

Lecture Notes in Chemistry 79

Evangelia A. Varella *Editor*

Conservation Science for the Cultural Heritage

Applications of Instrumental Analysis

 Springer

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Chapter 5

Applying the Techniques on Materials I

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5.6 Solid State NMR to Characterise Paper and Parchment Samples from a Sixteenth Century Book

A. Spinella, D. Capitani, S. Bastone, C. Di Stefano and E. Caponetti

Abstract In this chapter, a solid state Nuclear Magnetic Resonance (ss-NMR) study of a sixteenth century book is reported. Cross-Polarization Magic-Angle Spinning Nuclear Magnetic Resonance ($^{13}\text{C}\{^1\text{H}\}$ CP MAS NMR) spectra of paper samples collected from the book, allowed us to obtain information on its state of conservation. The physicochemical characterisation of two parchment samples collected from the cover book was performed through the evaluation of the changes in line width and intensity of signals in the spectra.

5.6.1 Introduction

Solid state NMR (ss-NMR) is a powerful tool for chemical characterisation of many types of materials and natural polymers based materials such as paper [88] and parchment [89].

The application of ss-NMR spectroscopy can provide valuable information to conservation scientists, conservators and archaeologists.

In this chapter, the characterisation and determination of the extent of degradation of a sixteenth century book is presented in order to show the potentiality of ss-NMR technique in this field.

The book under investigation consists of three different volumes bound together. The three volumes were printed in Paris in different years of the sixteenth century (1534, 1578, 1597) and bound together at the Monastery of San Martino delle Scale (near Palermo, Sicily), which was one of the most illustrious monastic libraries of Sicily and also a writing workshop producing good quality manuscripts not only for its own library but also for other Benedictine monasteries. The parchment cover was processed in the same monastery, and it shows typical incisions of Martinian binding [90]. For this reason, the book binding was dated between the late sixteenth and early seventeenth century as in the period after that time the monastery commissioned the bindings outside. An example of this kind of binding is shown in Fig. 5.18.

Paper is one of the most common, oldest and sometimes precious man-made materials. The main source of cellulose fibres for the paper production in Medieval Europe was rags made by cotton or flax. Modern paper is mostly made from wood cellulose that is difficult to purify. The chemical treatments involved in the purification process can cause significant damage to the cellulose fibres. An undamaged cellulose with long fibres is necessary in order to obtain strong papers with a good durability. For this reason, traditional Chinese and Japanese papers

Fig. 5.18 An example of Martinian binding



were made from cellulose fibres derived from local plants which had these characteristics.

The first paper was made in China in 105 AD from a suspension of cellulose fibres dispersed in water. The suspension was drained through a fine mesh so that the water drained away and the retained fibres formed a mat on the mesh. After drying the fibres stacked together forming a sheet of paper [91].

High quality paper is mainly a two-component material constituted by cellulose and by an almost equimolar amount of bound water, plus a variable amount of organic and inorganic additives and/or dia- and paramagnetic impurities. [92]. The paper structure can be schematically described as amorphous cellulose domains surrounding water pools, and the amorphous domains are surrounded by crystalline cellulose domains [93]. Some water molecules also permeate the cellulose fibres. A comprehensive microscopic view of paper could describe it as an interconnected structure of water, amorphous and crystalline cellulose domains on the nanometre scale [94].

On the basis of $^{13}\text{C}\{^1\text{H}\}$ CP MAS NMR spectra, Vanderhart and Atalla [95] found out that all native celluloses are a mixture of two crystalline modifications, called $I\alpha$ and $I\beta$, in different proportions. The two allomorphs are shown in Fig. 5.19.

The corresponding crystallographic units are characterised by one-chain triclinic and two-chain monoclinic unit cells, respectively. In fact, these crystalline forms have the same microfibril but in the monoclinic form, the cellobiose units stagger with a shift of a quarter of the crystallographic c -axis period, whereas the triclinic form exhibits a diagonal shift of the same amount. The ratio of the two phases depends on the origin of the cellulose. The $I\alpha$ form is dominant in cellulose produced by primitive organisms, such as the bacterium *Acetobacter xylinum* and the alga *Valonia macrophyssa*, whereas the $I\beta$ form dominates in cellulose produced by higher plants.

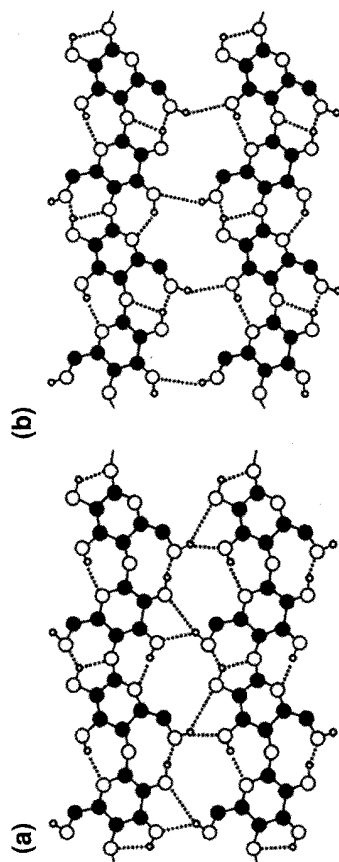


Fig. 5.19 Hydrogen bonding patterns in cellulose I α (a) and I β (b) (Sturcova et al., Copyright 2006 American Chemical Society)

Cellulose in a well-preserved paper has a high degree of polymerization and the amorphous domains surround small water pools. A measurable loss of bound water and an increase of the amorphous fraction accompany the destruction of the texture of the paper. Also paramagnetic impurities play an important role in the degradation of the paper, as they may act as catalysts and initiators of hydrolytic and/or oxidation reactions. Furthermore, paramagnetic centres undergo profound changes upon thermal or photochemical ageing of paper [96, 97].

Parchment is a material made from calfskin, sheepskin or goatskin. Parchment used for manuscripts, scrolls, charters, book covers and substrata for artworks makes a major contribution to the most valuable objects of European cultural heritage [98]. Large collections exist in varying degrees of preservation in public and private libraries, archives, museums and in different religious foundations. At the end of the Middle Age, parchment was joined by paper; however, it has continued to be used for special purposes, such as official documents and book bindings. It is manufactured from animal hide after strong alkaline removal of the epidermis and subcutaneous tissue collagen that is a fibrous protein constituting the main structural component of parchment. The structure of collagen is well described in terms of three individual protein strands in the α -helix conformation [99]. These strands are rigidly held by strong hydrogen bond between the hydroxyl group of hydroxyproline and the amino function of adjacent glycine units, forming a rod like crystalline phase embedded into an amorphous matrix.

The degradation of parchment involves structure loss of the intact fibre through different stages of alteration to a terminal stage when the fibre structure is close to a complete hydrolysis. When in contact with water or stored in wet conditions, the fibres transform into a gelatinous substance. To improve preservation and conservation treatments of patrimonial skin objects can be, therefore, useful to study the chemical composition and the physical-chemical state of aged collagen. Many collagen-based objects are valuable treasures due to the history they represent, and their preservation is still a challenge for museums and private collectors as well.

Table 5.11 Paper samples and position of sampling

Sample	Position
1	First page of the first volume
2	Last page of the first volume
3	First page of the second volume
4	Centre of the second volume
5	Last page of the second volume
6	First page of the third volume
7	Last page of the third volume

The development of specific analytical techniques may improve the procedures to authenticate patrimonial objects made from collagen-based materials as well as methods to study the impact of environmental factors.

5.6.2 Experimental

The sampling was performed in such a way to minimally damage the product and to obtain a representative number of samples. The paper sampling was carried out by taking two small (10 mg) stripes from each volume, one from the first and one from the last page. In addition, another sample was collected from the centre of the second volume. The two parchment samples were taken from the back of the cover.

^{13}C $\{^1\text{H}\}$ CP MAS NMR spectra were obtained at room temperature with a Bruker Avance II 400 MHz (9.4 T) spectrometer operating at 100.63 MHz for the ^{13}C nucleus, the spinning rate was 13 kHz. A total of 1,024 scans were collected, a contact time of 1.5 min and a repetition delay of 2 s were used. The Hartmann-Hahn shift external reference. Samples were placed in 4 mm zirconia rotors and sealed with KEL-F caps using silica as a filler to avoid inhomogeneities inside the rotor. Spectral deconvolution was performed using the DMFit software [101].

5.6.3 Analysis of NMR Spectra of Paper Samples

^{13}C $\{^1\text{H}\}$ CP MAS NMR spectra were acquired on the seven paper samples reported in Table 5.11:

As an example, the spectrum of sample 1 is shown in Fig. 5.20. The peak assignment of carbons of cellulose repetitive unit is also reported.

The weak signal observed at 172 ppm is ascribed to carbonyl carbons. In the range between 110 and 100 ppm, assigned to C1, three intense signals are observed; two external signals are due to C1 of $I\alpha$ polymorphous form, whereas the inner signal is due to C1 of $I\beta$ polymorphous form. A shoulder due to anomeric carbons of oligomers is also present. The signal at 88.7 ppm is due to C4 of crystalline cellulose and the signal at 83.8 ppm is due to C4 of amorphous

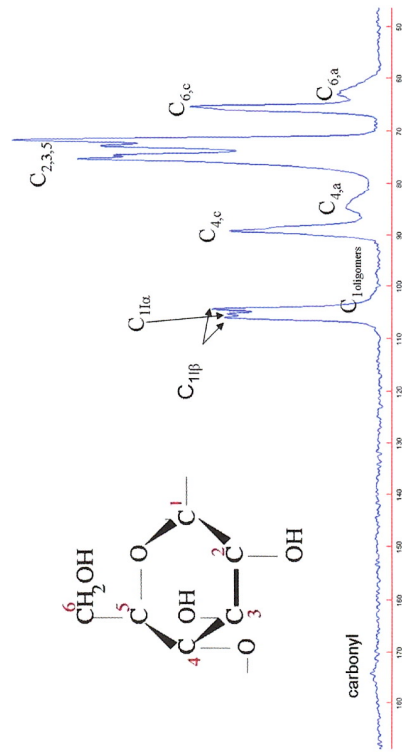


Fig. 5.20 $^{13}\text{C}\{^1\text{H}\}$ CP MAS NMR spectrum of sample 1

cellulose. Peaks between 80 and 70 ppm are assigned to C2, C3 and C5 carbons. The signal at 67 ppm is due to C6 of crystalline cellulose, whereas the one at 63 ppm is due to C6 of amorphous cellulose. The spectral deconvolution of the 110–55 ppm region is shown in Fig. 5.21.

Peak areas obtained from the spectral deconvolution of NMR spectra of all samples are reported in Table 5.12.

In the Table, the relative composition of $I\alpha$ and $I\beta$ polymorphous forms obtained from the deconvolution is also reported. This value is obtained from the ratio between the integral of $I\alpha$ ($I\alpha$ or $I\beta$) and the total integral of C1.

The deconvolution of C4 signal allowed us to determine the degree of crystallinity (DCry) of the cellulose which is strictly correlated to the state of degradation of the paper. The latter parameter is calculated from the ratio between the integral of C4,c signal and the total integral of C4 [102].

All samples show a rather good degree of crystallinity between 0.5 and 0.6. Samples 1, 3, 4, 6 and 7 also show the presence of a certain amount of cellulose oligomers possibly due to the hydrolysis of the glucosidic bonds. The lowest amount of oligomers is observed in samples 2 and 5. In samples 1, 2, 3 and 5, $I\beta$ is the predominant polymorphous form; whereas, in samples 4, 6 and 7, $I\alpha$ polymorphous form prevails. Sample 1 is the only one showing a carbonyl carbon signal due to cellulose oxidation.

It is worth noting that the ss-NMR results obtained by Horii et al. [103] for pure cellulose samples were in accordance with the ones obtained by X-ray Diffraction. The advantage of using NMR is that, to perform the analysis, a small quantity of sample without any previous preparation is needed; on the contrary, to measure the degree of crystallinity by XRD technique the sample must be milled. As it has been noted, the grinding often causes a decrease of the cellulose degree of crystallinity; nevertheless the milling is necessary to obtain homogeneous samples and reproducible measurements.

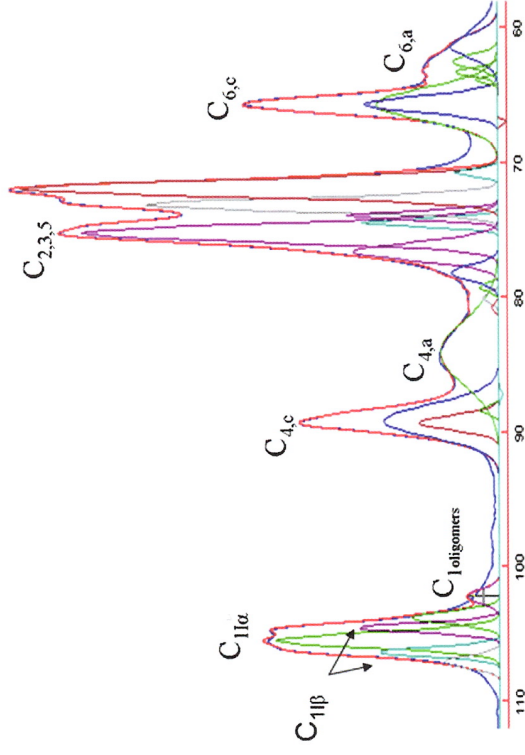


Fig. 5.21 Deconvolution of $^{13}\text{C}\{^1\text{H}\}$ CP MAS NMR spectrum of sample 1

Table 5.12 Peak areas of C1, α , C1, β , oligomers, C4,c and C4, α signals obtained from the spectral deconvolution

Sample	Carbonyl		C1, α		C1, β		C1, oligomers		C4,c 89 ppm	C4, α 84 ppm	DCry
	175 ppm	105 ppm	105 ppm	105 ppm	$I\alpha$	$I\beta$	103 ppm				
1	3.49	6.42 (44 %)	8.16 (56 %)	0.8	0.8	9.66	6.80	0.6	6.61	0.6	
2	–	6.41 (48 %)	6.86 (52 %)	0.9	0.1	10.21	6.61	0.6	9.29	0.5	
3	–	5.11 (35 %)	9.50 (65 %)	0.5	0.7	8.71	7.72	0.5	8.62	0.5	
4	–	7.58 (52 %)	7.01 (48 %)	1.1	0.9	10.05	5.90	0.6	8.33	0.6	
5	–	4.78 (36 %)	8.49 (64 %)	0.6	0.3	8.33	5.24	0.6	8.58	0.5	
6	–	5.50 (53 %)	4.88 (47 %)	1.1	0.6	8.33	5.24	0.6	8.58	0.5	
7	–	7.23 (57 %)	5.49 (43 %)	1.3	0.9	8.58	7.53	0.5			

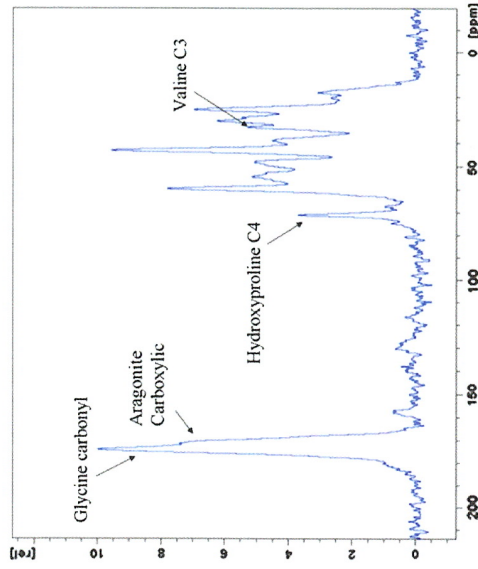
The relative composition of α and β polymorphous forms and the degree of crystallinity DCry are also reported

5.6.4 Analysis of NMR Spectra of Parchment Samples

$^{13}\text{C}\{^1\text{H}\}$ CP MAS NMR spectra were acquired on two parchment samples, A24 and A35, and on a sample of new parchment used as a reference. The spectrum of sample A24 is shown in Fig. 5.22.

The assignment of signals was carried out according to the literature [104]. The region from 185 to 160 ppm contains the carbonyl and carboxylic carbons of amino acid residues; in particular the most intense peak at 174 ppm is due to the

Fig. 5.22 ^{13}C $\{^1\text{H}\}$ CP MAS NMR spectrum of parchment sample A 24



glycine carbonyl carbon. The strong peak at 171 ppm is ascribed to aragonite. The weak peak at 169 ppm is ascribed to calcite. Aragonite and calcite, two common polymorphs of CaCO_3 present in most of the historical parchments are probably due to the reaction of the atmospheric CO_2 with $\text{Ca}(\text{OH})_2$ which was used to eliminate the fat from the skin. The weak peaks observed in the 127–138 ppm range are assigned to aromatic carbons of aromatic amino acids. The signals due to amino acids are observed in the aliphatic region (0–85 ppm), in particular the signal at 71 ppm is assigned to hydroxyproline C4, and the signal at 33 ppm is assigned to valine C3.

The spectrum of sample A35 reported in Fig. 5.23 shows the same features as those of sample A24; however, in the spectrum of sample A35, the peak at 33 ppm is more intense than expected. This peak is assigned to the methylene carbons (CH_2)_n of hydrocarbon chains of fatty acids. Besides, a rather sharp signal overlapped with the signal of C3 of alanine, is also observed at 18 ppm. This signal was ascribed to CH_3 groups of fatty acids. Furthermore, a peak at 185 ppm due to free fatty acids is present. It is not possible to determine the type of lipid present. The expanded aliphatic carbons region of both spectra is reported in Fig. 5.24.

A semiquantitative comparison of the lipid content in the two samples can be obtained from the ratio between the intensity of the resonance at 33 ppm (lipid), I_{33} , and the intensity of the resonance at 43 ppm I_{43} (collagen).

The I_{33}/I_{43} ratio for samples A24 and A35 was found to be 0.4 and 0.9, respectively. In the case of a new parchment sample collected as a reference, the I_{33}/I_{43} ratio was found to be 0.4, i.e. the same value obtained for sample A24. Ghioni suggested a relationship between the collagen degradation and the increase of lipid content [105]. He showed that new parchments contain small quantities of lipids usually acquired through microbial attack, besides it is possible that the lipid

Fig. 5.23 ^{13}C $\{^1\text{H}\}$ CP MAS NMR spectrum of parchment sample A 35

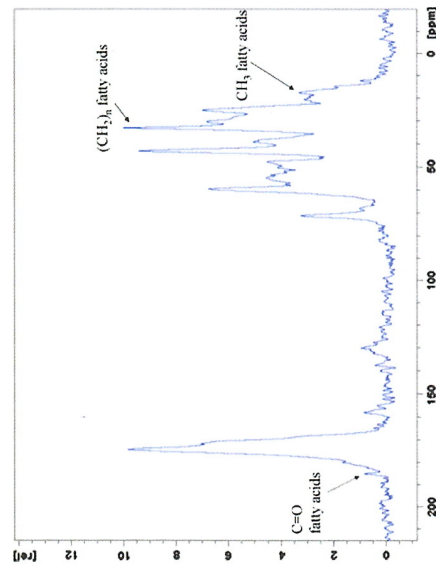
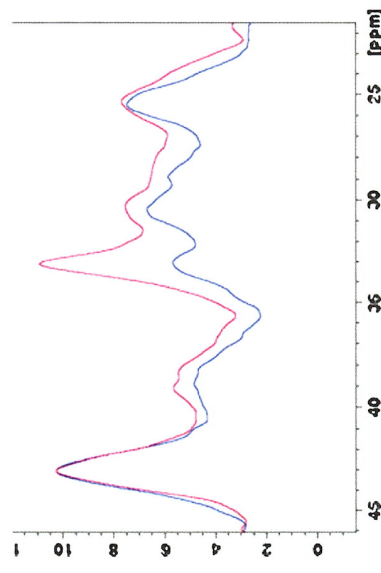


Fig. 5.24 Region between 20 and 45 ppm of the spectrum of sample A 24 (*bottom*) and sample A 35 (*top*)



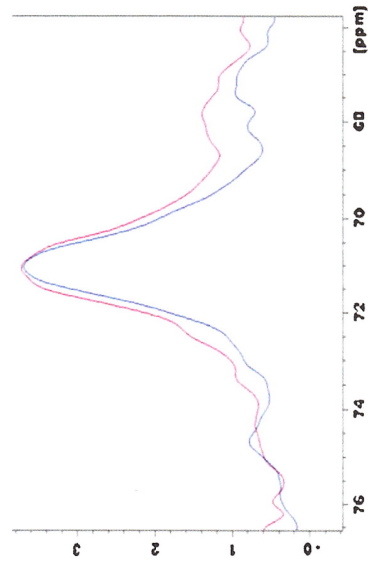
fraction of parchment causes with its peroxidation a generation of reactive oxygen species that can damage the collagen structure of parchment [105].

It has been shown that, as gelatinization evolves through hydrolysis of polypeptidic chains of collagen, a loss of structural order occurs causing a significant broadening of the lines in the solid state ^{13}C NMR spectra [104]. In this regard, a method which makes possible to investigate the state of conservation of parchment was introduced. This method consists in determining a parameter R obtained by measuring the line width of the peak at 71 ppm, due to C4-hydroxyproline. The spectral region between 66 and 76 ppm of samples A24 and A35 is reported in Fig. 5.25.

The parameter is calculated using the following equation [104]:

$$R = \frac{(\Delta\nu_{1/2} - \Delta\nu_{1/2}^c)}{(\Delta\nu_{1/2}^g - \Delta\nu_{1/2}^c)}$$

Fig. 5.25 Spectral region between 66 and 76 ppm of samples A24 (*bottom*) and A35 (*top*)



where $\Delta\nu_{1/2}$ is the line width of the signal at 71 ppm of the parchment under investigation, $\Delta\nu_{1/2}^0$ and $\Delta\nu_{1/2}^R$ are the line widths of the same signal measured in a sample of new parchment used as a reference and in gelatin, respectively.

By definition $R = 0$ in the case of new parchment, $0 \leq R \leq 1$ in the case of historical parchments in their pregelation state, with higher R values corresponding to a higher level of degradation. Compared to gelatin, hydrolyzed collagen is a mixture of smaller molecular weight polypeptides, as a consequence it can be considered as degraded gelatin, with $R > 1$ reflecting the extent of the disintegration relative to gelatin.

In samples A24 and A35, R was found to be 0.34 ± 0.03 and 0.47 ± 0.03 , respectively. As a consequence, it can be inferred that sample A24 is less degraded than sample A35.

This result is further confirmed by the presence of lipids that is evident in the NMR spectrum of the A35 sample. Lipids are not generally present in parchment; however, their presence may be a consequence of an incomplete cleaning or a bacterial degradation. This type of degradation can cause the breakdown of protein structures and the formation of protein–lipid aggregates. Furthermore, the contact with the skin of those who handled them, especially at the edges and the surface can be the cause of the observed lipids. Therefore, the source can be intrinsic, extrinsic or both.

The presence of lipids increases the speed of degradation of collagen because the lipid peroxidation in the presence of atmospheric SO_2 causes the hydrolysis of collagen. Therefore, this observation must be carefully taken into account especially in the consolidation phase of the material.

5.6.5 Conclusions

NMR technique is a powerful investigation tool in the field of Cultural Heritage artworks. Parameters which may be obtained with this technique are useful to get information on the state of conservation of various items, as shown in the case of

ancient paper and parchment. Analyses are performed on a small quantity (10 mg or less) of sample which can be utilised for further investigations as the method is not destructive. Besides, no treatment of samples is required.

The careful analysis of ^{13}C $\{^1\text{H}\}$ CP MAS NMR spectra produced information on the state of conservation of the sixteenth century book investigated.

It was demonstrated that the paper is in a good state of conservation, with only a small amount of oligomers due to the hydrolysis of cellulose. Only the first sample, taken from the first page of the book, shows an oxidative degradation. All samples show a good index of crystallinity.

Ss-NMR also provided information on the state of degradation of the two ancient parchment samples analysed. Parchment A35 is more degraded than parchment A24. The degradation observed in A35 is related to the presence of lipids clearly observable in the NMR spectrum.