increase in the denaturation temperature and the slight decrease in the DSC curve width at its half height have also been observed for parchments exposed to short-term hydrothermal ageing in the presence of visible light [16] and attributed to the formation of cross-links [9, 17]. It was found that over long periods of exposure of hydrothermal treatment, the cross-linking induced by exposure to visible light irradiation is overcome by destabilisation effects [5, 9]. Consequently, the damage profile of the artificially-aged vegetable tanned leather is influenced by the balance of these two processes: thermal stabilisation through cross-link formation and thermal destabilisation through cleavage of peptide bonds and intermediate states formation.

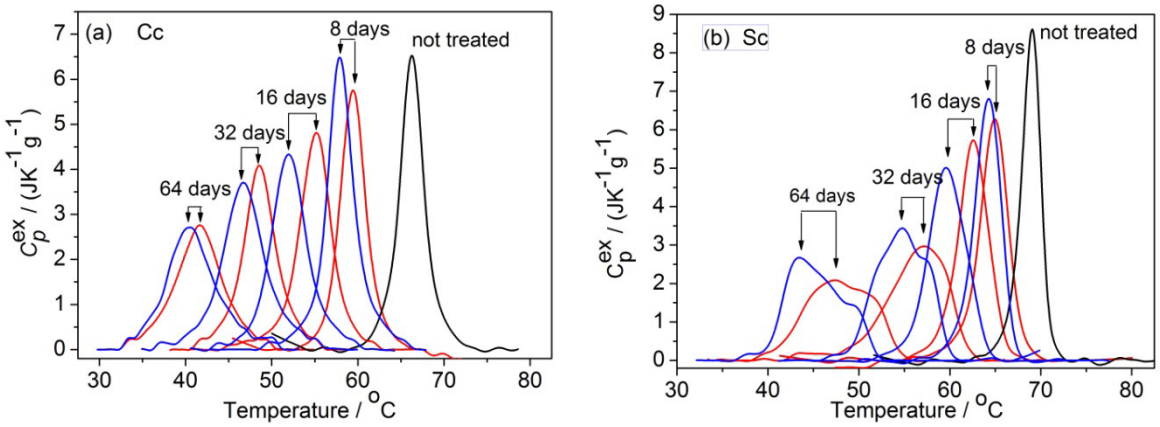


Figure 12. *DSC denaturation curves for two series of (a) chestnut calf leather and (b) chestnut sheep leather exposed to accelerate ageing at 70 °C and 30% RH, for 8 to 64 days in both dark (blue curves) and daylight (red curves) irradiation conditions.*

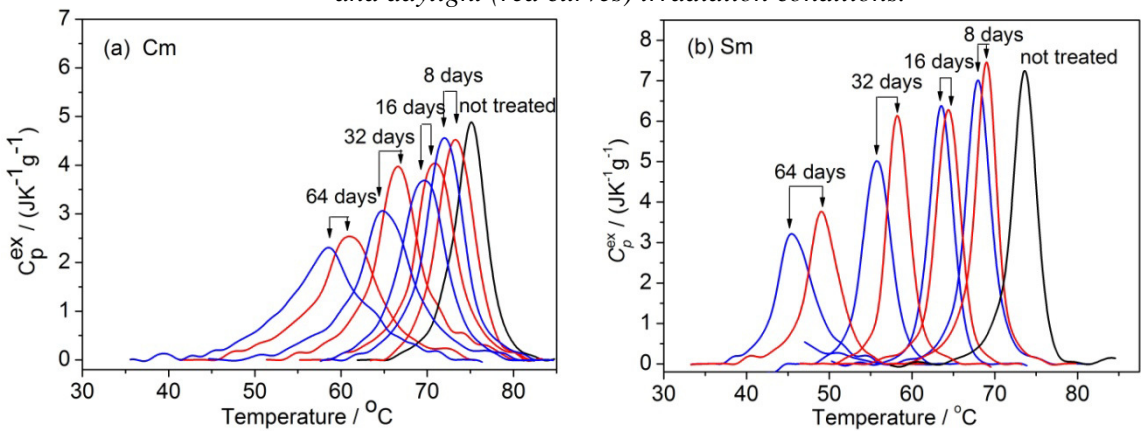


Figure 13. *DSC denaturation curves for two series of (a) mimosa calf leather and (b) mimosa sheep leather exposed to accelerate ageing at 70 °C and 30% RH, for 8 to 64 days in both dark (blue curves) and daylight irradiation (red curves) conditions.*

DSC analysis in nitrogen flow proved that the crystalline phase of collagen in parchment or vegetable-tanned leather is not affected by the applied artificial ageing treatments. The denaturation temperature of the crystalline phase of collagen (T_d) remains constant over the

periods of exposure of artificial ageing treatments: around (221.8 ± 2.4) °C for parchment and around (246.6 ± 4.3) °C for vegetable tanned leather.

Damage at molecular level of the collagen in artificially-aged parchments and vegetable tanned leathers were assessed by FTIR-ATR method. Positions and relative intensities of the main characteristic absorption bands of collagen reflect the changes occurring in the parchment structure due to various deterioration mechanisms [6]. The ratio between the intensities of amide I and amide II bands (A_I/A_{II}) is the indicator of the degree of hydrolytic cleavage of the peptide bonds of collagen. For a new parchment, A_I/A_{II} value is about 1 and increases with the increasing of the hydrolytic cleavage of peptide bonds [18]. The irreversible transformation of the native structure (triple helix) of collagen into disordered structure (gelatine) is indicated by the moving of A_{II} band to lower wave numbers. For a new parchment, the difference between the positions of A_I and A_{II} bands ($\Delta\nu$) is about 90-95 cm^{-1} and increases with the increasing formation of gelatinised structures [18]. Also, the alteration of the collagen triple helix integrity is indicated by the ratio between the intensities of amide III and 1450 cm^{-1} bands (A_{III}/A_{1450}) [19]. It has been shown that the A_{III}/A_{1450} value is about 1 for an intact collagen triple helix and about 0.5 for a denatured collagen [20]. Oxidation of the polypeptide chains of collagen is indicated in the spectra at about 1720 - 1740 cm^{-1} ($\nu_{C=O}$), the absorption band attributed to the formation of free carbonyl / carboxylic compounds [6, 18, 21].

The artificially ageing treatments induced a progressive hydrolytic cleavage of peptide bonds of collagen in parchment. This process was observed only for sheep leather tanned with condensed tannins (mimosa and quebracho) and exposed to 70 °C, 30 % RH and visible light. The oxidation of polypeptide chains of collagen was observed only in certain areas of the grain side of the parchment. The oxidation wasn't observed for vegetable tanned leather. The integrity of the native structure of collagen in parchment or leather was not affected by the applied artificial ageing treatments.

CHAPTER VI

DEVELOPMENT OF THE DAMAGE ASSESSMENT PROTOCOL FOR HISTORICAL LEATHERS AND PARCHMENTS

The results obtained for new and artificially-aged collagen-based materials allowed the development of a damage assessment protocol for historical leathers and parchments, which is shown schematically in Figure 14. The protocol application on heritage objects made from leather or parchment depends upon: (i) the possibility of sampling and quantity of sample and (ii) the access to the physico-chemical techniques. If the sampling is not possible, the damage assessment of a heritage object made from leather or parchment is achieved non-destructively through visual analysis and infrared spectroscopy with attenuated total reflection (FTIR-ATR) method. Valuable information on the level of damage of collagen-based materials can be obtained micro-destructively, by sampling. The micro-destructive version of the protocol includes the following methods: MHT, DSC in water, micro DSC and DSC in nitrogen flow. In ideal conditions, in which there is available about 5 mg of sample and access to all analytical equipments, the heritage object deterioration should be assessed both non-destructively (visual analysis and FTIR-ATR method) and micro-destructively (MHT, micro DSC and DSC in nitrogen flow methods), in order to obtain an accurate and complete image of the damage of collagen structure in parchment or leather.

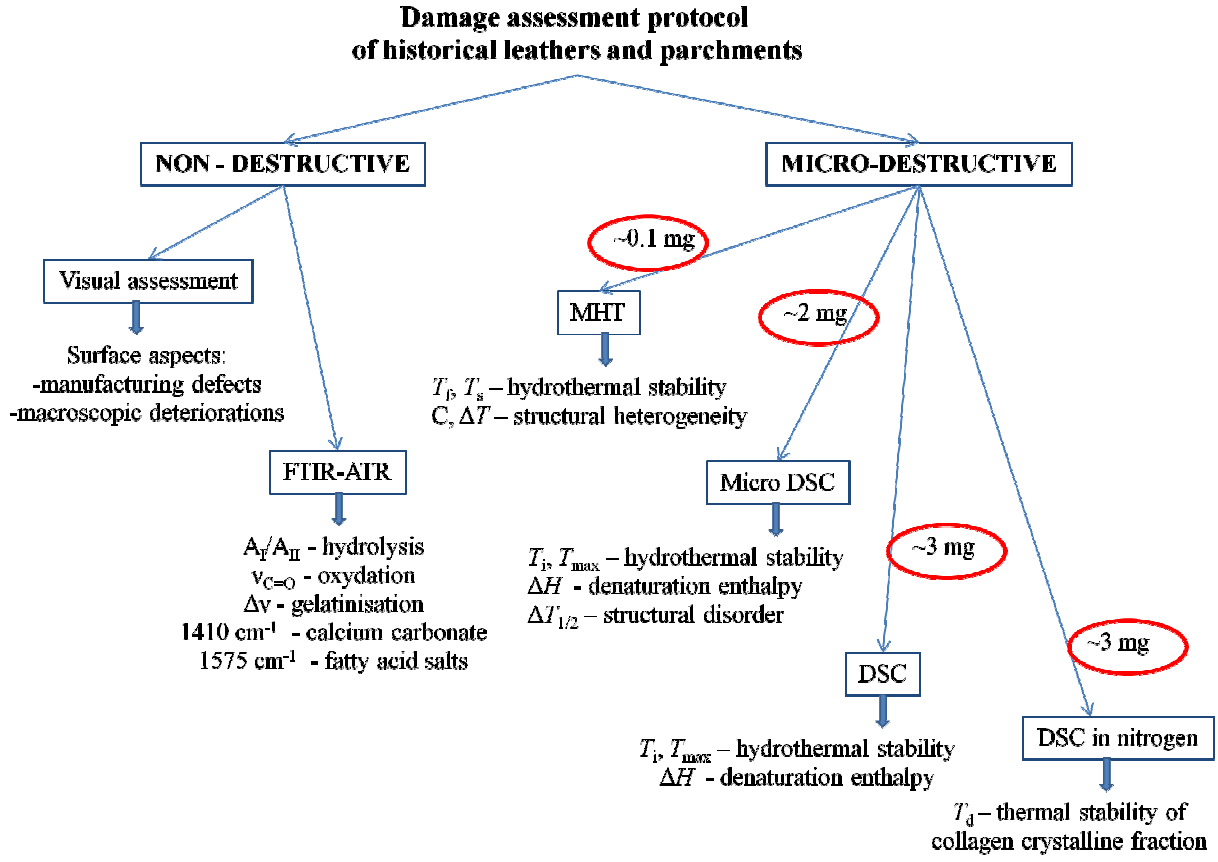


Figure 14. *Damage assessment protocol for historical leathers and parchments.*

CHAPTER VII
APPLICATION OF THE DAMAGE ASSESSMENT PROTOCOL FOR HISTORICAL LEATHERS AND PARCHMENTS

VII.1 The study of a parchment documents collection

33 documents written on parchment, issued by the Moldavian Chancery during the reign of Stephen the Great, representing formal acts who served as title of property or privilege and preserved at the Romanian Academy Library, were investigated according to the damage assessment protocol, by using visual analysis and FTIR-ATR and MHT methods. In Figure 15 are presented three of the investigated documents.

Macroscopic analysis of parchment aimed the observation of both the manufacture quality dependent features: colour, transparency, flexibility, the presence of deposits of calcium carbonate, veins and "bleedings" etc. and physical features related to deterioration: mechanical, thermal, caused by water/moisture, microbiological or caused by insects and rodents [22, 23]. It was found that the quality of the parchments used for writing royal chancery documents is very good. They have uniform thickness without obvious defects and a very good surface preparation of the written side having light colour and matte and velvety aspects.

Microscopic analysis of parchment aimed at identifying the animal species used for parchment manufacturing by observing the pattern of the hair follicles on the grain side. About half of the studied parchments were manufactured from calf hides (51 %), the rest being obtained from goat (27 %) and sheep (21 %) hides. Previous studies have shown a correlation between the geographical area and the animal species used in the manufacture of parchment. Due to the small number of the investigated documents it cannot be said precisely the origin of the Moldavian parchments. However, the high percentage of calf parchments, finished on both sides, suggests that they may have been imported from northern Europe. For goat and sheep parchments there may be assigned an Italian origin.

(a) (b) (c)

Figure 15. *Chancery documents: (a) P110 – parchment having white colour, well finished on the written side; (b) P170 – parchment having white-yellow colour, well finished on both sides and (c) P290 – a fake parchment having reddish brown colour and glassy appearance.*

The documents present mechanical deformations on extended areas, the lacunas on the folding lines due to mechanical stress, old brown stains and deposits of dirt, halos of moisture who trained dust, wax stains, holes caused by insects, lacunas caused by rodents, pink-purple spots due to accumulation of mould metabolites. In addition, they present degradation signs caused by physical and chemical factors, half of the documents showing thermal degradation (contracted areas) and degradation caused by water.

The FTIR-ATR method was used to evaluate deteriorations at molecular level: hydrolytic cleavage of peptide bonds, oxidation of polypeptide chains, gelatinisation, as well as to identify the materials added to parchment. Despite the poor appearance of the documents, the obtained results confirmed a good conservation state of the surface, at molecular level. All colected FTIR-ATR spectra showed no degradation by hydrolysis or gelatinisation, the indicators value being close to those of a new parchment ($A_I/A_{II} \leq 1$ and, respectively, $\Delta\nu \leq 95$ cm^{-1}) [6, 18]. However, the unwritten area of all parchments, which is in direct contact with the environment, is affected by the oxidative degradation processes (Figure 16). Small quantities of calcium carbonate (1410 cm^{-1} and 875 cm^{-1}) were identified in all cases, except for few areas of some documents, such as P119 (Figure 17) for which was found huge amount of $CaCO_3$. The presence of a small amount of $CaCO_3$ is an argument that supports the previous statement on the high quality of parchments. Also, for all investigated parchments, it was found the alumino-silicates band at 1026 cm^{-1}, which confirms the visual observations on the adhered dust to the surface (Figure 17).

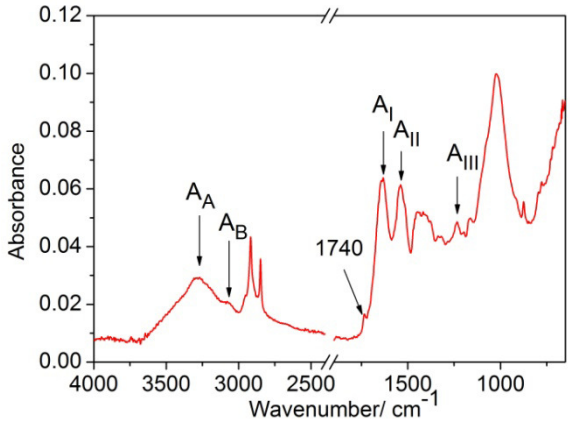


Figure 16. *FTIR-ATR spectra of the P159 document. The main absorption bands of collagen (A_A, A_B, A_I, A_{II} and A_{III}) and the absorption band of free carbonyl/ carboxylic compounds (1740 cm^{-1}) are indicated.*

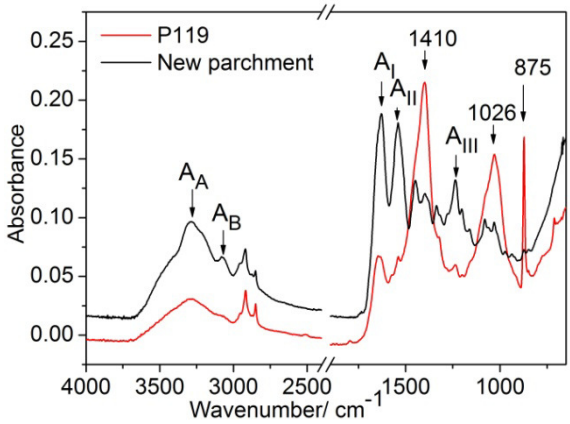


Figure 17. *FTIR-ATR spectra of the new parchment (black) and the P119 document (red). The main absorption bands of collagen (A_A, A_B, A_I, A_{II} and A_{III}), the absorption bands of calcium carbonate (1410 and 875 cm^{-1}) and alumino-silicates (1026 cm^{-1}) are indicated.*

The MHT method was used to determine the hydrothermal stability of 10 selected parchment documents. Only one micro-sample (a few parchment fibres) was taken from each document, from an area that appeared deteriorated. In Figure 18 are presented the shrinkage intervals of parchment samples as well as the indicators of hydrothermal stability. The shrinkage temperature (T_s) values of the investigated parchments, higher than 50^{o}C, indicate a hydrothermal stability comparable to that of the new parchment. However, the presence of the unstable and pre-gelatinised collagen populations indicated by the low values of the temperature at which the first shrinkage motion is observed (T_f), suggests a medium degree of deterioration.

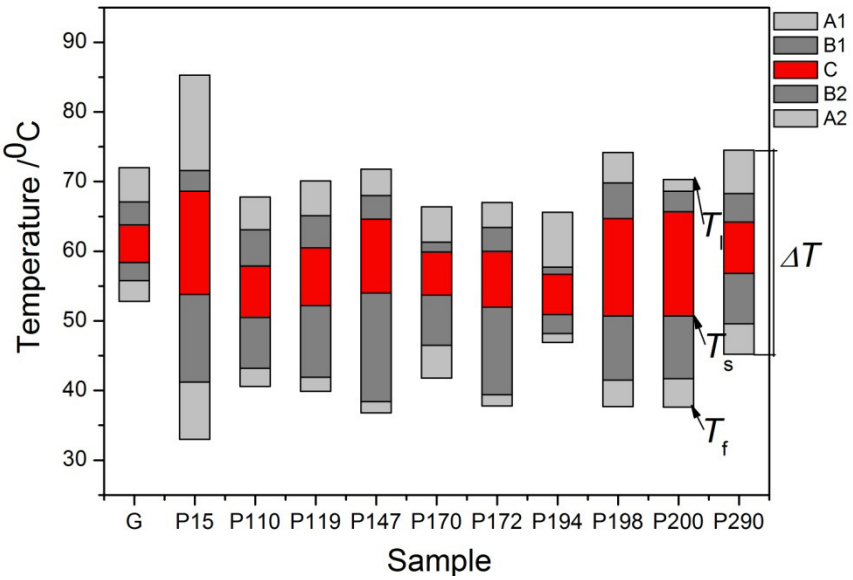


Figure 18. *Shrinkage intervals (A1, B1, C, B2, A2) for the new goat parchment and for 10 parchment documents issued by the Moldavian Chancery during the reign of Stephen the Great. Temperature at which the first shrinkage motion is observed (T_f), shrinkage temperature (T_s), temperature at which the last shrinkage motion is observed (T_l) and total shrinkage interval (ΔT) are indicated.*

Even if the parchments surface shows mechanical deterioration signs, visible to the naked eye, both the FTIR-ATR and MHT methods revealed a low collagen alteration. Unfortunately, the mechanical damage effects were reflected on the ink and leaded to reduced readability in folding areas and even material loss of the text.

VII.2 Marco Polo's Testament investigation

Marco Polo's Testament, written on parchment, dated from 1328, is preserved at the Marciana National Library in Venice. The visual analysis was used to identify the macroscopical, physico – chemical and morphological aspects of parchment as well as the animal skin type used for parchment manufacturing. The evaluation of the conservation state was made by using the FTIR-ATR and MHT methods.

The parchment used for writing the Marco Polo's Testament is goat skin in origin, is stiff and opaque, characterised by numerous visual imperfections which indicates an inferior quality of the manufacturing process. Despite the skilfulness of the parchment maker that mainly influenced its aesthetic appearance, the mechanical deteriorations and numerous water stains, the parchment surface is in relatively good condition, as confirmed by the FTIR-ATR analysis. However, the rather low hydrothermal stability indicated by the presence of the unstable and pre-gelatinised collagen populations suggests a high deterioration level of the layers beneath the surface.

VII.3 Damage assessment of a Byzantine manuscript

The conservation state of a Byzantine manuscript belonging to the Centre for Slavo–Byzantine Studies "Ivan Dujčev" in Sofia was evaluated according to the damage assessment protocol. The Byzantine manuscript is a palimpsest (Figure 19) whose earlier layer dates back to the end of the 9th or the beginning of the 10th century and contains Byzantine chants written with uncial script and the second layer is a Lectionary from the 13th century. The bookbinding is made

of wood covered with leather. Both the parchment and leather were evaluated by using the visual analysis and FTIR-ATR, MHT, DSC in water and DSC in nitrogen flow methods. Investigations were made on four parchment micro-samples and one leather micro-sample.

(a) (b)

Figure 19. *Byzantine manuscript from the Centre for Slavo–Byzantine Studies "Ivan Dujčev", Sofia: (a) front wooden board covered by leather and (b) a parchment sheet with stiff, pleated, of dark yellow colour and with glassy appearance.*

At macroscopic level, both the parchment and leather appear strongly deteriorated. The parchment sheets are stiff, pleated, of dark yellow colour and with glassy appearance (Figure 19b), while the leather is stiff and fragile (Figure 19a). According to FTIR-ATR analysis, the parchment micro-samples are affected by the hydrolysis of the collagen peptide bonds and gelatinisation. Gelatinisation is most probably prevailing at the sample surface as indicated by the visual assessment. The gelatinised and stiffed glassy surface of parchment behaves differently if exposed to even small variations of relative humidity and temperature. The interlayer tensions could peel off the surface layer and lead to the partial or total loss of writing and decorations. The rather high hydrothermal stability, as well as the good condition of the crystalline phase indicates a limited degree of hydrolysis of the layers beneath the surface. As compared to parchment, the crystalline phase of collagen in leather is slightly altered.

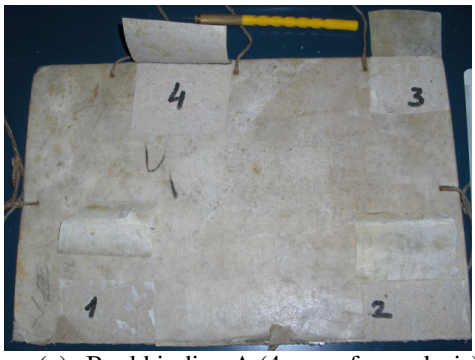
VII.4 Damage assessment of a military coat

The conservation state of a military coat, from XVI – XVII centuries, belonging to the History Museum of Braşov – Romania was evaluated according to the damage assessment protocol.

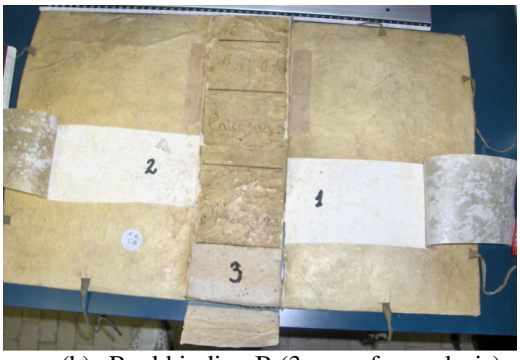
According to DSC and MHT analysis, the hydrothermal stability of most of the leathers samples taken from the military coat is very low, which suggest a high degree of deterioration. This observation is confirmed by the DSC in nitrogen flow analysis which showed a significant alteration of the collagen crystalline phase. Consequently, the MHT and DSC results contradict the visual observations which indicated that the conservation state of the military coat is a good one.

VII.5 Damage assessment of some parchment bookbindings

Two parchment bookbindings (Figure 20) from XIX century and donated by Archivio di Stato di Torino in scientific purpose were evaluated according to the damage assessment protocol. MHT, micro DSC and FTIR-ATR methods were used.



(a) Bookbinding A (4 areas for analysis)



(b) Bookbinding B (3 areas for analysis)

(c) Bookbinding B (spine stiffening)

(d) Bookbinding B – spine stiffening (3 areas for analysis)

Figure 20. *Parchment bookbindings images. The analysed areas are indicated.*

It was found that, at the artificial ageing of parchment, the native collagen population (N) is progressively transforming into unstable collagen structures (U) and even gelatine (G). The micro DSC curve is broadening, shortening and shifting to lower temperatures [3, 5]. The temperature range of denaturation transitions of collagen in parchment was divided in four intervals: (1) T_{max} > 58 °C – stabilized collagen (S); (2) 48 °C ≤ T_{max} ≤ 58 °C – native or stable collagen (N); (3) 35 °C ≤ T_{max} ≤ 48 °C – unstable collagen (U) and (4) T_{max} ≤ 35°C gelatine (G) [5]. The micro DSC curves deconvolution of parchment samples taken from the bookbindings revealed the co-existence of the collagen populations with distinct thermal stabilities (Figures 21 and 22). The collagen populations identified by deconvolution were allocated to one of the described intervals, depending on their T_{max}. The quantification of the collagen populations was made based on their enthalpy percent [24] (Table 1). The co-existence of the collagen populations with distinct thermal stabilities as well as the heterogeneity of the fibrilar structure is indicated also by the high $\Delta T_{1/2}$ values. In all cases, the fibrilar structure of collagen in parchment is affected, the ΔH values being at about 50 % smaller in comparision with that of the new parchment. However, the majority of the investigated parchment samples present a high percent, more than 90 %, of native (N) and stabilized (S) collagen populations which indicates a good structural and thermal stability. In all cases, we found small amount of unstable, gelatinised and pre-gelatinised structures of collagen. According to micro DSC results, the investigated parchment bookbindings have a medium degree of deterioration which was also confirmed by the MHT results.

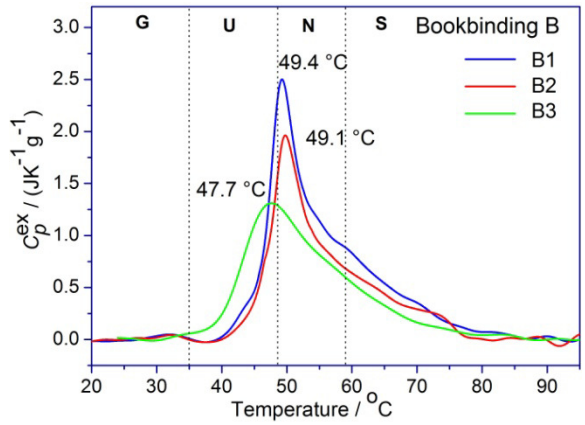


Figure 21. *Micro DSC curves associated with the thermal denaturation of collagen in parchment samples, taken from bookbinding B.*

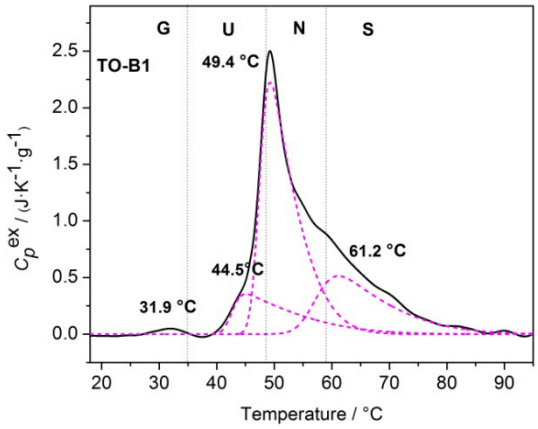


Figure 22. *Deconvolution of the micro DSC curve of the parchment sample B1, taken from bookbinding B.*

Table 1. *Thermal stability and proportions of the various collagen populations in parchment samples obtained by deconvolution of the full DSC peaks. The overall enthalpy (ΔH), peak half-width ($\Delta T_{1/2}$) and curve height (C_p^{ex}max) are also reported.*

Sample	ΔH / J·g^{-1}	$\Delta T_{1/2}$ / °C	C_p^{ex} max / J·K^{-1}·g^{-1}	G		U		N		S	
				T_{max}/ °C	% ΔH	T_{max}/ °C	% ΔH	T_{max}/ °C	% ΔH	T_{max}/ °C	% ΔH
New parchment	42.5 ± 2.4	3.6 ± 0.4	5.4 ± 0.5	-	-	-	-	57.9 ± 1.0	48	64.1 ± 0.8	52
Bookbinging A (4 areas for analysis)											
A1	27.5	12.0	1.6	-	-	36.2 47.2	10 61	-	-	60.3	29
A2	27.3	12.1	1.8	33.7	7	-	-	48.3	62	60.6	31
A3	30.9	10.8	2.3	33.6	6	-	-	50.2	64	59.6	30
A4	26.3	11.2	2.0	33.4	5	-	-	49.2	67	61.0	28
Bookbinging B (3 areas for analysis)											
B1	28.7	6.4	2.6	31.9	3	44.5	13	49.4	48	61.2	36
B2	26.6	7.9	2.4	32.6	4	-	-	49.1	61	62.5	35
B3	24.5	12.1	1.9	-		47.7 39.2	62 4	-	-	60.9	34
Bookbinging B – spine stiffening (3 for analysis)											
B4_a	20.5	5.6	2.2	-	-	-	-	51.0	65	58.3	35
B4_b	18.8	4.6	2.0	-	-	39.6	11	51.7	56	62.0	33
B4_c	21.0	6.0	2.0	-	-	39.3	10	51.8	56	61.6	34

FTIR-ATR results showed that the parchments surface is slightly affected by the hydrolysis process and only some areas present degradation by oxidation. Calcium carbonate and alumino-silicates were identified for all investigated parchments. In FTIR-ATR spectra of the samples taken from the spine stiffening of the bookbinding B, calcium oxalate was identified through its characteristic absorption bands at 1618 cm^{-1} and 1320 cm^{-1}. The calcium oxalate is, most probably, the consequence of a fungal attack [25].

VII.6 Damage assessment of some historical leathers

The conservation state of some historical leather objects belonging to national museums (Table 2) were evaluated according to the damage assessment protocol. Only one micro-sample was taken from each object, from those areas that appeared most deteriorated and was investigated by MHT and micro DSC methods.

Table 2. *List of the historical leathers.*

Sample symbol	Object	Dating	Belonging to
BU-1	Sword scabbard	XVIII century	National Military Museum
BU-2	Cuirass	XVI century	"Ferdinand I", Bucharest
BU-3	Sword hilt 1	1837	
BU-4	Sword hilt 2	XIX century	
BU-5	Scimitar scabbard	not dated	
BU-6	Belt	1860	
BU-7	Cordovan upholstery	not dated	National Museum "Cotroceni", Bucharest
BU-8	Bookbinding	XVIII century	National Museum of Romanian History, Bucharest
BU-9	Travel chest of Manuk Bey	XVIII century	History and Art Museum of Bucharest
BU-10	Tobacco box	XIX century	

Deconvolution of the most historical leathers curves revealed several collagen populations with distinct thermal stability, from collagen structures strongly linked to tannin molecules (T_{max}> 80 ° C) to collagen structures unlinked to tannin molecules (T_{max} <60 ° C) (Figure 23). Based on these findings, the temperature range of denaturation transitions of collagen in vegetable leather was divided in three intervals: (1) *leather-like* (L) interval (60 °C < T_{max}< 85 °C), where constrained collagen (i.e. linked to the tannin matrix) shows denaturation; (2) *parchment-like* (P) interval (40 °C < T_{max} < 60 °C), where fully de-tanned, unconstrained collagen shows denaturation and (3) *gelatine-like* (G) interval (T_{max} ≤ 40 °C), where gelatinised collagen shows thermal transition. The thermal transitions of the various collagen populations identified by deconvolution could be thus allocated to one of these intervals depending on their T_{max}. This categorisation enabled us to quantify the collagen populations within historical leather based on their enthalpy percent (Table 3).

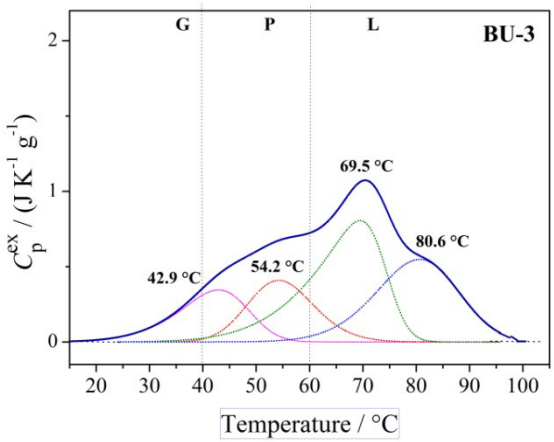


Figure 23. *Micro DSC curve associated with thermal denaturation of collagen from BU-3 historical leather sample.*

Table 3. *Thermal stability and proportions of the various collagen populations in historical leathers obtained by deconvolution of the full DSC peaks. The overall enthalpy (ΔH) and peak half-width ($\Delta T_{1/2}$) are also reported.*

			Population G		Population P		Population L	
Sample	ΔH / J·g^{-1}	$\Delta T_{1/2}$ / °C	T / °C	% ΔH	T / °C	% ΔH	T / °C	% ΔH
New leather (Sc)	26.8 ± 2.2	2.4 ± 0.2					69.1 ± 1.3	100
BU-1	37.8	39.8	-	-	39.5 47.5	12 40	66.0 84.4	35 13
BU-2	31.3	28.9	-	-	39.0 47.0	4 55	65.4 82.8	31 10
BU-3	36.6	35.7	-	-	42.9 54.2	14 16	69.5 80.6	42 28
BU-4	7.3	10.7	38.5	78	55.0	22	-	-
BU-5	7.1	11.0	-	-	45.2 50.0 55.4	12 75 13	-	-
BU-6	28.9	14.7	33.6	5	48.2 60.3	74 6	76.6 84.9	6 9
BU-7	9.8	10.6	33.7	66	42.6	17	85.1	17
BU-8	16.6	6.5	-	-	-	-	66.2 75.7	81 19
BU-9	18.5	4.8	20÷30	7	55.1	82	64.9	11
BU-10	44.6	10.4	-	-	44.2 51.1	10 42	69.0 84.4	39 9

It was found that historical leathers are complex blends of collagen with distinct thermal stability and gelatine structural states. Early identification of unstable, pre-gelatinised and gelatine structures can help to prevent eventual irreversible damage caused by environmental conditions or conservation / restoration treatments. Micro DSC analysis has allowed us to rank the historical leathers in 3 groups as follows: (i) stable (thermal stability decreases in the order BU-3 > BU-10 > BU-1 ≈ BU-2); moderately stable (thermal stability decreases in the order BU-8 > BU-9 ≈ BU-6 > BU-5) and unstable (BU-7 more unstable than BU-4). Additionally, the MHT and micro DSC indicators were correlated. T_f and T_s as well as C and ΔT intervals have been considered for the evaluation of the shrinkage activity of collagen in historical leathers. The obtained results confirm the high potential of a systematic analysis of historical leathers deterioration based on micro DSC and MHT methods.

CONCLUSIONS

Thermal microscopy (MHT), differential scanning calorimetry (DSC) and infrared spectroscopy (FTIR) proved to be extremely useful and appropriate to characterise artificial ageing of collagen in parchment and vegetable tanned leather, as well as for damage assessment of historical collagen-based materials. New and artificially-aged collagen-based materials (parchments and vegetable tanned leathers) as well as MHT, DSC in water, DSC in nitrogen flow, micro DSC and FTIR-ATR methods were used to test the capabilities of these techniques.

The obtained results for new and artificially-aged collagen-based materials allowed us to establish specific indicators, provided by each applied analytical method, for identification and monitoring of the collagen structure deterioration in parchment and vegetable tanned leather, as well as to evaluate the effects induced by the main environmental factors (heat, humidity and visible light).

Also, a damage assessment protocol for historical leathers and parchments was developed, based on the obtained results for new and artificially-aged collagen-based materials. The application of the damage assessment protocol for heritage objects made from leather and/ or parchment depends on both the possibility of sampling and amount of sample, as well as the access to analytical techniques. The non-destructive version of the protocol includes the using of the visual analysis and infrared spectroscopy with attenuated total reflection (FTIR-ATR) method. Visual assessment, at macroscopic and microscopic levels, provides information on the material's quality and on isolated deterioration features, while degradation at molecular level (hydrolytic cleavage of peptide bonds, oxidation of polypeptide chains, gelatinisation) and identification the materials added during the manufacturing or formed on ageing are obtained by using FTIR-ATR. The micro-destructive version of the protocol includes the use of the MHT, DSC in water, micro DSC and DSC in nitrogen flow methods, which provide quantitative and qualitative information on leather and/ or parchment deterioration. If a very small amount of sample (0.1 mg) is available, the MHT method could be applied in order to evaluate the shrinkage activity of parchment or leather fibres. Complementary and precise information on the hydrothermal stability of collagen at mesoscopic level and the thermal stability of collagen crystalline phase, as well as the proportions of the unstable, pre-gelatinised and gelatinised collagen populations can be obtain if 2-5 mg of sample and DSC equipment are available. Based on this information, the "sensitivity" of the historical materials at environmental conditions can be established.

The utility of the damage assessment protocol for historical leathers and parchments was confirmed by the obtained results for heritage objects. The application of the protocol to heritage objects made from leather and/ or parchment (documents issued by the Moldavian Chancery during the reign of Stephen the Great – XV century; Marco Polo's Testament – XIV century; a Byzantine palimpsest from IX century, rewritten in XIII century; a military coat dated XVI – XVII centuries etc.) allowed the conservation state evaluation and thus contributed to making the right decisions on the optimal conditions for storage and exposure, as well as to selection of the conservation or restoration treatments.

It has been shown that the application of the damage assessment protocol for heritage objects made from leather and/ or parchment contributes to the knowledge, conservation and valorification of cultural heritage in Romania and abroad.

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Dissemination of the results:

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