S. VIEL[1,2,✉]
D. CAPITANI[1]
N. PROIETTI[1,3]
F. ZIARELLI[4]
A.L. SEGRE[1]

NMR spectroscopy applied to the Cultural Heritage: a preliminary study on ancient wood characterisation

[1] Istituto di Metodologie Chimiche, CNR, Via Salaria km 29.3, C.P.10, 00016 Monterotondo Staz. (Roma), Italy
[2] UMR 6516 Synthèse, Chiralité et Catalyse, Université d'Aix–Marseille III, Av. Escadrille Normandie Niémen, 13397 Marseille, France
[3] CAMPEC, Via G. Porzio, Centro Direzionale, Isola F4, Napoli, Italy
[4] Centre Régional de RMN, case 511, Av. Escadrille Normandie Niémen, 13397 Marseille, France

ABSTRACT High and low resolution solid state NMR methods have been applied to characterise a few samples of ancient wood. In an ancient larch wood sample, by applying ^{1}H low resolution NMR methods as a function of the temperature, the average pore size and its distribution have been determined. In addition, high resolution NMR techniques have allowed addressing of the question of the proximity of water pools to cellulose and lignin. In particular, a model can be hypothesized in which water pools are surrounded by thin layers of amorphous cellulose and/or lignin while the crystalline domains of cellulose surround the layers of amorphous cellulose. Preliminary results obtained using a fully non invasive and portable NMR unilateral relaxometer, the Eureka-Mouse10 (EM10), are reported. This instrumentation is shown to be perfectly suitable for characterizing degradation in ancient wood samples.

PACS 76.60 k

1 Introduction

Most materials of interest to Cultural Heritage, such as ancient paper, wood, and some rocks, can be treated as porous systems with water located in the pores. Recently, the average pore size and the porosity distribution in good quality paper has been determined by applying high and low resolution solid state NMR methods [1]. In particular, high resolution NMR techniques have allowed evaluation of the average size of water pools in Linters paper. It has also been demonstrated that water pools are surrounded by amorphous cellulose which, in turn, is surrounded by crystalline cellulose in the polymorphous forms I_α and I_β.

Spin-spin relaxation time T_2 is sensitive to degradation and its measurement allows differentiation between paper in a good state of preservation and degraded paper [2].

With the aim of characterizing ancient wood that exhibits a very low water content, we present here some preliminary results obtained by applying both high and low resolution NMR methods. Ancient wood is considered as a porous sys-

tem with water confined in small pores within a rigid polymeric matrix made of cellulose, hemicellulose, and lignin [3]. The question of the proximity of the water pools to cellulose and lignin is also addressed.

Finally, results achieved with a unidirectional NMR instrument, the Eureka-Mouse10 (EM10), are also reported. Standard NMR methods are particularly suitable to study materials and are generally considered as non invasive. However, they do require some sampling which might be prohibited when studying rare and precious materials. This sampling can be avoided by using a unilateral NMR instrument such as the EM10. The EM10 is a portable, fully non invasive NMR instrument that allows measuring of common NMR parameters such as the spin density, spin–spin, and spin–lattice relaxation times. In order to perform the NMR measurements, the sensor can be positioned near to intact objects at different places. Therefore, this instrument is perfectly suitable for studying items that are part of the Cultural Heritage.

2 Experimental

In this study, two ancient samples of larch wood and fir wood (samples A and B, respectively) from the trussed rafter of the Castle of Valentino, XV century, (Turin) were considered; both samples were courtesy of Prof. Bartolini Cestari. In addition, some measurements were performed on a modern fir wood sample (sample C).

[13]C CP-MAS and 2D WISE experiments were recorded on a Bruker ASX200 spectrometer operating at 200 MHz on a broad band probehead with a spinning rate of 8 kHz. [1]H dipolar filtered ^{13}C CP-MAS spectra were recorded on a Bruker Avance400 spectrometer operating at 400 MHz on a 7 mm broad band probehead with a spinning rate of 4 kHz. All spectra were recorded with a contact time of 300 μs. The ^{1}H low-resolution NMR spectra at variable temperature were recorded at 65 MHz on a commercial spectrometer Spinmaster from STELAR, Mede (PV) Italy. The temperature within the 200–300 K range was controlled by means of a variable temperature unit supplied by STELAR (VTC91 unit) and equipped with a standard Bruker nitrogen evaporator system.

Relaxometric measurements were also performed with the EM10 prototype at 18.153 MHz.

✉ Fax: +39-06/9067-2477, E-mail: viel@imc.cnr.it

3   Results and discussion

3.1   [1] H *low resolution NMR*

The Free Induction Decay (FID) of a sample of ancient wood shows two components. The fast decaying component is due to the rigid polymeric matrix while the slow decaying one is due to water (Fig. 1a).

After a Fourier transformation, the rigid polymeric component appears as a broad resonance with a line width at half height $\Delta_{1/2} \cong 50 \, \text{kHz}$, while the water component gives a rather sharp resonance with $\Delta_{1/2} \cong 1.5 \, \text{kHz}$ (Fig. 1b).

By lowering the temperature the water resonance progressively broadens and completely disappears at about 200 K when the water is fully frozen (Fig. 2); the freezing process is slow and continuous.

Since the lowering of the melting point is associated with a confinement in small cavities or pores, the melting temperature of water can be related to its confinement in small pores [6]. Therefore, wood can be treated as a porous system containing a mobile species, water, embedded into a rigid polymeric matrix made of cellulose, hemicellulose, and lignin. Information on the pore size distribution can be obtained by measuring the water resonance intensity as a function of the temperature (IT method) [7].

According to the IT method, the intensity of the water resonance is reported against the inverse temperature $X = 1000/T$ as shown in Fig. 3a.

As a function of X, the water resonance intensity can be written as:

$$I(X) = \sum_{i=1}^{N} \frac{I_{0i}}{\sqrt{\pi}} \int_{0}^{(X-X_{ci})/\sqrt{2}\Delta_i} \exp\left(-u^2\right) \mathrm{d}u \qquad (1)$$

where i denotes the number of transitions, $I(X)$ represents the amount of mobile water at the inverse temperature, I_{0i} is
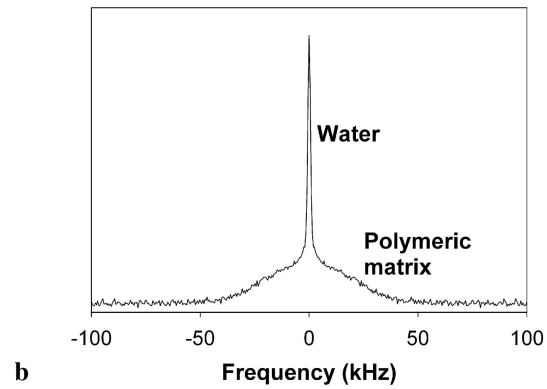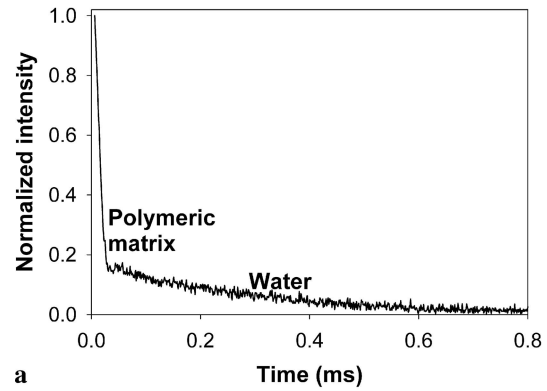

FIGURE 1   **a** Free induction decay and **b** Fourier transformed spectrum of sample A

the amount of water confined in the pore, X_{ci} is the inverse temperature of the phase transition, and Δ_i is the width of the transition curve; note that the more Δ_i tends to zero the sharper the relative phase transition.

Parameters I_{0i}, X_{ci}, and Δ_i are obtained by fitting the experimental data to equation (1); the solid line through the experimental points in Fig. 3a is obtained by applying the best
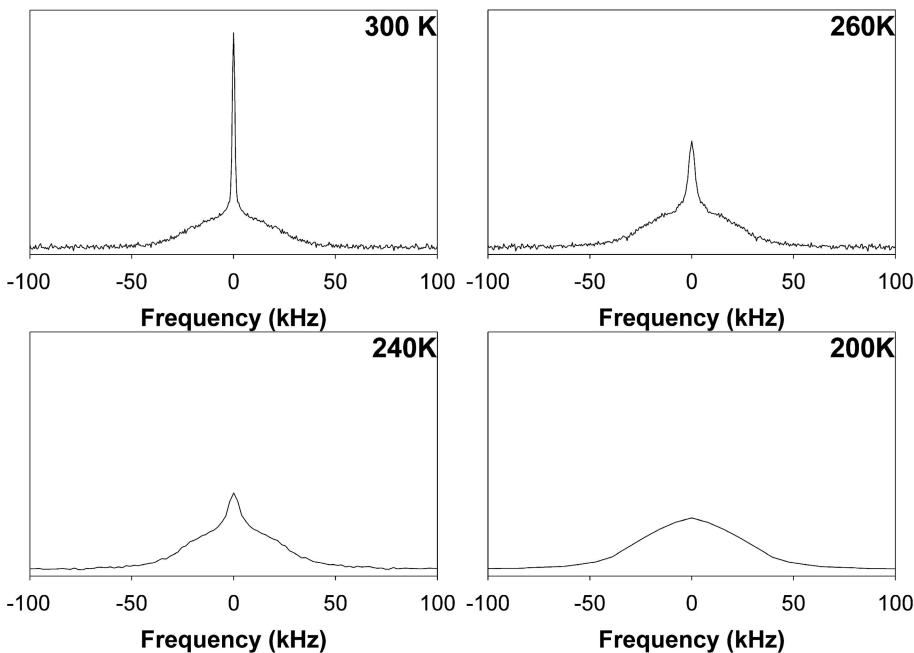

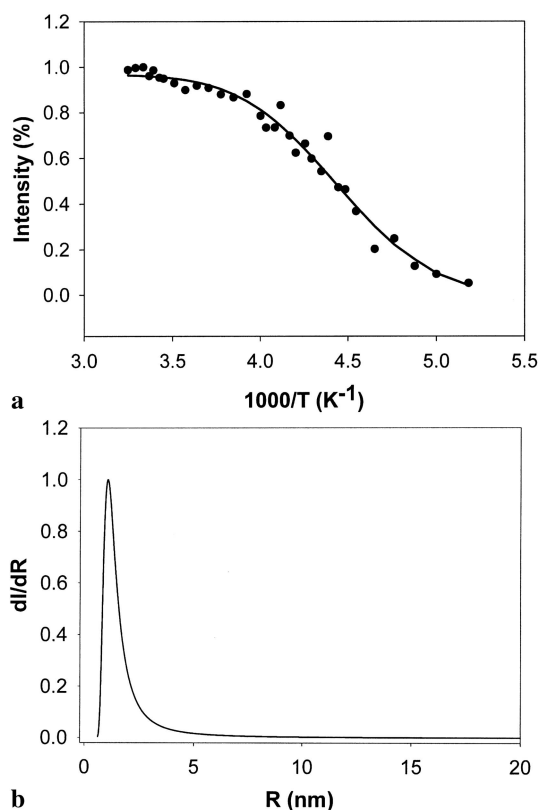**FIGURE 2**   65 MHz ^{1}H spectra of sample A recorded at various temperatures

FIGURE 3   **a** IT plot and **b** pore size distribution of sample A

fit procedure. In our case only one transition ($N = 1$) is observed as defined with: $I_{01} = 100$; $X_{c1} = 4.44$; $\Delta_1 = 0.44$. The pore radius is hereafter referred to as R. Since

$$\frac{dI}{dR} = \frac{dI}{dX}\frac{dX}{dR}$$

and

$$\frac{dI}{dX} = \sum_{i=1}^{N} \frac{I_{0i}}{\sqrt{2\pi}\,\Delta_i} \exp\left[-\left(\frac{X - X_{ci}}{\sqrt{2}\Delta_i}\right)^2\right]$$

the pore size distribution can be obtained according to:

$$\frac{dI}{dR} = \sum_{i=1}^{N} \frac{I_{0i}}{1000 K_f \Delta_i \sqrt{2\pi}}(XT_0 - 1000)^2$$
$$\times \exp\left[\frac{-(X - X_{ci})^2}{\Delta_i \sqrt{2\pi}}\right]$$

where T_0 denotes the freezing temperature of the free water and K_f is a constant equal to $676\,\mathrm{K\,Å^{-1}}$ [1, 7]. As reported in Fig. 3b, the pore size distribution shows a well defined maximum at $R \cong 1$ nm; the distribution is strongly asymmetric, abruptly decreasing for values lower than about 1 nm and smoothly for values higher than 1 nm. It is worth noticing that a net loss of bound water is observed in ancient, deteriorated wood. The residual bound water that is detected is the one tightly confined in small, closed pores. As a consequence the pore size distribution that is reported in Fig. 3b corresponds to

the distribution of these small, inaccessible cavities. Note that the IT method can be applied to porous systems with a pore size R ranging from few tenths of nanometers up to a few tens of nanometers. For higher R values the method is not sensitive and other experimental techniques must be applied. Finally, it must be pointed out that, in order to obtain information on the distribution of open pores, the sample must be re-hydrated.

3.2   *High-Resolution Solid State NMR*

3.2.1 [1]H *Dipolar Filtered* [13]C *CP-MAS Spectra.* As previously shown in Fig. 1b, the proton wide line spectrum of a piece of ancient wood shows a rather sharp resonance due to the water, and a broad resonance due to the rigid polymeric matrix. The line width of a ^{1}H wide line spectrum characterises the strength of the dipolar coupling among protons and, in turn, the molecular mobility. Therefore, this marked difference in the line width allows the use of the "Dipolar Filter" technique [8]. In this experiment, applying a dipolar filter on the ^{1}H channel, the selection of the magnetization of the mobile component is achieved. As a result, at very short mixing time τ, the carbon signals corresponding to the broad ^{1}H component are filtered whereas, at long mixing times, i.e. when the spin diffusion process is complete, the conventional ^{13}C CP-MAS spectrum is obtained.

The ^{13}C CP-MAS spectrum of a piece of ancient wood is reported in Fig. 4a.

The resonances at 89.7 and 65.8 ppm are respectively due to C_4 and C_6 of crystalline cellulose (C_{4c}, C_{6c}), while those at 83.7 and 62.7 ppm are respectively due to C_4 and C_6 of cellulose in an amorphous environment (C_{4a}, C_{6a}). Therefore, the dipolar filter experiment allows addressing of the question of the proximity of water pools to the amorphous or to the crystalline domain. In fact, at very short mixing times, the carbon resonances belonging to the environment which is closer to the water pools can be selected. While it has already been reported that water pools are surrounded by the amorphous domains of cellulose [1], no information is presently available about their position with respect to the lignin component.

The ^{1}H dipolar filtered ^{13}C CP-MAS spectra recorded at different mixing times are shown in Fig. 4b. At a very short mixing time, $\tau = 0.05$ ms, the resonance due to C_{6a} is more intense than the resonance due to C_{6c}. By increasing the mixing time, the intensity of the C_{6c} resonance grows and eventually becomes more intense than the resonance due to its amorphous counterpart, C_{6a}. A similar behavior is observed to a lesser extent in the intensity of the resonances due to C_{4a} and C_{4c}, respectively. Therefore, at very short mixing times, allowing a polarization transfer from the ^{1}H signal of the water to the ^{13}C signals of the polymeric matrix, an indirect selection of the amorphous domain has been achieved. It is worth noticing that even at very short mixing times the resonances due to the aromatic and carboxylic components of lignin resonating in the 140–180 ppm range and the resonance due to the methoxyl group of lignin observed at 56 ppm, show an intensity similar to that observed at long mixing times. Therefore, even at a very short mixing time, an indirect selection of the lignin component is obtained. A model can be hypothesized in which water pools are surrounded by thin layers of amorph-
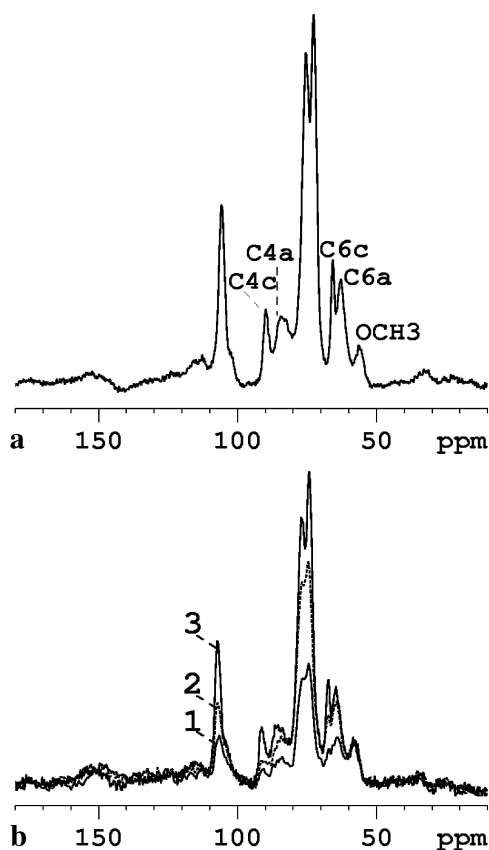
FIGURE 4 **a** 400 MHz ^{13}C CP-MAS spectrum of sample A. **b** 400 MHz ^{1}H dipolar-filtered ^{13}C CP-MAS spectra recorded on sample A with a mixing time of 0.05 ms (1), 0.5 ms (2), and 50 ms (3), respectively; all three spectra are normalized on the resonance of the methoxyl group of lignin at 56 ppm

FIGURE 5 2D WISE spectra recorded at 200 MHz on sample A with a mixing time of 0.05 ms (**a**) and 40 ms (**b**)

ous cellulose and/or lignin while the crystalline domains of cellulose surround the layers of amorphous cellulose.

3.2.2 2D WISE. For every resolved carbon resonance the 2D WISE experiment displays a proton wide line spectrum reflecting the size of the dipolar coupling of the protons in the proximity of the respective carbon nucleus. Thus the wideline ^{1}H NMR spectrum, fully overlapped in the conventional one dimensional ^{1}H spectrum, is spread out according to the chemical shifts of the corresponding carbon nucleus [9]. With the insertion of a mixing time allowing spin diffusion among different proton spin pools, the WISE experiment can provide information on the domain size.

In the 2D map of a WISE experiment recorded without any mixing time on a piece of ancient wood, only the broad resonance due to the polymeric matrix can be observed (data not shown). The same is observed in the WISE map recorded with a very short mixing time $\tau = 0.05$ ms as shown in Fig. 5a.

The absence of the narrow water line points out that the heteronuclear dipolar interaction between the mobile water protons and the carbon resonances of the polymeric matrix is missing when the mixing time is very short. Hence, at very short mixing times, the distance between the water protons and the carbon nuclei of the polymeric matrix is too large for observing the water resonance. By lengthening the time during which the spin diffusion process operates, the dynamic
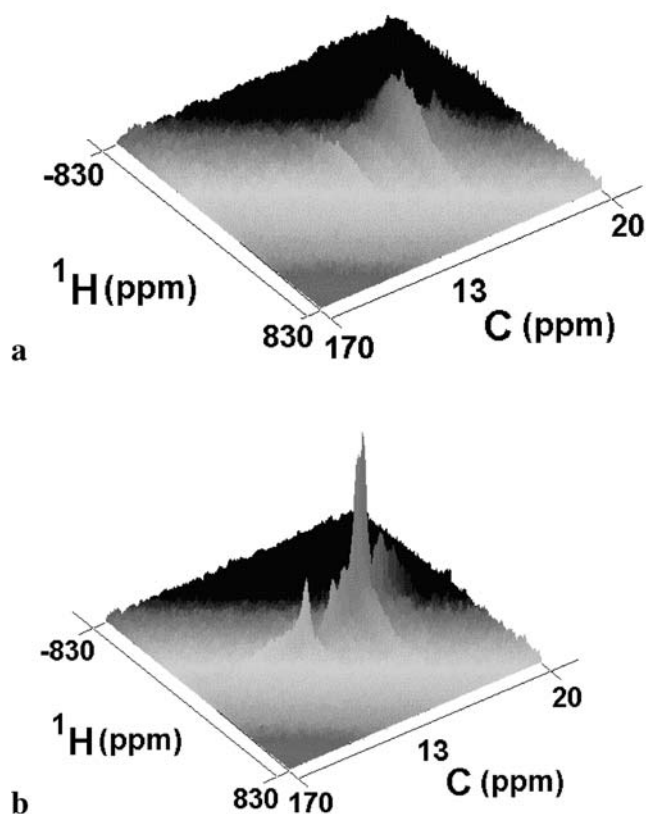
information is transferred from the water protons to the carbon nuclei. In fact, at a long mixing time $\tau = 40$ ms, in the full spin diffusion regime, the narrow line appears in the ^{1}H projection of the WISE map (Fig. 5b).

3.3 Measurements with the EM10

In the past few years NMR relaxometric methods have been proved to be valuable in assessing paper quality, especially in relation to the early detection of enzymatic attack [2]. The most sensible parameter is the spin-spin relaxation time T_2; in fact, after the enzymatic attack, a net decrease in a T_2 component was observed. The same behavior has been identified by measuring samples of paper, cotton, and wax before and after oxidation processes: in all degraded materials the long T_2 component is definitively shortened.

It must be taken into account that, even though the amount of sample required for performing the relaxometric measurements is low (≈ 20 mg), the NMR method as applied in the standard way cannot be fully considered as non destructive; in fact, sampling of objects belonging to the Cultural Heritage should be absolutely avoided.

The advantage of using the EM10 instrument is that the magnetic field is applied to the sample from one side only. The probe connected with the proper portable instrumentation can measure the spin density, the spin lattice relaxation time and also the ^{1}H T_2 relaxation in a fully non destructive way, i.e. fully preserving the integrity of the object under investigation.

FIGURE 6   EM10 magnet and probehead during on-site measurements at "Torre delle Milizie" in Rome (Italy)
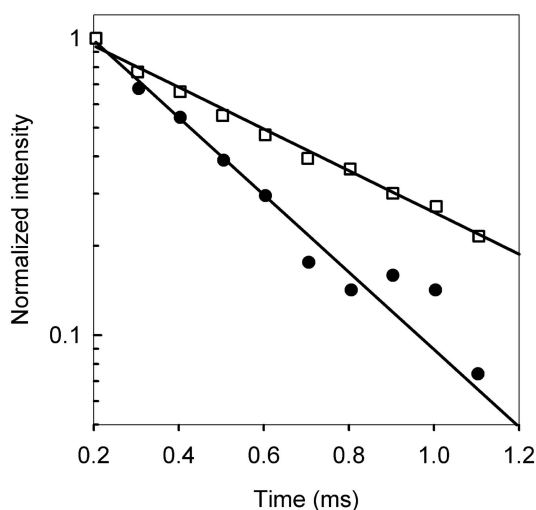

FIGURE 7   Semi-log representation of the echo decay of ancient, *filled circles*, and modern, *empty squares*, fir wood samples (samples B and C, respectively). The *lines* through the experimental data points are drawn manually to guide the eye

A picture of the EM10 magnet and probehead as used during on-site measurements is shown in Fig. 6.

The information acquired by this NMR instrumentation is well suited for soft matter and wet porous materials. Therefore, paper, wood, wet frescos, ceramics, and rocks can be investigated.

Here, we report some preliminary results obtained with the EM10 on ancient and modern fir wood samples. In Fig. 7 the echo decay obtained applying a CPMG sequence with the EM10 apparatus is reported for sample B, ancient fir, and C, modern fir.

For the sake of clarity, all data are shown in a semi-logarithmic scale. The line through the experimental echoes was drawn manually to guide the eye: it illustrates how the decay is faster in the ancient sample than in the case of the modern one. Note that in the ancient, degraded wood sam-

ple the T_2 value is clearly shortened, which is in agreement with previous findings on paper, wax and cotton. This decrease in T_2 reflects a net loss of bound water, i.e. a loss of water in pores. Efforts are currently dedicated to increase the signal-to-noise ratio of the EM10 and to perform in situ measurements, with the aim of standardizing the experimental conditions.

4   Conclusion

Small pores containing water are present in ancient wood samples. These water cavities are surrounded by amorphous cellulose and lignin, while crystalline cellulose does not touch the water filled pores. The average dimension of the pores is about 1 nm and their distribution is found to be strongly asymmetric, decreasing abruptly for values lower than 1 nm, and smoothly for values larger than 1 nm. Degradation in wood is characterised by a decrease in the spin-spin relaxation time, observable either by the micro-invasive NMR relaxation techniques or by the fully non invasive measurements achieved with a unilateral NMR instrument. Experimental values obtained with both techniques are in agreement demonstrating that, indeed, unilateral NMR relaxometric measurements constitute a perfect non-invasive method for assessing the state of conservation of items belonging to the Cultural Heritage.

It is worth noticing that the reported study on ancient wood can give information about the state of wood wedges used in the restoration of ancient choirs and stairs.

The unidirectional NMR method and the EM10 instrumentation are suitable for exploring the state of humidity of ancient buildings before and during restoration. For instance, using the unidirectional NMR instrumentation in the "Criptoportico of Colle Oppio", 60–80 a.C., the humidity of the walls of the recently discovered fresco, was found to be lower than the humidity of the fresco itself [5].

REFERENCES

1 D. Capitani, N. Proietti, F. Ziarelli, A.L. Segre: Macromolecules **35**, 5536 (2002)
2 D. Capitani, M.C. Emanuele, A.L. Segre, C. Fanelli, A.A. Fabbri, D. Attanasio, B. Focher, G. Capretti: Nordic Pulp Paper Res. J. **13**, 95 (1998)
3 S.L. Maunu: Prog. Nucl. Magn. Reson. Spectrosc. **40**, 151 (2002)
4 B. Blümich, S. Anferova, S. Sharma, A.L. Segre, C. Federici: J. Magn. Reson. **161**, 204 (2003)
5 B. Blümich, S. Anferova, K. Kremer, S. Sharma, V. Hermann, A.L. Segre: Spectroscopy **18**, 18 (2003)
6 J.H. Strange, M. Rahman, E.G. Smith: Phys. Rev. Lett. **71**, 3589 (1993)
7 R. Schimdt, E.W. Hansen, M. Stöcker, D. Akporiaye, O.H. Ellestad: J. Am. Chem. Soc. **117**, 4049 (1995)
8 J. Clauss, K. Schmidt-Rhor, A. Adam, C. Boeffel, H.W. Spiess: Macromolecules **25**, 5208 (1992)
9 K. Schmidt-Rhor, J. Clauss, H.W. Spiess: Macromolecules **25**, 3273 (1992)