

^1H HR-MAS NMR reveals the degradation mechanisms of ancient documents

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Summary. — Water is highly important in regulating the physico-chemical properties of every biological system it interacts with. In this frame, paper is mainly constituted by an almost equimolar ratio of cellulose and water and the degradation mechanism of ancient documents is linked with the action of hydration water. Indeed, the interaction between water and cellulose within the fibres structure is the cause of breaking of the hydrogen bonds and of the consequent swelling of the cellulose fibres. In this paper, Nuclear Magnetic Resonance (NMR) spectroscopy results obtained by means of the High-Resolution Magic Angle Spinning (HR-MAS) technique have been shown for modern paper samples made of pure cellulose not aged and artificially aged as well as on ancient paper samples. These results show that water gradually affects the cellulose degradation in aged samples providing fundamental information for industrial applications and for the preservation and restoration of cultural heritage materials based on cellulose such as ancient paper or textiles.

1. – Introduction

Liquid water is essential for life processes in all biological systems [1, 2]. In fact, biological water accomplishes delicate biochemical tasks in its interaction with biosystems, *e.g.*, proteins, triggering their functioning and properties. It is well-known that the proper flexibility necessary to activate the biofunctionality of macromolecules is induced by the surface water molecules (hydration water) and, specifically, by the formed network of hydrogen bonds (HBs) [3-5].

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Among the systems made of biopolymers whose properties change due to the presence of water, paper is probably the most common and employed material: in fact, since ancient times, it has been used to put in storage and disseminate knowledge [6]. Paper was invented in the 2nd century A.D. in China and it spread from the Far East to the Western world in the Middle Ages and for centuries afterward, increasing the number of documents accumulated in archives and libraries all over the world [7, 8]. Paper can be considered as mainly constituted by cellulose fibers in an almost equimolar amount with bound water [9, 10] and small quantities of contaminants and organic or inorganic substances [11, 12].

Cellulose is a biocompound made up of many glucose (β -D-glucopyranose) units attached end to end with β -(1,4)-glycosidic bonds [6]. The cellulose chains have a strong tendency to aggregate into highly ordered structural entities through an extended network of intra- and intermolecular HBs [6, 13]. These bonds are affected by several factors such as the presence of H₂O molecules that contribute to degradation mechanisms. In fact, the oxygen contained in water molecules, attacking the hydroxyl groups in a glucopyranose molecule and influencing, in addition, the glycosidic bond, causes the cellulose cleavage and depolymerization [14, 15].

Therefore, if from a macroscopic point of view, paper degradation is revealed by yellowing and/or discoloration, from a microscopic perspective, these phenomena are mainly due to i) acidic hydrolysis of β -(1,4)-glycosidic bonds and ii) oxidative attacks on β -D-glucopyranose units of cellulose polymers [6, 16, 17], processes that are coupled since hydrolysis accelerates the oxidation and vice versa. Acid hydrolysis acts on the mechanical properties of paper sheets weakening them because it reduces the dimensions of cellulose chains. The breaking of glycosidic bonds is catalyzed either by the activity of enzymes that have the capacity to hydrolyze cellulose or by hydronium cations (H₃O⁺) produced through the acidic hydrolysis of internal components, *e.g.*, alum [6]. For what concerns the oxidative processes of cellulose, it generally begins with the transformation of individual β -D-glucopyranose units to fairly unstable oxidized derivatives, which induces, *e.g.*, ring opening [6] and depolymerization [14, 18]. As a consequence, depolymerization can be enhanced by using acidic oxidation products and/or light products of the degradation [19]. Other microscopic processes in paper degradation are chemical crosslink in the wood polymers, crystallization and hornification (physical volume relaxation), which are manifested in color changes in the paper, processes that follow i) and ii) [20].

For what concerns cultural heritage, one of the most obvious difficulties in the study of the degradation of ancient documents is that researchers do not know exactly the history of the studied sample and the environmental conditions to which it has been subject over the years. Anyway, artificial and monitored high-temperature aging gives the possibility to supervise the change of environmental conditions, providing useful information for ancient paper conservation [16].

Nuclear Magnetic Resonance (NMR) spectroscopy is an absorption technique in which the radio-frequency (RF) waves are absorbed by the studied nucleus in the presence of an external magnetic field resulting in spin changes at nuclear level. This technique allows for studying distinct environments experienced by the resonant nuclei and to discriminate between the same nuclei in different chemical environments [21]. Previously, several NMR experiments have been performed to study paper and cellulose-based materials [11]. Note that non-liquid samples are characterized by strong anisotropic interactions that provoke line-broadening effects, limiting the NMR spectral resolution and sensitivity. One of the most used experimental techniques to overcome this difficulty is known as ¹H High-Resolution Magic Angle Spinning (HR-MAS), in which the sample is put in a rotor,

and spun at the magic angle of about 54.74° by few thousands of Hertz resulting in highly resolved NMR spectra [22]. Today, rotors can spin at frequencies between about 1 and 100 kHz: at higher frequencies the signal results more enhanced and with higher resolution. Recently, this technique is causing a lot of interest because of its versatility, allowing the investigation of microquantities of semi-solid compounds, in different fields, particularly in medicine, biology, and food science [23-25]. In particular, it is possible to get structural and/or dynamical information on all the components that constitute the investigated sample without any chemical treatment. Indeed, the HR-MAS technique permits to study cellulosic materials and their interaction with water.

In this paper, HR-MAS NMR research studies on the degradation processes of ancient documents have been reported. In particular, the interaction between water and cellulose has been studied in detail, by performing lineshape analysis of the NMR spectra and by evaluating the spin-lattice relaxation time, T_1 , which takes into account the interactions between the spins and their environments. In this way, the swelling of cellulose fibers and, consequently, the degradation reactions can be properly examined.

2. – Materials and methods

NMR measurements were performed by means of a Bruker Avance spectrometer operating at 700 MHz (proton Larmor frequency) using the HR-MAS technique [26,27]. The experiments were executed acquiring 512 transients of a standard 90° pulse sequence and the duration of the hard pulse was $7\ \mu\text{s}$. Free induction decays were Fourier transformed by means of Bruker Xwinnmr 3.5 software packages. For further experimental details refer to [26].

To properly investigate the degradation process, the NMR data of both modern and ancient samples have been analyzed. The modern paper ones (labeled “P2”) were made of unbleached cotton linters with low amounts of inorganic elements and no additives, and were furnished by the Netherlands Organization for Applied Scientific Research (TNO). These samples were artificially aged in air within a climatic chamber at relative humidity (RH) of 59% and a temperature of 90°C for different numbers of days. The reactor (climatic chamber) and the conditions employed were chosen to spot the influence of moisture and according to the ASTM standard [28]. Specifically, the chamber used is a “Humidity chamber HCP246”, produced by Memmert, with a volume of 246l. To better identify the samples used, the not aged sample was marked with the label “P2REF”, instead the aged samples were denoted with “P2C90xx”, where “xx” indicates the number of aging days. Data shown in this paper for the modern samples regard P2REF and the P2C9048 (aged for 48 days) samples. Concerning ancient samples, they were made of cotton and linen cellulose fibers and were not printed. These samples were produced in the 15th century in European countries, *e.g.*, France and Italy. In fact, two ancient samples, both made in Perpignan, France, in 1413, were labeled A1 and B1, and they were in good and intermediate conservation condition, respectively. A third sample, made in Milan, Italy, in 1430 and labeled A3, was in bad conservation condition (because it was water-stained).

The considered samples present varied levels of degradation related to their concentration of chromophores, which are oxidized groups able to absorb ultraviolet and visible photons. For example, the concentration of chromophores is negligible for the non-aged P2REF sample, instead, it is 0.8 mmol/100 g of cellulose for P2C9048 and 3.1 mmol/100 g of cellulose for A1 [29,30].

The NMR measurements were carried out using paper samples with distinct values of moisture content [26,27]. The moisture content, also called the hydration level h , can

be expressed as

$$(1) \quad h = \frac{m_{water}}{m_{drypaper}},$$

where m_{water} is the mass of water and $m_{drypaper}$ is the mass of the completely dry sample. The values of m_{water} are obtained as difference between the total sample weight and the value of $m_{drypaper}$ [26]. Samples with hydration level of about 2%, 8%, 14%, 20%, and 40% have been investigated. The samples were initially maintained in an oven at 105 °C for 4 hours to obtain a moisture content of approximately 2% (labeled as “quasi-dry”). Then, the desired hydration was attained by putting the dry samples in a closed chamber with RH = 100% for several hours at room temperature [26]. Note that the reached h level was estimated by weighing the samples before and after each measurement and verifying that the weight remained stable within 5%, and by the use of the NMR signal itself [26, 27].

The MAS NMR spectra were analyzed performing a line-shape analysis [27]. The best fit is obtained with a pseudo-Voigt function of the following form:

$$(2) \quad y = A \left[\mu \frac{2}{\pi} \frac{\omega_L}{4(x-x_c)^2 + w_L^2} + (1-\mu) \frac{\sqrt{4 \ln 2}}{\sqrt{\pi} w_G} e^{-\frac{4 \ln 2}{w_G^2} (x-x_c)^2} \right],$$

where A is the amplitude, μ is the weight of the Lorentzian component, x_c is the peak position and w_L and w_G are the corresponding width of the Lorentzian and Gaussian contributions of water and cellulose protons, respectively [9, 27, 31]. In particular, plotting the total sample weight *vs.* $A * \mu$ (area of the Lorentzian component ascribed to water), it is possible to calculate the mass of dry cellulose by evaluating the y -axis intercept, having also a more precise information of the real hydration of the investigated samples [26].

The relaxation time T_1 was measured by using the standard inversion recovery pulse sequence [32]: the delay (τ) between the two pulses was varied from 3 μ s to 100 seconds, the repetition time was adjusted to 20 s and the number of scans was 16 for each delay time [26]. The obtained relaxation time was not a single exponential, but it can be considered as a multi-exponential (an usual behavior of water proton spin-lattice relaxation times found in biological tissues [33]) composed by three exponential functions. Specifically, these three components were associated to i) free water (component a, the slowest), ii) bound water (component b, the intermediate one), and iii) cellulose protons (component c, the fastest) [9, 26, 34, 35].

3. – Results and discussion

Figure 1 shows an example of the HR-MAS spectrum of the P2C9048 paper sample at 2% of hydration, shown with the corresponding spectral deconvolution in terms of the pseudo-Voigt function indicated in eq. (2). The total fit described by this function is reported as a black solid line, whereas the Lorentzian and Gaussian contributes are shown as dashed and dotted lines, respectively.

As stated before, this procedure is useful, *e.g.*, to recognize the signal due to water and get precise information of the real hydration of the investigated samples and/or the presence of degradation. In fact, as confirmed in refs. [27] and [36] in which detailed studies for this purpose have been reported, the Lorentzian contribute clearly changes its shape by raising the hydration of the sample, whereas the Gaussian function gives

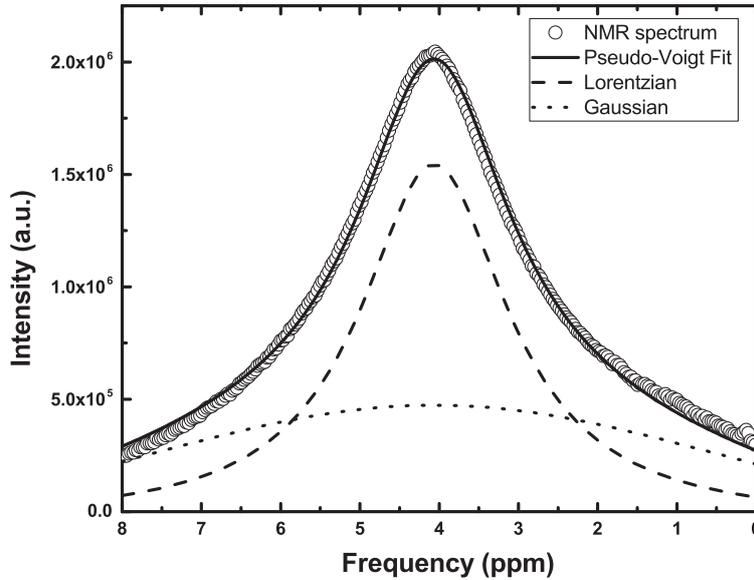


Fig. 1. – The proton HR-MAS NMR spectra of P2C9048 samples at hydration level of 2%. The total fit with the pseudo-Voigt function (solid line) and the two components, Lorentzian (dashed line) and Gaussian (dotted line), are reported. Figure adapted from [27].

essentially the same contribution as the Lorentzian one only for the sample with the lowest hydration, turning into a negligible background at higher h values. Furthermore, it is possible to relate the increased presence of degradation by-products with the hydration level of paper. In particular, it has been shown that the not aged sample weakly affects the water behavior, whereas, for aged samples, the amphiphilic character of cellulose surfaces and of the several by-products certifies the complex dependence on hydration.

For what concerns the dynamical analysis, the behavior of the relaxation time T_1 as a function of the hydration level and of the number of degradation days has been reported in figs. 2 and 3, respectively.

As already stated in the “Materials and methods” section, the T_1 data can be decomposed into three different components (denoted as T_{1a} , T_{1b} , and T_{1c}), which display different behaviors as a function of moisture content of the cellulose, allowing for properly studying the hydration in artificially aged paper [26,37]. Figure 2 shows the T_{1a} and T_{1c} components for P2REF and P2C9048 samples.

T_{1a} (fig. 2, top) represents the slowest part of the multi-exponential function previously introduced. This component describes the interaction between the liquid-vapor phase water molecules and voids or pores in the structure of cellulose fibers. The addition of water leads to the transition from the liquid-vapor phase to the liquid phase with an increment of the interactions between water and cellulose. This behavior is due to the increase of the solvent accessible volume with increasing h caused by cellulose fibers swelling. Therefore, T_{1a} has an exponential-like behavior as a function of h [26]. The T_{1b} component (not reported here, see ref. [26]) is attributed to non-freezing water and is characterized by a constant trend as a function of h . In fig. 2 (bottom) the T_{1c} component that has been attributed to that of cellulose protons [26,37,38] is displayed and has the smallest value, which slowly increases with increasing h . In fact, raising

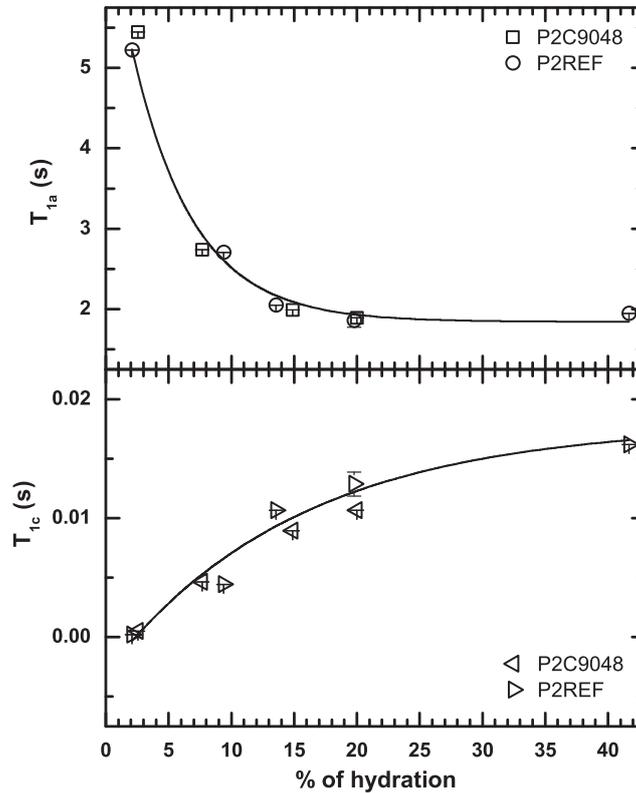


Fig. 2. – The T_1 data of the T_{1a} (top) and T_{1c} (bottom) contributions as a function of the hydration for both P2REF and P2C9048 samples. Lines are a guide for the eye. Reproduced from ref. [26] with permission from the PCCP Owner Societies.

the hydration level, the cellulose fibers swelling increases, allowing an enhancement of mobility of cellulose protons.

The trend of T_1 components *vs.* the number of degradation days is reported in fig. 3, including modern (*e.g.*, P2REF and P2C samples at different artificial degradation days) and naturally aged samples (denoted as A1, B1, and A3) [26].

The T_{1a} component initially raises of about 20% up to 12 degradation days and then decreases linearly as shown in the figure. This behavior is very intriguing and can be considered as a result of the first step of the degradation process associated with the break of HBs enhancing the fibers accessible volume [26]. After 12 degradation days, the data show that there is a drop of the hydrophilic character due to the progressive change of hydroxyl groups into carbonylic ones: this transformation is well evident for ancient samples where T_{1a} decreases as a function of the degradation. T_{1a} values for ancient samples have been defined by means of a linear fit of T_{1a} data of samples with more than 12 degradation days. Consequently, it has been possible to identify the equivalent artificial degradation days for A1, B1 and A3 samples by placing them on the extrapolation of the performed fit. In the same way, a linear fit was executed for T_{1b} data also showing that the ancient samples were spontaneously very well fitted by this linear fit. Regarding T_{1c} data, there is an agreement among the values of modern and ancient samples, and

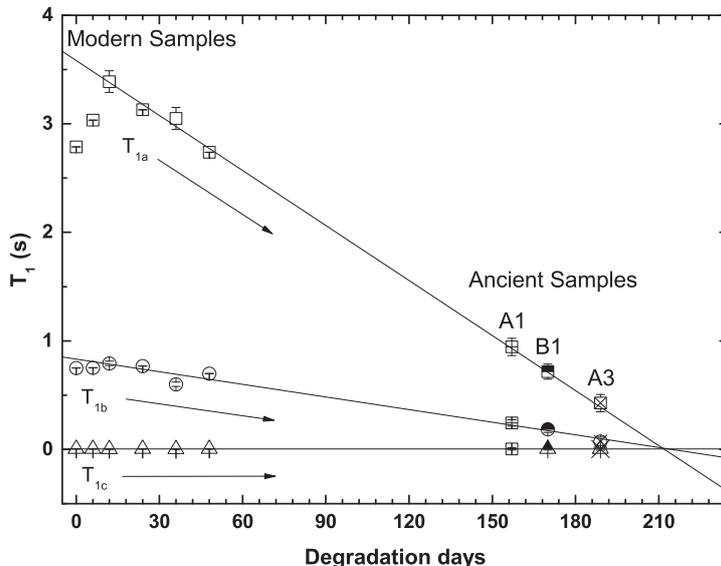


Fig. 3. – The three components of the relaxation time T_1 as a function of the degradation days. The data from modern aged samples (empty symbols) and the extrapolated value for ancient samples (crossed and half-filled symbols) are reported. The linear fits cross each others at about 212 artificial degradation days (see the text). Figure adapted from [26].

they lied, in fact, on a straight line with slope ≈ 0 . Furthermore, as shown in fig. 3, the linear evolution of each contribution crosses each other with a very good approximation at about 212 degradation days, *ergo* this can be considered as an indication of the limit of artificial degradation. This point corresponds to the levelling-off of the degree of depolymerization of cellulose polymers [39] when paper loses most of its mechanical properties and begins to crumble.

4. – Conclusions

In this work, the results obtained on the degradation processes of ancient and modern, aged and not aged, paper by means of ^1H HR-MAS NMR spectroscopy have been shown, focusing, in particular, on the analysis of NMR lineshapes and relaxation time T_1 . In fact, these investigations reveal the degradation mechanisms of ancient documents allowing to understand how the cellulose fibers swell and/or how fast the kinetics of degradation are.

Here, the best-fits of the MAS NMR spectra of the P2C9048 sample have been reported. This analysis was performed by means of the pseudo-Voigt function expressed in eq. (2) in which the Lorentzian part belongs to the water signal and the Gaussian contribution to that of cellulose. Furthermore, the data of the longitudinal relaxation time T_1 were displayed as a function of the hydration level h and of the number of degradation days. The HR-MAS technique is a powerful tool to discriminate the signals of the singular components and also permits to identify three different proton contributions to the overall relaxation indicating the dynamics belonging to free water, bound water, and cellulose. In this way, for example, it is possible to relate the degradation level of paper with its hydration level. In fact, our overall results indicate that hydration and degradation processes are interconnected and strictly dependent upon each other. Specifically,

the addition of water provokes the modification of the structure of cellulose by accelerating the oxidative and hydrolytic mechanisms. These processes lead to a consumption of the amorphous regions and, consequently, to the swelling of the cellulose fibers. In this frame, the degradation of paper can be considered as a two-step process: water penetrates within the amorphous regions that indeed are progressively consumed acting as a powerful catalyst of hydrolytic and oxidative reactions with a consequent production of different by-products with distinct complexities. These by-products influence the properties of the water molecules present within the cellulose fibers. The dependence of the water dynamics on hydration and aging can suggest appropriate strategies for the preservation and restoration of cultural heritage materials based on cellulose such as ancient documents [40,41]. These findings could be improved further in order to lead to a recognized approach for the determination of the local status of water molecules, that triggers the degradation process of cellulose, both in fundamental research and industrial applications.

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