Badea *et al. Herit Sci* (2019) 7:68
https://doi.org/10.1186/s40494-019-0310-x

Heritage Science

RESEARCH ARTICLE Open Access

The effect of halloysite nanotubes dispersions on vegetable-tanned leather thermal stability

Elena Badea[1,2*], Cristina Carşote[3], Emanuel Hadîmbu[1], Claudiu Şendrea[1] and Maria-Cristina Lupaş[1]

Abstract

Leather artefacts in historical collections and old leather bookbindings in archives and libraries frequently show a variety of visible changes which alter their appearance and ability to be used, such as powdery surface, weakened structure, complete or partial loss of the grain layer. However, historical leather stabilization and consolidation yet represent a challenging problem for conservators due to the sensitivity of collagen to the action of most of the conservation and restoration formulations currently used. In this paper we report our recent research concerning the development of halloysite nanoparticles dispersions for enhancing the hydrothermal stability of weakened historical vegetable tanned leather. The effects of halloysite nanotubes (HNTs) dispersed in various aqueous and non-aqueous mediums on the thermal stability of collagen in historical leather were tested using thermal microscopy, thermogravimetry and micro differential scanning calorimetry. Unilateral nuclear magnetic resonance was used to evaluate the changes in water dynamics due to collagen matrix interaction with dispersion media. The treated samples were also characterized using infrared spectroscopy and scanning electron microscopy. The analytical assessment confirmed the leather thermal stability increase and a partial recovery of fibres cohesion, thus validating the use of HNT dispersions as a basic tool for the preservation of collagen-based materials. Further functionalisation and encapsulation using antimicrobials, fungicides and pH adjustment nanoparticles will deliver novel and more durable HNTs-based conservation treatments.

Keywords: Historical leather, Halloysite nanotubes, PEG 400, Beeswax, Urea, Thermal stability, Unilateral NMR, SEM, ATR-FTIR

Introduction

For millennia, man has used animal hides in its day by day life to protect himself against the hostile or extreme natural elements or other physical dangers by building shelters (e.g. tents, yurts) and producing footwear, garments and military accessories (e.g. cuirasses, shields, helmets, harnesses, etc.). With further technological development, skins and hides found additional uses as writing material or as elements of musical instruments, household tools, entertainment items (e.g. puppets and toys). In the Middle Ages, the production of artistic objects (e.g. travel chests, jewelry boxes, luxury bookbindings) and furniture accessories (e.g. furniture cordovan leather, tooled, painted and gilt leather wall hangings) has grown to meet the aesthetic taste of the upper classes. Nowadays, such artistic, historical and archaeological objects are preserved in museums, libraries or archives and keep alive our history, culture and tradition. Keeping these objects in good condition is a struggle that demands continuous attention and cure from the conservation community. However, there are still no satisfactory solutions to remedy leather deterioration and the currently applied conservation methods offer temporary mitigation solutions, without guaranteeing a real and durable effectiveness. A survey of leather conservation practices conducted in 2008 highlighted that solvent set tissue

*Correspondence: elena.badea@unito.it
[1] Advanced Research for Cultural Heritage (ARCH Lab) Group, National Research & Development Institute for Textiles and Leather, ICPI Division, Ion Minulescu 93, 031215 Bucharest, Romania
Full list of author information is available at the end of the article

hinge repair, consolidation via application of cocktails of microcrystalline waxes and cellulose ethers, and the use of molded cave paper as a substitute for leather were widely accepted and utilized while traditional treatments such as the application of leather dressings and cleaning with saddle soap were also still used [1]. It should be underlined that deterioration is an inevitable long-term effect triggered by environment-induced reactions and physical wear and tear caused by handling and use. Deterioration is also influenced by the manufacturing technology, namely the chemical ingredients used in the various steps of leather making, from dehairing to tanning and finishing. In fact, a marked disparity between the performance of early (vegetable tanned) and current/industrial (chrome tanned) leather was observed as early as 1850 when the first investigation associated the leather red rot with sulfur dioxide present in the atmosphere [2]. The performance of chrome tanned leather improved from 1930 onwards and the evolving leather technology provided new products with certain desired properties. However, the long-term durability of modern leather is not known since there is little commercial interest in long periods of durability and the market of leather for art, design and archive purposes is very small. There is thus a serious risk that modern and contemporary artworks made of/with chrome leather undergo faster degradation processes than ancient and medieval artworks. The subject of consolidating deteriorated leather has however received relatively little attention despite that the traditional materials and methods used for leather preservation [3, 4] involves risks to the artefacts due to the sensitivity of destabilized collagen to the action of solvents and chemicals. In addition, many commercial care products used in everyday practices for cleaning and softening have been found not effective in preserving leather and even harmful [5, 6]. In recent years, the use of nanoparticles in art conservation has started to be explored. In 2016, Baglioni et al. [7] reported the use of chemical hydrogels loaded with an o/w nanostructured cleaning fluid to remove surface patinas of dirt and salts from the surface of historical leather objects, while in 2018 Bicchieri et al. described the restoration of leather cover of eighteenth century book using nanocollagen [8]. Hybrid systems based on HNTs and hydroxypropyl cellulose revealed as successful consolidants for paper [9], whereas waterlogged archaeological woods were successfully consolidated through the immersion method using acetone dispersions of HNTs and sustainable polymers [10, 11]. Pickering emulsions based on halloysite nanotubes and ionic biopolymers (chitosan and pectin) were developed for wax layer removal from marble surfaces, opening up a new sustainable approach in preparing cleaning formulations for conservation of cultural heritage [12]. Given the crushing number of red rotted/weakened leather artefacts, especially bookbindings, as well as the current lack of treatment options beyond surface consolidation, the use of HNTs for preservation of weak and friable leather it seemed to us a very promising subject.

Halloysite is a natural emerging nanoclay that has attracted a growing scientific and industrial interest because of its abundance, biocompatibility and safety as well as its versatile characteristics including the large specific area, high dispersion, high porosity, no swelling and the tunable surface chemistry [13–18]. Halloysite is a two layered aluminosilicate ($Al_2Si_2O_5(OH)_4 \cdot 2H_2O$) with a predominantly hollow tubular structure in submicron range. Halloysite dimensions depend on the specific geological origin, the length ranging from 1 to 3 μm, while the external and internal diameters range between (50–200) and (10–70) nm, respectively [19, 20]. It was showed that halloysite nanotubes (HNTs) uniformly distributed within the polymer matrix induced an enhancement of tensile strength and vapor barrier properties that can be attributed to the large HNTs specific surface [19, 21]. Addition of nanotubes generated a thermal stabilization of the polymers due to the entrapment of the degraded products into the HNTs lumen [22].

Based on these considerations and on the fact that leather deterioration induces a progressive thermal destabilization of collagen [23–25], in the present study we opted for testing the application of HNTs in the form of nanoparticles dispersed in various aqueous and non-aqueous media in order to develop a product that could improve the thermal stability of damaged leather. Besides stabilizing leather, such formulation should also cause no further damage to the collagen matrix and be safe for the conservators too. Relying on both recent literature data and conservation practices, we selected beeswax alcoholic solution, PEG 400 and a hydroalcoholic solution of urea and sodium chloride to disperse the nanotubes and facilitate filling up the structure or blocking the pores of the historical leather. Beeswax is a no toxic and cheap biological material which mainly consists of alkanes, alkenes, free higher fatty acids, monoesters, diesters and hydroxymonoesters, while fatty alcohols and hydroxydiesters are minor constituents. It is stable from the chemistry point of view and water-repellent. Moreover, literature reports that wax slows down the wood photodegradation [26] whereas wax/HNTs nanocomposites possess enhanced thermo-mechanical properties with respect to the pristine wax due to the inorganic skeleton conferred to wax by the nanotubes [10]. Moreover, waxes dressing formula (i.e. SC6000 microcrystalline wax, Renaissance Wax, British Museum and other commercially produced dressings made of lanolin, neat foot oil and beeswax) are still highly preferred by conservators

for leather consolidation, lubrication and cosmetic surface treatments [1]. The urea-sodium chloride hydro-alcoholic solution was successfully used for restoring the fire injured parchment codices of the National University Library of Turin (BNUTO). It was found that wet treatment followed by drying under moderate tension allowed partial recovery of the native properties of the collagen molecules [27]. Moreover, the urea-sodium chloride hydro-alcoholic solution showed particularly effective softening and stabilizing effects compared with traditional softening agents such as iso-propanol, various alcoholic mixtures, lanolin and Bibliobalsamo®crema (Italy) [28]. Regarding the use of PEG, it is worth mentioning that a treatment still currently used for water-logged leather consists in impregnation with PEG 400 followed by freeze-drying [29]. Moreover, Ershad-Langroudi and Mirmontahai [30] reported an increase of thermal stability of a historical leather bookbinding treated with PEG 400 and hydroxyapatite nanoparticles. Finally, the HNTs can load active chemical agents into the lumen of the nanotubes and allow their release in an efficient and controlled manner [31, 32]. So, in case that the investigated HNTs dispersions will exhibit good stabilising effects on collagen, the encapsulation of antimicrobials, fungicides and pH adjustment nanoparticles will further enhance the durability of HNTs-based conservation treatments. Moreover, through the functionalization of HNTs surface, it is possible to modulate their physico-chemical properties, increasing their ease of dispersion and manipulation.

In conclusion, this work was aimed at achieving deeper comprehension of the effects of various aqueous and nonaqueous dispersions of HNTs on historical leather stability. The hydrothermal stability of collagen matrix, one of the most critical aspects which should be considered in the development of any restoration treatment for historical leather, was evaluated at macroscopic, microscopic and nanoscopic levels using appropriate methods and techniques such as standard method for determination of shrinkage temperature, Micro Hot Table (MHT) method and micro Differential Scanning Calorimetry. Unilateral nuclear magnetic resonance was used to evaluate the changes in water dynamics due to collagen matrix interaction with dispersion media. The treated samples were also characterized using infrared spectroscopy and scanning electron microscopy.

Materials and methods

Chemicals

Halloysite nanotubes (Sigma Aldrich), urea (98%, Sigma Aldrich), NaCl (> 99%, Sigma Aldrich), ethanol absolute (99.9%, Chemical Company), polyethylene glycol (PEG) 400 (Alfa Aesar), 1-chlorobutane (> 99%, Alfa Aesar) and natural cosmetic beeswax (EcoNatura) were used for the preparation of nanoparticles dispersions.

Preparation of the dispersions

It is essential for raw halloysite to disperse as they are aggregations with varying diameter (30–70 nm) and length (1–3 μm) under natural conditions. After dispersed, both a higher specific surface and a greater cavity area of halloysite nanotubes (HNTs) could be obtained.

HNTs 0.5% (w/w) was added to PEG and stirred at 4000 rpm until the complete homogenization was reached.

The hydroalcoholic solution of urea and sodium chloride (BNUTO solution) was prepared by mixing water 48% (w/w), ethanol 48% (w/w), urea 2% (w/w) and NaCl 2% (w/w). HNTs 0.5% (w/w) was added to BNUTO solution and stirred at 4000 rpm until the complete homogenization was reached.

Beeswax and 1-chlorobutane were mixed with a weight ratio beeswax/1-chlorbutane of 2/98 (g g^{-1}) under stirring and kept at 40 °C overnight. Then 0.5% of HNTs was added while maintaining the stirring for a further 24-h period until complete homogenization was reached.

Before performing the treatments, all dispersions were conditioned for 10 min in an ultrasound bath. The three dispersions will be referred to as HNT-beeswax, HNT-PEG and HNT-BNUTO.

Treatment of historical leather

The samples used in this study were from a bookbinding dated 1811 which was donated for research purposes by the Vaslui County Museum after decision was taken to replace it with a new cover. Four samples of (5 × 5) cm were cut from the same area of the binding to limit as far as possible the effects of the lack of homogeneity of leather properties.

The microscopic examination of both grain and flesh sides of leather was carried out at (50 and 200)X magnification to ascertain the heterogeneity of the deterioration as well as to determine the animal species of the skin used to manufacture the leather. A portable digital microscope Dino-Lite AD7013MZ with a resolution of 1.3 Megapixels was used. The tannin type was detected by ATR-FTIR analysis based on the vegetable tannins characteristic absorption bands [33].

The nanoparticles dispersions were applied by the immersion method. The dispersion was transferred into a Petri dish placed on the shaker plate and the samples were kept immersed for 10 min under shaking at 100 rpm speed. To remove the solvents, the samples were firstly dried in the oven at 25 °C for 60 min and then left at ambient temperature for at least 48 h, except for the

HNT-PEG treated sample which required a much longer drying time.

pH measurement

Surface pH measurements on leather samples before and after treatments were taken using a Nahita model 903 benchtop pH meter equipped with a flat membrane ceramic electrode 901. Each measurement spot (1×1 cm^2) was damped with a droplet of water using a pipette. After 2-min extraction, the electrode was placed on the surface and the pH of the water film was measured. The pH value was obtained as the average between 2 measurements (being the leather surface highly water sensitive, it was not possible to carry out numerous measurements on each spot).

Scanning Electron Microscopy (SEM) analysis

Surface examination of the leather morphology before and after treatments as well as monitoring of HNTs within leather surface fibrils was made using a FEI Quanta 200 scanning electron microscope. Samples were mounted on Al stubs and observed at increasing magnification (from 500× to 16000×) and at different locations for each sample. SEM observation of the grain surface of samples were performed in low vacuum mode without the need of a conductive layer, at 15 keV accelerating voltage with a tungsten filament and working distance of 10 mm.

Infrared spectroscopy in attenuated total reflection mode (ATR-FTIR) analysis

For the ATR-FTIR analysis, an Alpha portable spectrometer (Bruker Optics) equipped with a Platinum diamond ATR was used. Spectra were acquired on both sides of leather samples (flesh and grain), before and after the treatments, in the range (4000 to 400) cm^{-1} by performing 32 scans with a resolution of 4 cm^{-1}. OPUS 7.0 software was used for processing and evaluating the spectra.

Measurement of dynamic contact angle

Wettability was determined by dynamic contact angle measurements carried out with a goniometer CAM100 (KSV Instruments Ltd.) at room temperature using deionized water (2.0 µL), the sample being placed horizontally. To determine the dynamics of contact angle change with time, the shape of the liquid droplet was recorded by the camera starting from second zero (placing the drop on the sample) to the 120th second with 3 s intervals. For each sample, three measurements from different spots were taken, and the average of these values was reported as a contact angle. Initial contact angle ($t = 1$ s) and final contact angle ($t = 120$ s) were used for the analysis.

Measurement of hydrothermal stability

The hydrothermal stability of collagen represents a reliable measure of the degree of deterioration of leather. It can be measured by various methods commensurate with different levels of collagen hierarchic organization, from molecules that successively pack into fibrils, to fibres/fibre bundles and tissue (e.g. skin) level. Regardless of the method, the process to be measured is collagen denaturation, a time-dependent irreversible transformation of the native triple helical structure into uncoiled structures. Differential Scanning Calorimetry (DSC) technique has the ability to extract the hydrothermal properties of molecular and fibrillar collagen and check its integrity in different collagen-based materials, whereas micro hot table (MHT) method provides the shrinking behavior (deformation) of collagen fibres. The standard method SR EN ISO 3380-2003 used in leather industry measures the shrinkage temperature of leather material. We have measured the hydrothermal stability of leather before and after treatments at macroscopic, microscopic and nanoscopic levels using the standard method SR EN ISO 3380-2003, micro hot table (MHT) method and micro differential scanning calorimetry (micro DSC) technique, respectively.

The standard test method SR EN ISO 3380-2003 provides the temperature at which a thoroughly wetted leather specimen of 1 cm width and 5 cm length experiences shrinkage when it is heated in water at a rate of 2 °C·min^{-1}. The value of the so-called shrinkage temperature T_s is commonly used in leather industry as an indicator of the type of tannage or degree of tannage, or both. More recently T_s is considered a fine measure of the deterioration of ancient leathers [34]. The standard tests for T_s were performed using a Giuliani IG/TG/Theiss apparatus (Giuliani Tecnologie S.R.L.).

The standard method had been modified to be applicable to micro-samples and used in the conservation field. The resulted MHT method is based on the combination of microscopy and thermal analysis and requires only a few micro-fibres for measuring their shrinking activity throughout the shrinking interval of collagen fibres. Based on the shrinking activity one can assess the level of damage in historical collagen-based materials such as leather and parchment [23, 34]. The collagen fibres shrinking activity was measured using a Linkam LTS120 stage equipped with a temperature controller and Linksys32 temperature control software which enables full PC programming of temperature. The shrinking motion was digitally recorded with a SMZ 745 Nikon stereomicroscope equipped with a Nikon D90 digital camera. Besides the shrinkage temperature, the temperature at which the very first shrinking motion is observed T_f and temperature of the very last observed shrinking motion T_l were

measured. The main shrinking interval ΔC (in which most of the fibres simultaneously shrink) was also measured, while the total shrinking interval was calculated as $\Delta T = T_{\overline{1}} T_{f}$.

The collagen fibrils hydrothermal stability was measured using a high-sensitivity SETARAM Micro-DSC III calorimeter. Micro-samples of about 2.0 mg suspended in 0.5 M acetate buffer (pH = 5.0) were heated in the temperature range (5 to 95) °C at 0.5 K min^{-1} heating rate as described earlier [25]. From the experimental data, the temperatures at the onset (T_{onset}) and at the peak (T_{max}) as well as the specific enthalpy of fibrillar collagen denaturation (ΔH) were calculated. The temperature span of the denaturation transition was reported as peak width at half height ($\Delta T_{1/2}$). DSC multiple peaks of historical leather were deconvoluted using the PeakFit asymmetric Gaussian fit function to improve the fit of the asymmetry in the peaks [25, 35]. The collagen populations evidenced by deconvolution were classified in function of their thermal stability (T_{max}). Assuming that the percent contribution to the overall denaturation enthalpy of each endotherm is proportional to the percentage of the corresponding collagen population, a quantitative description of damage is also provided [25, 36].

Determination of stability at thermo-oxidation by thermogravimetry (TG) analysis

TG analysis consists in the measurement of the mass change of a sample while it is subjected to a temperature regime. It is commonly used for materials characterization through the analysis of characteristic decomposition patterns. As modern instruments require samples less than 1 mg for an accurate determination, TG analysis is suitable for the investigation of artefacts. TG analysis was carried out with a STA 409PC Luxx (Netzsch) in oxygen flow, in the temperature range (25 to 700) °C, at 10 K min^{-1} heating rate. The mass of the analyzed samples was in the range (4 to 6) mg. From the first derivative of TG curve (DTG curve), the rate (d %Δm/dt) of the first process of thermo-oxidation was calculated as previously reported [24].

Single sided ^{1}H Nuclear Magnetic Resonance analysis

Non-invasive NMR relaxometric characterization of leather before and after treatment was performed at room temperature using an NMR MOUSE PM2 (Magritek GmbH) controlled by a Kea 2 spectrometer (Magritek GmbH) operating at 27 MHz ^{1}H resonance frequency. This system can measure proton relaxation times. Effective ^{1}H spin–spin relaxation T_{2eff} measurements have been measured using the Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence with an echo-time (TE) of about 25 μs. The experimental CPMG curves were best analyzed by a combination of double exponential functions [37, 38].

Results and discussions

The effect of HNTs dispersions on collagen matrix hydrothermal stability

The parameters used for assessing the shrinking behaviour and thermo-oxidative stability of historical leather before and after treatment are listed in Table 1, while the calorimetric parameters associated with denaturation of fibrillar collagen in the historical leather before and after treatment are reported in Table 2.

At macromolecular level, the values of shrinkage temperature T_s measured using the standard method SR EN ISO 3380-2003 showed an increase of the hydrothermal stability for all treated samples. This behavior is similar to that we observed in new vegetable tanned leather treated with the same types of dispersions [39]. In terms of shrinking behavior of collagen microfibres, the most important effects were the increase of the T_f and T_s temperatures and the simultaneous decrease of the ΔC and ΔT intervals (Table 1 and Fig. 1). Wide ΔT and ΔC intervals have been associated to a broad

Table 1 Characteristic parameters of collagen shrinking and rates of thermal oxidative degradation for untreated and treated historical samples

Sample	Hydrothermal stability (shrinking behavior)				Thermo-oxidation rate
	Standard method	MHT method			TG/DTG
	T_s (°C)	T_f (°C)	T_s (°C)	T_l (°C)	d %Δm/dt (min^{-1})
Untreated	68 ± 1	36.2 ± 0.8	68.6 ± 1.2	86 ± 2.3	4.78
HNT-beeswax	72 ± 2	42.4 ± 1.3	71.3 ± 2.8	84 ± 3.1	1.96
HNT-PEG	74 ± 1	44.8 ± 1.0	73.7 ± 1.6	83 ± 1.9	3.54
HNT-BNUTO	70 ± 1	45.1 ± 0.9	69.4 ± 1.5	82 ± 2.0	3.89

For both standard and MHT methods, at least three measurements with fresh samples were performed for each sample

Uncertainty is the standard deviation

Table 2 Characteristic parameters of collagen denaturation for historical samples before and after treatment

Sample	Micro DSC (collagen fibrils denaturation)							
	Overall DSC peak parameters				Population 1		Population 2	
	T_{onset} (°C)	T_{max} (°C)	ΔH (J·g^{-1})	$\Delta T_{1/2}$ (°C)	T_{max1} (°C)	%	T_{max2} (°C)	%
Untreated	63.1 ± 1.5	70.9 ± 1.8	17.0 ± 1.9	7.0 ± 0.5	68.2 ± 0.5	49	70.9 ± 0.8	51
HNT-beeswax	65.9 ± 1.7	72.5 ± 0.6	17.2 ± 1.2	5.5 ± 0.3	69.1 ± 1.1	26	72.5 ± 0.6	74
HNT-PEG	64.6 ± 1.0	72.3 ± 0.5	16.1 ± 1.6	3.1 ± 0.8	68.0 ± 0.6	15	72.3 ± 0.5	85
HNT-BNUTO	64.2 ± 2.2	72.4 ± 1.2	18.2 ± 1.4	6.6 ± 0.4	69.1 ± 1.3	30	72.4 ± 1.2	70

The percentage of each collagen population is calculated based on its enthalpy contribution to the overall denaturation enthalpy. At least three measurements with fresh samples were performed for each sample

Uncertainty is the standard deviation

Overall enthalpies of denaturation were corrected based on the mass variation of the sample after treatment

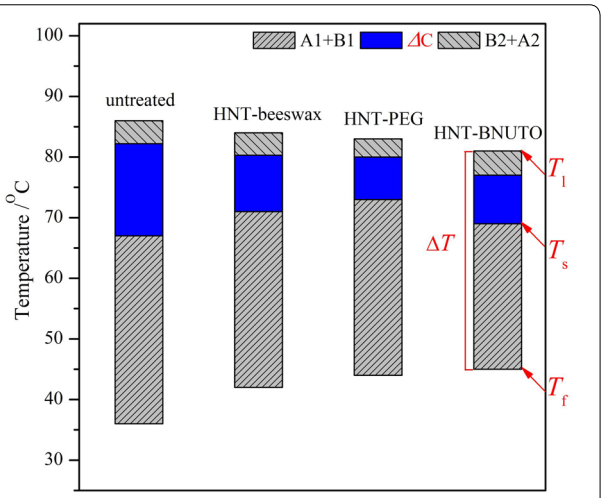

Fig. 1 Stacked column charts displaying the shrinking intervals for collagen fibres for the investigated historical leather before and after the treatments. The main shrinkage temperatures (T_f, T_s and T_i) and intervals (ΔC and Δ*T*) are indicated

distribution of collagen populations characterised by low to high thermal stability [38, 40, 41]. A high level of non-uniformity of deterioration is generally responsible for a high degree of leather heterogeneity [23]. Conversely, the narrowing of Δ*T* and ΔC intervals could be explained by an increase in the microfibre's cohesiveness. At the macroscale, the organization of collagen fibres into thicker fibres accompanied with increasing mesh size is a key feature of the structure of many tissues/materials. The observed hydrothermal stabilization could therefore be attributed to collagen bundles size and cohesiveness increase through collagen fibres association.

At fibrillar level, the calorimetric parameters associated with denaturation of collagen reported in Table 2 confirm the increase of the hydrothermal stability after treatments. Indeed, slightly higher values of T_{max} (temperature of denaturation of collagen fibrils) and T_{onset} (temperature at which first conformational changes occur within the fibrils) accompanied by the narrowing of the overall denaturation peak were observed after the treatments. The decrease of the peak width at half height $\Delta T_{1/2}$, a parameter that gives a measure of the breadth of the distribution of molecular thermal stabilities, suggests a more homogeneous thermal behavior of treated leather, in agreement with the shrinking behavior of collagen microfibres evaluated by the MHT method.

In addition to the MHT method, which only measures intensive properties such as temperature, DSC also directly measures an extensive property, namely the enthalpy of denaturation ΔH. This quantitative parameter is the energy required to disrupt the interactions stabilizing the secondary and tertiary structures of the triple helix. It was shown to be very useful in quantifying the effects of damaging factors on chemically unmodified collagen in parchment [36, 37, 42]. Recently, Carșote et al. [25, 35] have shown that micro DSC offers both qualitative and quantitative information to also characterize chemically modified collagen in historical leather.

Historical leather is a mix of collagen populations which structurally form a composite material: collagen with various degrees of tanning (chemically modified), fully de-tanned (chemically unmodified) collagen and gelatinized collagen. As a consequence, its hydrothermal stability behavior cannot be fully understood by simply analyzing the variation of some extensive parameters such as shrinking temperatures and intervals. The additional input from micro DSC consists of identification, separation and quantification of the various populations of collagen. This provides a bulk measure of the distribution of molecular thermal stabilities and a more

comprehensive picture of the structural integrity of collagen in leather. The endothermic peaks of collagen denaturation before and after treatments presented in Fig. 2 showed a multiple character as expected. Two peaks with $T_{max} > 65$ °C, indicating a certain degree of tanning, were singled out by appropriate deconvolution. It should be stressed that both T_{max} and ΔH values strongly depend on the tannin type and tanning technology (including the percent of tannin and tanning uniformity), factors that greatly varied depending on the craftmanship, geographical location and historical period. It is therefore not possible to define a range of standard T_{onset}, T_{max} and ΔH values for hypothetical undamaged old leather. In fact, very scattered values were reported by different authors [43, 44] for newly obtained vegetable-tanned leathers, i.e. T_{onset} varies from 63 to 88 °C and T_{max} from 66 to 90 °C, while values from 11.5 to 28 $J \cdot g^{-1}$ were found for ΔH. This could be ascribed, on one hand, to the experimental conditions which resulted in various hydration levels, influencing both the enthalpy and temperature of denaturation, and, on the other hand, to the tanning technology that determines the thermal stability of leather and its collagen content. The variation of the percentage of collagen in leather makes it impossible to compare the experimental specific enthalpy of denaturation obtained by different authors due to the extensive character of this property: ΔH it is calculated by taking the total enthalpy of the system measured as the area under the denaturation peak and dividing it by the total mass of the system. Hence, to avoid the errors due to the mass sample variation after the treatment, all samples were weighted before and after the treatment. Although no significant changes in the overall enthalpy of denaturation following treatments were observed, there was a noticeable redistribution of the partial enthalpy of the two collagen populations (Table 2). Accordingly, the hydrothermal stabilization was not only due to the increase in denaturation/shrinkage temperature, but also to the increase of the weight of the collagen population with higher thermal stability (Table 2). Could we attribute this thermal stabilization to the association of collagen microfibres and fibres? When collagen fibres deteriorate, the fibre bundles are observed to become thinner and start to lose cohesion: open spaces appear between the bundles,

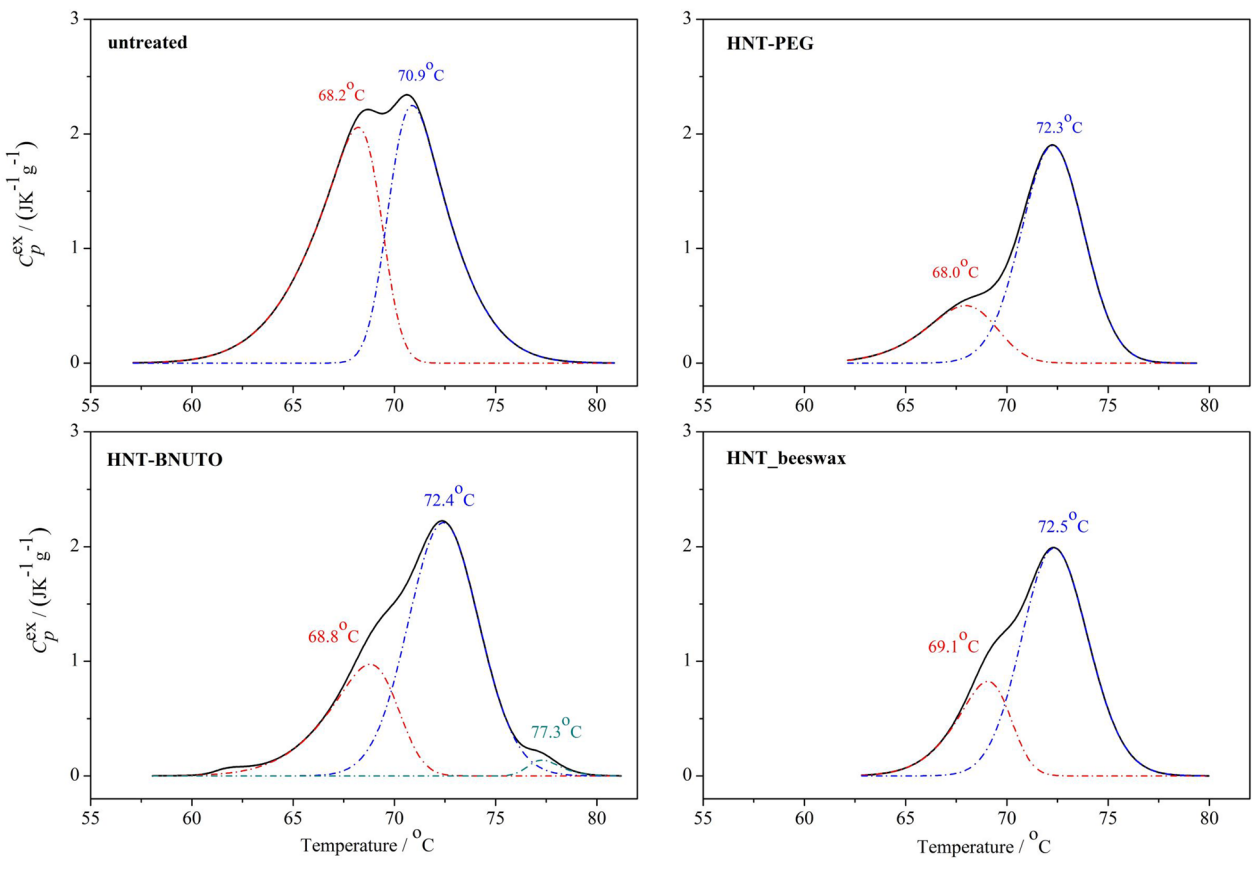

Fig. 2 Deconvolution of DSC denaturation multi-component peaks for the untreated and treated historical leather samples illustrating two collagen populations with distinct thermal stability

which are interspersed with crisscrossed, tangled, thinner collagen fibres. Collagen fibres bundles start to split apart and lose orientation as shown by SEM images in Fig. 3. Contrary, the treatments caused a rounding/swelling of the fibers and a reduction of the spaces between them with a slight superficial melting effect (Fig. 4).

In fact, a method to directly tune the self-assembly of collagen molecules and fibre growth using unreactive hydrophilic polyethylene glycol (PEG) chains was reported [45]. The underlying mechanism known as macromolecular crowding affects fibre diameter and organization having thus large effects on conformational stability and structural properties of fibrous proteins [46]. Based on these evidences, we can hypothesize that the stabilization effect determined by the HNT-BNUTO treatment is the result of two synergistic actions: the formation of urea-mediated hydrogen-bond network and sodium chloride-induced desolvation [47–49]. Regarding the thermal stabilisation induced by the HNT-beeswax treatment this could be attributed to the filling and blocking of leather pores as a result of HNT-beeswax adhesion onto porous leather surface.

Taken together, the increase of the hydrothermal stability of all treated samples could be explained by the association of collagen fibrils into thicker fibres and that of fibres into thicker fibre bundles thereby causing partial recovery of fibrillar cohesion, a key feature of the structure of collagen-based tissues that can be "manipulated" to partially recover the stability of deteriorated leather.

The effect of HNTs dispersions on collagen matrix thermo-oxidative stability

The rates d %Δm/dt of the pyrolytic thermo-oxidation process for untreated and treated samples are reported in Table 1, while the R_{max} values normalised by the corresponding average value of the untreated leather are illustrated in Fig. 5. The rate of the thermo-oxidation process corresponds to the maximum of the thermo-oxidation peak (peak II) in the DTG curves (Fig. 6). Generally, the non-isothermal degradation of leather occurs through three processes accompanied by mass loss: water loss at $T < 150$ °C, pyrolytic thermo-oxidation and decomposition of the material. Previous studies on various collagen-based materials reported by Budrugeac et al. [50–52] shown that the rate d %Δm/dt of the pyrolytic thermo-oxidation process well correlates with the collagen matrix cross-linking degree being thus a good indicator of the damage level in historical collagen-based materials. They observed two different thermo-oxidative behaviours of historical leather: low rates of the thermo-oxidation process were associated to a parchment-like behaviour (i.e. leather fully de-tanned), while the high rates of the thermo-oxidation process were related to an advanced deterioration degree of leather [52]. Of the results obtained it is deduced that all treatments have an oxidation rate reduction effect.

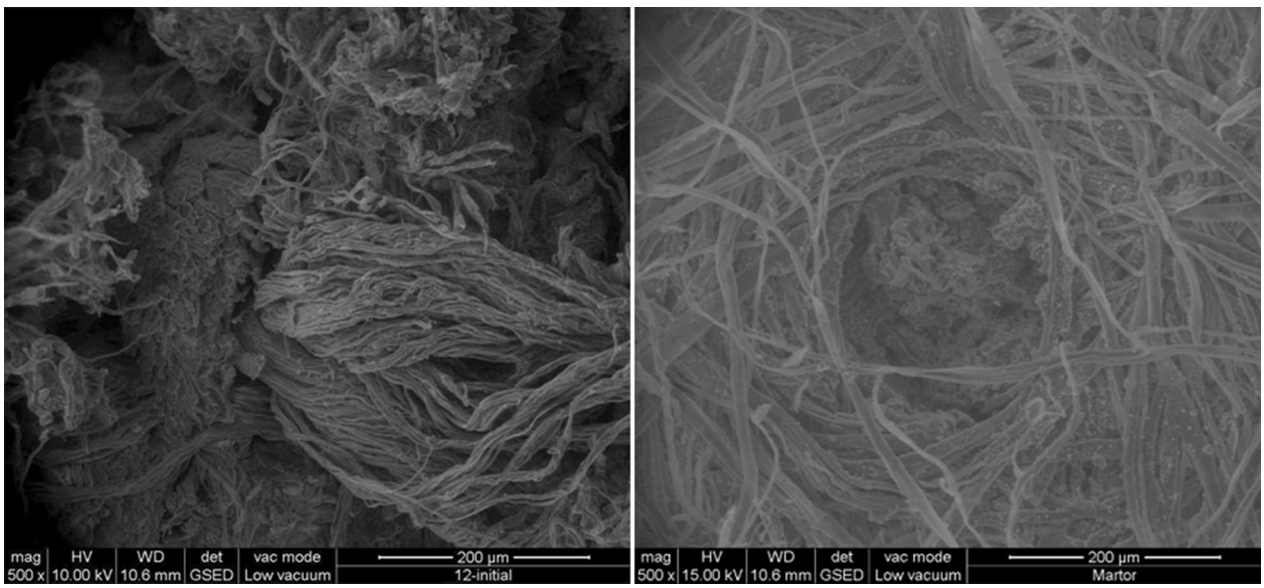

Fig. 3 SEM images of highly oriented fibres bundles in new vegetable calf leather (left side) and fibres cohesion loss and crisscrossing in historical leather (right side). Magnification ×500

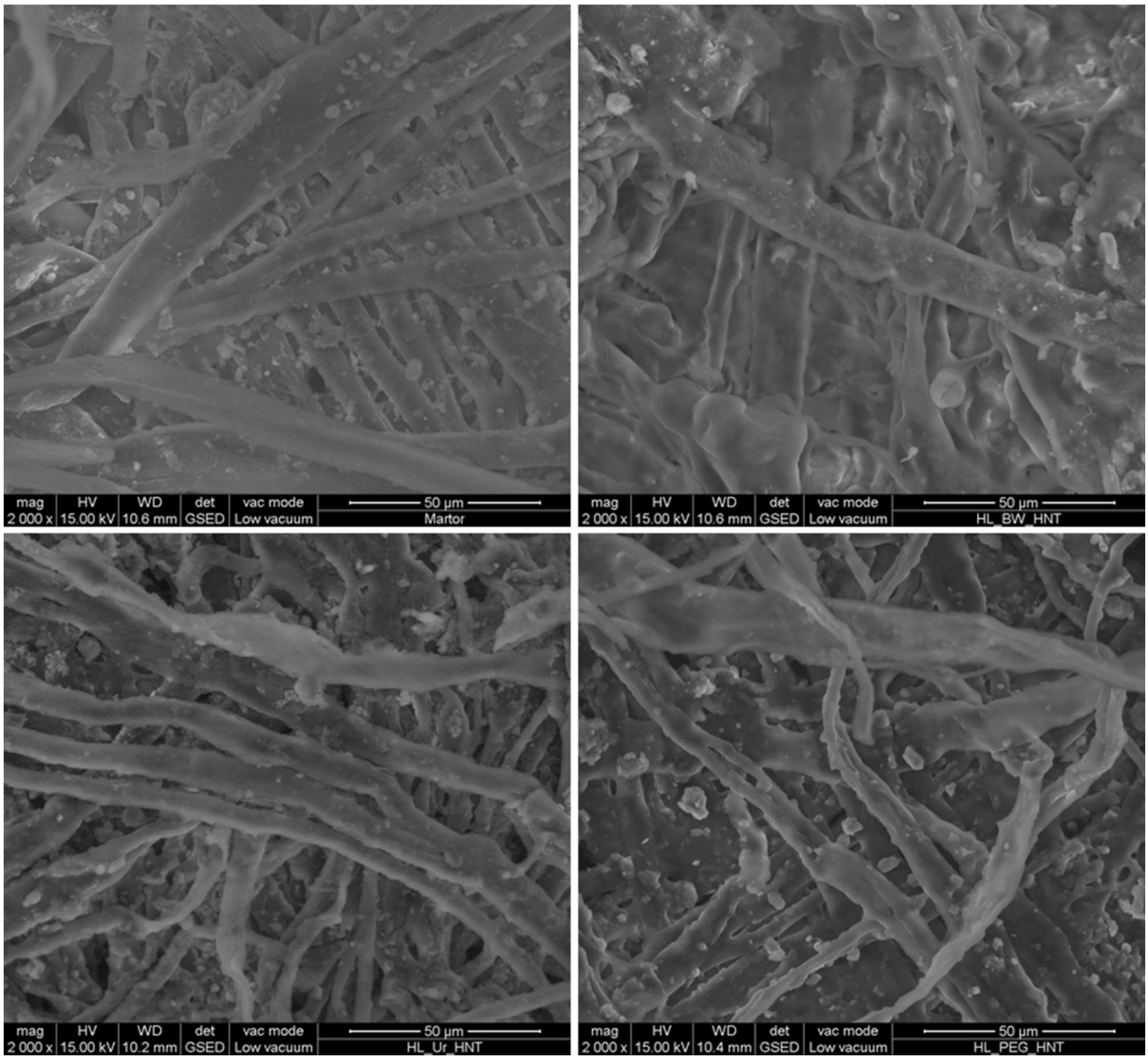

Fig. 4 SEM images of untreated (top left side) and treated leather: HNT-PEG treated sample (top right side): swollen fibres and melted surface; HNT-beeswax treated sample (bottom left side): fibres with rounded edges and partially melted; HNT-BNUTO treated sample (bottom right side): slightly swollen fibres and partially melted. Magnification ×2000

The effect of HNTs dispersions on water dynamics in collagen matrix

The interaction of collagen with water at the various levels of its hierarchical organisation confers a great stability to the collagen-based materials. The overall model of water hydration involves the monomolecular layer in which the water molecules are H-bonded to the helical structures and several outer polymolecular layers which are considerably more mobile [53]. Badea et al. [38] showed that in complex systems like those formed between the collagen and tannin molecules, the structure of water surrounding collagen is strongly affected by the tannin type. Changes in the water dynamics due to the collagen-tannin matrix interaction with the HNT dispersions were monitored with the help of the transversal relaxation T_2 time measurements (Table 3). Two relaxation components were detected through the exponential fitting of the transversal decays. The short relaxing component $T_{2\text{eff\_short}}$ was assigned to the rigid and semi-rigid fraction and the long relaxing component $T_{2\text{eff\_long}}$ was assigned to the mobile-amorphous fraction [54–56]. From the results reported in Table 3 we can note that

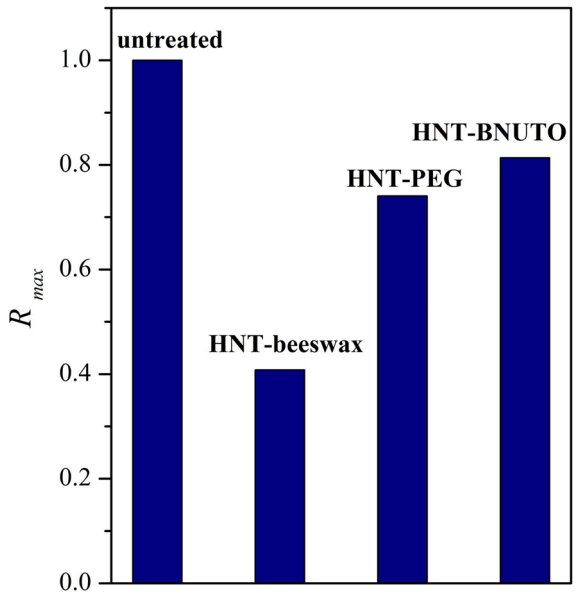

Fig. 5 R_{max} variation after leather treatment. R_{max} is the rate (d %Δm/dt) of the first process of thermal oxidation normalized by the corresponding value of the untreated leather

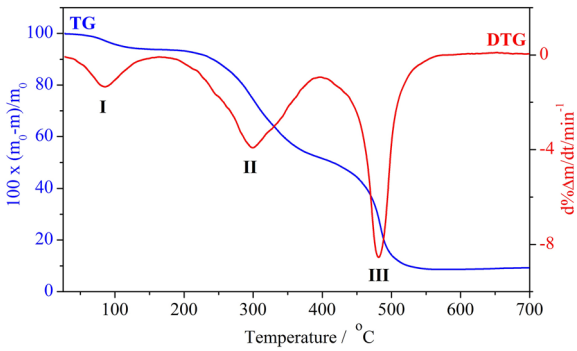

Fig. 6 TG and DTG curves showing the typical decomposition pattern for a new vegetable-tanned leather characterised by three successive exothermic processes: water loss, pyrolytic thermal oxidation and thermal decomposition

Table 3 Exponential fitting of transversal decays for historical leather before and after treatment compared to the corresponding values obtained for new vegetable tanned leather

Colagen-based material	T_{2eff_long} (ms)	T_{2eff}_short (ms)
New vegetable leather	2.32 ± 0.38	0.25 ± 0.02
Untreated	4.92	1.38
HNT-beeswax	8.17	1.63
HNT-PEG	1.52	0.01
HNT-BNUTO	1.53	0.03

both the long and short components are relaxing more slowly in the historical leather than in new leather. This could be attributed to an increase in the bulk water content [57] since deterioration of the collagen native structure causes increased mobility of water and consequentially increased water content within the collagen matrix. In fact, in the unfolded collagen structure more polar groups are exposed to water, and can therefore bind to significantly more water molecule that the native collagen [37]. The decrease of T_{2eff_long} value after both HNT-PEG and HNT-BNUTO treatments could therefore indicate a reduction in the "pools" of bulk water in treated leathers through substitution with PEG or urea. Besides, the decrease of the short relaxing component T_{2eff_short} may be related to a partial recovery of structural order in the rigid/semi-rigid phase due to microfibres association as a result of PEG/urea-mediated crosslinking. This agrees with the hydrothermal behaviour of HNT-PEG and HNT-BNUTO treated samples.

On the other hand, both T_{2eff_long} and T_{2eff_short} values increased after the HNT-beeswax treatment. A higher transversal relaxation time is generally related to an increased mobility of the chains, thus a less rigid system [58]. Hence, the relaxation behavior of leather treated with HNT-beeswax could be associated with some beeswax components such as fatty acids esters and carboxylic fatty acids acting as plasticizers and fluidifiers.

The effect of the beeswax's hydrophobic components should be also taken into consideration to explain the very large values of both T_{2eff} components. Appolonia et al. reported T_2 higher amplitudes for travertine after its treatment with hydrophobic products generally used for restoration [59]. The probable mechanism they proposed is that water molecules in the large pores can no longer mix with molecules in smaller neighboring pores, with higher surface areas and shorter relaxation times, which may not be occupied in the treated sample.

Application of the nanoparticles to leather

ATR-FTIR was carried out for both flesh and grain sides of the historical leather bookbinding, before and after the treatments. The spectra of the untreated sample show the main bands of collagen and those assigned to tannins. The main absorption bands of collagen, namely amide A (A_A): 3280 cm^{-1} (νN–H), amide B (A_B): 3070 cm^{-1} (δNH_2 overtone), amide I (A_I): 1635 cm^{-1} and amide II (A_{II}): 1541 cm^{-1} [60], are present in both the spectra of flesh and grain sides of historical leather (Fig. 7a). The infrared bands assigned to tannins of vegetable origin are generally overlapping with those corresponding to collagen bands in the (1800 – 1000) cm^{-1} spectral region. The second derivative of both flesh and grain sides spectra allowed us to identify the band at 1713 cm^{-1} assigned to

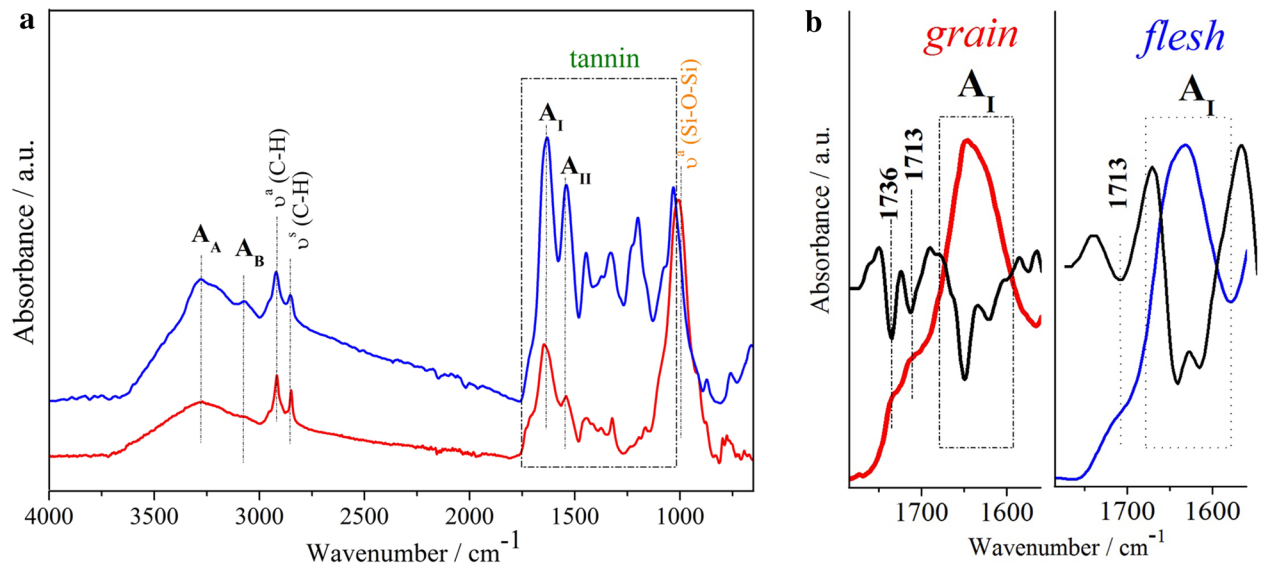

Fig. 7 **a** ATR-FTIR spectra of both flesh (blue line) and grain (red line) sides of the historical leather bookbinding. The bands assigned to the asymmetric and symmetric stretching vibration of C–H bonds of aliphatic CH_2 group (collagen/fatty acid backbone), as well as the band corresponding to the asymmetric stretching vibration of Si–O–Si (silicates from dust/dirt) are highlighted. **b** ATR-FTIR spectra in the (1780–1560) cm^{-1} domain of both grain (red line) and flesh (blue line) sides of the historical leather bookbinding. The band at 1713 cm^{-1} assigned to the stretching vibration of the carbonyl function (C=O) in hydrolysable tannin, as well as 1736 cm^{-1} band assigned to the stretching vibration of ester carbonyl functional groups of triglycerides (O–C=O) are highlighted

the stretching vibration of the carbonyl function typical for hydrolysable tannins [33, 61] (Fig. 7b). The spectra of both the grain and flesh sides of untreated leather showed the bands at ~2920 and 2850 cm^{-1} (Fig. 7a) corresponding to the ν^a(C–H) and ν^s(C–H) groups. The band at 1736 cm^{-1} visible only in the spectrum taken on leather grain side (Fig. 7b) could be ascribed to the presence of waxy or fatty materials, which could have been used to finish or preserve the leather [7, 62]. In addition, the high intensity band at 1025 cm^{-1} indicates the presence of silicates from dirt adhering to the surface of leather.

In the spectra of the treated samples (Fig. 8), the absorption bands at 3623 and 3695 cm^{-1} (assigned to the stretching vibration of inner O–H and the O–H located at the inner-surface of the halloysite nanotubes), and those at 1027 and 912 cm^{-1} (corresponding to the stretching vibration of Si–O and deformation vibration of Al–O, respectively) [63, 64] confirmed the presence of the HNT nanotubes. The presence of the nanoparticles on the surface was also evidenced by SEM analysis (Fig. 9).

The contact angle measurement showed that a hydrophobic effect is generated by the HNT-beeswax treatment as expected, while the other treatments caused an increase of water absorption of leather (Fig. 10).

The pH measurement showed an average of (4.6 ± 0.3) for the historical leather indicating the absence of the red

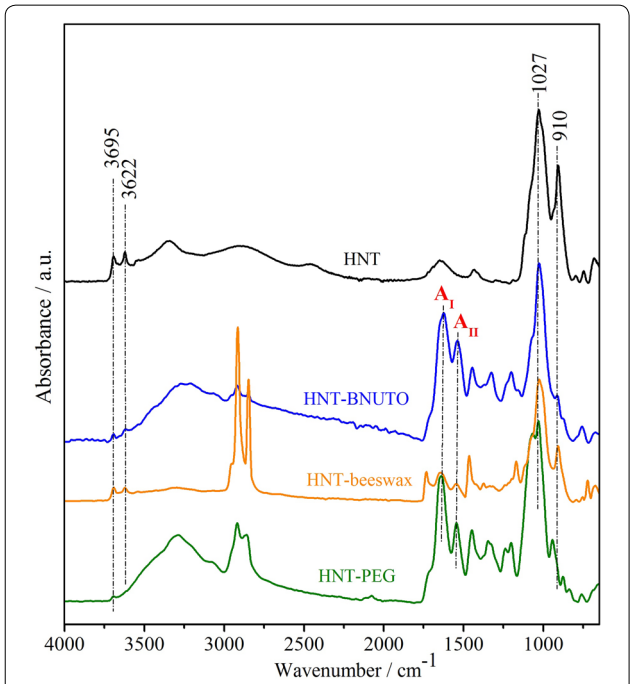

Fig. 8 ATR-FTIR spectra of the treated leather samples as compared with HNTs spectrum. The main absorption bands of HNTs (3695 cm^{-1}, 3622 cm^{-1}, 1027 cm^{-1} and 910 cm^{-1}) as well as those corresponding to collagen (A_I and A_{II}) are highlighted

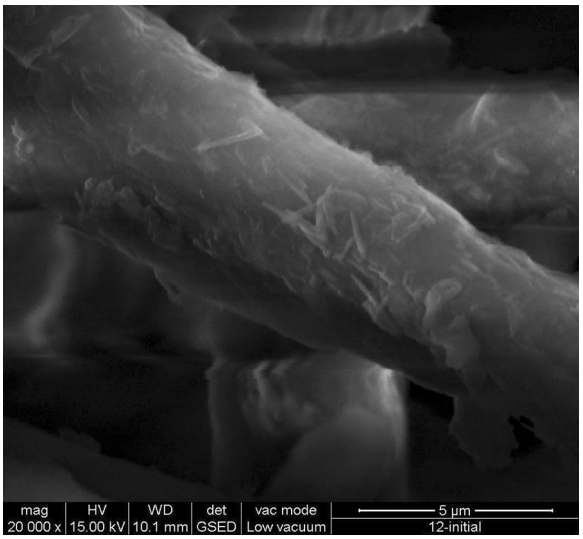

Fig. 9 SEM images showing the presence of halloysite nanoparticles on leather surface of the treated samples. Magnification ×8000

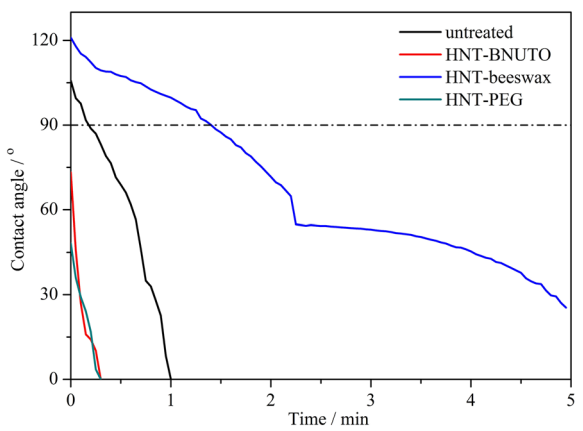

Fig. 10 The contact angle of the untreated leather decreases with a constant rate until the water droplet is totally absorbed (around 1 min). For both HNT-PEG and HNT-BNUTO the water droplet is fully absorbed in 20 s. For HNT-beeswax treatment, a two-rate process is observed: a stepper absorption rate in the first 2 min, followed by a lower rate up to 5 min

rot deterioration even though the binding appeared fragile in some areas. Its appearance could be mainly ascribable to the usage of the book over time and to insect attack. After the treatment the values of pH remained in the range (4.4–4.7).

Conclusions

Halloysite nanoparticles dispersed in various aqueous and non-aqueous dispersion media were used for the first time to treat a historical bookbinding. The dispersion media were selected so as to avoid detrimental effects to collagen-tannin matrix in leather. The treatments were applied by total immersion of leather samples for 10 min followed by air drying. Results from MHT method and micro DSC technique showed a hydrothermal stabilization evidenced by increased T_{first}/T_{onset} and T_s/T_{max} values. A redistribution of the partial enthalpy of the two collagen populations due to the increase of the weight of the most stable collagen population was also observed. Accordingly, narrower ΔC and ΔT intervals, as well as $\Delta T_{1/2}$ values suggested a more homogeneous thermal behavior, in agreement with the unilateral NMR analysis which indicated a partial recovery of collagen structural order. The obtained results match both microscopic and macroscopic levels and could be attributed to partial recovery of the hydrothermal stability of the collagen-tannin matrix. This effect was related to the association of collagen fibrils into larger fibres and fibre bundles and consequentially recovery of fibrillar cohesion.

The results of this research may open new perspectives in the conservation of collagen-based artefacts by functionalisation and/or loading the halloysite nanoparticles inner lumen with antimicrobials, fungicides and pH adjustment nanoparticles.

Abbreviations

ATR-FTIR: Infrared Spectroscopy in Attenuated Total Reflection mode; BNUTO: Biblioteca Nazionale Universitaria di Torino (The National University Library of Turin); DSC: differential scanning calorimetry; HNTs: halloysite nanotubes; MHT method: micro hot table method; NMR: nuclear magnetic resonance; PEG: polyethylene glycol; SEM: scanning electron microscopy; TG: thermogravimetry.

Acknowledgements

We thank Dr. Petru Budrugeac from the National Institute for Research and Development in Electrical Engineering ICPE-CA, Bucharest, for discussions and allowing us to obtain TG/DTG data in his laboratory.

Authors' contributions

EB conceived the study, carried out interpretation of the results, and drafted the manuscript. EH prepared the HNTs dispersions, performed the leather treatments, as well as MHT measurements and SEM analysis. CC performed the ATR-FTIR measurements. CC and EH helped to draft the manuscript. CS performed the unilateral NMR analysis. MCL performed the micro DSC measurements. All authors read and approved the final manuscript.

Funding

This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, through the *Nucleu* Programme, Project No. PN 19 17 05 01, Grant Number 4N/2019.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1] Advanced Research for Cultural Heritage (ARCH Lab) Group, National Research & Development Institute for Textiles and Leather, ICPI Division, Ion Minulescu 93, 031215 Bucharest, Romania. [2] Department of Chemistry, Faculty of Sciences, University of Craiova, Calea Bucureşti 107 I, 200585 Craiova,

Romania. [3] National Museum of Romanian History, Calea Victoriei 12, 030026 Bucharest, Romania.

Received: 10 June 2019 Accepted: 24 August 2019
Published online: 03 September 2019

References

1. Hain Teper J, Straw M. A survey of current leather conservation practices. Book Paper Group Annual. 2011;30:131–51.
2. Haines BM. Deterioration in leather bookbindings-our present state of knowledge. Br Libr J. 1977;3(1):59–70.
3. Dignard C, Mason J, Caring for leather, skin and fur. Part of CCI's Preventive conservation guidelines for collections online resource. https://www.canada.ca/en/conservation-institute/services/preventive-conservation/guidelines-collections/caring-leather-skin-fur.html.
4. Raphael T, Mccrady E. Leather dressing: to dress or not to dress. Leather Conserv News. 1983;2:2–3.
5. Stambolov T, Van Soest HAB, Hallebeek PB. Conservation of Leather. Stud Conserv. 1984;29:21–31.
6. Mahony CC. https://escholarship.org/uc/item/79m1h3pwpeer reviewed dissertation. UCLA Electronic Theses and Dissertations. 2014:https://escholarship.org/uc/item/79m1h3pw.
7. Baglioni M, Bartoletti A, Bozec L, Chelazzi D, Giorgi R, Odlyha M, Pianorsi D, Poggi G, Baglioni P. Nanomaterials for the cleaning and ph adjustment of vegetable-tanned leather. Appl Phys A. 2016;122:114.
8. Bicchieri M, Valentini F, Pascalicchio F, Riccardi ML, Colaizzi P, Del Re C, Talamo M. The solution to an unresolved problem: newly synthesised nanocollagen for the preservation of leather. J Cult Herit. 2018;33:1–9.
9. Cavallaro G, Lazzara G, Milioto S, Parisi F. Halloysite nanotubes as sustainable nanofiller for paper consolidation and protection. J Therm Anal Calorim. 2014;117:1293–8.
10. Cavallaro G, Lazzara G, Milioto S, Parisi F, Sparacino V. Thermal and dynamic mechanical properties of beeswax-halloysite nanocomposites for consolidating waterlogged archaeological woods. Polym Degrad Stab. 2015;120:220–5.
11. Cavallaro G, Lazzara G, Milioto S, Parisi F, Ruisi F. Nanocomposites based on esterified colophony and halloysite clay nanotubes as consolidants for waterlogged archaeological woods. Cellulose. 2017;24:3367–76.
12. Cavallaro G, Milioto S, Nigamatzyanova L, Akhatova F, Fakhrullin R, Lazzara G. Pickering emulsion gels based on halloysite nanotubes and ionic biopolymers: properties and cleaning action on marble surface. ACS Appl Nano Mater. 2019;2:3169–76.
13. Lvov Y, Abdullayev E. Functional polymer–clay nanotube composites with sustained release of chemical agents. Prog Polym Sci. 2013;38:1690–719.
14. Lvov Y, Wang W, Zhang L, Fakhrullin R. Halloysite clay nanotubes for loading and sustained release of functional compounds. Adv Mater. 2016;28:1227–50.
15. Kryuchkova M, Danilushkina A, Lvov Y, Fakhrullin R. Evaluation of toxicity of nanoclays and grapheme oxide in vivo: a *Paramecium caudatum* study. Environ Sci Nano. 2016;3:442–52.
16. Kamble R, Ghag M, Gaikawad S, Panda BK. Halloysite nanotubes and applications: a review. J Adv Sci Res. 2012;3(2):25–9.
17. Xueping W, Cun L, Huijie Q, Xianlong Z, Jianjian D, Qingxin Z, Liang Z, Yucheng W, Xinhua P. Synthesis and adsorption properties of halloysite/carbon nanocomposites and halloysite-derived carbon nanotubes. Appl Clay Sci. 2016;119:284–93.
18. Pasbakhsh P, Churchman GJ, Keeling J. Characterisation of properties of various halloysites relevant to their use as nanotubes and microfibre fillers. Appl Clay Sci. 2013;74:47–57.
19. Gorrasi G, Pantani R, Murariu M, Dubois P. PLA/halloysite nanocomposite films: water vapor barrier properties and specific key characteristics. Macromol Mater Eng. 2014;299:104–15.
20. Cavallaro G, Chiappisi L, Pasbakhsh P, Gradzielski M, Lazzara G. A structural comparison of halloysite nanotubes of different origin by Small-Angle Neutron Scattering (SANS) and Electric Birefringence. Appl Clay Sci. 2018;160:71–80.
21. Gorrasi G. Dispersion of halloysite loaded with natural antimicrobials into pectins: characterization and controlled release analysis. Carbohydr Polym. 2015;127:47–53.
22. Du M, Guo B, Jia D. Thermal stability and flame retardant effects of halloysite nanotubes on polypropylene. Eur Polym J. 2006;42:1362–9.
23. Larsen R, Vest M, Nielsen K. Determination of hydrothermal stability (shrinkage temperature) of historical leathers by Micro Hot Table technique. J Soc Leather Technol Chem. 1993;77:151–6.
24. Budrugeac P, Cucos A, Miu L. The use of thermal analysis methods for authentication and conservation state determination of historical and/or cultural objects manufactured from leather. J Therm Anal Calorim. 2010;104:439–50.
25. Carşote C, Badea E, Miu L, Della Gatta G. Study of the effect of tannins and animal species on the thermal stability of vegetable leather by differential scanning calorimetry. J Therm Anal Calorim. 2016;124(3):1255–66.
26. Lesar B, Pavlič M, Petrič M, Škapin AS, Humar M. Wax treatment of wood slows photodegradation. Polym Degrad Stab. 2011;96:1271–8.
27. Fessas D, Schiraldi A, Tenni R, Vitellaro Zuccarello L, Bairati A, Facchini A. Calorimetric, biochemical and morphological investigations to validate a restoration method of fire injured ancient parchment. Termochim Acta. 2000;348:129–37.
28. Badea E, Miu L, Carsote C, Petroviciu I, Granziero R, Perona S, Cacchia V, Pittari B, Laurora C, Della Gatta G, Cucos A, Budrugeac P, Vitale Brovarone A. Validazione di interventi di recupero conservativo di manufatti in pergamena. In: Lo Stato dell'arte 12. Florence: Nardini; 2014, p. 463–70.
29. Johnson J, Wills B. Leather wet and dry: current treatments in the conservation of waterlogged and desiccated archaeological leather. J Am Inst Conservat. 2002;41(2):189–91.
30. Ershad-Langroudi A, Mirmontahai A. Thermal analysis on historical leather bookbinding treated with PEG and hydroxyapatite nanoparticles. J Therm Anal Cal. 2015;120(2):1119–27.
31. Cavallaro G, Lazzara G, Milioto S, Parisi F, Evtugyn V, Rozhina E, Fakhrullin R. Nanohydrogel formation within the halloysite lumen for triggered and sustained release. ACS Appl Mater Interfaces. 2018;10:8265–73.
32. Lazzara G, Cavallaro G, Panchal A, Fakhrullin R, Stavitskaya A, Vinokurov V, Lvov Y. An assembly of organic-inorganic composites using halloysite clay nanotubes. Curr Opin Colloid Interface Sci. 2018;35:42–50.
33. Falcão L, Araújo EM. Tannins characterization in historic leathers by complementary analytical techniques ATR-FTIR, UV–Vis and chemical tests. J Cult Herit. 2013;14:499–508.
34. Larsen R, Sommer DV, Axelsson K. Scientific approach in conservation and restoration of leather and parchments objects in archives and libraries. In: Engel P, editor. New approaches to book and paper conservation-restoration. Horn/Wien: Verlag Berger; 2011. p. 239–58.
35. Carsote C, Badea E. Micro differential scanning calorimetry and micro hot table method for quantifying deterioration of historical leather. Herit Sci. 2019;7:48.
36. Badea E, Della Gatta G, Usacheva T. Effects of temperature and relative humidity on fibrillar collagen within parchment: a micro differential scanning calorimetry (micro DSC) study. Polym Degrad Stab. 2012;97:346–53.
37. Sendrea C, Badea E, Adams A. Unilateral NMR and micro DSC study of artificially aged parchments. Rev Chim. 2017;68(8):1780–5.
38. Badea E, Şendrea C, Carşote C, Adams A, Blümich B, Iovu H. Unilateral NMR and thermal microscopy studies of vegetable tanned leather exposed to dehydrothermal treatment and light irradiation. Microchem J. 2016;129:158–65.
39. Hadîmbu E, Badea E, Carşote C, Şendrea C, Miu L. Halloysite nanotubes as innovative consolidants for historical leather. In: Proceedings book of the 7th international conference on advanced materials and systems (ICAMS 2018). Bucharest: Certex; 2018. p. 189–94. https://doi.org/10.24164/icams-2018.ii.2.
40. Sendrea C, Carşote C, Radu M, Badea E, Miu L. The effect of gamma irradiation on shrinkage activity of collagen in vegetable tanned leather. Rev Chim. 2017;68(7):1535–8.
41. Carsote C, Kövari L, Albu C, Hadîmbu E, Badea E, Miu L, Dumitrescu G. Bindings of rare books from the collections of the Romanian Academy Library—a multidisciplinary study. Leather and Footwear Journal. 2018;18(4):307–20.
42. Badea E, Usacheva T, Gatta G. The use of differential scanning calorimetry to characterise collagen deterioration in parchment. Rossiiskii Khimicheskii Zhurnal-Zhurnal Rossiiskogo Khimicheskogo Obshchestva im. D. I. Mendeleeva (Russian Chemistry Journal). 2015;59(1):28–41.
43. Chahine C. Changes in hydrothermal stability of leather and parchment with deterioration: a DSC study. Thermochim Acta. 2000;365:101–10.

44. Budrugeac P, Miu L. The suitability of DSC method for damage assessment and certification of historical leathers and parchments. J Cult Herit. 2008;9:146–53.
45. Liang Y, Kong H, Wah Tong Y. Generation of cell-instructive collagen gels through thermodynamic control. ACS Macro Lett. 2013;2:1077–81.
46. Dewavrin JY, Hamzavi N, Shim VP, Raghunath M. Tuning the architecture of three-dimensional collagen hydrogels by physiological macromolecular crowding. Acta Biomater. 2014;10:4351–9.
47. Usha R, Ramasami T. Effect of hydrogen-bond-breaking reagent (Urea) on the dimensional stability of rat tail tendon (RTT) collagen fiber. J Appl Polym Sci. 2002;84:975–82.
48. Komsa-Penkova R, Koynova R, Kostov G, Tenchov BG. Thermal stability of calf skin collagen type I in salt solutions. Biochim Biophys Acta. 1996;1297(2):171–81.
49. Li Xinxin, Wang Ya-nan, Li Jing, Shi Bi. Effect of sodium chloride on structure of collagen fiber network in pickling and tanning. J Am Leather Chem As. 2016;111(6):230–7.
50. Budrugeac P, Miu L, Bocu V, Wortmann FJ, Popescu C. Thermal degradation of collagen-based materials that are supports of cultural and historical objects. J Therm Anal Calorim. 2003;72:1057–65.
51. Budrugeac P, Cucos A, Miu L. The use of thermal analysis methods for authentication and conservation state determination of historical and/or cultural objects manufactured from leather. J Therm Anal Calorim. 2011;104:439–50.
52. Budrugeac P, Carşote C, Miu L. Application of thermal analysis methods for damage assessment of leather in an old military coat belonging to the History Museum of Braşov-Romania. J Therm Anal Calorim. 2017;127(1):765–72.
53. Bonvin MG, Goodman M, Boelens R, Kaptein R. Hydration dynamics of the collagen triple helix by NMR. J Mol Biol. 2000;300:1185–91.
54. Popescu C, Budrugeac P, Wortmann FJ, Miu L, Demco DE, Baias M. Assessment of collagen-based materials which are supports of cultural and historical objects. Polym Degad Stab. 2008;93:97.
55. Fathima NN, Baias M, Blümich B, Ramasami T. Stucture and dynamics of water in native and tanned collagen fibers: effect of crosslinking. Int J Biol Macromol. 2010;47:590–6.
56. Sendrea C, Carsote C, Badea E, Adams A, Niculescu M, Iovu H. Non-invasive characterisation of collagen-based materials by NMR-MOUSE and ATR-FTIR. UPB Sci Bull. 2016;78(3):27–38.
57. Baum T, Joseph GB, Karampinos DC, Jungmann PM, Link TM, Bauer JS. Cartilage and meniscal t2 relaxation time as non-invasive biomarker for knee osteoarthritis and cartilage repair procedures. Osteoarthr Cartil. 2013;21(10):1474–84.
58. Besghini D, Mauri M, Simonutti R. Time domain NMR in polymer science: from the laboratory to the industry. Appl Sci. 2019;9:1801.
59. Appolonia L, Borgia GC, Bortolotti V, Brown RJS, Fantazzini P, Rezzaro G. Effects of hydrophobic treatments of stone on pore water studied by continuous distribution analysis of NMR relaxation times. Magn Reson Imaging. 2001;19(3):509–12. https://doi.org/10.1016/s0730-725x(01)00280-6.
60. Barth A. Infrared spectroscopy of proteins. Biochem Biophys Acta. 2007;1767:1073–101.
61. Falcão L, Araújo EM. Application of ATR–FTIR spectroscopy to the analysis of tannins inhistoric leathers: the case study of the upholstery from the 19th century Portuguese Royal Train. Vib Spectrosc. 2004;74:98–103.
62. Vichi A, Eliazyan G, Kazarian SG. Study of the degradation and conservation of historical leather book covers with Macro attenuated total reflection-Fourier Transform infrared spectroscopic imaging. ACS Omega. 2018;3:7150–7.
63. Joussein E, Petit S, Churchman J, Theng B, Righi D, Delvaux B. Halloysite clay minerals—a review. Clay Miner. 2005;40:383–426.
64. Tang Z, Wei Q, Lin T, Guo B, Jia D. The use of a hybrid consisting of tubular clay and graphene as a reinforcement for elastomers. RSC Adv. 2013;3:17057.

Publisher's Note