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The X-ray irradiation as disinfection treatment: the state-of-the-art

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Abstract **Collagen-based materials constitute a significant part of the archival and library heritage. Among all the animal skin-derived substrates, parchment was the principal writing support employed in the western world until the invention and diffusion of paper and printing. However, parchment can suffer biodeterioration processes if preserved under altered conditions. Recently, the possibility of using X-ray irradiation to respond to the demand for new effective and 'green' methods for the mass disinfection of the archival and library heritage has opened up new and interesting perspectives. Different studies have been carried out to characterise eventually induced deterioration as well as the effectiveness of the irradiation treatment employed for disinfection purposes on different series of samples. In particular, modern parchment samples before and after the irradiation treatment, artificially aged parchment and historical and natural biodeteriorated parchment have been investigated after being exposed to the irradiation treatment.**

In the present work, a brief review and some new results and perspective have been presented.

I. INTRODUCTION

A significant part of the archival and library heritage is made of collagen-based materials. Substrates such as leather, parchment, or alum-tawed skin, derived from animal skin like goat, sheep, cattle, , have been used through the centuries because of their peculiar physical properties. These are mainly due to the characteristic fibrillary structure of the collagen molecule (the main protein present in the pelt), its orientation in fibrils and bundles of fibrils to compose the fibres and thus, its complex fibrillary networking [1].

In particular, parchment was widely used in the western world as a writing support at least until the invention and diffusion of paper and printing. The peculiar process of parchment manufacturing, and in particular the stretching step which the pelt is subjected to, make this support different from leather and alum-tawed skin. A stable and resistant material is produced because of the particular reorganisation of the fibre network in the dermal layer that gives to it peculiar physicochemical properties and mechanical characteristics [2-3].

However, as well as all the collagen-based materials, parchment can undergo biodeterioration processes if it is preserved under altered conditions of temperature and humidity. Since the Antiquity, for the library collections the problems of the disinfection and pest control were highlighted; it suffices to know that Pliny the Elder already suggests remedies to control the effects of insects on papyri in his *Naturalis Historia*. Even without a not yet complete awareness of the microbiological nature of the biodeterioration (18^{th} century), the conservators tried some attempts to stop microbiological infections [4]. Only at the start of the twentieth century, when library materials start to be taken in greater consideration, systematic protocols of disinfection start to be considered and the remediation treatments to applied [5].

Different treatments have been used in the 20^{th} century in order to respond to the demand for disinfection of huge quantities of material employing chemicals that are often dangerous for the operator as well as to the treated materials, such as the fumigation with ethylene oxide [6]. The limitations to their use set in recent years by the European Community [7-8] have prompted the researchers to find new approaches to the disinfection of this kind of Cultural Heritage (CH). Similarly, a number of chemical agents normally used for disinfection of paper, such as the use of bath or spray solution of quaternary ammonium salts, begin to be abandoned with the consequence that the only approved disinfectant treatment remains the ethanol hydroalcoholic solution [9]. In a general tendency to a green approach to restoration and, specifically, to the disinfection, in recent years the use on natural and biocompatible agents has been tested. In particular, sustainable treatments have been proposed such as fumigation with essential oils and plant extracts [10-11]. Disinfection based on UV exposure has also been proposed but has never applied due to the capability of the radiation to cause depolymerisation processes in the molecule exposed to that [12]. The use of ionising radiation, such as the gamma rays, seems to be a very promising aid in the library and archival disinfection, but it remain very difficult to apply due to the security measures related to access to nuclear plants and radioactive sources [13-14]. The X-ray radiation seems to be a good compromise since they can be produced by the conversion of accelerated electron beams, and thus not radioactive sources are not necessary. It is necessary to point out that each new restoration material or proposed treatment to be applied to the original cultural heritage, must have some peculiar characteristic that it is required to evaluate before it could be applied to the original items. Clearly, every new proposed approach must have the capability to not alter the mechanical properties of the treated material and to be efficient in preventing future deterioration, but, especially in the last years, it must also to be environmentally friendly and safe for the restorer to use. In a general scenario of reduction of chemicals employed in the restoration field, the possibility of employing X-ray irradiation to respond to the demand for new effective methods for the disinfection of large volumes of archival and library heritage opens up new and interesting perspectives.

Although the control of the microclimate in conservation environments remains the main and best method for the prevention of infections of microbiological nature [15], in recent years, the application of X-rays has been investigated and tested to evaluate the possibility of being employed as a disinfection protocol. In particular, Vadrucci *et al*. [16] detected in 350 Gy the so-called threshold dose able to inhibit the bacterial growth and, at the same time, not induce further deterioration processes in the collagen matrix of modern parchment substrates. The short- and long-term efficiency and the possible induced deterioration of the treatment in function of the irradiation dose has been thoroughly addressed with the help of different spectroscopic analysis and advanced microscopic techniques also on leather [17]. The chemical stability of the leather due to the tanning process is probably the main cause of the ability of this material to maintain unaltered response at higher X-rays doses with respect to the parchment. In the case of the parchment substrate, different studies have been carried out in analysing modern parchment samples before and after irradiation treatment [18] and after an artificially induced ageing process [19] in order to evaluate the effects of the radiation in the long terms of preservation. Both the research works confirm the threshold dose identified in the previous one also thanks to different techniques and analytical method of investigation, confirming the possibility to safely employ the method. In particular, the combined effect of the irradiation treatment and the ageing process on the collagen matrix has also been investigated. The samples, irradiated and then artificially aged by thermo-hygrometrically altered conditions of conservation, have revealed the primary role of the ageing in inducing deterioration processes with respect to the once induced by the X-rays doses [19].

As reported in Vadrucci *et al.* [20], the impact of irradiation on naturally aged and biodeteriorated parchment has been recently investigated. In order to analyse the irradiation effects produced on historical biodeteriorated parchment, a sample derived from a discarded book cover belonging to an archival registry (from around the 16th century), from a private collection, has been irradiated with increasing doses of X-rays and then analysed. The samples, which came from an area highly damaged by biodeteriogen attack, were analysed from the microbiological point of view to obtain information on the bacterial and fungal species involved in the infection. Therefore, the species were isolated and characterized, and used to evaluate the effectiveness of antimicrobial treatment on the microorganisms isolated on the specific substrate.

Today, the irradiation protocol has been applied to a series of modern parchment samples previously artificially aged to simulate different stages of partially deteriorated artefacts like those that it is possible to find in the context of the original library or archival heritage. The aged samples have been then irradiated with increasing doses of X-rays in order to evaluate the effect eventually induce by the radiation on the partially deteriorated collagen matrix of the aged samples with respect to the effect produced on the unaged one.

In the research here presented, the proposed irradiation protocol has been performed by a high energy X-ray beam produced by the REX source (Removable Electrons to X rays). The facility (based on a 5-MeV S-band electron linear accelerator equipped with an interchangeable X-ray converter) allows the use of volumetrically penetrating radiation (photons and electrons) without the safety risks of the radioactive sources [21].

Different techniques have been used to assess the eventual alteration in the stability of the collagen protein within the parchment. Attenuated total reflectance Fourier-Transformed Infrared spectroscopy (ATR-FTIR) spectroscopy, which allows, in a non-destructive way, to monitor changes in the protein secondary structures through the analysis of the variation in the Amide I and II bands, has been demonstrated to be a valid tool to characterise the changes in the protein structure of collagen [22]. On the other hand, the Light Transmission Analysis (LTA), although being a microdestructive technique, turns out to be fundamental to characterize eventual variation in the hydrothermal stability of the collagen matrix [23]. The preliminary research studies presented in the following show the potentiality of the method that, although is still in a preliminary step and thus still hardly available, could constitute a valid possibility in the mass treatment of these kind of heritage.

II. THE IRRADIATION PROTOCOL

A disinfestation treatment by X-ray irradiation was applied using a REX source. The process developed is an unconventional method in CH applications and is completely sustainable and safe both for the objects to be treated and for the environment. It does not use toxic substances and does not use radioactive sources; therefore, it is safe and less complicated also from an authorisation and radiation protection point of view. To inhibit the biodegradation of parchments and leather, a high energy X-ray beam produced by the REX source was used. The facility, available at the ENEA Research Centre of Frascati (RM), allows the use of volumetrically penetrating radiation (photons and electrons) without the safety risks of conventionally used radioactive sources. REX is based on a 5 MeV S-band electron linear accelerator equipped with an interchangeable X-ray converter. The instrumentation can deliver radiation with intensity and fluence modulated for a specific purpose and performs online dosimetry [21]. For this work, a tungsten converter specifically designed to provide a uniform X-ray distribution over large treatment areas was used.

III. METHODS

A. SAMPLES PREPARATION

The analysis were carried out on samples of modern parchment prepared using a traditional method by the National Research & Development Institute for Textiles and Leather of Romania [24] and on a historical parchment affected by biodeterioration coming from a discard cover of an archival registry (approximately 16th century). The modern parchment samples were artificially aged in a specifically designed oven able to keep them in an environment with altered conditions of temperature (80°C) and relative humidity (75%). The ageing was performed for different times (2, 4, 8, 16 and 32 days) with the aim of reproducing the possible different ageing of the original CH objects with respect to the control un-aged sample (0 days). The control sample and the aged ones were submitted to the irradiation protocol with different increasing X-rays doses (from 0 Gy of the control sample to 4000 Gy) to allow the characterization of the effects of the radiation in the variously aged samples (Fig.1).

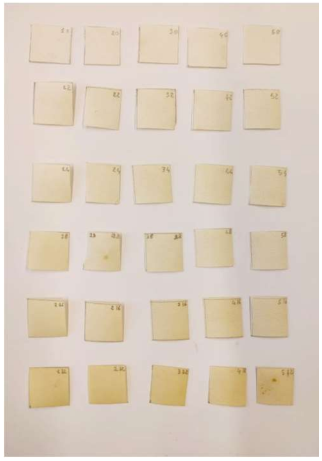

Fig. 1. Modern parchment samples after the ageing process and irradiation treatment. From top to bottom, the ageing increases (0, 2, 4, 8, 16 and 32 days)*; from left to right, the irradiation doses passes from 350 Gy to 4000 Gy (350, 500, 1000, 2000, 4000 Gy).*

B. LTA

Light Transmission Analysis (LTA) has proven to be a reliable technique for determining the so-called hydrothermal denaturation temperature (Td) of collagen structured in parchment or leather fibres. At the base of the experimental technique, there is the evaluation and characterisation of the hydrothermal stability of the collagen-based material, a peculiar feature of this kind of material usually investigated with a number of techniques like Micro Hot Table (MHT), Differential Scanning Calorimetry (DSC), Adiabatic Scanning Calorimetry (ASC) [23, 25]. The mentioned deterioration process takes place in a collagen-based sample when its fibers are heated in water. Being strictly related to the breaking of the molecules hydrogen bonds, clearly, in damaged or partially deteriorated parchment samples where the molecular bonding results in already weakened, to complete the hydrothermal denaturation process a smaller amount of thermal energy is required, and thus, the denaturation will occur at lower temperature with respect to a well-preserved sample.

In the experimental LTA set-up the process is artificially induced placing a 0.1 mm thick suspension of parchment fibres in water in a hoven with a constant rate of temperature increase of approximately 1.8 °C / min, from room temperature up to 90 °C. The sample is obtained by mechanically pulping a 1mm^2 of parchment by a scalpel in condition of full water excess. To determine the descriptive parameter of the process, Td, a light source provided by a He-Ne laser beam, crosses the suspension while the light emerging from the cell is collected by a lens and focused onto a photodiode that generates the LTA signal. During this induced deterioration process, the collagen fibres structure is modified from a typical hierarchical one, with the well ordered triple helix fibres, to an amorphous one. The latter structure has a sensibly smaller capability to scatter the light coming from the laser beam resulting in an increase of the transmitted signal with respect to the scattered one. The larger the increase of the transmission signal, the greater the quantity of the parchment samples that has completed its transformation at that given temperature. The signal derivative allows one to identify the temperature at which this process reach its maximum rate of activity that can be identified as the so-called denaturation temperature.

The experimental setup allows also the real time evaluation of the changes in the sample morphology induced by the hydrothermal denaturation process by means of an integrated polarisation microscopy.

C. ATR-FTIR

FTIR absorption spectra were acquired with the Thermo-Scientific instrument (model Is50) (Thermo Scientific Inc., Madison WI USA) in Attenuated Total Reflectance (ATR) mode using a single reflection diamond cell. Spectra were recorded from 4000 to 750 cm^{-1}, averaging over 32 scans with a resolution of 2 cm^{-1}. All experiments were performed in triplicate, yielding consistent and reproducible results. Deconvolution was performed in the 1360−1800 cm^{-1} region on normalized absorbance ATR-FTIR spectra by means of Voigt bands of full width at half maximum (FWHM) of 25 cm^{-1} maximum, using OMNIC software [22].

IV. RESULTS

At first, it is possible to say that the LTA results on aged parchment confirm the threshold dose identified in the previous studies. In fact, for all the aged series of samples, up to the irradiation dose of 350 Gy, there are no recordable substantial changes in the values of the T_d with respect to the un-irradiated one (ΔT_d <0.5°C for all aged samples). At larger doses of irradiation, the T_d values show a general tendency to progressively decrease, remaining, in some cases, within the maximum uncertainty range of the data (±0.5°C). It is worth to note that the combined effect of ageing and irradiation tends to saturate with increasing of the induced deterioration. So that, we can surely say that with the increasing of the ageing, the effect of high doses of irradiation induce only slight differences in the recorded denaturation temperatures being this saturation effect particularly evident for samples aged more than 8 days.

Similarly, ATR-FTIR spectroscopy returns the same tendency for the Amide I and Amide II intensity ratio (Fig. 2).

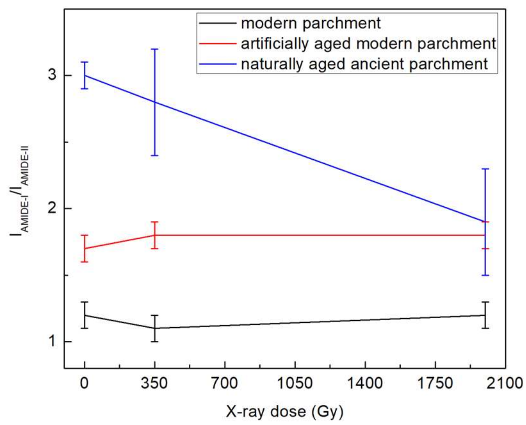

Fig. 2. Amide-I – Amide II intensity ratio Vs irradiation dose in unaged, artificially aged and naturally aged samples.

This parameter reflects the degradation processes induced by the treatment in the collagen molecule. When trying to discriminate the effect of the irradiation from the one induced by artificial ageing, appears that the variation in the intensity ratio as a function of the increasing doses of X-rays remains, in most of the cases, in the range of uncertainty of the measurement. On the contrary, the effect of the ageing, which influences the hydrolysis degree of the molecule and, thus, the measured parameter, is appreciable.

V. CONCLUSIONS AND PERSPECTIVE

At present, disinfection treatment based on X-ray irradiation seems to be a valid alternative to the use of chemicals for the treatment of parchment substrate. Although the method involves mobilization of the artefacts and the methodology results still not easily accessible to everybody, the basic research on its application, effects and effectiveness shows potentialities in particular for the mass treatment of large quantity of library and archival heritage. The satisfying results obtained in the case of modern and ancient parchment, confirmed by the most recent analysis performed on artificially aged samples, encourage continuing the research to test the effect of the radiation on all the materials which make up the book and the archival document.

In particular, the further step of this research regards, necessarily, the evaluation of the radiation-induced effect on inks and inked parchment samples.

From the introduction of parchment in Late Antiquity, the use of iron gall ink starts to replace the use of carbon-based ones, since the better capability of iron gall ink to persist on the parchment leaf. However, the chemical composition

of the iron gall ink (essentially made by the combination of iron sulphate (II) with tannins in water and Arabic gum as a medium to produce a suspension) constitutes an important external factor of deterioration for the writing supports. It is mainly due to its acid pH, the formation of gallic acids due to tannins and all the negative consequences due to the presence of iron ions remaining on the support surface [26].
The effect of this kind of ink has mainly been studied in the case of paper manuscripts, as this kind of support suffers the most negative effects leading to its complete corrosion and the total loss of the writings.
Due to the greatest resistance of the parchment support to acid corrosion, less attention has been devoted to the ink on parchment and its potential contribution to the deterioration of this type of artefacts. However, some studies revealed a possible degradation of ancient parchments due to the diffusion of iron-based inks into the unwritten part of the supports [27-28].
In order to confirm the safe applicability of our X-ray-based disinfection method even in the case of written parchment supports, further research is necessary to explore and characterise all the possible variations induced in the molecular structure of the collagen protein constituting artefacts where inks have been applied for writing or decorating purposes.

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