Water and cellulose: hydration and degradation processes seen by NMR

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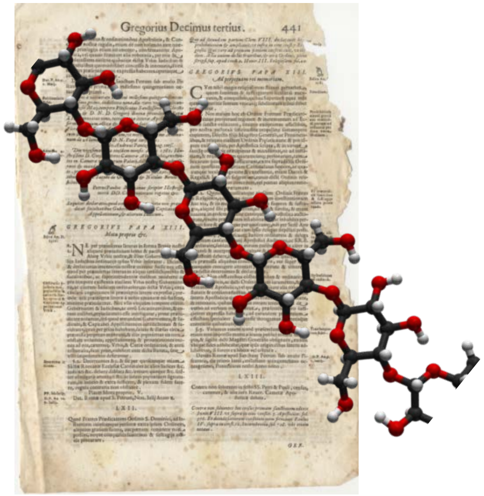
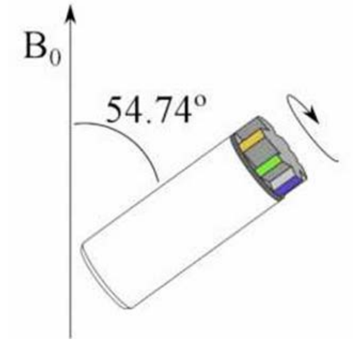




Water and Water Systems

22nd July– 31st July 2016, Erice (Italy)

SoNS SCHOOL OF NEUTRON SCATTERING FRANCESCO PAOLO RICCI

OUTLINE

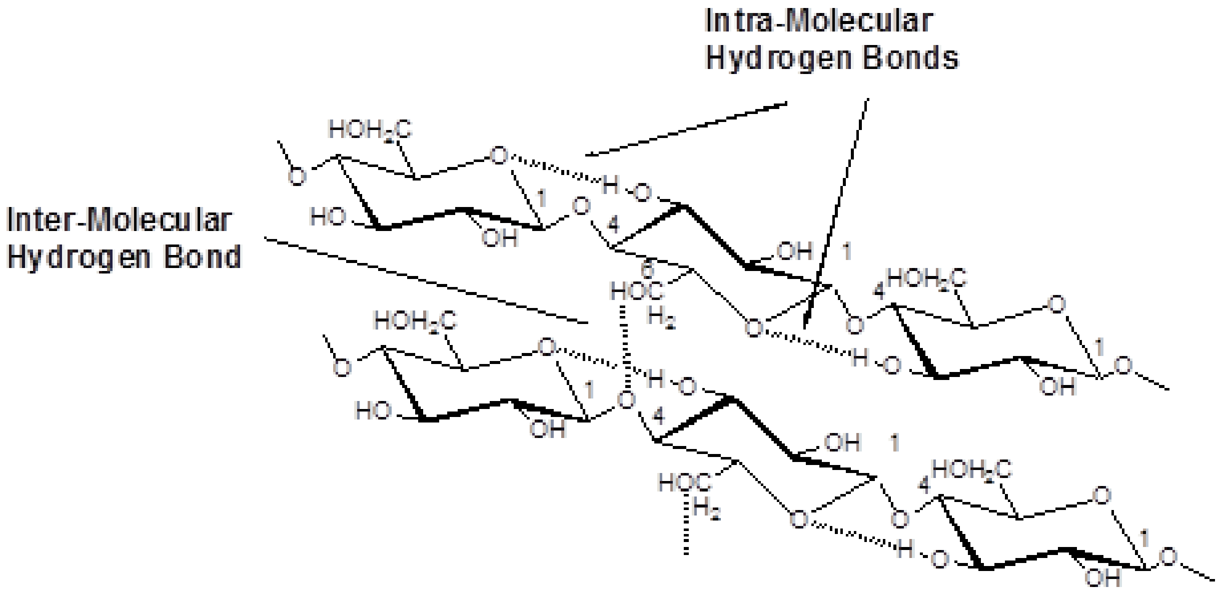
- The system: ancient and modern paper
- The topic: paper hydration and degradation
- The motivation: comprehension of water cellulose interactions for cultural heritage preservation and artefacts authentication
- The experimental technique: proton HR-MAS NMR
- Results and Discussion

Paper and cellulose

SEM image of cellulose fibers in ancient paper

Cellulose is the most abundant biopolymer on Earth and is mainly produced by the cotton plants.

Cellulose is a linear homopolymer composed of β-D-glucopyranose units $(C_6H_{10}O_5)_n$, which are linked together by β-(1,4)-glycosidic bonds. The cellulose chains have a strong tendency to aggregate to highly ordered structural entities through an extended network of intra and intermolecular hydrogen bonds.



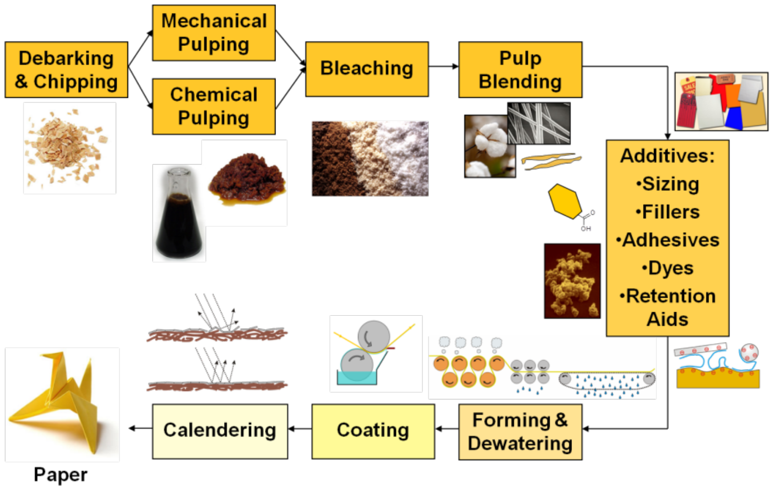
Paper and cellulose

The first paper was invented by ancient Egyptian by using papyrus trees.

Nowadays, paper is produced from a dilute suspension of cellulose fibers in water, that are then drained through a sieve, pressed and dried, to obtain a sheet composed of a network of randomly interwoven fibers.

Paper composition varies depending on the production period and technology employed.

Elemental fibrils, basic components of the cellulose supramolecular structure, include assembly of highly ordered (crystalline) domains and of disordered (amorphous-like) regions. Crystalline domains constitute from around 60 to 70%, of total cellulose material depending on its origin and history.



Debarking & Chipping

Mechanical Pulping

Chemical Pulping

Bleaching

Pulp Blending

Additives:
- Sizing
- Fillers
- Adhesives
- Dyes
- Retention Aids

Forming & Dewatering

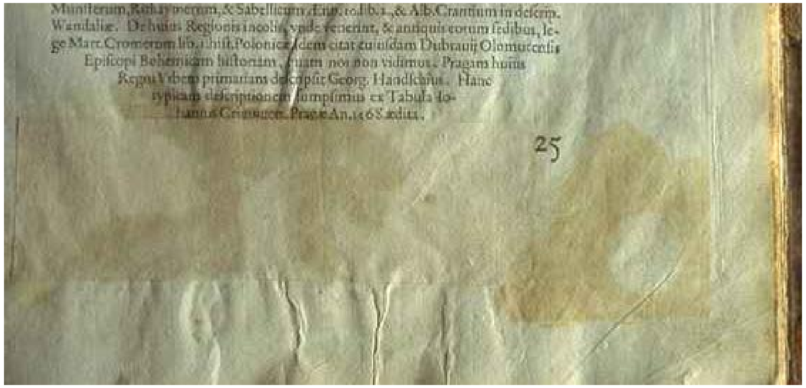
Coating

Calendering

Paper

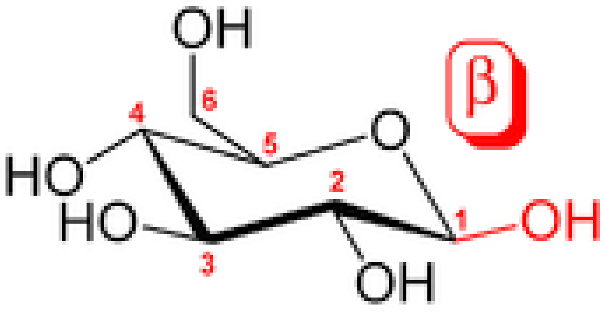
Paper degradation

On macroscopic scale, paper degradation reveals as weakening of mechanical properties of the sheets and by widespread or localized yellowing and/or discoloration.



From a microscopic perspective three are the most important degradation processes:

i) Recrystallization;
ii) acid hydrolysis of β-D-(l,4)-glycosidic bonds, and
iii) oxidation of the β-D-glucopyranose units;



Environmental conditions were somehow reproduced by using, as reference model, paper samples that were artificially aged at 59% of RH and 90°C for different days.

Cultural heritage preservation and artefacts authentication

The study of light products of paper degradation formed during natural aging is necessary to understand degradation pathways and to achieve further progress in preservation and conservation of cellulosic materials. Recognition and description of light products (degradation markers) can also be used to evaluate the paper degradation stage and its authentication.

Extraction processes were used to separate specific classes of chemical compounds, such as low molar mass aliphatic organic acids, and aromatic (phenolic and furanic), or hydroxyl-benzoquinones.

A non-destructive method is based on collecting the volatile organic compounds (VOCs) emitted by paper during its degradation, which are believed to be responsible for the characteristic smell of old books.

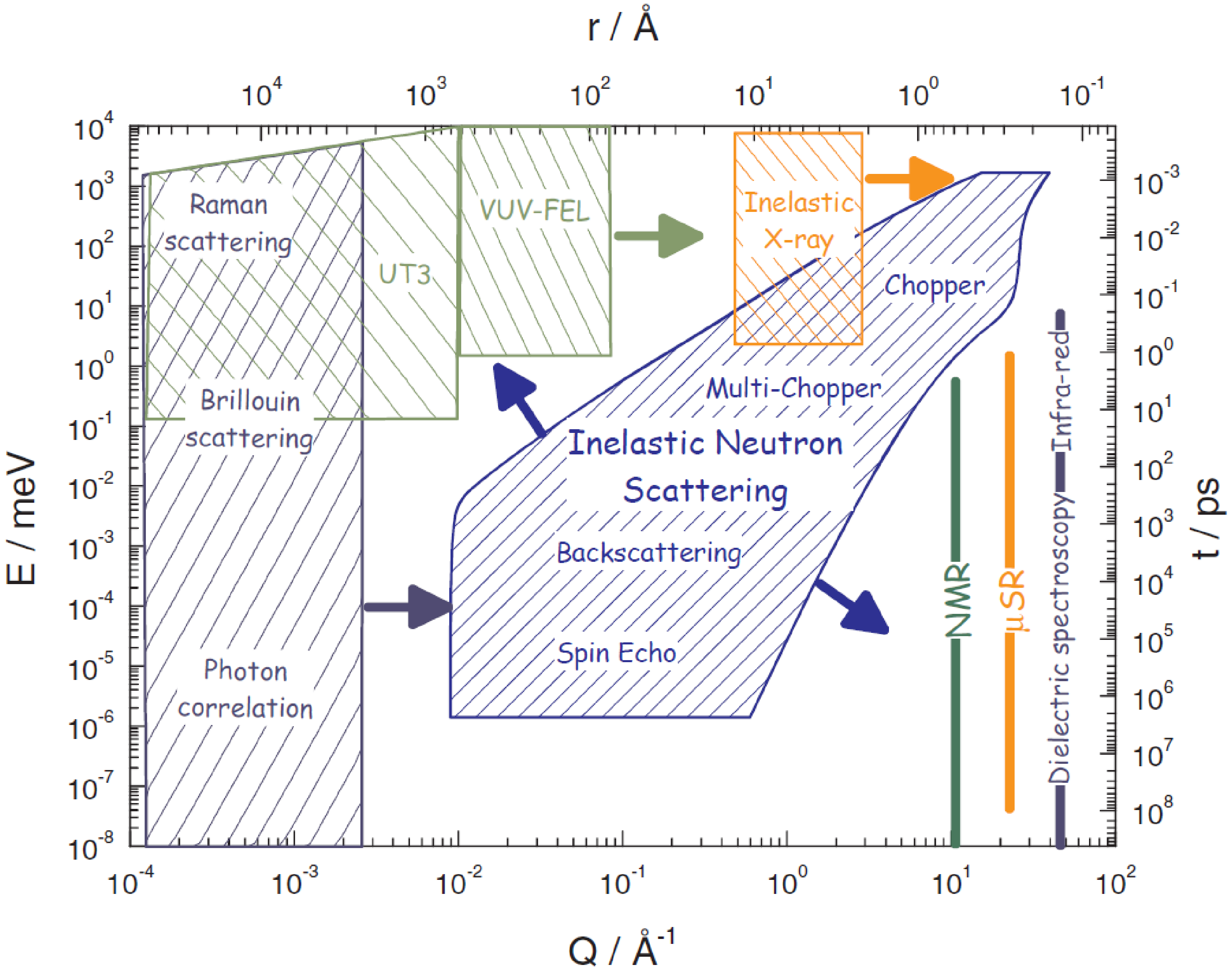
Gibson *et al. Chemistry Central Journal* 2012, **6**:42
http://journal.chemistrycentral.com/content/6/1/42

Chemistry Central Journal

RESEARCH ARTICLE Open Access

Measurement of volatile organic compounds emitted in libraries and archives: an inferential indicator of paper decay?

Lorraine T Gibson[1*], Abdunaser Ewlad-Ahmed[1], Barry Knight[2], Velson Horie[2], Gemma Mitchell[1] and Claire J Robertson[1]


r / Å
10^4
10^3
10^2
10^1
10^0
10^-1
E / meV
10^4
10^3
10^2
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10^-1
10^-2
10^-3
10^-4
10^-5
10^-6
10^-7
10^-8
Raman scattering
UT3
VUV-FEL
Inelastic X-ray
Chopper
Brillouin scattering
Multi-Chopper
Inelastic Neutron Scattering
Backscattering
Spin Echo
Photon correlation
NMR
µSR
Dielectric spectroscopy
Infra-red
t / ps
10^-3
10^-2
10^-1
10^0
10^1
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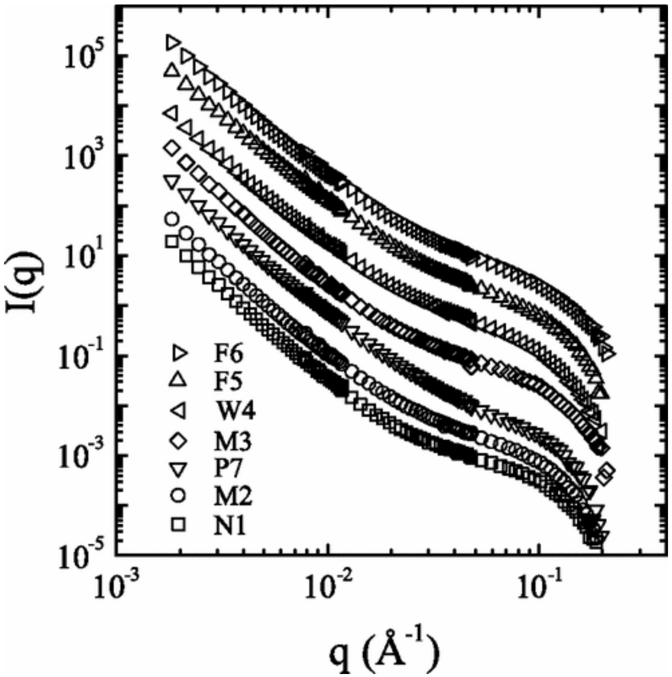
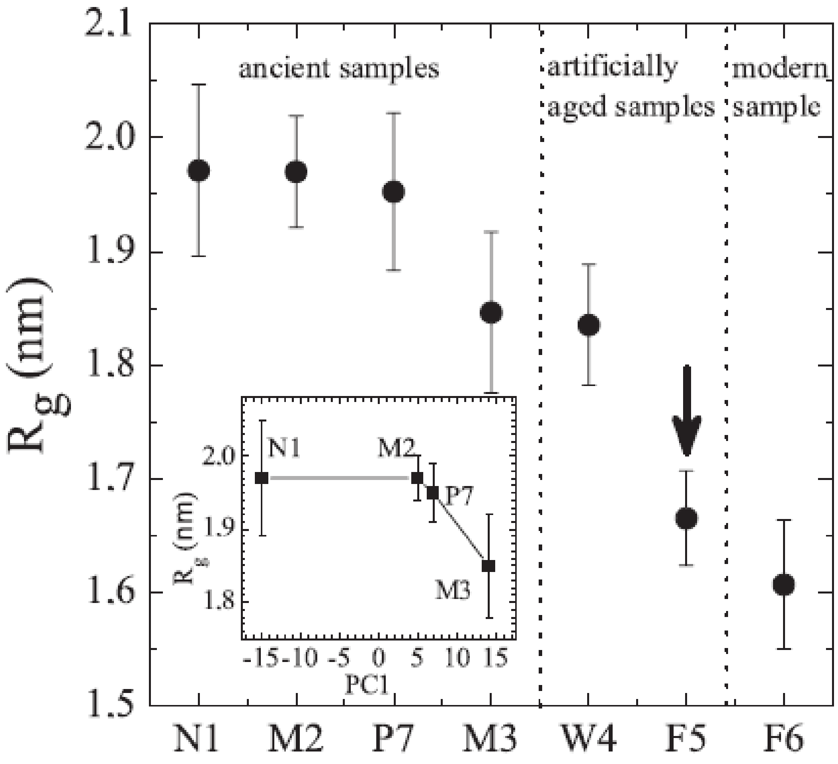
10^4
10^5
10^6
10^7
10^8
10^-4
10^-3
10^-2
10^-1
10^0
10^1
10^2
Q / Å^-1

A previous Neutron scattering study

Neutron scattering intensity versus scattering vector *q* from all the samples exposed to D2O atmosphere. Curves are arbitrarily shifted for clarity.



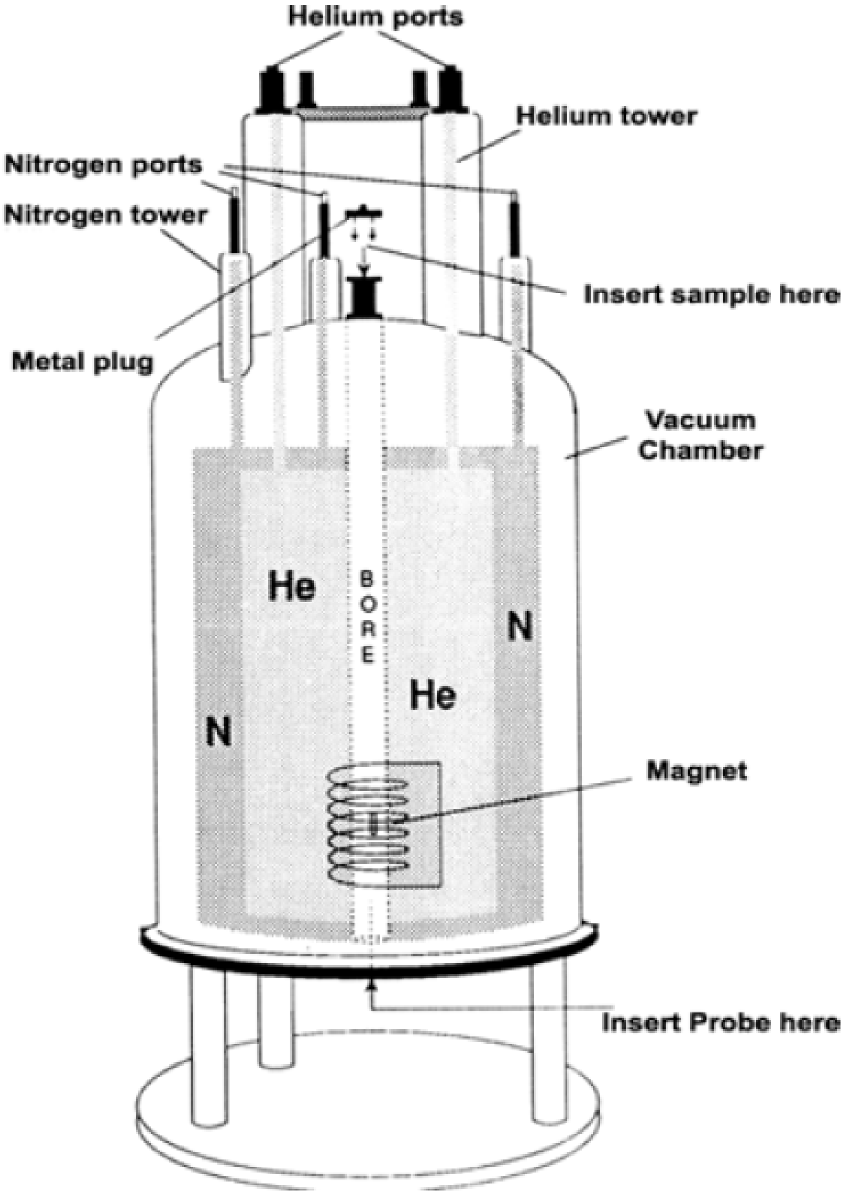


The mean radius of the pore sizes obtained in the Guinier approximation by

$$I(q) = I(0)/[1 + (qR_g)^2/3]^2$$

Missori et al. Phys. Rev. Lett. **97**, 238001 (2006)

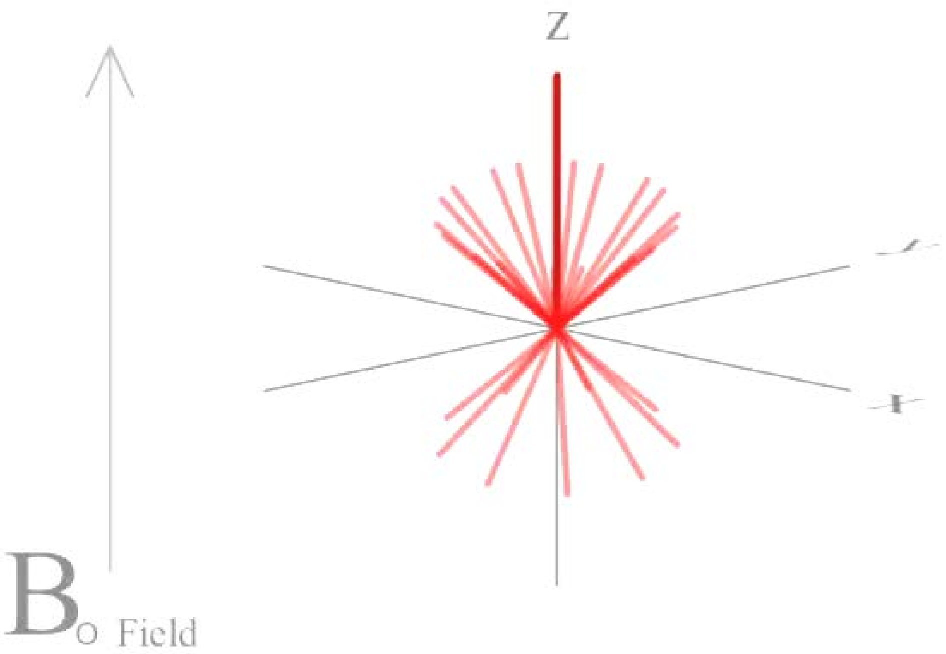
NUCLEAR MAGNETIC RESONANCE



NMR spectroscopy is an absorption technique in which the radio-frequency (RF) waves absorbed by the studied nucleus in the presence of an external magnetic field resulting in spin changes at nuclear level.

The Larmor frequency of each nucleus depends on the magnetic field:

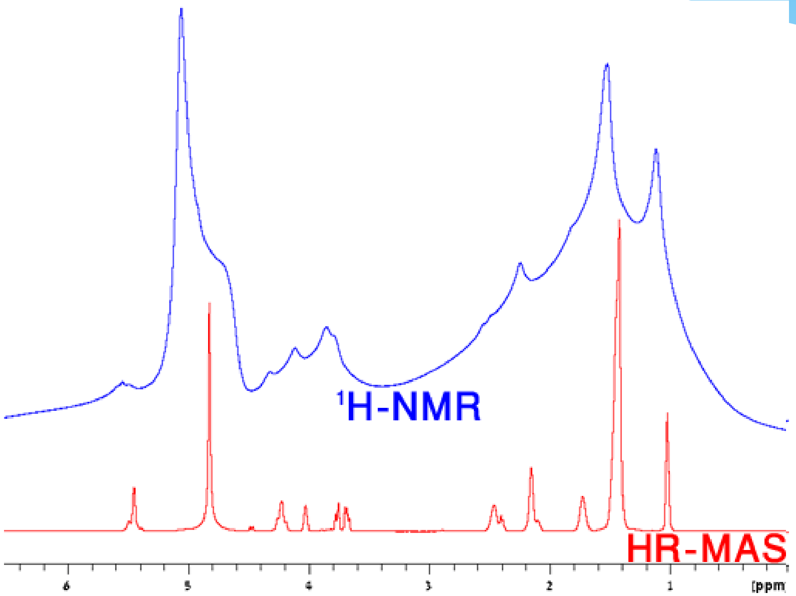
$$\omega_0 \equiv \gamma B_0$$

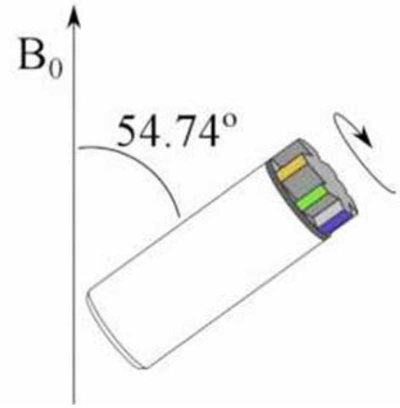


The magnitude of the Magnetization depends essentially on the magnetic field and on the nuclear gyromagnetic ratio (prop to the nuclear magnetic moment):

$$\frac{N_+}{N_-} = e^{-\Delta E/kT} = e^{-\hbar\omega_0/kT} = e^{-\hbar\gamma B_0/kT}$$

HR-MAS NMR

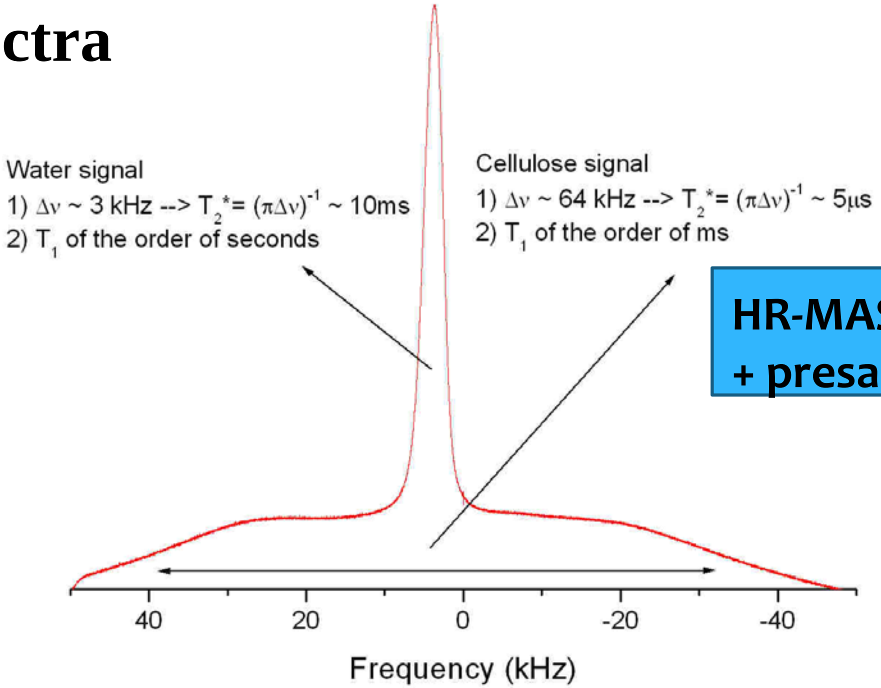




50 μL rotor

By tilting samples of a precise angle with respect to the direction of the applied magnetic field and by spinning at a rate greater than or equal to the magnitude of the anisotropic interaction (few thousands of Hertz), the Hamiltonian term corresponding to dipolar interactions ($3\cos^2\theta-1$) vanishes and NMR peaks become narrower resulting in High Resolution quality spectra also for semi-solid systems.

Modern paper spectra
Cotton linter (P2)



Water signal

1) $\Delta\nu$ ~ 3 kHz --> $T_2^* = (\pi\Delta\nu)^{-1}$ ~ 10ms

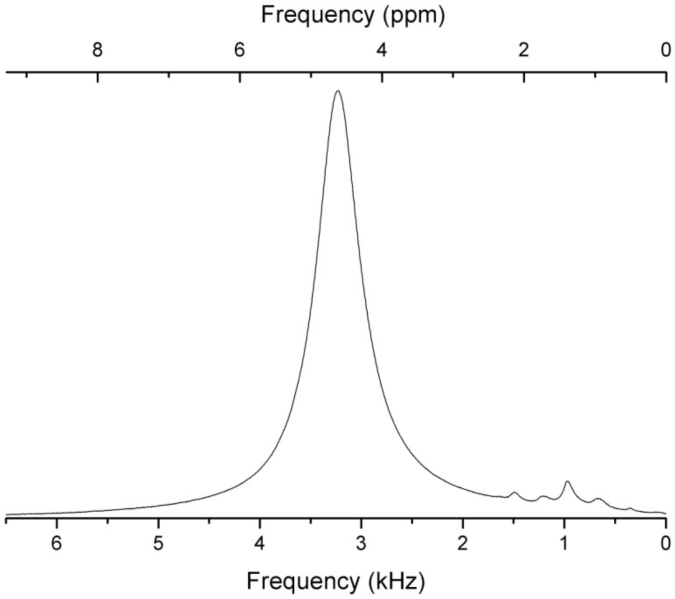
2) T_1 of the order of seconds

Cellulose signal

1) $\Delta\nu$ ~ 64 kHz --> $T_2^* = (\pi\Delta\nu)^{-1}$ ~ 5µs

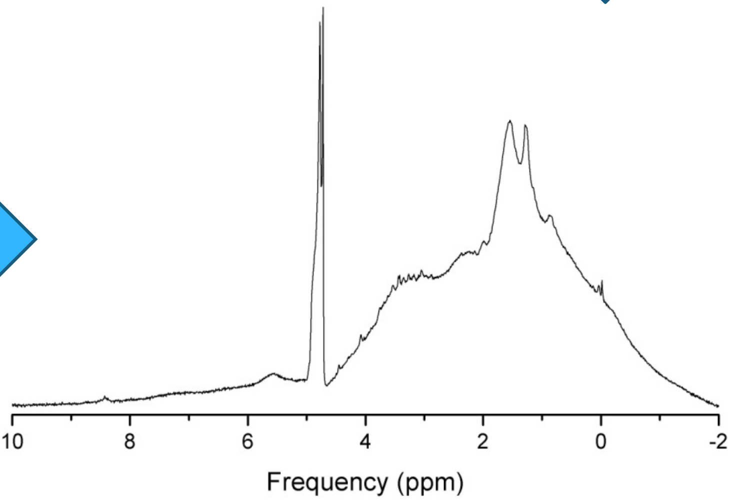
2) T_1 of the order of ms

HR-MAS



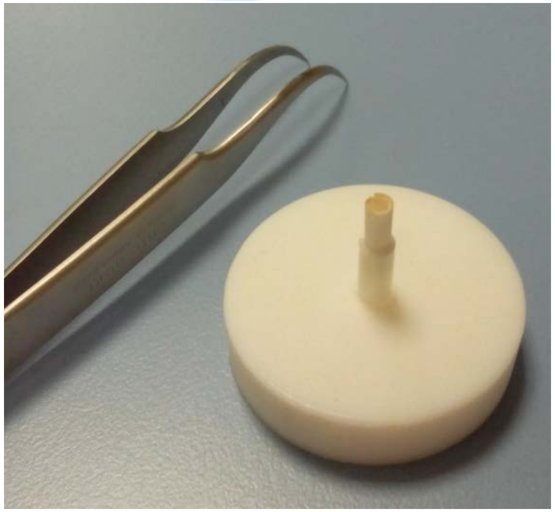
D_2O dilution + presat

HR-MAS + D_2O dilution + presat



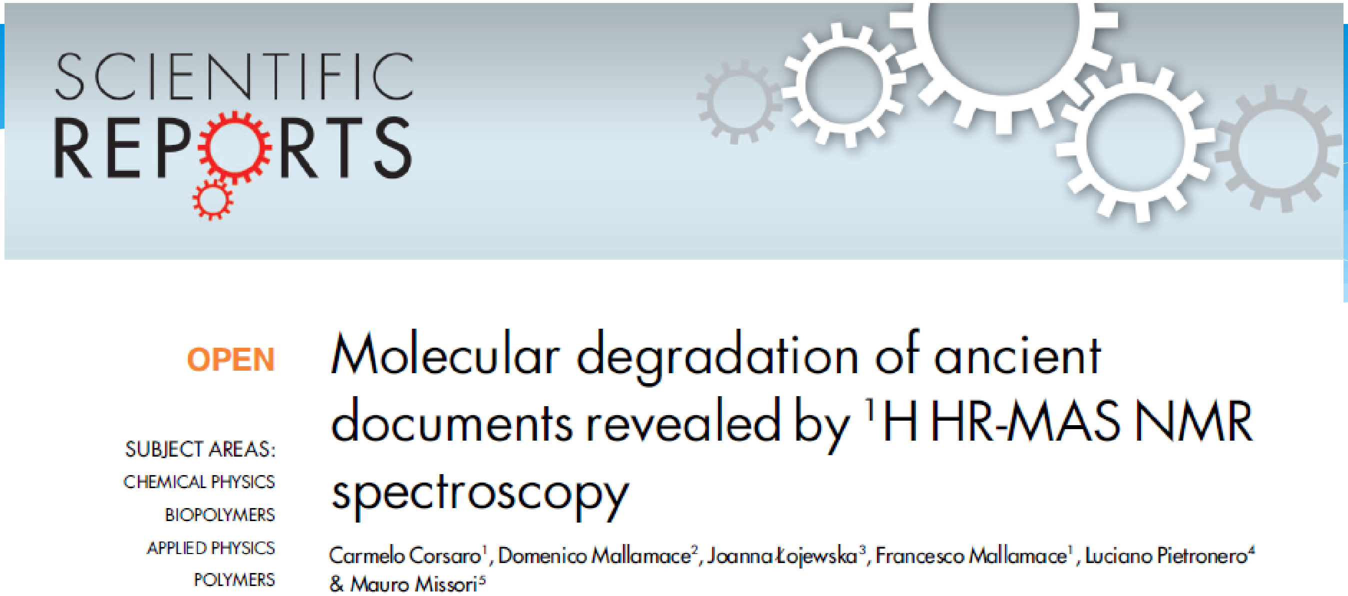
Experiments (1)

Small rectangular pieces, 0.7 x 1.8 cm^2, of about 10mg in weight, of different papers were rolled up, placed in the rotor together with 30µl of D_2O, sealed (with the corresponding insert) and spun at 7000 Hz at the magic angle to increase the spectral resolution.



Model samples, obtained from Netherlands Organization for Applied Scientific Research (TNO), are made of unbleached cotton linters, containing very low inorganic ingredients (ash content < 0.005% in weight) and no additives or lignin, with high mean degree of polymerization. These samples are named <u>P2 and were artificially aged in air in a climatic chamber at relative humidity of 59% and temperature of 90°C for 24 and 48 days (P2C9024 and P2C9048)</u>.

Ancient samples used in this research consist of small pieces of paper, produced in the 15th century in European countries named <u>A1 and B1 (both made in Perpignan, France, in 1413) and A3 (made in Milan, Italy, in 1430)</u> in bad conservation conditions (within a water stain).



SCIENTIFIC REPORTS

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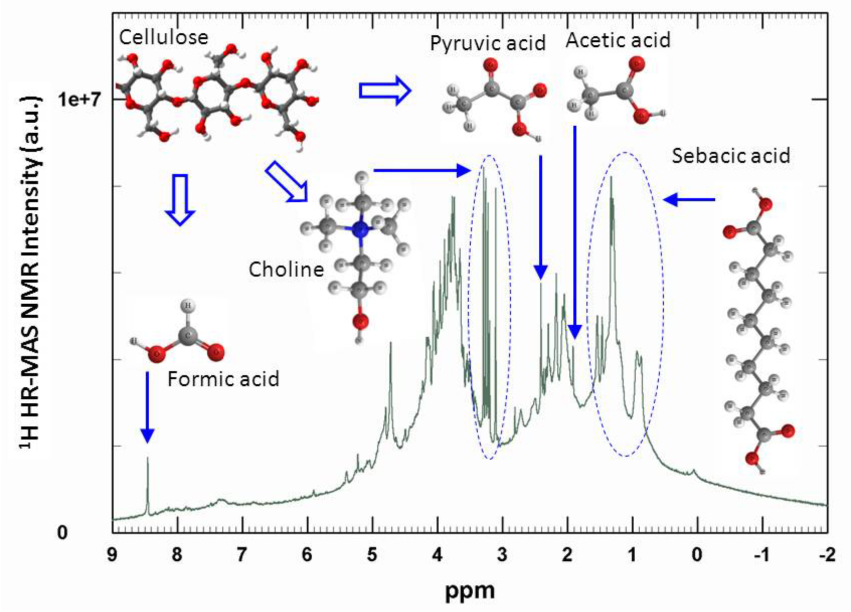
Molecular degradation of ancient documents revealed by ^{1}H HR-MAS NMR spectroscopy

SUBJECT AREAS:
CHEMICAL PHYSICS
BIOPOLYMERS
APPLIED PHYSICS
POLYMERS

Carmelo Corsaro[1], Domenico Mallamace[2], Joanna Łojewska[3], Francesco Mallamace[1], Luciano Pietronero[4] & Mauro Missori[5]

Scientific Reports **3**, Article number: 2896 (2013) doi:10.1038/srep02896



Proton HR-MAS spectrum of an ancient sample made in Milan in 1430

Products of cellulose degradation were individually detected in solid paper samples by means proton HR-MAS NMR in both ancient and, as reference, artificially aged paper samples. Carboxylic acids, in addition to more complex dicarboxylic and hydroxy-carboxylic acids, were found in all samples studied. Since these products can catalyze further degradation, their knowledge is fundamental to improve conservation strategies of historical documents. Furthermore, the identification of compounds used in ancient production techniques, also suggests for artifacts dating, authentication and provenance.

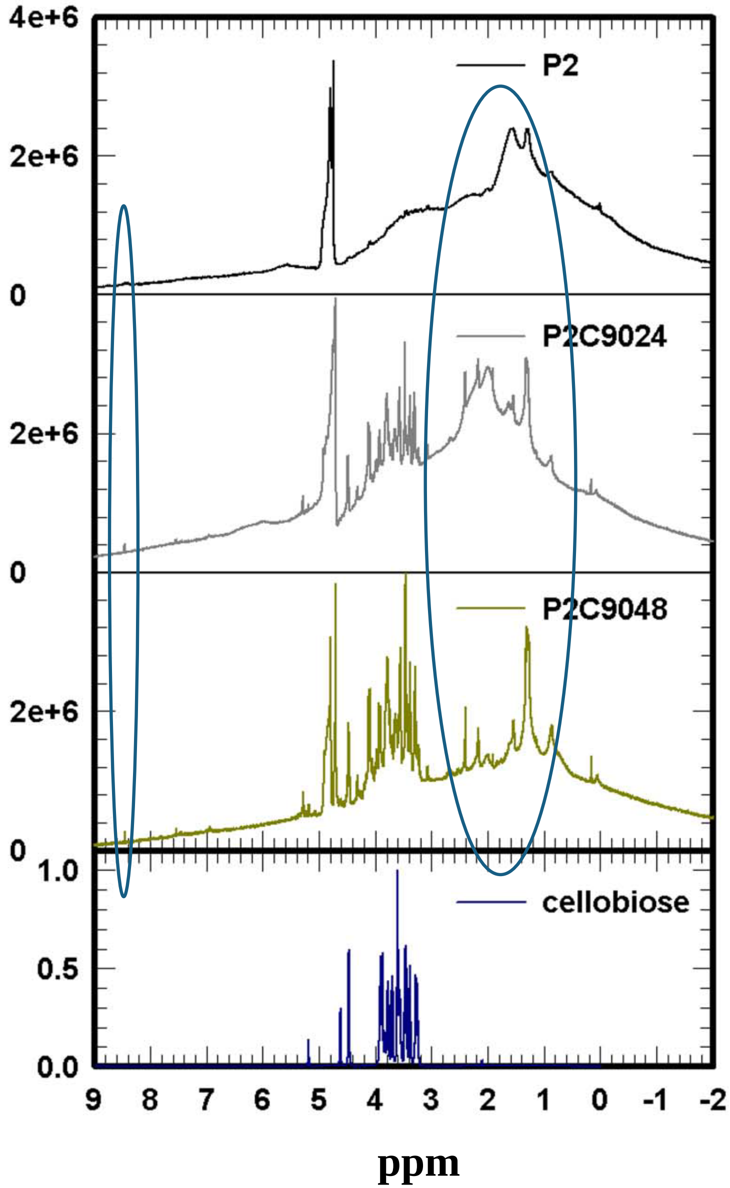
Artificial aging of modern paper

Upon artificial aging of P2 samples, as the cellulose structure swells, making the cellulose fibers more accessible to water and oxygen, the increased molecular mobility makes evident new features in the spectra.

Sharp peaks at 1.91 and 8.45 ppm can be attributed to the simplest carboxylic acids, acetic and formic acids, respectively.

The spectral features in the region 0.8-2.5 ppm can be attributed to more complex carboxylic acids such as succinic and pyruvic acids. The presence of these acidic compounds reinforces the hypothesis that the degradation processes are initially oxidative in nature.

The strong increase of the peaks corresponding to cellobiose indicates an enhanced presence of water within the cellulose fibers. This is accompanied by the cleavage of intra-molecular hydrogen bonding in cellulose, which at this stage of degradation makes more degraded cellulose samples more accessible to oxygen active species and thus prone to further oxidation.

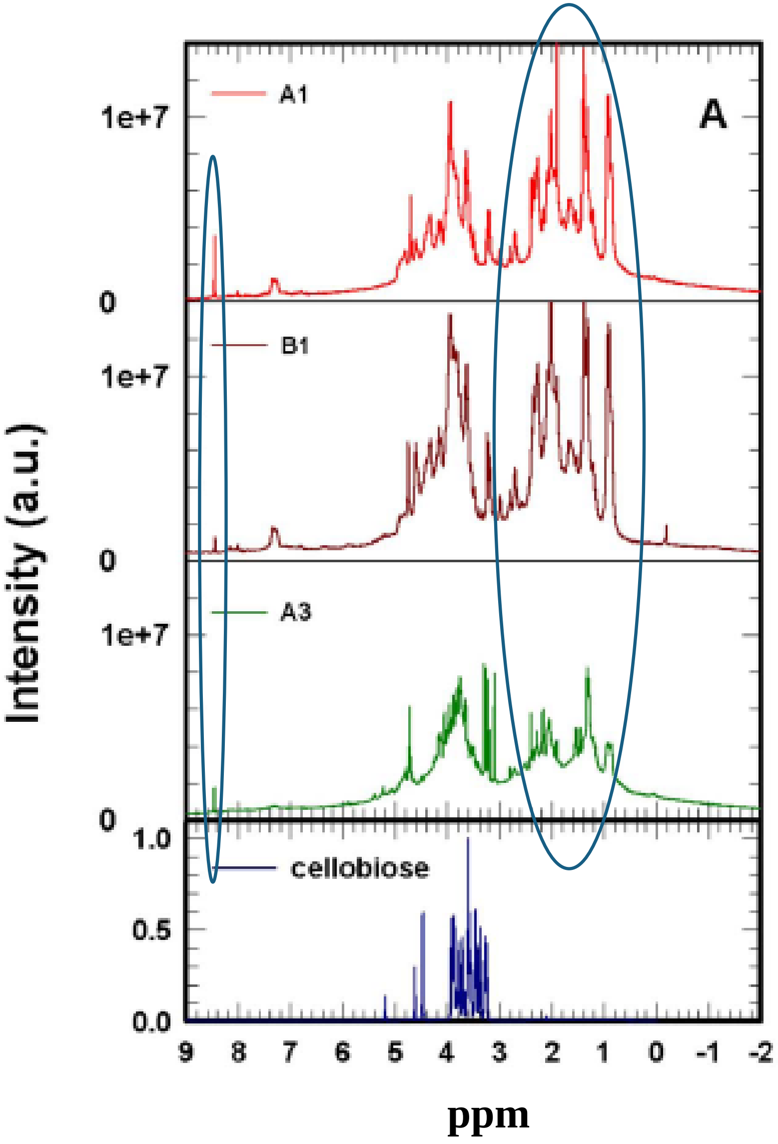


Corsaro et al. Scientific Reports **3**, Article number: 2896 (2013)

Ancient paper 1

All compounds detected in modern artificially aged samples are also found in ancient paper samples A1, B1 and A3, although the bands assigned to them show slightly different shapes and intensities. 1H NMR spectra of ancient samples A1, B1 and A3 are characterized by a flat background similar to that observed for the most degraded modern sample P2C9048. This confirms that degradation processes affect amorphous regions which become disrupted, then more mobile, so providing well resolved sharp peaks in 1H NMR spectra.

In ancient samples, the characteristics peaks of cellobiose are less evident. Most intense peaks are likely due to hydroxy-carboxylic acids, such as glucaric and lactic acids. Glucaric acid is derived from the oxidation of D-glupyranose, which can be also oxidized to pyruvic acid and finally transformed in lactic acid. Both these compounds are identified in our samples.



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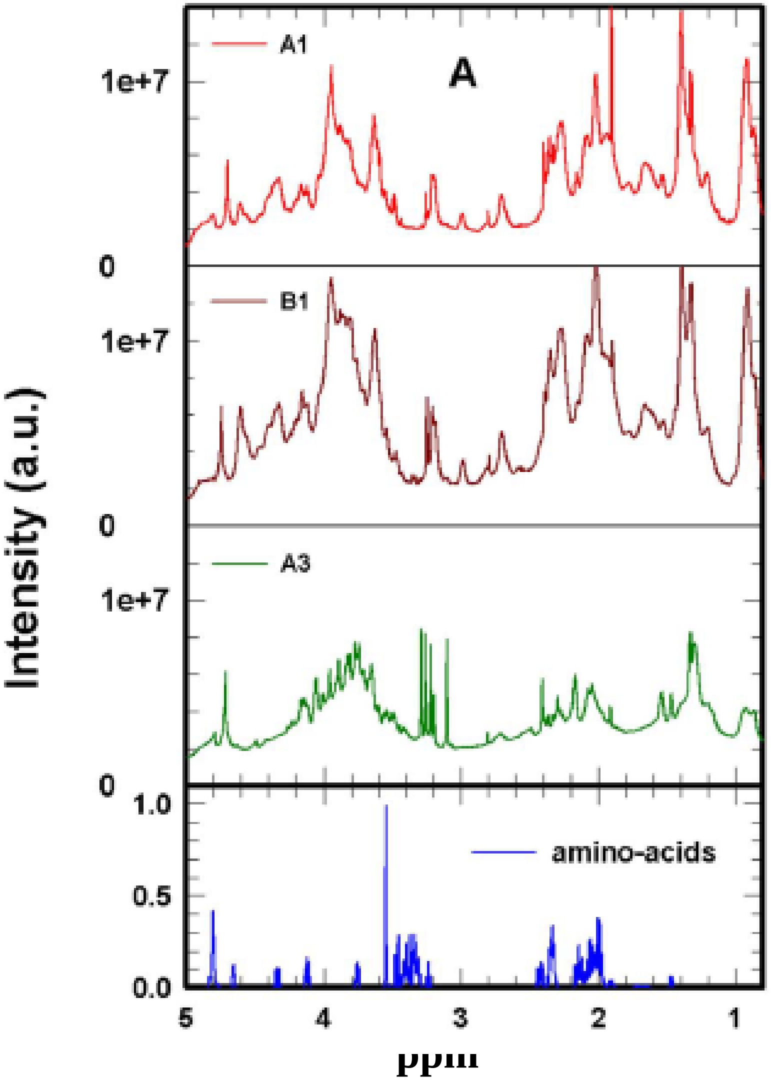
Ancient paper 2

Ancient samples show bands which could be assigned to several amino-acids. In particular, the peak at about 1.46 ppm can be assigned to alanine (Ala) whereas the broad one at about 1.6–1.75 ppm to arginine (Arg). Other peaks from 1.96 to 2.20 ppm and from 2.30 to 2.44 ppm are assigned to proline (Pro), glutamic acid (Glu) and hydroxyproline (Hyp). Characteristic peaks of phenylalanine can be also identified at about 3.1, 3.3, 4.05 and around 7.3 ppm, some of them originate evident TOCSY cross-peaks at (3.1, 3.3) and (3.1, 4.05) ppm. The presence of these amino-acids gives evidence of ancient production methods, which employed glue from animal connective tissues (gelatin, i.e. denatured collagen) to improve paper writing quality.

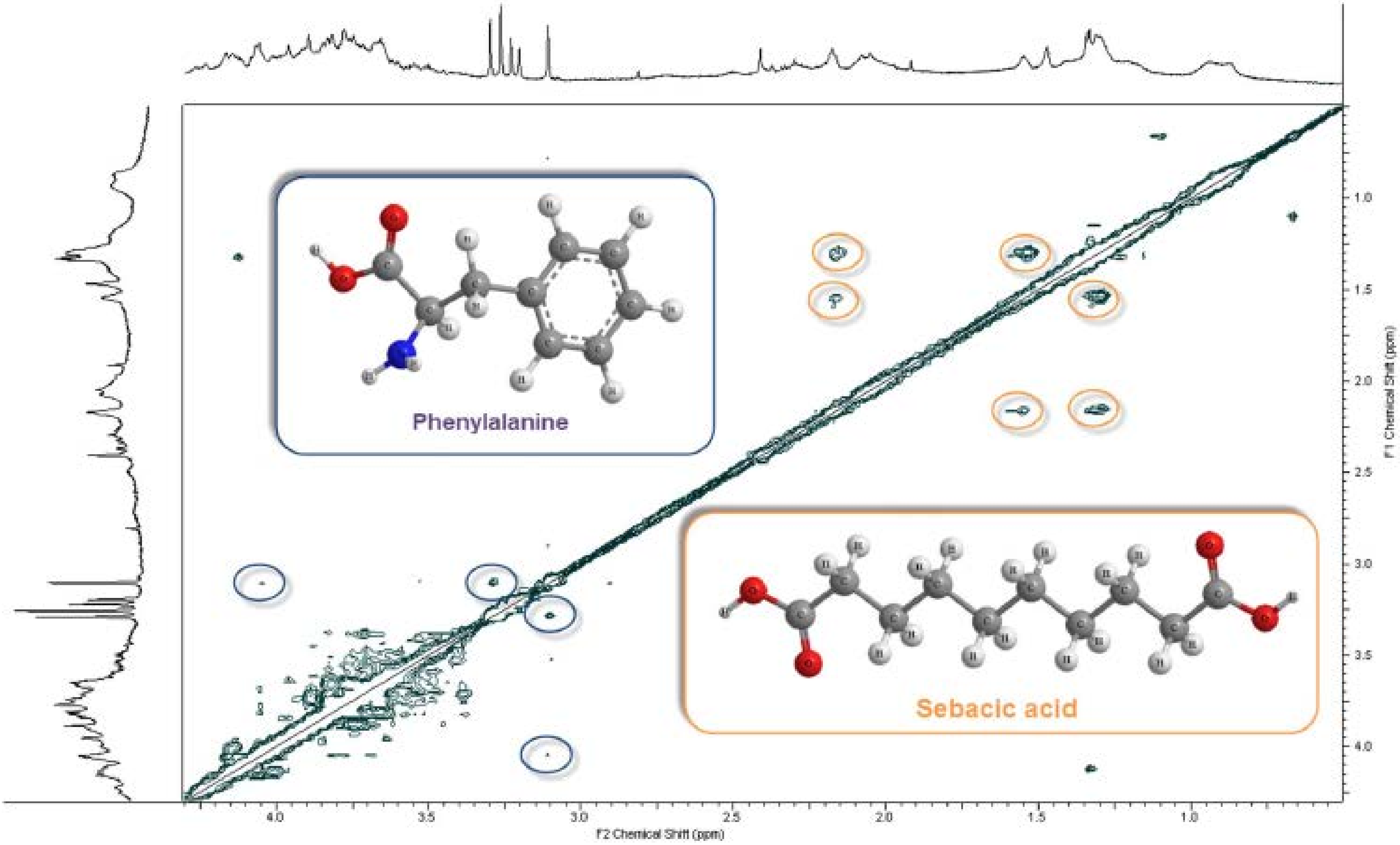
For a visual comparison between spectra of ancient paper and collagen, we used a weighted linear combination, I, of single spectra (in aqueous solution) of most abundant amino-acids in collagen (Gly, Pro, Ala, Hyp, Glu, Asp and Arg), obtained from on-line NMR databases (HMDB and BMRB).



$$I = 0.329Gly + 0.126Pro + 0.109Ala + 0.095Hyp + 0.074Glu + 0.047Asp + 0.049Arg$$

Corsaro et al. Scientific Reports **3**, Article number: 2896 (2013)

Ancient paper 3: TOCSY on A3 sample



The highlighted cross peaks belong to sebacic acid and phenylalanine.

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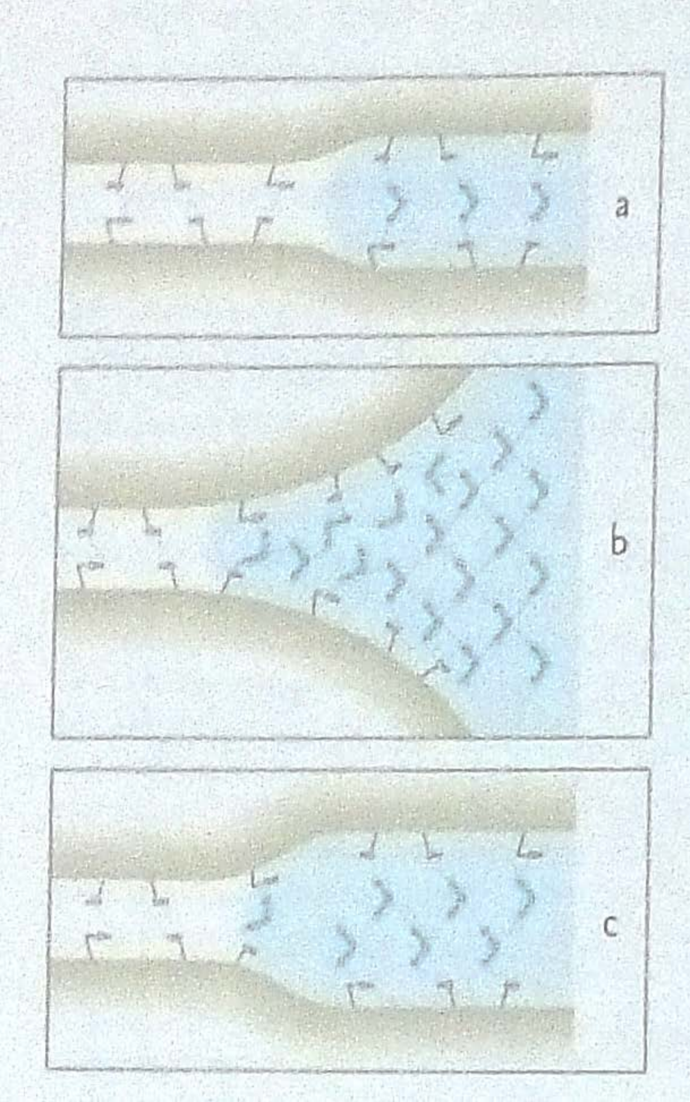
Next studies: ageing and hydration processes studied by relaxation times

Water content shifts the kinetic equilibrium of chemical reactions such as acidic hydrolysis and oxidative processes causing the formation of different by-products. The study of the dynamics of water as a function of the moisture content for artificially aged paper is essential for the understanding of the deterioration pathway of cellulosic materials.

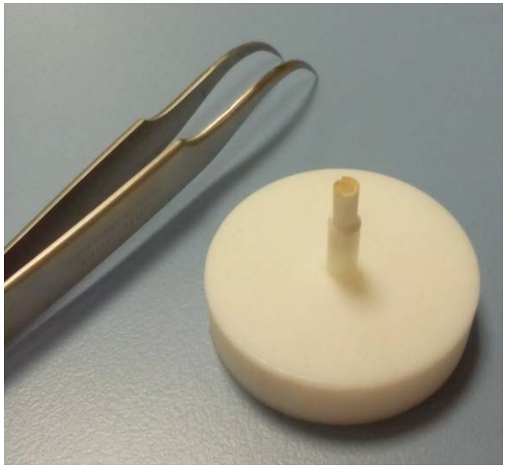
Two types of water are generally identified within cellulosic materials:
a) free water or "easy-to-remove" and
b) bound water or "hard-to-remove" (that includes freezing and non-freezing water).

During drying, these types of water are removed in sequence according to the strength of their interaction with the fibres, with some overlap at the boundary between two types.



Experiments (2)

Model samples are named P2 and were artificially aged in air in a climatic chamber at relative humidity of 59% and temperature of 90°C for 6, 12, 24, 36 and 48 days. The not aged P2 sample, which can be considered as a reference, is named P2REF.

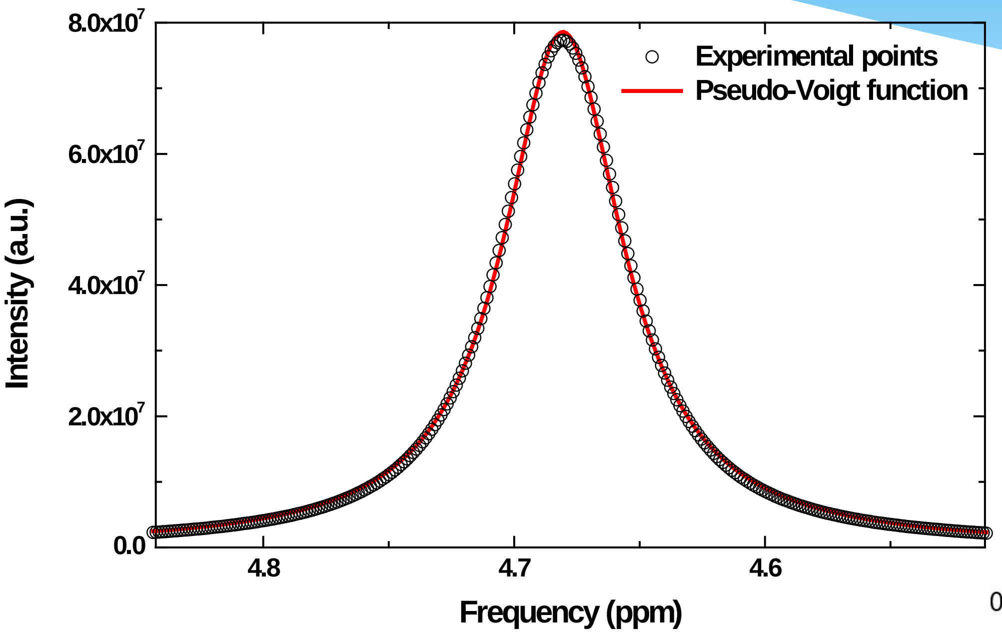


Ancient samples used in this research consist of small pieces of paper, produced in the 15th century in European countries named A1 and B1 (both made in Perpignan, France, in 1413) and A3 (made in Milan, Italy, in 1430) in bad conservation conditions (within a water stain).

Small rectangular pieces, 0.7 x 1.8 cm^2, of about 10mg in weight, were dried in a oven at 105°C for 4 hours. The dry sample were firstly measured and then hydrated in a closer chamber with 100% relative humidity for several hours. In our study we have considered values for moisture content (or hydration, h) of approximately 2%, 8%, 14%, 20% and 40%.

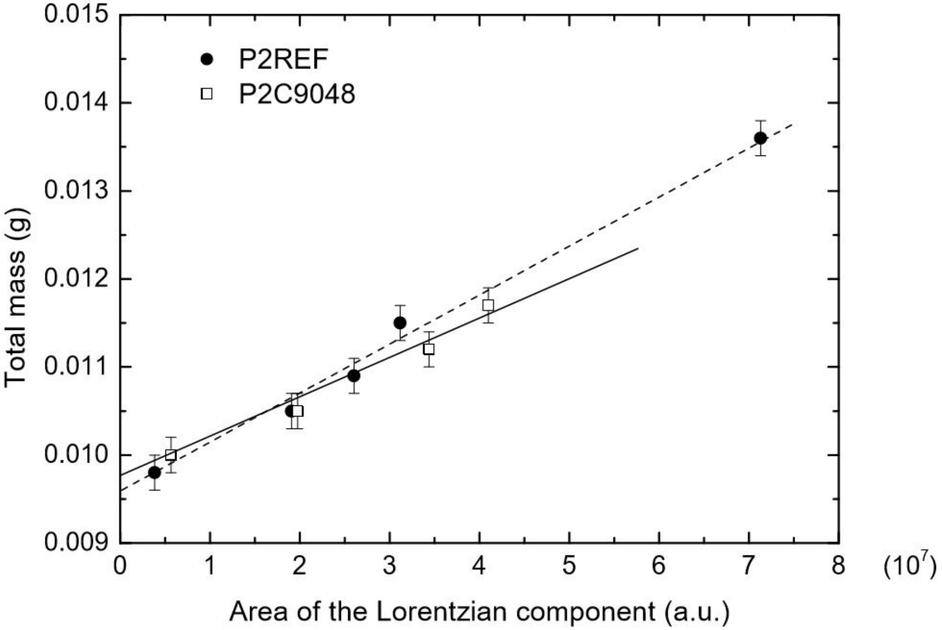
$$h = \frac{m_{H_2O}}{m_{dry\,paper}}$$

Interpretation of NMR signal



The water spectral band can be well fitted with a Pseudo-Voigt function, in which the Gaussian curve represents the cellulose background and the Lorentzian one the water content.

The total sample weight (cellulose + water) as a function of the area of the Lorentzian contribution of the water NMR band. The linear fit for both P2REF (dashed) and P2C9048 (solid) samples provides, as the y-axis intercept, the dry mass 0.0096g and 0.0097g, respectively.



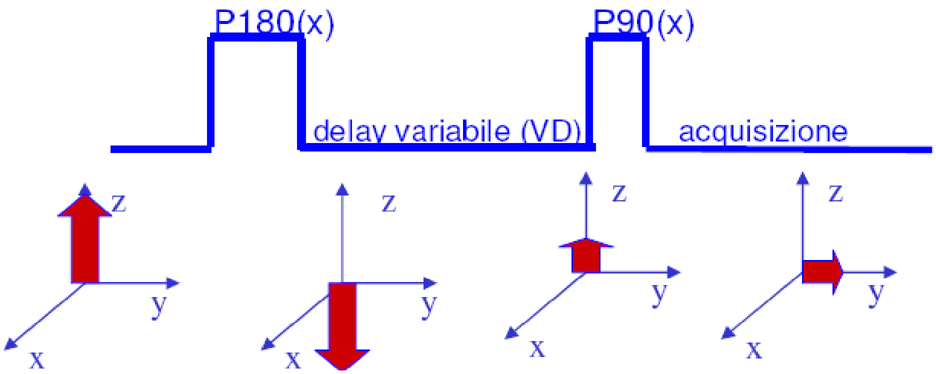
How to measure relaxation times

With proper pulse sequences it is possible to measure dynamic quantities such as the characteristic relaxation times of the nuclear magnetization.

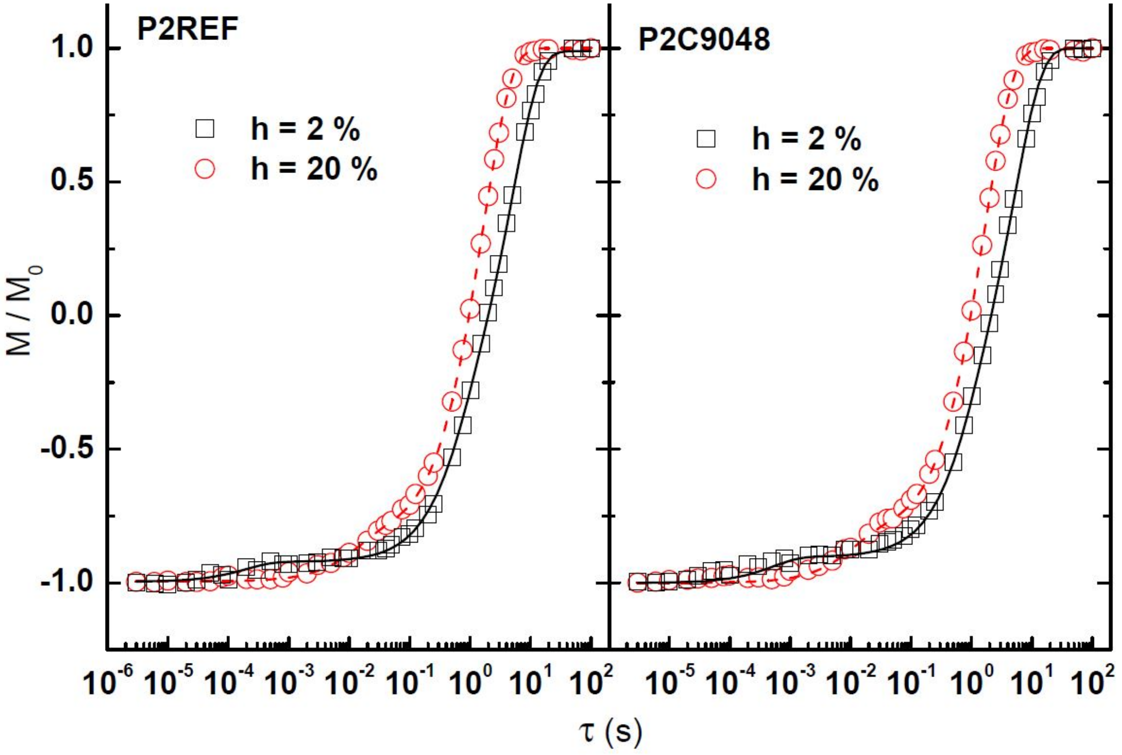
The longitudinal or spin-lattice relaxation time, T_1, represents the time required for the longitudinal component of the magnetization to recover its equilibrium value after the application of the perturbing pulse sequence. It is a measure of the dipolar interactions of the investigated spins with their surrounding. Its value ranges from tens to thousands of milliseconds for protons in hydrogenated compounds, and usually becomes smaller at lower temperatures.

The transversal or spin-spin relaxation time, T_2, is the time required for the transverse component of the magnetization to vanish from the plane orthogonal to the static field direction. It measures the dipolar interactions between spins belonging to the same species (i.e. the strength of interplay among the same species). A weaker interaction corresponds to a longer T_2.

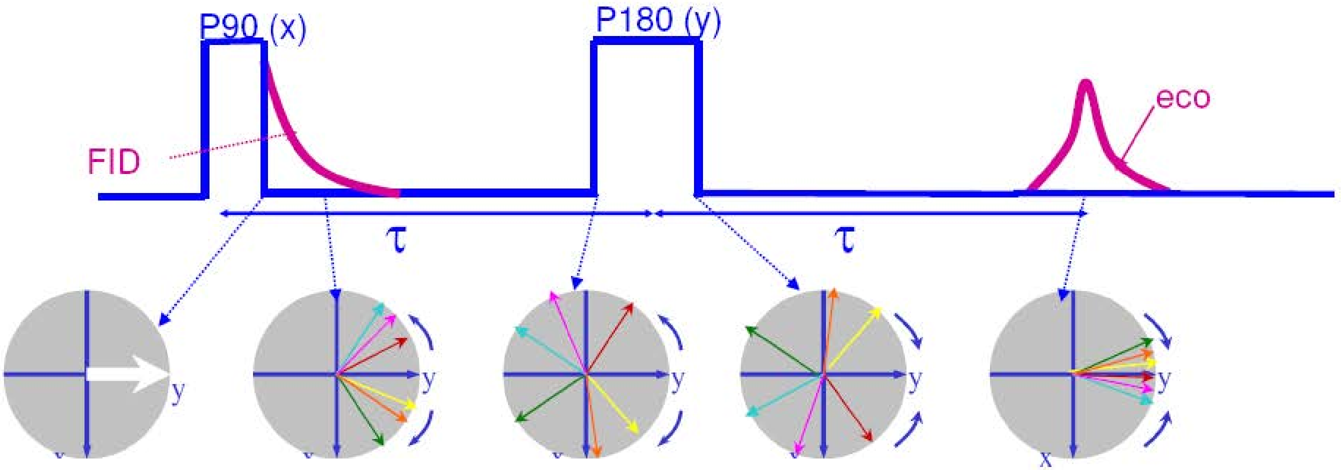
Inversion Recovery & Spin-Lattice relaxation time



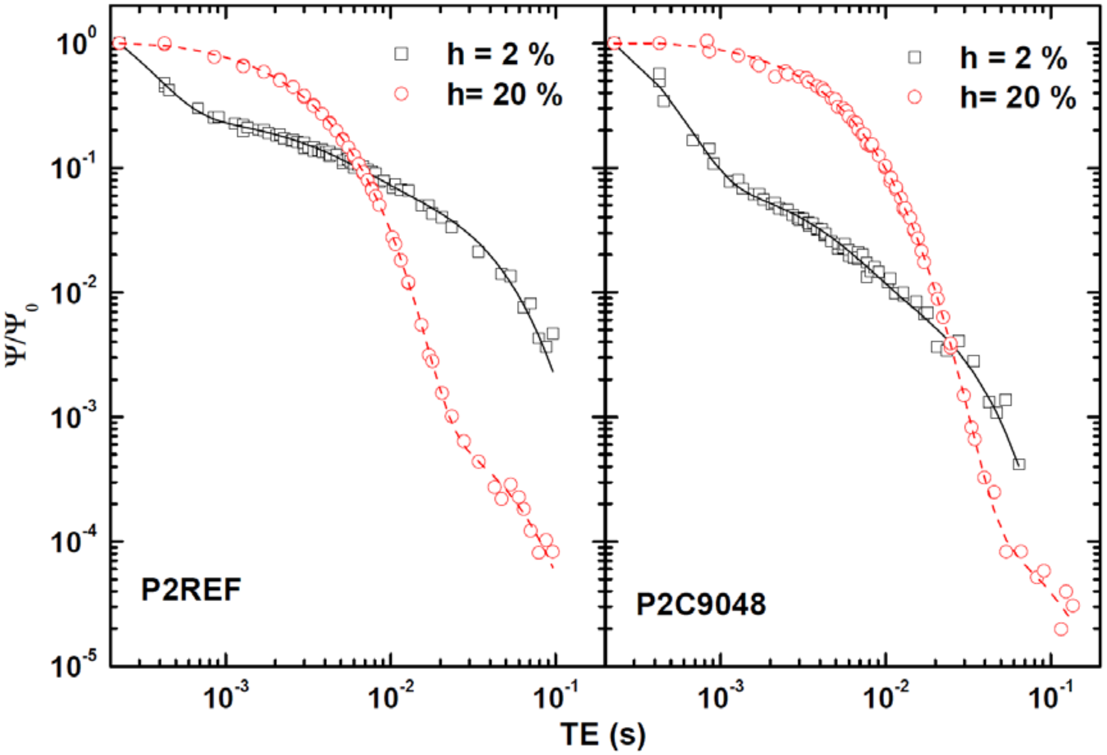
$$\frac{M}{M_0} = 1 - [A_1 \exp(-\frac{\tau}{T_{1a}}) + A_2 \exp(-\frac{\tau}{T_{1b}}) + A_3 \exp(-\frac{\tau}{T_{1c}})]$$



Hahn’s echo:

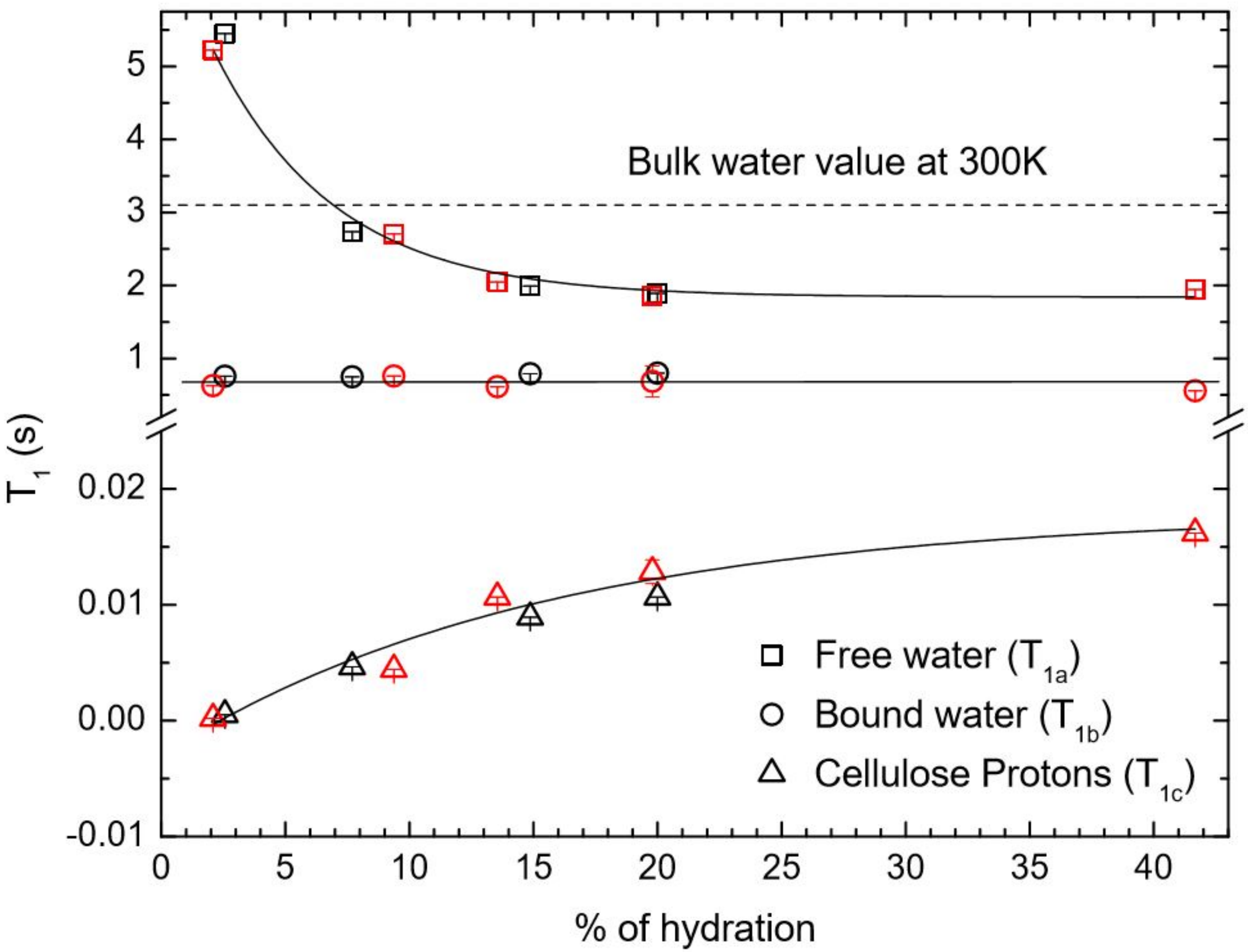


$$\frac{\Psi}{\Psi_0} = A_1 \exp\left(-\frac{TE}{T_{2a}}\right) + A_2 \exp\left(-\frac{TE}{T_{2b}}\right) + A_3 \exp\left(-\frac{TE}{T_{2c}}\right)$$



Spin-Lattice relaxation time vs. hydration

P2REF, P2C9048



Spin-Lattice relaxation time vs. hydration

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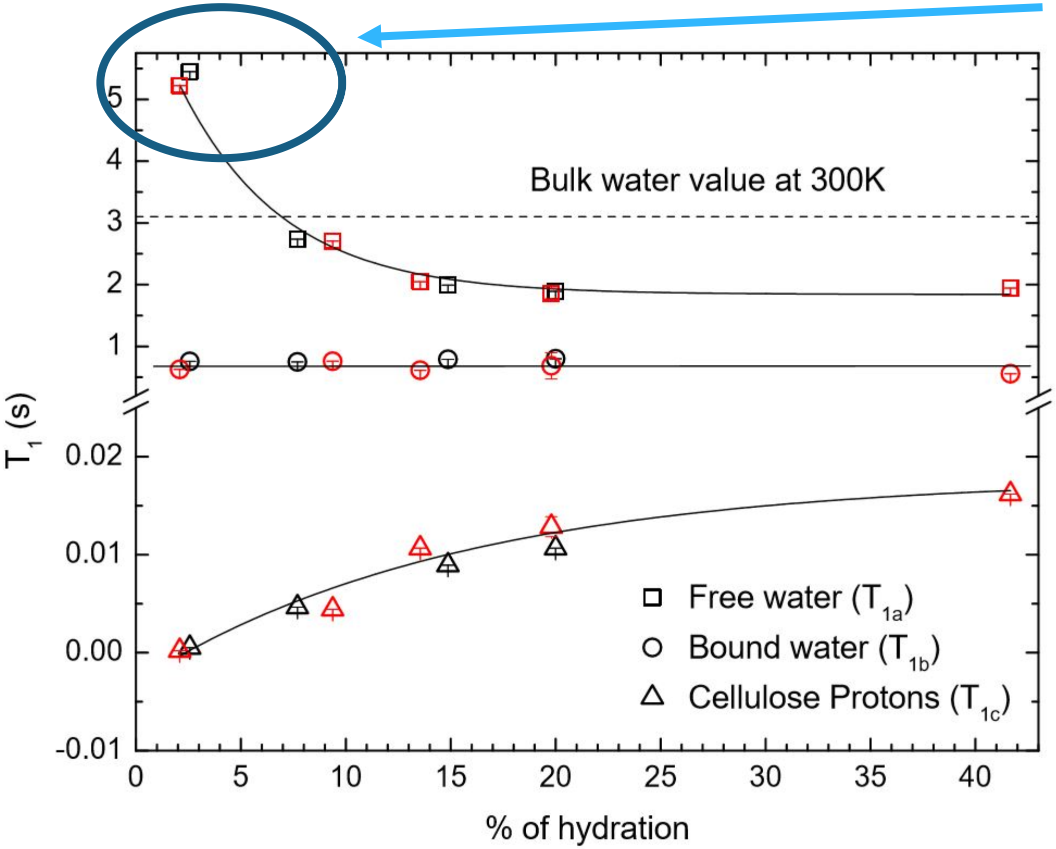
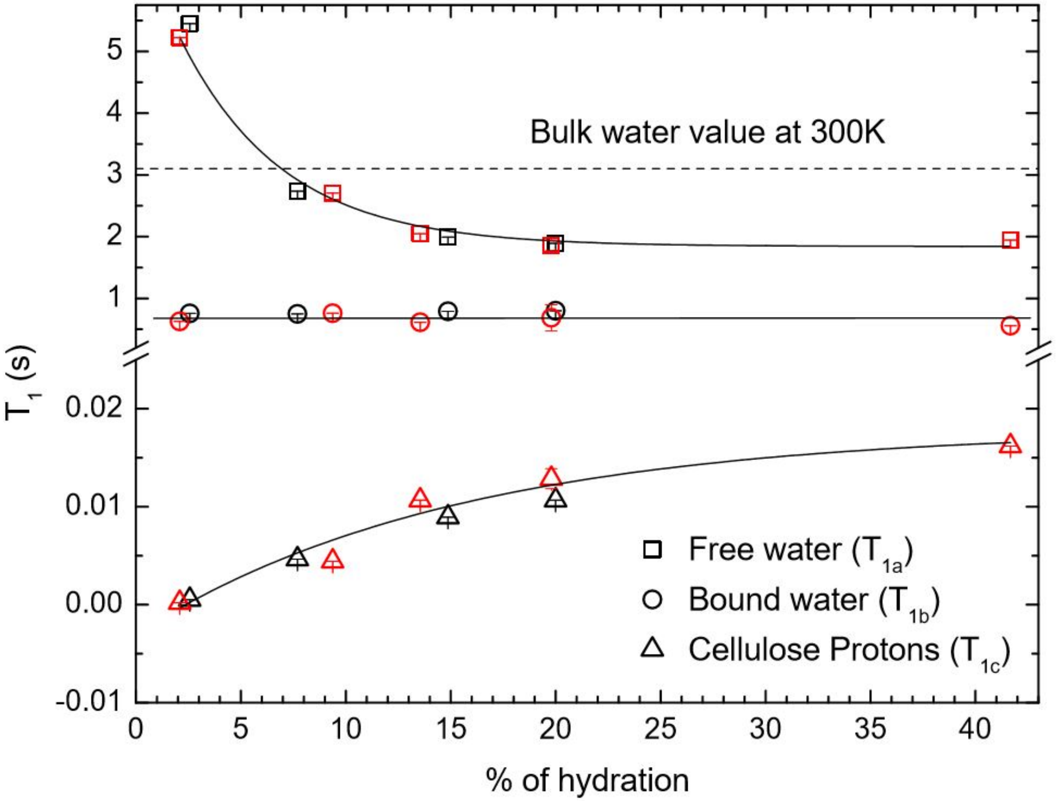


For the smallest hydration, T1a shows the longest value even longer of bulk water due to the presence of vapour-phase water molecules trapped in the voids or pores in the structure of cellulose fibres. These water vapour molecules interact very weakly with the system and are characterized by an higher mobility with respect to that of bulk liquid water.

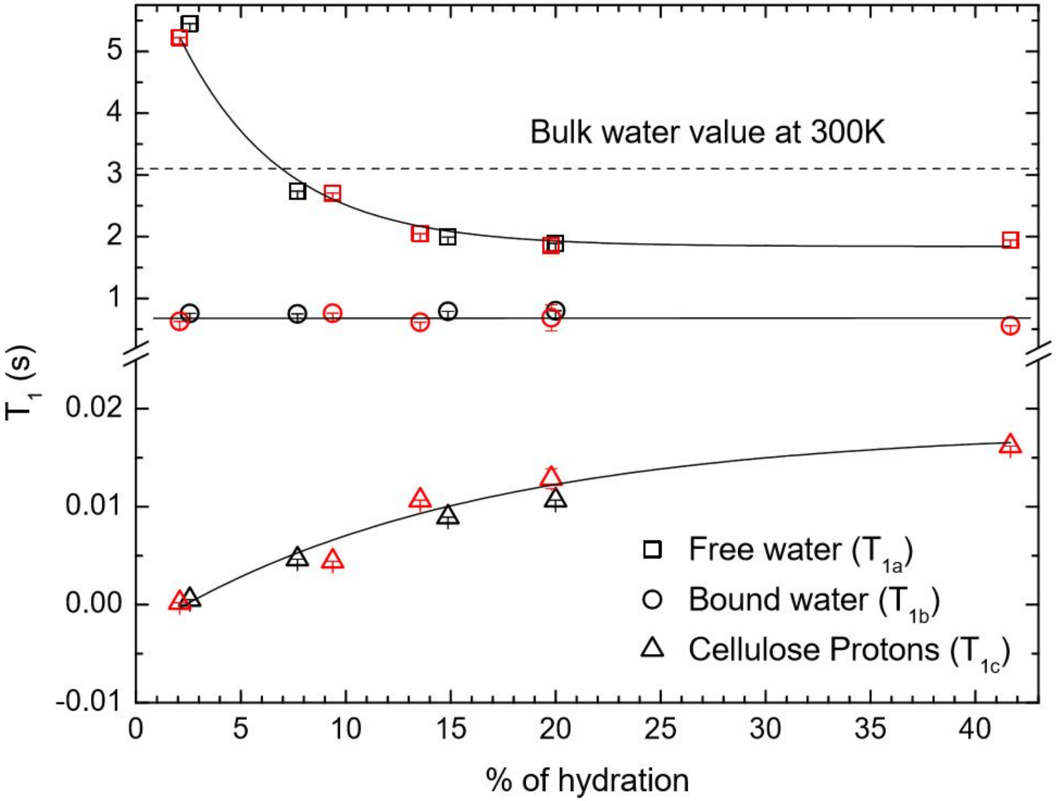
Spin-Lattice relaxation time vs. hydration

P2REF, P2C9048



The addition of water leads to the transition from the liquid-vapour phase to the liquid phase with an increment of the interactions between water and cellulose. This behaviour is due to the increase of the solvent accessible volume with increasing *h* caused by cellulose fibres swelling.

Spin-Lattice relaxation time vs. hydration

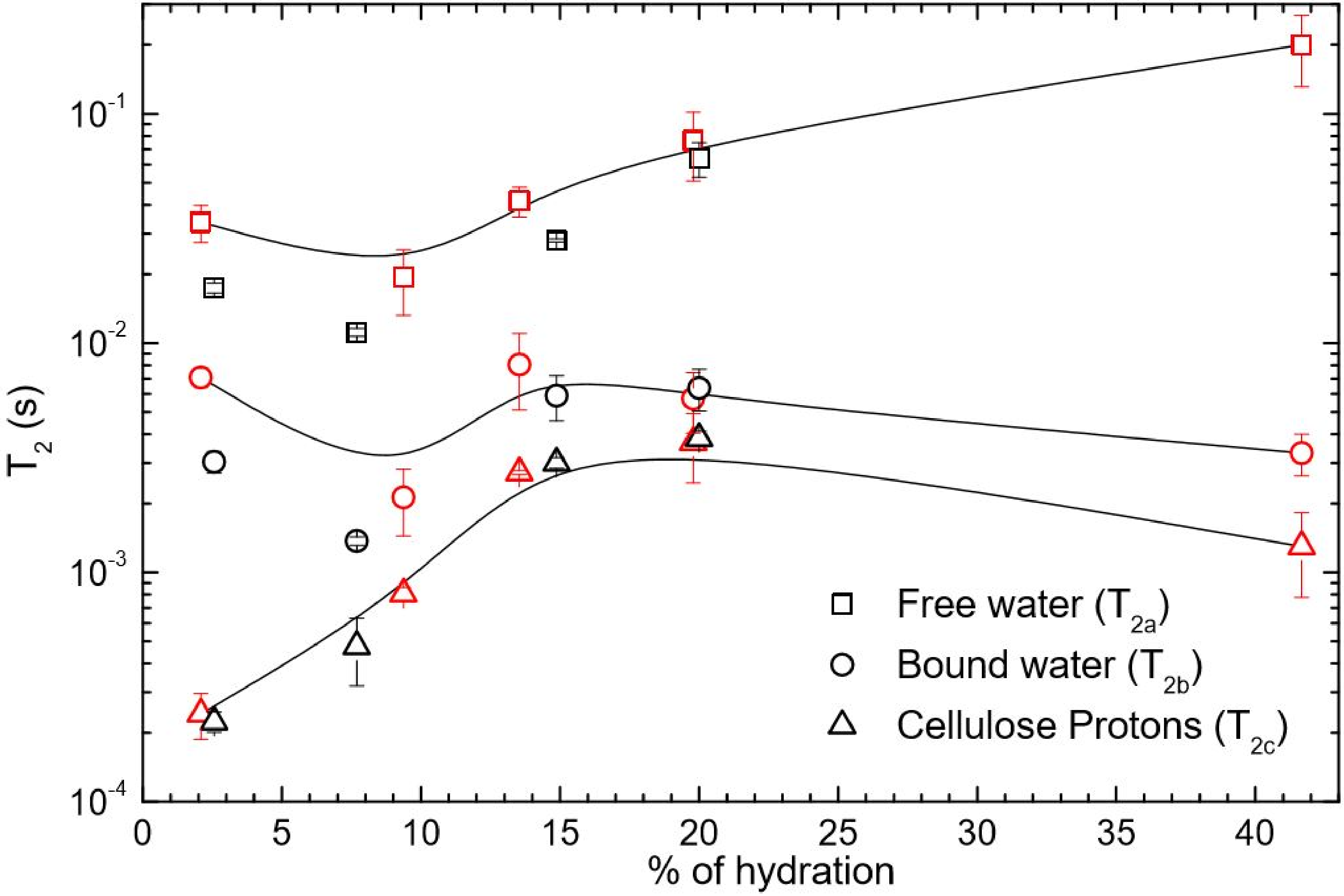


T1b has a constant trend as a function of *h*, *because bound* water steadily interacts with cellulose.

T1c has the smallest values and it slowly increases with *h*. because of cellulose fibres swelling with increasing *h*, allowing an enhancement of mobility of cellulose protons.

Spin-spin relaxation time vs. hydration

P2REF, P2C9048



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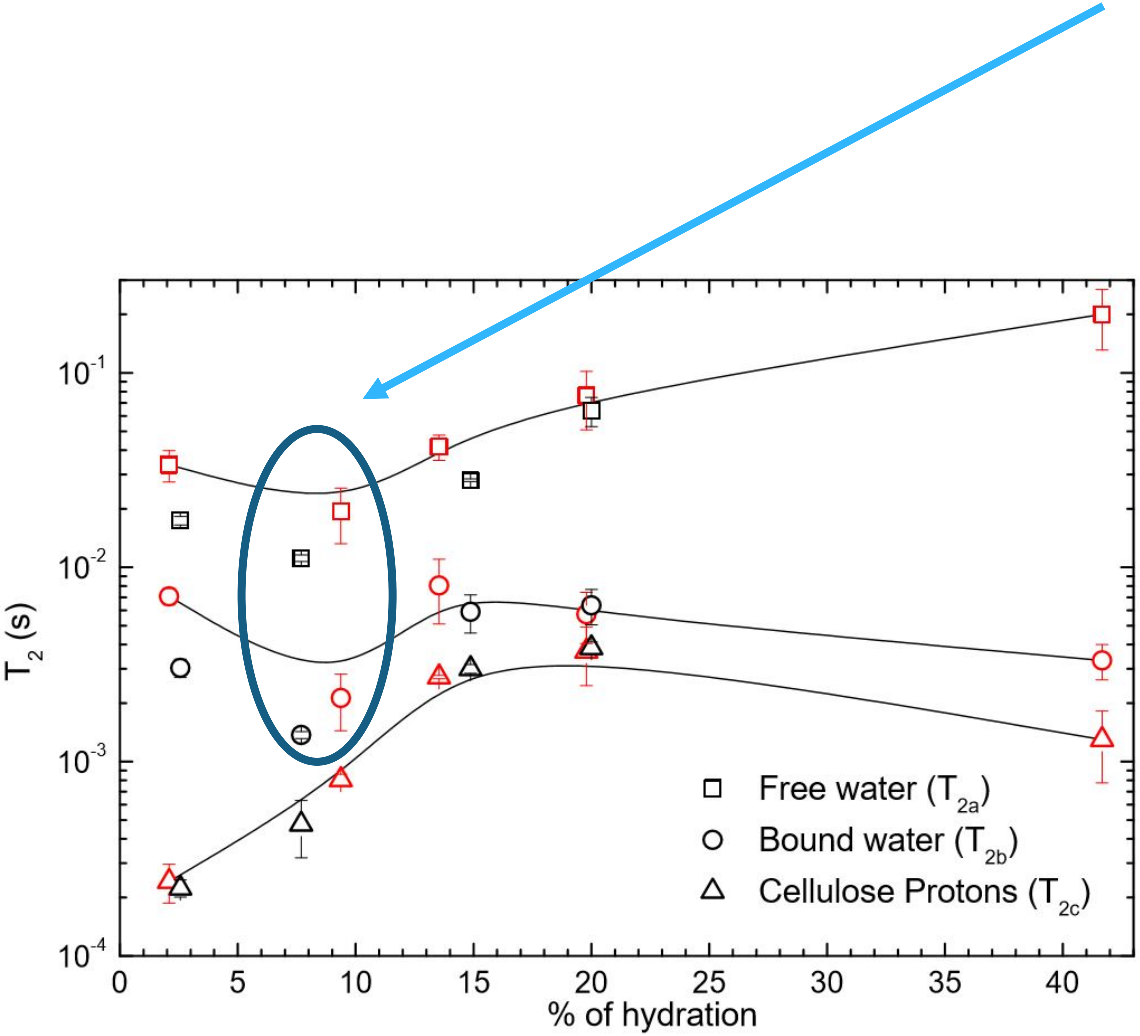

T_{2a} shows a minimum at ambient hydration confirming that this is the equilibrium condition for our samples made of pure cotton cellulose. For higher hydration, T_{2a} increases with *h* going towards the bulk water value. Also T_{2b} shows a minimum at ambient hydration, however its value decreases at higher moisture content because the mobility of bound water, is hampered by the increasing number of water molecules in the bulk condition.

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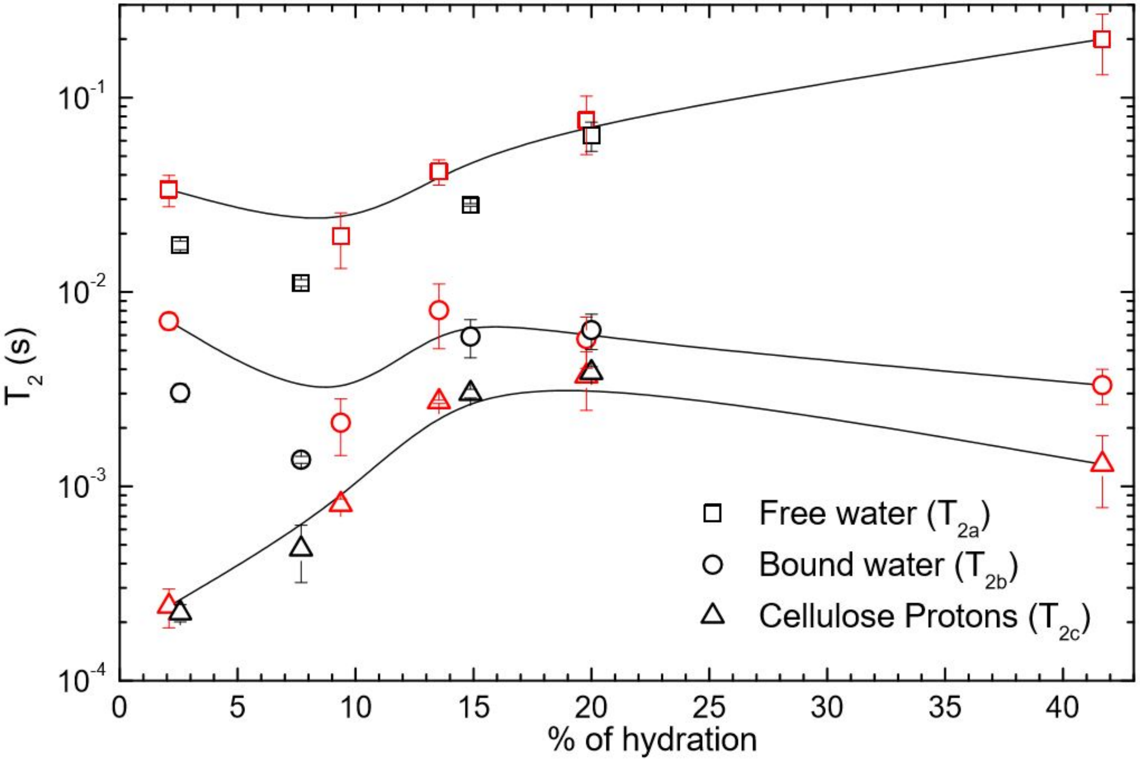
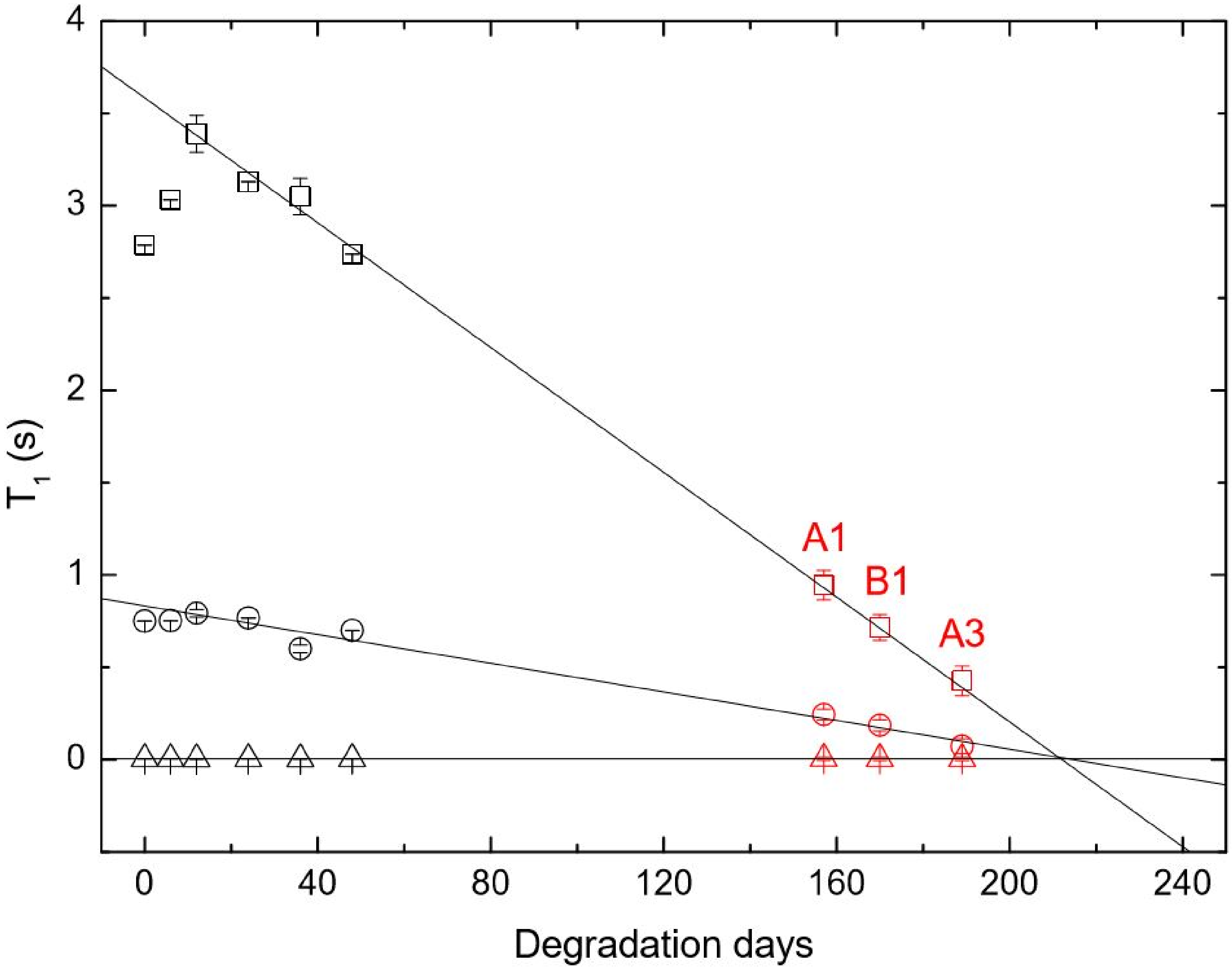
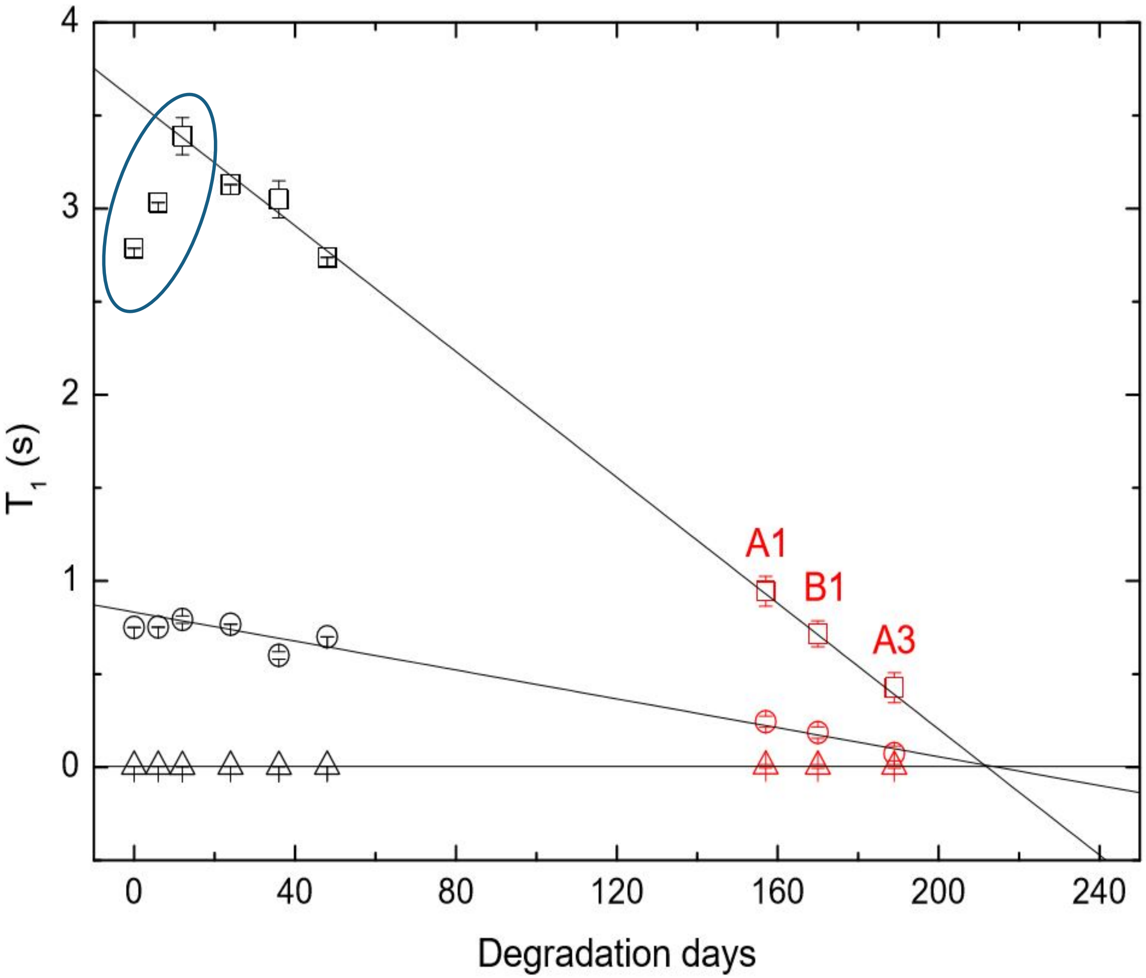


Finally, T_{2c} increases up to a moisture content value of about 20% and then decreases on increasing *h*. This is why the mobility of cellulose protons increases with *h* up to the fibre saturation point.

Spin-Lattice relaxation time vs. degradation



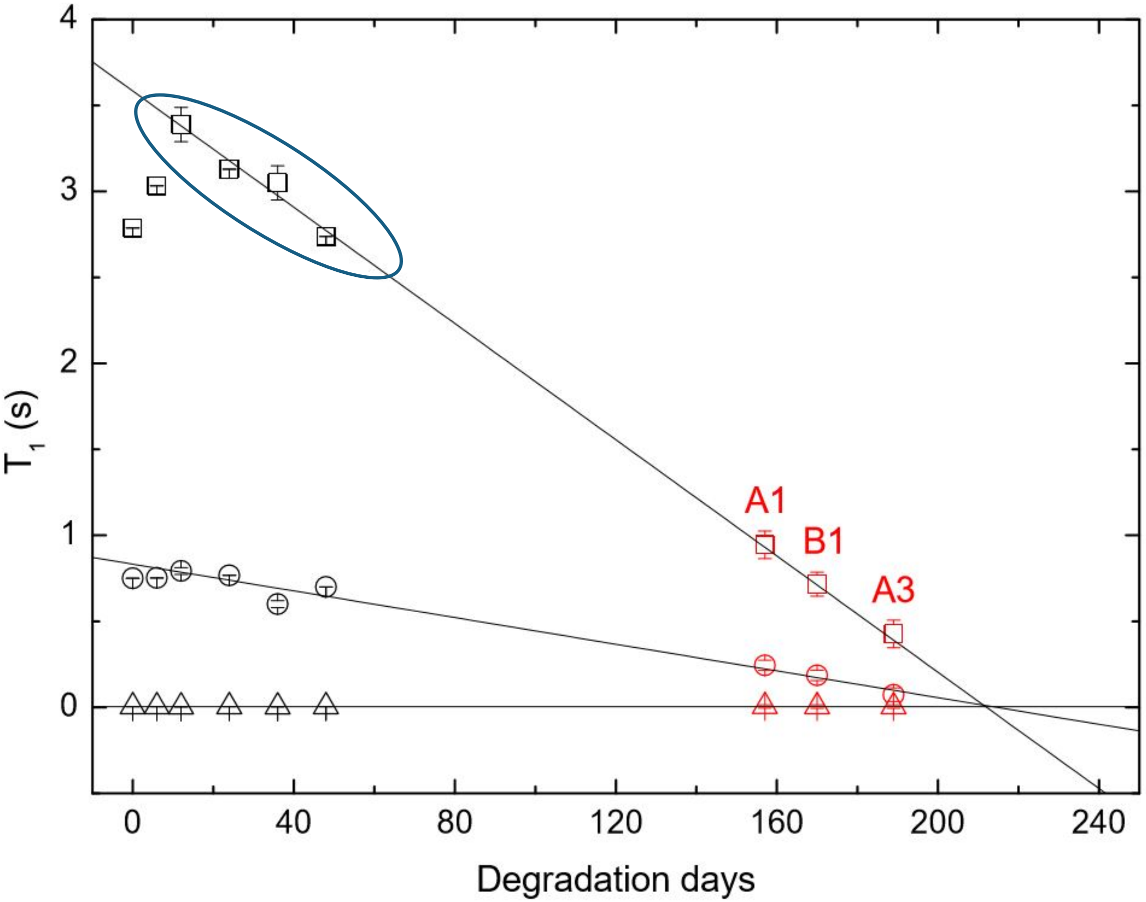
Spin-Lattice relaxation time vs. degradation



T_{1a} firstly increases of about 20% up to 12 degradation days and then decreases linearly: the initial consequence of degradation is the rupture of hydrogen bonds linking cellulose polymers mostly within amorphous regions, so enhancing the fibres' accessible volume.

Spin-Lattice relaxation time vs. degradation

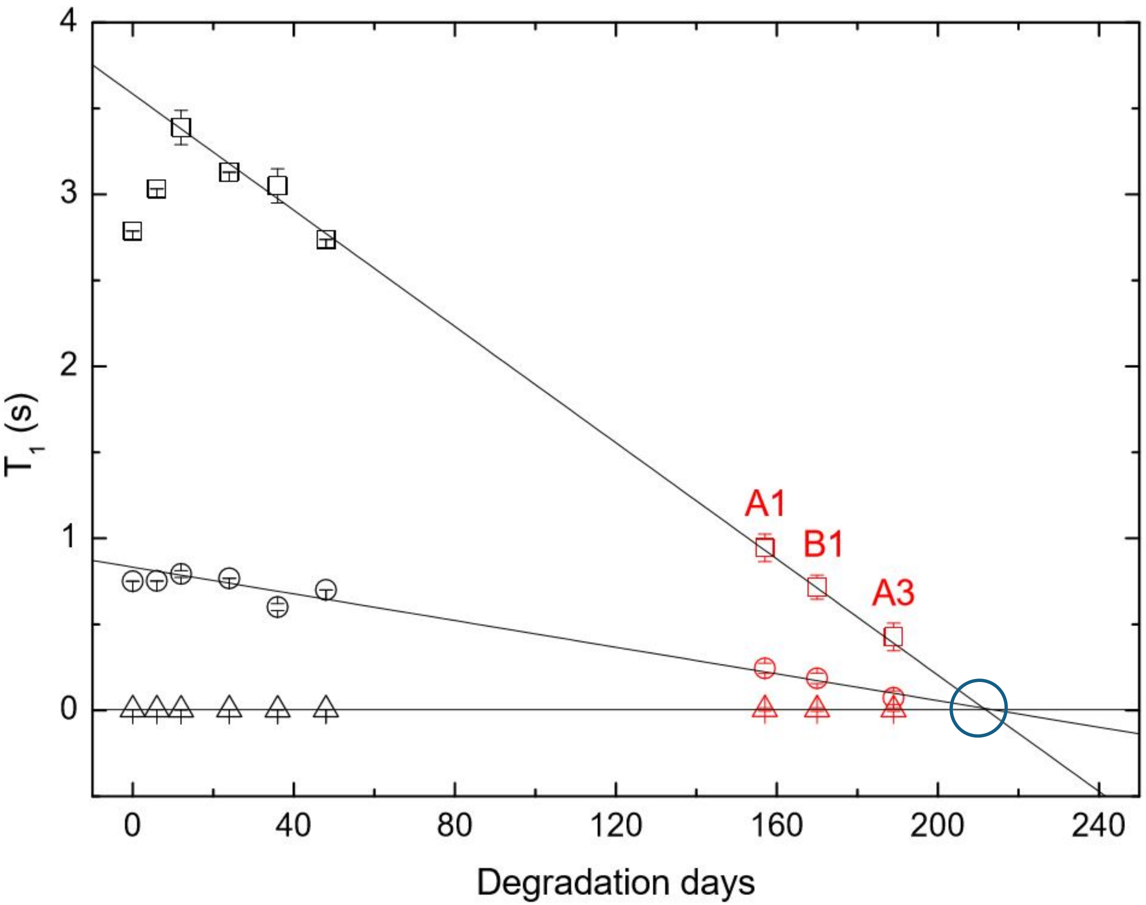
After 12 degradation days our data indicate that the newly accessible regions lost their hydrophilic character due to the progressive transformation of hydroxyl groups into carbonylic groups. The values of the degradation days for the naturally aged samples were estimated by performing a linear fit on T_{1a} data of samples with more than 12 degradation days.



T_{1a} values of A1, B1 and A3 samples were placed on the extrapolation of the linear fit and equivalent artificial degradation days with our aging conditions were associated to ancient samples: 157, 170 and 189, respectively.

Spin-Lattice relaxation time vs. degradation

Another linear fit was performed for T_{1b} data of samples with more than 12 degradation days. Interestingly T_{1b} data for ancient samples were spontaneously very well fitted by this linear fit. T_{1c} shows similar values for both modern and ancient samples (straight line with slope 0).



The linear evolution of each contribution cross each others at about 212 artificial degradation days that should be an indication of the limit of artificial degradation. At this limit all amorphous regions are consumed, and this corresponds to the levelling-off of the degree of depolymerization of cellulose polymers when paper loses most of its mechanical properties and begin to crumble.

Summary (1)

We were able to identify the presence of carboxylic acids in all studied paper samples.

The presence of the simplest carboxylic acids, acetic and formic acids, together with the cellobiose-like fragments in modern aged samples, is an indication of coupling between hydrolytic and oxidative degradation routes.

The detection of amino acids in ancient paper opens the way to detailed studies of paper artifacts dating and authentication.

Our data give a strong evidence that the interactions between water and cellulose act over time to progressively break hydrogen bonds between cellulose polymers. This results in the swelling of the cellulose fibres.

The responsible is the formation of hydrogen bonds between water and cellulose molecules that locally modify the fibres’ structure by “consuming” the amorphous regions.

Summary (2)

Degradation is a two-step process: water penetrates within the amorphous region that indeed are progressively destroyed.

Water acts as a powerful catalyst of hydrolytic and oxidative reactions with a consequent transformation of cellulose polymers into low molecular weight products.

T1 evolution encompasses both modern artificially aged and ancient paper providing therefore a non-destructive method for estimating the degradation of cellulosic materials in terms of an equivalent time of artificial aging.

Therefore, T1 measurements in particular could be used as a benchmark for the assessment of the state of degradation of cellulose products of industrial interest or ancient artefacts such as paper and textile as well as for eventual treatment.