

## QUANTITATIVE MULTISPECTRAL IMAGING FOR THE DETECTION OF PARCHMENT AGEING CAUSED BY LIGHT: A COMPARISON WITH ATR-FTIR, GC-MS AND TGA ANALYSES

Marcello MANFREDI<sup>1\*</sup>, Greg BEARMAN<sup>2</sup>,  
Fenella FRANCE<sup>3</sup>, Pnina SHOR<sup>4</sup>, Emilio MARENGO<sup>1</sup>

<sup>1</sup> University of Easter Piedmont, Viale T. Michel 11, 15121 Alessandria, Italy;

<sup>2</sup> ANE Image, Pasadena CA, USA, Consultant to the IAA for Imaging Technologies of the Dead Sea Scrolls;

<sup>3</sup> Preservation Research and Testing Division, Library of Congress, Washington, DC, USA;

<sup>4</sup> Director of the Dead Sea Scrolls Digitization Projects, Israel Antiquities Authority, Jerusalem, ISRAEL;

### Abstract

*In this paper a non-invasive quantitative multispectral imaging technique was used for monitoring the degradation of parchment caused by light exposure. The parchment samples were aged for 24, 48, 72 and 120 hours in a weather-ometer ageing chamber to simulate a long term sunlight exposure. The effect of light exposure when a ultra violet (UV) filter is used was investigated. The degradation was monitored by using Light Emitting Diode (LED) multispectral imaging coupled to multivariate statistical methods and infrared spectroscopy, gas chromatography-mass spectrometry (GC-MS) and thermo-gravimetric analysis (TGA) techniques in order to elucidate the mechanism of the parchment ageing and to correlate chemical and imaging data. A description of the effect of light exposure is given: the degradation of collagenous phase and thermo-oxidative phenomena are not involved in the photo-oxidation process and, as expected, the UV radiation accelerates the ageing effects. Moreover the non-invasive imaging methods used for the detection of ageing and the monitoring of the conservation state of the parchment surface were able to identify the non-visible degradation long before possible detection by other common analytical techniques.*

**Keywords:** parchment; monitoring degradation; LED quantitative imaging; light ageing.

### Introduction

Parchment has been used as a writing material for more than 2000 years due to its strong and durable properties. Historic and highly significant manuscripts such as the Dead Sea Scrolls, and a large volume of museum patrimony, were written on parchment. Parchment is a semi-tanned skin made of insoluble proteins (collagen) and water. The proteins are grouped together, forming fibers of considerable physical consistency and excellent hydration capacity. Water is the only binding agent, which forms intermolecular bonds that join the fibers by means of hydrogen-bond bridges.

There are many studies concerning the application of various analytical methods in the characterization of parchment and ageing phenomena, some non-destructive (optical, scanning

\* Corresponding author: [marcello.manfredi@unipmn.it](mailto:marcello.manfredi@unipmn.it); Tel.: +39 333 4722270

electron microscopy, X-ray diffraction, spectral methods: infrared, ultraviolet-visible spectroscopy) and others destructive (thermo-gravimetric analysis (TGA), differential thermal analysis (DTA), differential scanning calorimetry (DSC), differential mechanical analysis (DMA), thermo-mechanical analysis (TMA) [1-3].

The method of thermal analysis was found to be very useful for the assessment of the degradation degree of parchments and leathers [4-5]. Most previous studies focused on the thermal ageing of collagen to investigate the variation induced by ageing on the thermodynamic parameters associated with both parchment denaturation and softening of collagen crystalline fraction [6-9].

It is known that photochemical reactions depend upon the structure of polymer chains, the presence of chromophoric groups, impurities, temperature, and pressure and irradiation wavelength. Moreover, parchment mass decrement, activation energy and entropy changes provide some information about the changes of water content and about the structural transformation of the protein constituting the parchment after light irradiation [10].

The analysis of the formation and release of volatile compounds from parchment was used as a tool for evaluating the parchment stability. The content of volatile aldehydes that can be produced by auto oxidation of parchment lipids was investigated using gas chromatography analysis of the atmosphere surrounding the parchment during its oxidation. It has been observed that the presence of lipids may cause the oxidative degradation of collagen [11]. The relative content of lipids strongly affects the decrease of shrinkage temperature of collagen during the degradation, and thus its thermo-mechanical properties, confirming the importance of lipid oxidation during the parchment degradation [12].

The effects of drying and heating of collagen were analyzed by X-ray diffraction [13] and a new method using micro focus X-ray beams was proposed for investigating variations in the state of conservation within a parchment sample [14]. The challenge with this method was not simple to use and only allowed analysis of a very small region of the parchment under examination.

Another non-invasive method for quantitative characterization of parchment deterioration based on optical spectral measurement in the UV-VIS field was proposed, but the technique had the limitation of not providing data in a timely manner [15].

While much is known about parchment structure, its ageing phenomena and conditions that can cause damage, there is a lack of knowledge about how to monitor the parchment degradation processes in order to prevent irreversible damage. This degradation relate in particular to ageing effects caused by light exposure of parchment documents of great historical importance (like the Dead Sea Scrolls).

The effect of exposure of paper and parchment to UV radiation was investigated using ATR-FT-IR spectroscopy and multivariate statistical tools [16].

Raman spectroscopy was utilized for the assessment of changes in parchment glue and protein-based paint media after artificial ageing with exposure to visible light, with data indicating changes of absorption bands in the fingerprint region associated with amino acid oxidation [17]. It is known that light degradation promotes oxidation of parchment and often it's yellowing [18].

Multispectral imaging techniques have been widely used in cultural heritage and archeological fields. In a previous work the suitability of LED imaging coupled to multivariate statistics for monitoring the conservation state of parchment was tested. The method was used to detect parchment changes after degradation with humidity and temperature [19, 20]. Reflectance Transformation Imaging (RTI), a surface imaging method, was employed coupled

to statistics for the non-invasive detection of morphological changes in paintings and drawings [21, 22].

This study presents the use of quantitative multispectral imaging for monitoring the degradation of parchment caused by light exposure. The effect of light exposure when a UV filter is used was investigated. The degradation was also monitored by ATR-FTIR, GC-MS and TGA techniques in order to understand the ageing mechanisms of parchment and to correlate chemical and imaging information.

### ***The LED multispectral imaging method***

Imaging techniques are widely used in the cultural heritage field often to recover illegible text in manuscripts and to view non-visible features in paintings and frescoes, and these techniques can be advantageously applied to monitor the conservation state of cultural heritage materials.

In a previous work we developed a quantitative LED multispectral imaging method coupled to multivariate statistics for the automatic detection of a degradation process on cultural heritage surfaces, including its localization and identification before the human eye can visibly detect any change or damage [19, 20]. This method explicitly requires a stable and repeatable spectral imager that provides reliable quantitative results.

The method consisted of the following steps:

- a. Sample characterization: characterization of the parchment was accomplished by recording a set of replicated multispectral images of the parchment samples before artificial ageing is applied (normally at least 10 images are required).
- b. Alignment of replicated multispectral images: it was important that image cubes collected during different imaging sessions were accurately aligned since the proposed approach for analysis was a pixel by pixel strategy.
- c. Statistical elaboration: principal component analysis (PCA) of the baseline data set: this enabled the description of the initial conservation state by means of the relevant principal components (PCs) that take into account the systematic relationships present in the data. The multivariate control charts are calculated using the scores of the relevant PCs obtained in the characterization phase. For each pixel the upper (UCL) and lower (LCL) control limits are calculated (mean value of the score of each pixel plus three times its standard deviation for UCL and minus three times its standard deviation for LCL) within which the parchment can be considered unchanged.
- d. Parchment monitoring: the multispectral image of the parchment acquired along the artificial ageing is aligned to the characterization images and then projected onto the principal component space previously obtained. The results are compared with the control limits calculated during the characterization step, to identify which pixels exceed the control limits.

## **Experimental**

### ***Materials***

Our experiments were carried out on goat parchment samples made by an Orthodox Rabbi from Jerusalem (Israel). The samples were characterized and monitored over the ageing using LED multispectral imaging, ATR-FTIR, GC-MS and TGA and then aged for 24, 48, 72

and 120 hours in the weather-ometer ageing chamber (Ci4000 Weather-Ometer™, Atlas, Chicago, USA) with a light exposure of 700Watt/m<sup>2</sup> to simulate light exposure (Relative Humidity of 50% and Temperature of 30°C). One sample was aged using a UV filter to measure the effect of visible light.

### **Methods**

#### *LED multispectral imaging*

LED multispectral images of parchment were acquired at 1200 dpi with an Eureka Vision LED system from MegaVision, Santa Barbara, CA, USA. The system has a 39-megapixel Kodak CCD monochrome sensor array and the LED emissions are centered on the following wavelengths: 365, 450, 465, 505, 535, 592, 625, 638, 700, 735, 780, 870 and 940nm. Following our previous papers [19, 20], the monitoring of the conservation state of the parchments by means of LED Hyper spectral imaging and multivariate analysis was performed.

#### *ATR-FT-IR*

Fourier transform infrared spectral analysis of parchment was conducted using ATR-FT-IR. All spectra were obtained using a Thermo Nicolet 670 FT-IR Spectrometer. Sample spectra were obtained in absorption mode over a wavelength of 400cm<sup>-1</sup> to 4000cm<sup>-1</sup> at 64 scans per sample and a resolution of 4cm<sup>-1</sup>. Background readings of air were established prior to the data collection. The background was subsequently subtracted from each spectrum before data treatment. All data was recorded using OMNIC Spectra Software version 7.3 SP1 (Thermo Fisher Scientific Inc, USA).

#### *GC-MS*

A strip of 4x13mm of each sample was subjected to a pre-oxidation treatment in a 1.5mL sealed vial in a thermo stated water bath at 85°C for 30min; after 15min a headspace sampling was performed using SPME fibers (Supelco, Bellefonte) with a Carboxen/PDMS stationary phase, thickness 75µm, for 15min at 85°C and for 30min at room temperature. Before each sampling the fiber was pre-conditioned at 250°C for 15min. An Agilent Technologies 6890N gas chromatography, coupled to an Agilent Technologies 5975B quadrupole mass spectrometer equipped with a Agilent cooled injection system CIS 4 and a 30-m Agilent 19091S-433 HP 5-MS column, I.D. 0.25mm and 0.25µm stationary phase thickness was used. The mobile phase used was helium (99.999%) at a flow of 1.3mL min<sup>-1</sup>. The following oven conditions were used: 1 min at 40°C, then heating to 130°C at the rate of 5°C min<sup>-1</sup>, after which the temperature was kept constant for 10 min.

Ionization was performed using standard EI mode applying 70eV at 230°C. The interface was heated to 270°C and the quadrupole analyzer to 150°C. The detection was performed using total ion current and the identification of the most abundant volatiles was performed using the NIST mass spectra library.

#### *TGA*

TGA and differential thermo-gravimetric analysis (DTG) measurements were performed on parchment samples using synthetic air (purity better than 99.999%, 20mL min<sup>-1</sup> flow rate) in the temperature range 25-500°C at 10°C min<sup>-1</sup> heating rate, with a Perkin Elmer Pyris 1 TGA.

TGA was used to assess the content of water in parchment samples. The non-isothermal thermo-oxidative degradation of the parchment occurs through successive processes accompanied by loss in physical mass of the sample.

## Results and discussion

In order to understand the ageing mechanism of parchment samples caused by light irradiation, and to correlate this information with the degradation detected by quantitative imaging technique (LED multispectral imaging), we characterized and monitored the ageing of the parchments using three techniques: attenuated total reflectance Fourier Transform Infrared spectroscopy (ATR-FT-IR) to identify the formation/disappearance of chemical species in parchment samples; solid-phase micro-extraction and gas chromatography-mass spectrometry (SPME-GC-MS) to analyze the presence of volatiles that provide an indication of lipid auto oxidation in parchment, leading to the degradation of the collagenous phase; and thermo gravimetric analysis (TGA) to assess sample water release from parchment correlated with the thermo-oxidative degradation of parchment.

The parchment samples were aged for 24, 48, 72 and 120 hours in the weather-ometer ageing chamber with a light exposure of  $700\text{Watt/m}^2$  to simulate light exposure. One sample was aged using a UV filter to measure the effect of only the visible light.

After the initial characterization performed before the artificial ageing, the parchment samples were analyzed after each ageing step and the results were compared with the baseline characterization.

The LED multispectral imaging characterization was performed following the methods described in our previous work [19, 20]. The result is a model of the state of conservation of the parchment before exposure to light and consequent degradation. The outcome of the monitoring of the sample during the ageing periods is a control chart composed of an image where each pixel represents the position of each pixel of the parchment. The pixels are pseudo-colored according to whether they represent a changed (out of control points) or an unchanged area. In this paper blue and red pixels are the changed pixels of the parchment, where blue pixels represent regions that are darkening while red pixels represent regions that are whitening. Green pixels correspond to unchanged regions, or in control points.

### *Parchment characterization*

Figure 1 shows the ATR-FTIR spectra of the parchment sample before light ageing. The strong and broad band centered near  $3281\text{cm}^{-1}$  is assigned to the stretching of OH and NH groups variously hydrogen bonded. NH groups are confirmed by the broad absorption between  $500$  and  $600\text{cm}^{-1}$  associated with the out of plane motions of the NH group. The characteristic doublet near  $1632\text{cm}^{-1}$  (amide I) and  $1540\text{cm}^{-1}$  (amide II) indicated the presence of the amide group  $-\text{CONH}-$ . At  $2952$ ,  $2921$  and  $2851\text{cm}^{-1}$  there are the medium-weak band originating from the C-H stretching modes of  $-\text{CH}_2-$  and  $-\text{CH}_3-$  groups occurring in the amino acid residues of the polypeptide chains.

At  $1447$  and  $873\text{cm}^{-1}$  can be seen the characteristic band of  $\text{CaCO}_3$  that comes from the preparation process to which the parchments were subjected.

Peaks at  $1034$ ,  $1199$  and  $1331\text{cm}^{-1}$  are characteristic of tannins, noted by the producer to be part of the manufacturing procedures for this parchment. The first peak is due to the superimposition between the stretching modes of the ester  $-\text{C}-\text{O}-\text{C}-$  and of the carboxylic  $\text{C}-\text{O}-$  while the second is due to the superimposition between the stretching mode of  $\text{C}-\text{O}-\text{C}$  and the ending mode of  $\text{C}-\text{H}$ . Tannin peaks cover the amide III band near  $1239\text{cm}^{-1}$ .

The content of volatile aldehydes provides an indication of lipid auto oxidation of the parchment that leads to the degradation of the collagenous phase. Results indicated secondary peroxidation products that probably originate from a structural rearrangement of the primary

peroxidation products. The majority of chain-cleavage products are molecules belonging to short chain aldehydes: hexanal, heptanal, octanal, nonanal, decanal and 2-decenal (Fig. 2).

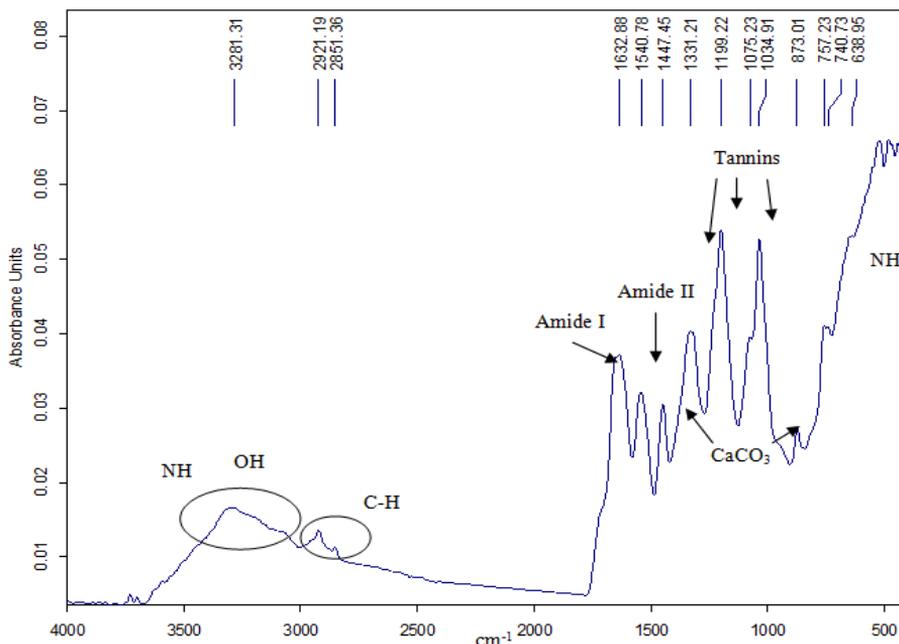


Fig. 1. ATR spectra of goat parchment, recto.

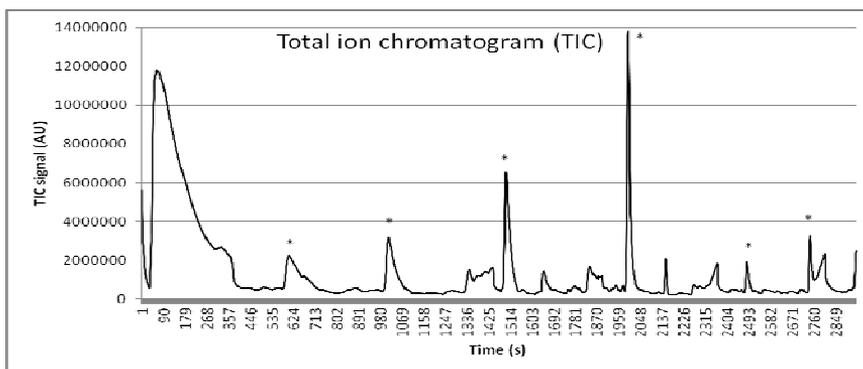


Fig. 2. Total ion chromatogram of volatile compounds desorbed from a goat parchment sample: the identified compounds are marked with \*.

The non-isothermal thermo-oxidative degradation of the parchment occurs through successive processes accompanied by mass loss to the parchment sample. TGA was used to assess the sample water release.

Figure 3a shows the TGA of the parchment sample: in the first process (I in Fig. 3b) water was completely lost (dehydration). The next two steps (II and III in Fig. 3b) consist of the pyrolytic decomposition of the parchment.

The dehydration temperature was 43°C and the two characteristic pyrolytic temperatures were 260°C and 341°C.

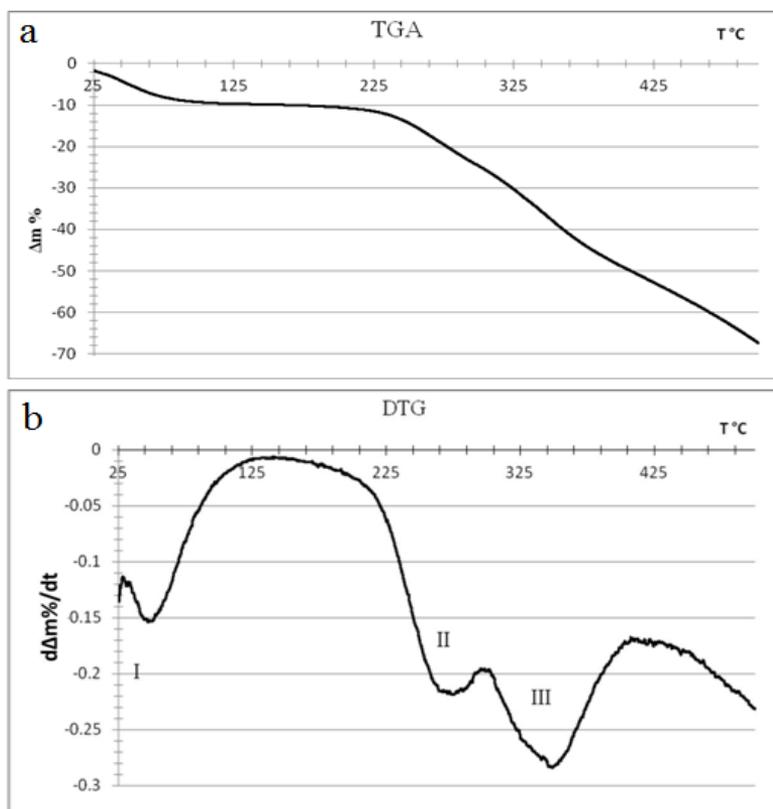
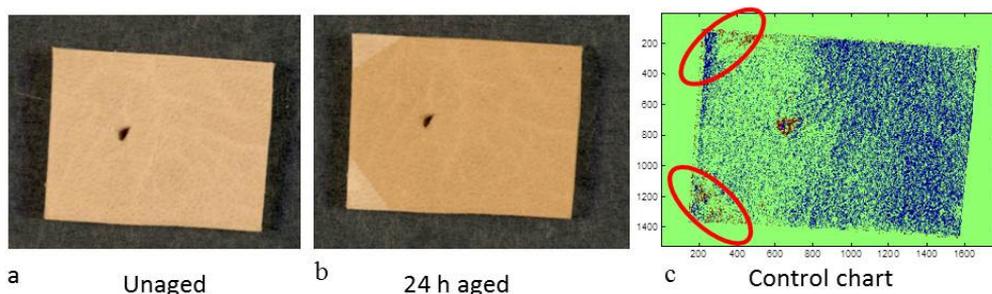


Fig. 3. TGA (a) and DTG (b) curves of the goat parchment analyzed in synthetic air atmosphere

### *Light ageing*

Figure 4 shows the color images of the parchment sample before ageing (a) and after the 24 hours ageing with light (b): both images were collected using the LED multispectral imaging system used for the monitoring. The control chart of the first PC of the parchment aged for 24 hours is represented in figure 4c where blue and red pixels in the charts indicate the region of the parchment that could be considered statistically changed. Wide areas of the image contain blue pixels, the pixels representing areas that are darkening. In the upper and lower left corner there are two regions with both red and blue pixels. These regions were used to attach the parchment to the sample holder in the aging chamber using tape, so the surface was damaged and in some areas it became lighter. The area on the right of the control chart shows more out of control pixels than the area on the left. This is mainly due to a different exposure angle of the sample, since the right side of the sample was not fixed to the sample holder and slightly detached from it, changing the exposure angle with respect to the illuminant. The red circle in the middle area of the chart represents a hole that was made before the characterization step. The large number of blue pixels explains the ability of the methodology to assess the presence of an ageing effect.



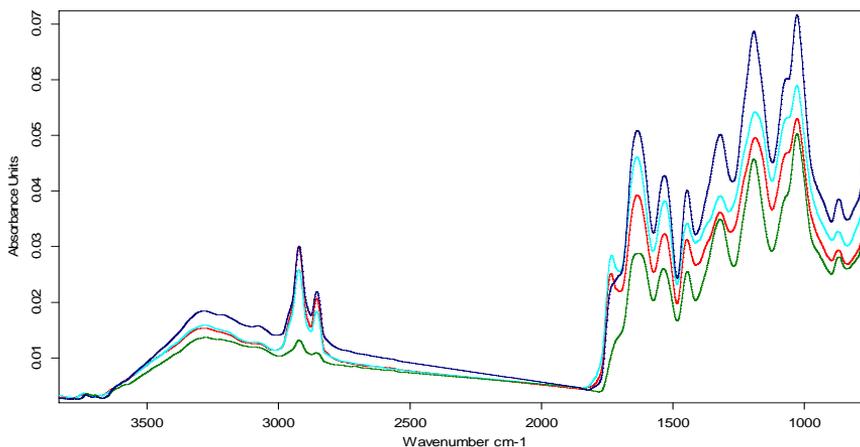
**Fig. 4.** Color images of parchment sample before (a) and after 24 hours ageing (b); control chart of the parchment aged for 24 hours: the blue pixels represent the regions that can be considered changed at a statistically relevant level (1% significance). The ageing detected by the LED multispectral imaging is clearly visible by looking at the color image in figure 1a and b.

The infrared analysis of the parchment sample aged by exposure for 24 hours to the accelerated light aging does not show any change (Fig. 5 blue line). The ageing for 48 and 72 hours showed a new band at  $1733\text{cm}^{-1}$  that slightly increases from 48 to 72 ageing hours. This band could be originated by a C=O groups. Moreover, the band at  $2922\text{cm}^{-1}$  and  $2855\text{cm}^{-1}$  increased their intensity. The degradation detected by the LED multispectral imaging after 24 hours was not detected by the infrared analysis.

The analysis of the content of volatile compounds released by parchment samples that can be useful to provide an indication of the degradation does not show significant results. The content of the five aldehydes identified in the samples during the characterization step did not significantly decrease during the degradation. The residual content of the five aldehydes during ageing did not significantly change.

Moreover, the dehydration and pyrolytic temperatures of parchment did not significantly change throughout the ageing period, as shown in table 1.

Since GC-MS and TGA analyses were not able to detect any change caused by the light ageing, this indicates that thermo-oxidative phenomena and degradation of collagenous phase related to the auto oxidation of lipids are not involved in the physical-chemical ageing process caused by light irradiation.



**Fig. 5.** ATR spectra of the goat parchment: unaged (green), 24 (blue), 48 (red) and 72 hours aged (light blue). The infrared analysis of the parchment sample, aged by 24 hours of sun light exposure does not show any change.

**Table 1.** Dehydration/pyrolytic temperatures and  $\Delta m\%$  of parchment samples at different ageing steps.

Ageing hours	Dehydration T °C (I)	$\Delta m\%$ (I)	Pyrolytic decomposition T °C (II)	$\Delta m\%$ (II)	Pyrolytic decomposition T °C (III)	$\Delta m\%$ (III)
0	48	5.4%	253	10.2%	334	13.0%
24	48	5.3%	251	10.0%	338	14.1 %
48	50	5.9%	255	11.1%	336	13.3%
72	45	5.3%	253	9.8%	336	12.9%

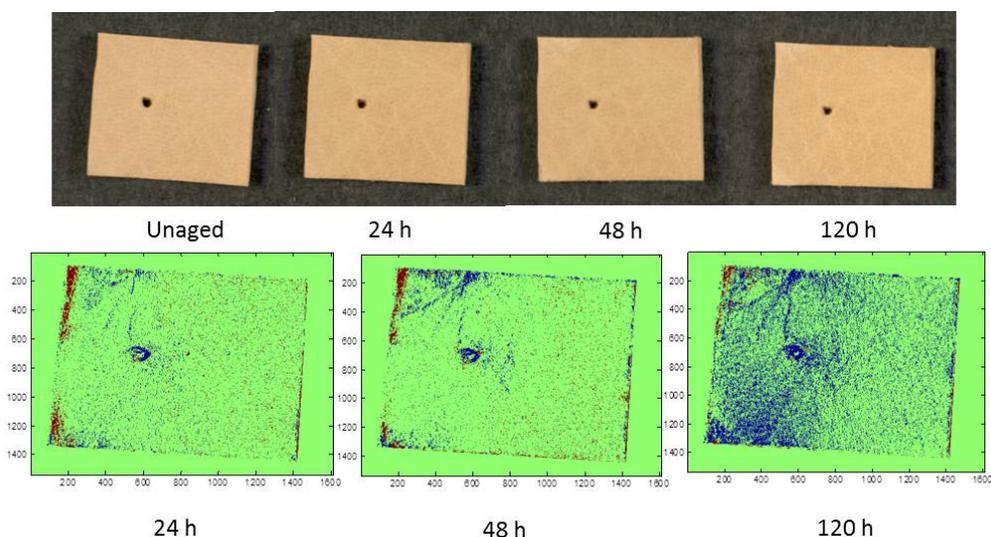
*Light ageing with UV filter*

Figure 6 shows the color images of the parchment sample before ageing (a), and after the 24 (b), 48 (c) and 120 (d) hours of exposure to light with a UV filter. All the images were collected using the LED multispectral imaging system used for the monitoring.

The control charts of the first PC of the parchment aged for 24 (a), 48 (b) and 120 (c) hours are represented in figure 6 in the lower side where blue and red pixels in the charts indicate the points of the parchment that can be considered changed at a statistically relevant level.

The effect of the light on the parchment when a UV filter was used was significantly reduced compared to the UV and visible aging conditions, and can be expected due to the reduced energy levels of the radiation spectrum. The results were visibly observable from the color images and by looking at the number of out of control pixels that the LED multispectral imaging detected after 24 hours of ageing: the change was not visible to the human eye.

The LED multispectral imaging method was able to detect the progress of the degradation, measured by the increase in level of out of control pixels measured after the ageing.



**Fig. 6.** Color images of the parchment sample before ageing (a) and after the 24 (b), 48 (c) and 120 (d) hours of degradation with light with a UV filter; control charts of the first PC of the parchment aged for 24 (a), 48 (b) and 120 (c) hours: blue and red pixels in the charts indicate the point of the parchment that can be considered changed at a statistically relevant level (1% significance).

The ATR, GC-MS and TGA analyses did not highlight any significant change along the ageing.

## Conclusions

We used a quantitative multispectral imaging technique coupled to statistics, ATR-FTIR, GC-MS and TGA to monitor the degradation of parchment samples caused by light, in order to understand the ageing mechanism and to correlate this information with the degradation detected by quantitative imaging. Parchment samples were aged for 24, 48, 72 and 120 hours simulating a sunlight exposure. The degradation was produced after exposure to light both with and without using a UV filter.

The degradation that occurred with UV and visible aging was detected by the LED multispectral imaging technique after 24 hours of accelerated light aging and the effect was clearly visible comparing color images before and after the degradation process. The large number of out of control pixels present in the control chart confirmed the ability of the methodology to assess the presence of an ageing process affecting the monitored surface. The infrared analysis after 24 ageing hours did not show any change, it was only after 48 hours of aging that a new band appeared at  $1733\text{cm}^{-1}$ , originated from the presence of a C=O group, where the bands at  $2922$  and  $2855\text{cm}^{-1}$  increased their intensity. The content of the five aldehydes identified in parchment by GC-MS did not decrease during the degradation and the TGA showed that the sample water release during the degradation did not change.

The effect of the light irradiation of the parchment when a UV filter was used was significantly smaller than when a UV filter is not used, due to the reduced energy input from the consequent irradiation at higher wavelengths. The number of out of control pixels that the LED multispectral imaging detected after 24 hours of ageing was smaller and the color images appeared optically similar from a visual inspection. However, the LED multispectral imaging method was able to detect the progress of the degradation because the out of control pixels increased during the ageing. The ATR, GC-MS and TGA analyses did not show any significant change along the ageing.

In general, the fact that the GC-MS and TGA analyses were not able to detect any change caused by the light irradiation allowed confirmation that thermo-oxidative phenomena and degradation of collagenous phase related to the auto oxidation of lipids are not involved in the chemical-physical ageing process caused by the light irradiation. Moreover we can affirm that UV light, as previously observed, definitely promotes the ageing of parchment. The use of UV filter when parchment materials are exhibited is strongly recommended.

In conclusion we can state that the LED multispectral imaging method was able to detect the ageing effect of light irradiation before other common analytical techniques.

## Acknowledgements

This research is part of “The Dead Sea Scrolls Digitization Project”, “The Leon Levy Dead Sea Scrolls Digital Library”, of the Israel Antiquities Authority (IAA).

## References

- [1] B. Dolgin, V. Bulatov, I. Schechter, *A complex analytical method for parchment characterization*, **Review Analytical Chemistry**, **28**(3-4), 2009, pp. 151-307.
- [2] E. Mannucci, R. Pastorelli, G. Zerbi, C. Bottani and A. Facchini, *Recovery of ancient parchment: characterization by vibrational spectroscopy*, **Journal of Raman Spectroscopy**, **31**(12), 2000, pp. 1089–1097.
- [3] E. Badea, L. Miu, P. Budrugaec, M. Giurginca, N. Badea and G. Della Gatta, *Study of deterioration of historical parchments by various thermal analysis techniques complemented by SEM, FTIR, UV-Vis-NIR and unilateral NMR investigations*, **Journal of Thermal Analysis and Calorimetry**, **91**(1), 2008, pp. 17–27.
- [4] P. Budrugaec, L. Miu, *The suitability of DSC method for damage assessment and certification of historical leathers and parchments*, **Journal of cultural heritage**, **9**, 2008, pp. 146-153.
- [5] D. Fessas, M. Signorelli, A. Schiraldi, C. Kennedy, T. Wess, B. Hassel, K. Nielsen, *Thermal analysis on parchments I: DSC and TGA combined approach for heat damage assessment*, **Thermochimica Acta**, **447**, 2006, pp. 30-35.
- [6] P. Budrugaec, E. Badea, G. Della Gatta, L. Miu, A. Comanescu, *DSC study of deterioration caused by environmental chemical pollutants to parchment, a collagen based material*, **Thermochimica Acta**, **500**, 2010, pp. 51-62.
- [7] C. Chahine, *Changes in hydrothermal stability of leather and parchment with deterioration: a DSC study*, **Thermochimica Acta**, **365**(1-2), 2000, pp. 101-110.
- [8] Y. Wang, J. Guo, H. Chen, Z. Shan, *Influence of containing moisture on hydrothermal stability of modified collagen thermal characteristics analysis by DSC*, **Journal of Thermal Analysis and Calorimetry**, **99**, 2010, pp. 295-300.
- [9] B. Roduit and M. Odlyha, *Prediction of thermal stability of fresh and aged parchment*, **Journal of Thermal Analysis and Calorimetry**, **85**(1), 2006, pp. 157–164.
- [10] A. Kaminska, A. Sionkowska, *Effect of UV radiation on the infrared spectra of collagen*, **Polymer Degradation and Stability**, **51**, 1996, pp. 15-19.
- [11] M. Stirlic, I. Kralj Cigic, I. Rabin, J. Kolar, B. Pihlar, M. Cassar, *Autoxidation of lipids in parchment*, **Polymer Degradation and Stability**, **94**, 2009, pp. 886-890.
- [12] A. Možir, M. Strlić, T. Trafela, I. Kralj Cigić, J. Kolar, V. Deselnicu, G. de Bruin, *On oxidative degradation of parchment and its non-destructive characterisation and dating*, **Applied Physics A**, **104**, 2011, pp. 211–217.
- [13] T. Weiss, J. Orgel, *Changes in collagen structure: drying, dehydrothermal treatment and relation to long term deterioration*, **Thermochimica Acta**, **365**(1), 2000, pp.119-128.
- [14] C. Kennedy, J. Hiller, D. Lammie, M. Drakopoulos, M. Vest, M. Cooper, W. Adderley, T. Wess, *Microfocus X-ray diffraction of historical parchment reveals variations in structural features through parchment cross sections*, **Nano Letters**, **4**(8), 2004, pp. 1373-1380.
- [15] B. Dolgin, V. Bulatov, I. Schechter, *Non-destructive assessment of parchment deterioration by optical methods*, **Analytical Bioanalytical Chemistry**, **388**(5), 2007, pp. 1885–1896.
- [16] E. Robotti, M. Bobba, A. Panepinto, E. Marengo, *Monitoring of the surface of paper samples exposed to UV light by ATR-FT-IR spectroscopy and use of multivariate control charts*, **Analytical Bioanalytical Chemistry**, **388**(5), 2007, pp. 1249–1263.

- [17] A. Nevin, I. Osticioli, D. Anglos, A. Burnstock, S. Cather, E. Castellucci, *The analysis of naturally and artificially aged protein based paint media using Raman spectroscopy combined with Principal Component Analysis*, **Journal of Raman Spectroscopy**, **39**, 2008, pp. 993–1000.
- [18] T. Teddy, L. Miu, M. Giurginca, A. Meghea, *Evaluation of degradation of parchment and leather by FT-IR and UV-VIS-NIR spectroscopy*, **Revista de Chimie**, **57**(5), 2006, pp. 466-471.
- [19] E. Marengo, M. Manfredi, O. Zerbinati, E. Robotti, E. Mazzucco, F. Gosetti, G. Bearman, F. France, P. Shor, *Development of a technique based on multi-spectral imaging for monitoring the conservation of cultural heritage objects*, **Analytical Chemistry**, **83**(17), 2011, pp. 6609–6618.
- [20] E. Marengo, M. Manfredi, O. Zerbinati, E. Robotti, E. Mazzucco, F. Gosetti, G. Bearman, F. France, P. Shor, *Technique based on LED multispectral imaging and multivariate analysis for monitoring the conservation state of the dead sea scrolls*, **Analytica Chimica Acta**, **706**(2), 2011, pp. 229– 237.
- [21] M. Manfredi, G. Bearman, G. Williamson, D. Kronkright, E. Doehne, M. Jacobs, E. Marengo, *A new quantitative method for the non-invasive documentation of morphological damage in paintings using RTI surface normal*, **Sensors**, **14**(7), 2014, pp. 12271-12284.
- [22] M. Manfredi, G. Williamson, D. Kronkright, E. Doehne, M. Jacobs, E. Marengo, G. Bearman, *Measuring changes in cultural heritage objects with Reflectance Transformation Imaging*, **Proceedings of the Digital Heritage 2013**, 28 October - 1 November 2013, Marseille, France, Vol. 1, 2013, Article number 6743730, pp. 189-192.

---

Received: June, 13, 2014

Accepted: December, 04, 2014