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Dedicated to the memory of
Professor Eugen Segal (1933-2013)

CHARACTERIZATION OF A BYZANTINE MANUSCRIPT BY INFRARED SPECTROSCOPY AND THERMAL ANALYSIS

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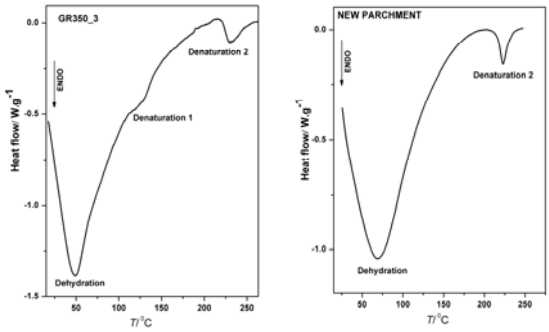
Historical leather is a complex biological material, and due to various methods of production or tannins used, unknown environmental histories of objects and heterogeneous composition and stratigraphy, it represents a particular analytical challenge. Recent analytical and technological improvements have allowed to reveal the causes of degradations and evaluate the level of damage of such materials. This information provides invaluable clues to helping conservators to determine the initial criteria of the conservation or restoration. In this paper a Byzantine parchment manuscript and its leather bookbinding from "Ivan Dujčev" Centre for Slavo-Byzantine Studies, Sofia, Bulgaria, were studied using optical microscopy, infrared spectroscoy (FTIR), differential scanning calorimetry (DSC) and Micro Hot Table method. Analyses were performed on several micro-samples of parchment as well as on a micro-sample of leather. Markers for identifying the main degradation and criteria for the assessment of damage in historical parchment and leather developed in recent national and European projects were applied for evaluating the state of conservation of both the manuscript and bookbinding. The results are discussed in terms of their implications for conservation science.



INTRODUCTION

Museums, libraries, archives and religious institutions preserve significant collections of leather and parchment objects (scrolls, manuscript, codices, bookbindings, furniture, clothing and footwear, wallpaper, upholstery, storage vessels and boxes etc.) providing an infinite source of

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information of historical and cultural interest. It is vital therefore that these documents and artefacts to remain well preserved. Conservators were often used to characterise a parchment or leather object based on a visual assessment focussing on changes in texture, colour, flexibility and the form of the sample to be analysed. However, deterioration cannot always be detected using the naked-eye or optical microscopy. Inappropriate treatment or storage conditions can lead to further damage of the documents and objects. The strong need to prevent their irreversible quality and material loss relies on a detailed knowledge of collagen structure in parchments and leather. Collagen is the main component of both leather and parchment. In spite of its intrinsic structural strength and resilience, it is subjected to inherent ageing and degradation promoted by environmental factors (light, heat, relative humidity, atmosferic polutants), biological agents (bacteria, fungi, insects, molds), natural catastrophic events (floods, earthquakes) and wars. Due to the irreplaceable value and uniqueness of the historical document and objects, and artefacts, non-destructive or micro-destructive analytical techniques are mainly considered by conservators to assess their healthiness. A considerable number of information has been acquired in the last two decades about the degradation pathways in collagen-based materials within national (PERGAMO, CEEX 1165/2006, PELRESTAURO, PN 91012/2007, Italian project – OPERA, CIPE 2004 D39) and European (STEP-CT-90-0105, ENVIRONMENT EV5V-CT-94-0514, IDAP, EVK4-CT200100061, MEMORI, 265132 4.10.201049) research projects. Hydrolysis, oxidation, leading to gelatinisation and the external factors that trigger these mechanisms were studied using optical microscopy and collagen fiber shrinkage measurement by Micro Hot Table (MHT) method,[1-4] thermogravimetry (TG/DTG) and differential scanning calorimetry (DSC),[4-10] infrared spectroscopy (FTIR),[11-14] Raman spectroscopy,[15,16] X-ray diffraction, X-ray scattering and Micro-X-ray fluorescence,[17-19] nuclear magnetic resonance (NMR),[12,20,21] scanning electron microscopy (SEM),[12] and atomic force microscopy (AFM).[22]

In this paper, the qualitative and quantitative aspects of the damage of a Byzantine parchment manuscript including its leather bookbinding were studied through optical microscopy, Attenuated Total Reflection-Infrared Spectroscopy (ATR-FTIR), Micro Hot Table method (MHT) and Differential Scanning Calorimetry (DSC). The manuscript belongs to "Ivan Dujčev" Centre for Slavo-Byzantine Studies Sofia, Bulgaria. Several micro-samples were taken by the conservators from the manuscript and only one micro-sample from its bookbinding. As a conservative approach to restoration is favored nowadays, this multi-scale analytical approach aims at helping conservators to determine the causes of degradation of both the manuscript and bookbinding and decide the initial criteria of their restoration process.

EXPERIMENTAL

Materials

The Byzantine manuscript is a palimpsest (Fig. 1) whose earlier layer dates back to the end of the 9th or the beginning of the 10th century. It contains Byzantine chants written with uncial script (i.e. entirely in capital letters). The second layer is a Lectionary from the 13th century. The bookbinding is made of wooden boards covered with leather. Both the manuscript and its bookbinding appear strongly damaged. The parchment sheets are stiff, pleated, of dark yellow or brown colour and with glassy appearance. The bookbinding is stiff and brittle. Investigations were made on four parchment micro-samples (GR350_1, GR350_2, GR350_3, GR350_5) and one leather micro-sample (GR350_6) provided by the Restoration Laboratory of "Ivan Dujčev" Centre for Slavo-Byzantine Studies.

Optical Microscopy

A Leica S4E stereomicroscope with magnifying power of (20 – 200)x was used for the optical observations.

Attenuated Total Reflection-Infrared Spectroscopy (ATR-FTIR)

ATR-FTIR measurements were performed with a portable spectrometer Alpha Bruker Optics equipped with a diamond crystal. During the experiments, 32 scans were coadded to achieve an acceptable signal-to-noise ratio, with wavenumber ranging from 4000 to 400 cm^{-1}. All the spectra were recorded at a resolution of 4 cm^{-1}. ATR-FTIR spectra were evaluated using Opus 6.5. software. Three measurements were averaged for each sample. Spectra were collected directly from the samples.

Differential scanning calorimetry (DSC)

The DSC measurements were performed with a DSC 204 F1 Phoenix instrument from Netzsch, Germany, on both hydrated and dry samples as follows:

A micro-sample of about 1-5 mg was introduced in an aluminium pan, 35 μL deionised water was added and the pan was hermetically sealed and kept for 24 hours at room temperature. Measurements were performed at 10 K min^{-1} heating rate in the temperature rage (25 to 110) ^{o}C.

A micro-sample of about 1-5 mg placed in an open crucible was heated from (25 to 280) ^{o}C in nitrogen flow (gas purity higher than 99.999%; 20 mL min^{-1}) at 10 K min^{-1} scanning rate.

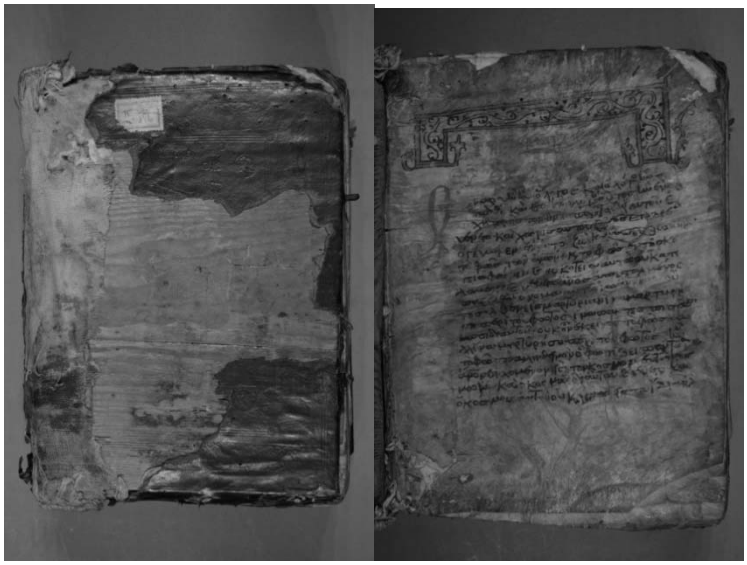

Fig. 1 – Byzantine manuscript from "Ivan Dujčev" Centre for Slavo-Byzantine Studies, Sofia: front wooden board covered by leather and a parchment sheet with stiff, pleated, of dark yellow or brown colour and with glassy appearance.

Micro Hot Table method (MHT)

The shrinkage temperature of collagen fibres from parchment and leather was measured with a home-made equipment made of a CALORIS Hot Table controlled by a temperature processor and coupled with a Leica S4E stereomicroscope. The magnification used was 40x. A sample of about 10–15 fibres was throughly wetted with deionised water for 10 minutes on a microscope slide. The fibers were then separated under the microscope using needles, covered with a second microscope slide, placed on the hot table and heated with a 2 °C min^{-1} rate. A F.L.T.K. 1.1.X home-made software was used for data collection.

RESULTS AND DICUSSION

Visible alterations

The microscopic examination of parchment samples enabled us to visualise the pattern of hair follicules and thus identify the animal skin used, *i.e.* goat skin. Further information as partial gelatinisation of the grain surface (Fig. 2), spots of calcium carbonate as well as early repairs carried out berofe writing using goat parchment (Fig. 3) were obtained.

Molecular alterations

The collagen based materials spectra are characterized by the following typical bands: Amide A (~3300 cm^{-1}) and Amide B (~3080 cm^{-1}) associated with stretching of peptide N-H groups involved in inter-chain hydrogen bonding; Amide I (~1631 cm^{-1}), corresponding to C=O stretching vibration of peptide bonds along the polypeptide backbone with a small contribution from N-H in-plane bending; Amide II (~1536 cm^{-1}), associated with N-H bending and C-N stretching vibration; Amide III (~1232 cm^{-1}), associated with N-H in-plane bending and CH_2 wagging vibration of glycine backbone and proline side chain. In case of parchment, the peaks of carbonate are frequently visible at 1445 cm^{-1} and 875 cm^{-1} (Fig. 4).

According to the literature,[11-14] the following criteria were considered to evaluate the molecular alteration of collagen in parchment and leather: (i) separation of the amide I and amide II bands ($\Delta\nu$), related to gelatinisation; (ii) amide I/ amide II ratio (A_I/A_{II}), related to hydrolysis; (iii) carbonyl groups band around 1740 cm^{-1}, due to oxidation.

Previously it was found that $\Delta\upsilon \approx 100$ cm^{-1} and $A_I/A_{II} \approx 1$ for new undamaged parchments.[11,12] Consequently, the ATR-FTIR values reported in Table 1 showed that all samples were degraded by hydrolysis (A_I/A_{II} ratio varies from 1.19 to 1.48) and gelatinisation ($\Delta\nu$ values varies from 106 to 110 cm^{-1}). The carbonyl band at 1740 cm^{-1} was not found suggesting that oxidative degradation was insignificant. All parchment samples displayed the carbonate ion bands around 1445 cm^{-1} and 875 cm^{-1}. The presence of carbonate is due to the traditional procedure of parchment manufacturing which includes soaking of skin in a lime bath. Generally, the lime removal by extensive washing is not complete and the remaining calcium hydroxide transforms into calcium carbonate by reacting with the carbon dioxide from atmosphere.

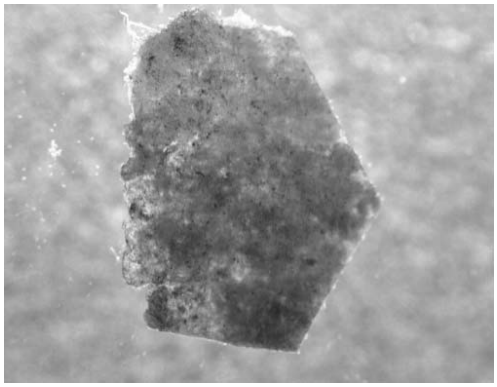

Fig. 2 – Parchment sample observed at 20x magnification displaying both glassy and matt areas.

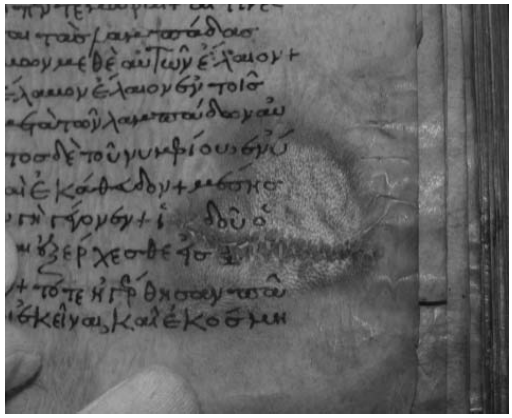

Fig. 3 – Repair carried out before writing, most probably after the fabrication of parchment.

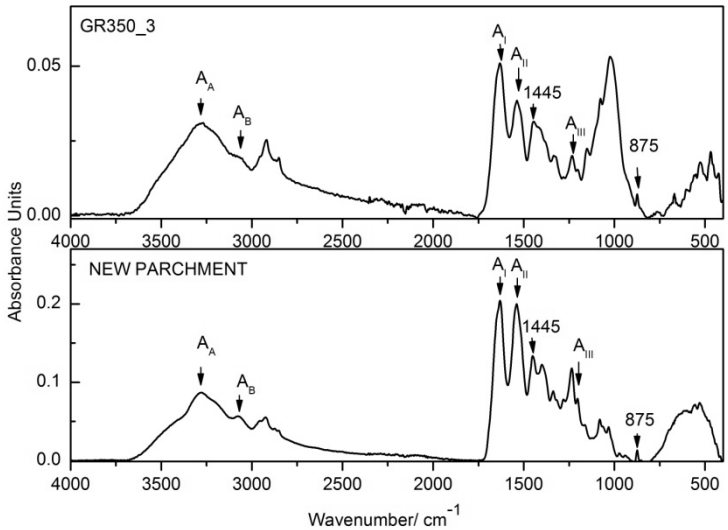

Fig. 4 – ATR-FTIR spectrum for the GR350_3 parchment sample compared with a typical new parchment spectrum.

Table 1

ATR-FTIR spectra characteristics for the investigated samples

Sample	**Δν (cm^{-1})**	**A_I / A_{II}**	**$\nu_{C=O}$**
GR350_1	107	1.19	Absent
GR350_2	108	1.48	Absent
GR350_3	106	1.36	Absent
GR350_5	110	1.48	Absent
GR350_6	110	1.25	Absent

Hydrothermal stability of collagen fibers

The hydrothermal stability of both parchment and leather was characterized by both the MHT method and DSC analysis of hydrated samples as previously described.[4,7] MHT method determines the temperature of shrinkage T_s of collagen fibers, whereas DSC analysis measures the extrapolated onset temperature T_{onset} of the DSC peak associated with collagen thermal denaturation (Fig. 5). It is worth to mention that collagen, when heated in water, converts from a highly ordered triple-helical structure to a random-coil structure over a defined temperature interval.[23] This phenomenon is called thermal denaturation and can be observed through a stereomicroscope using reflected light as a motion of the collagen fibers called shrinkage.[4] Accordingly, the temperatures measured by MHT and DSC characterise the same proces, *e.g.* thermal denaturation of collagen, and should be thus practically equal.[6,8] Fig. 6 shows the values of T_s and T_{onset} for both parchment and leather samples. The rather small difference between these values can be ascribed to the different heating rates used for MHT (2 K·min^{-1}) and DSC (10 K.min^{-1}) measurements. In fact, T_s(MHT) < T_{onset}(DSC), but the order of the change is the same. However, the heterogeneity that characterises historical samples could increase this difference.

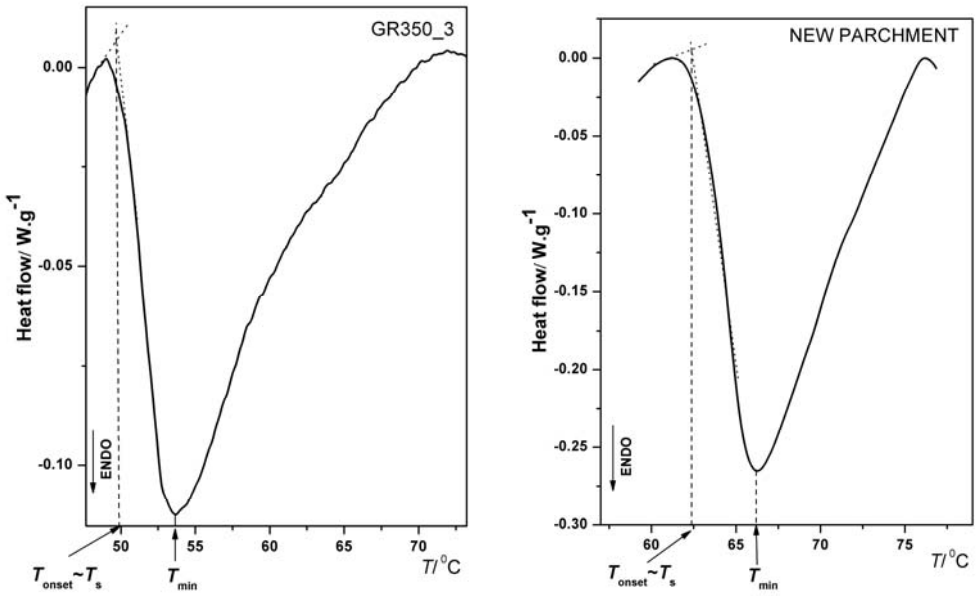

Fig. 5 – DSC curve for the parchment sample GR350_3 obtained in sealed crucible compared with a typical DSC curve for a new parchment. Samples were hydrated before being measured.

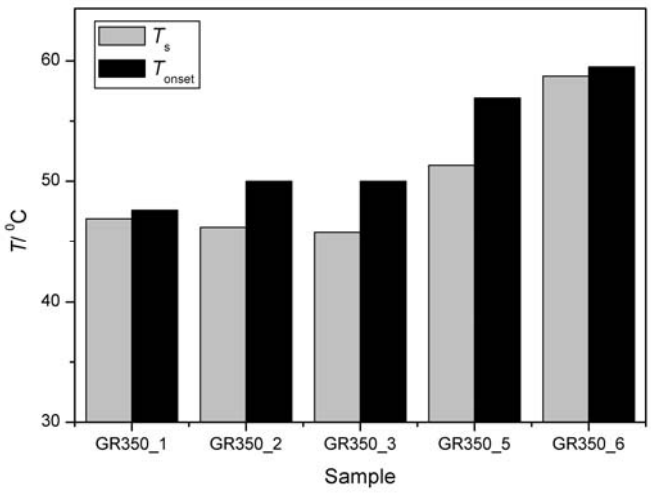

Fig. 6 – Comparison between T_s measured by MHT method and T_{onset} measured by DSC analysis in hydrated state for the investigated parchment and leather samples.

We have previously obtained an average T_s = (57.6±2.3) °C by analysing 8 goat parchments newly manufactured at the INCDTP-ICPI of Bucharest, using a traditional recipe.[8] This T_s value, higher than those of GR350_1, GR350_2 and GR350_3 samples, and comparable to that of GR350_5 sample, suggests a medium damage level for the first samples and almost no damage for the last one.

As far as new vegetable tanned leather is concerned, we determined an average T_s = (80.5±4.0) °C for 19 new vegetable tanned leathers manufactured at the INCDTP-ICPI of Bucharest, and a much lower T_s = (61.4±10.5) °C, for 76 historical leather samples.[8] The later value, close to that of recently manufactured parchments, could be explained by the two-step mechanism of natural ageing of leather *i.e.* breaking of the tannin-collagen cross-links followed by splitting of collagen macromolecules. The leather sample from the manuscript bookbinding displayed a T_s value in the same range (*e.g.* T_s = 58.7 °C) indicating a mediu-to-high degree of damage. Moreover, the T_s and T_{onset} closeness (T_{onset} = 59.5 °C) suggests a rather homogeneous.[4]

Denaturation of collagen crystalline (rigid) fraction

DSC curves associated to thermal transitions which tipically occur in parchment and leather samples measured in open crucibles and gas flow display a broad endothermic peak followed by smaller endotherms (Fig. 7). The broad peak in the temperature range (50 – 120) °C is associated with thermal dehydration of the sample. The first endotherm at about 129 °C was related to collagen matrix thermal denaturation[5,8], whereas the second peak at T > 220 °C was ascribed to thermal denaturation of the crystalline collagen embedded in the amorphous matrix.[8] Temperatures of denaturation of both the amorphous collagen matrix T_{d1} and crystalline fraction T_{d2} for the investigated samples are listed in Table 2.

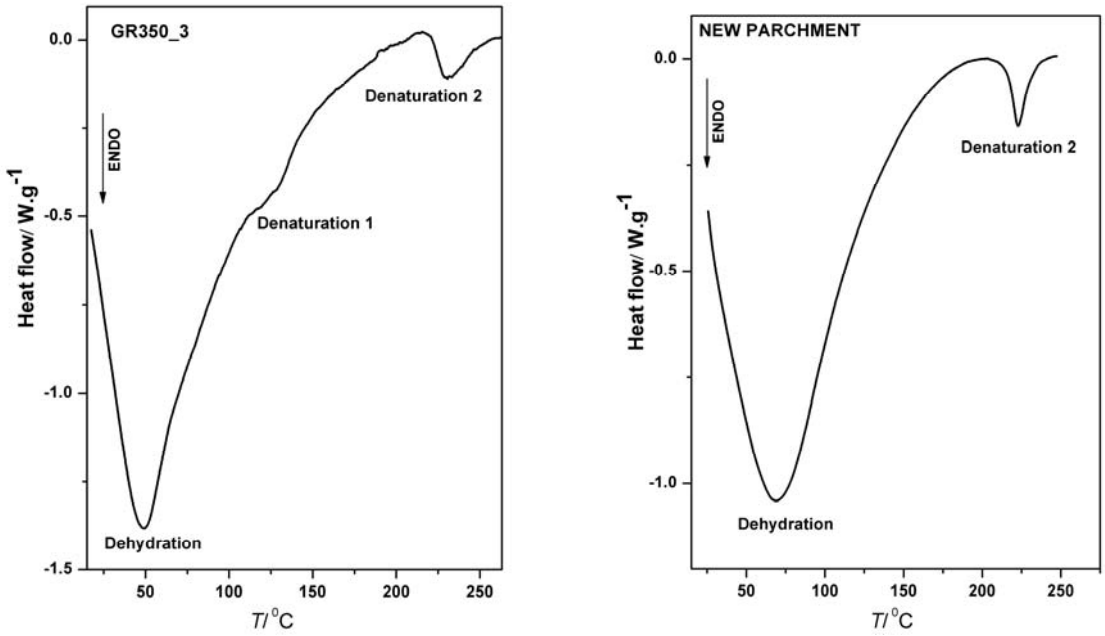

Fig. 7 – DSC curve obtained in open crucibles
and nitrogen flow for GR350_3 parchment sample compared to a typical DSC curve for a new parchment.

Table 2

Temperature of denaturation of the amorphous collagen matrix (T_{d1})
and crystalline fraction (T_{d2}) for the investigated samples

Sample	T_{d1}/^{0}C	T_{d2}/^{0}C
GR350_1	128.8	220.6
GR350_2	-	231.6
GR350_3	129.5	230.5
GR350_5	129.3	244.8
GR350_6	128.1	237.7

In new materials as well as artificially aged samples, the dehydration peak is broader and often overlaps the peak of denaturation of the collagen matrix. In this case, the natural ageing has most probably provoked the loss of tightly bonded water and the formation of cross-links in the structure of collagen. Sinergically, these processes produced an increase of thermal stability of both parchment and leather confirmed by the persistence of the collagen fractions which denaturate at a temperature close to that of native collagen already reported. [5,8]

It was shown that T_{d2} correlates well with the deterioration of the crystalline (rigid) fraction of collagen in both historical parchments and leathers.[6,8] In fact T_{d2} displays practically constant values in the new parchments and leather. We found average values of T_{d2} = (227.4±6.8) °C, for 31 recently manufactured parchments, and T_{d2} = (245.5±5.4) °C, for 19 recently obtained vegetable tanned leathers. On ageing these values behaved differently, *i.e.* T_{d2} practically did not vary for 63 historical parchments, T_{d2} = (232.6±5.1) °C, while it sharply decreased for 76 historical leathers, T_{d2} = (219.9±11.6) °C.[8] Accordingly, the crystalline collagen appears to be intact in all parchment samples while slightly damaged in the leather GR350_6 sample.

CONCLUSIONS

A multi-scale investigation was performed to characterise degradation and evaluate damage of a Byzantine manuscript from "Ivan Dujčev" Centre for Slavo-Byzantine Studies, Sofia, Bulgaria. Four parchment micro-samples from the internal sheets and a leather micro-sample from the bookbinding were analyzed by optical microscopy, ATR–FTIR, DSC and MHT method. In summary, the conclusions are the followings: (i) The main degradation patterns identified through ATR–FTIR analysis were hydrolysis and consequent gelatinization. Gelatinization is most probably prevailing at the sample surface as indicated by the visual assessment. The rather high hydrothermal stability of the sample mass measured by DSC, as well as the good condition of the crystalline zone indicates a limited degree of hydrolysis of the layers beneath surface; (ii) Both parchment and leather samples undergone strong dehydration and cross-linking prior to eventual macromolecules cleavage, which resulted in a tightly intertwinement of collagen fibers. These fibers show a denaturation temperature close to that of not damaged fibers as indicated by the DSC analysis in open crucibles and nitrogen flow.

Surface gelatinization produces a stiff glassy surface which behaves differently if exposed to even small variations of relative humidity and temperature by comparison with the fiber mass underneath. This is a highly risky condition for manuscripts carrying texts and illuminations since the interlayer tensions could peel off the surface layer and lead to the partial or total loss of writing and decorations.

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