High-Resolution Solid-State CPMAS NMR Study of Archaeological Woods

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High-resolution nuclear magnetic resonance of carbon-13 was employed to characterize archaeological wood samples from the 11th century excavation site in the lake Paladru at Charavines, France. Structural and chemical modifications were assessed by comparing the structural features of archaeological wood of beech with modern wood. The main results concern the strong decrease of a sugar moiety with a complete disappearance of hemicelluloses. During aging in a water environment, the β-O-4 interunit linkages of lignins have not been degraded. The analysis of the NMR spectra reveals that the residues occurring during depletion of sugars were not recombined inside the lignocellulosic matrix. Finally, in this article we illustrate the possibility of analyzing small amounts (4−6 mg) of starting material within reasonable experimental time using modern equipment.

The characterization and the preservation of archaeological woods have been active domains for fundamental and applied research since wooden artifacts are of first importance for recording human activity and technology through the ages. The most commonly used conservation treatment for waterlogged wood is the poly(ethylene glycol) (PEG) impregnation process which relies on the formation of hydrogen bonds between PEG and the carbohydrates of the wood.[1] Therefore, the content of carbohydrates in the starting archaeological material to be restored may significantly impact the success of any PEG treatment.[2]

Wood component analyses rely mostly on chemical methods requiring extensive chemical modifications necessary to obtain soluble fractions of the different components. Their compositions appear to be method dependent to some extent. A more attractive strategy for the characterization of such materials is to use techniques that are capable of providing detailed information directly on solid samples. High-resolution ^{13}C solid-state NMR with cross polarization and magic angle spinning (CPMAS) is such a technique and has been the object of several recent reviews.[3,4] Nevertheless, its impact on archaeology until recently has been minimal.[5,6] However, a few applications of solid-state NMR in the field of archaeology are given in two recent reviews in which the potential applications of the technique for quantitative analyses were also mentioned.[7,8] The aim of the present work is to show that by performing such quantitative treatments of spectra based on integration of specific areas of the different components, one can really obtain an objective view on the chemical and structural degradation of the material after aging in a water environment. To illustrate the strategy, archaeological and modern beech samples were analyzed. They were collected from the 11th century excavation site in the lake Paladru at Charavines, France.[9] Finally, we also present results obtained on modern 2.5-mm CPMAS probes, commercially available. Such probes require only minor amounts of starting material, 4−7 mg of dry wood to fill 11-μL rotors, whereas 300−400 mg of material is necessary to fill, for instance, 340-μL rotors of 7-mm CPMAS probes that have been widely used for structural studies of woods and wood products.

EXPERIMENTAL SECTION

Sample Preparation. Beech archaeological samples were collected from the 11th century excavation site in the lake Paladru at Charavines, France. Timbers for housing are made of oak and beech. Two archaeological beech samples with different degrees of deterioration as indicated by their water contents, respectively, 82% and 91%, were retained for NMR analyses. Water contents based on wet wood were obtained by weighing the wood sample before and after oven drying at 105 °C.

NMR Experiments. The samples were prepared by grinding a predetermined amount of dry wood in order to reduce them into powders. 7-mm-diameter (340-μL) cylindrical double-bearing rotors made of zirconia were filled with the dried sample (300−

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400 mg) retained with Kel-F end caps. Spectra were also recorded on a 2.5-mm-diameter (11-μL) cylindrical double-bearing rotor of zirconia filled with only 4.5 mg of powder.

High-resolution solid-state ^{13}C NMR spectra using proton dipolar decoupling (DD), magic angle spinning (MAS) and cross-polarization (CP) were recorded on a Bruker Avance 400-MHz spectrometer operating at 100.6 MHz at room temperature. The ^{1}H radio frequency field strength was set to give a 90° pulse duration around 2.5 μs. For each spectrum, 1600 scans were collected. When the 2.5-mm probe was used, 8000 transients were coadded. The chemical shift values were measured with respect to tetramethylsilane via glycine as a secondary substitution reference with the carbonyl signal set at 176.03 ppm. The amplitude of the ^{13}C NMR signal in a CP experiment was dependent on the contact times.[10] To get quantitative data, dynamic studies of the carbon magnetization buildup were performed by varying the contact values from 10 μs to 20 ms. From the variable contact time experiments, the different signal intensities were plotted versus the contact times, t_{cp}, and fit to the classical and simplified equation,

$$M = M_0(1 - \exp(-t_{cp}/T_{CH})) \exp(-t_{cp}/T_{1\rho H})$$

where M is the measured intensity, M_0 is the initial intensity of magnetization, and T_{CH} and $T_{1\rho H}$ are two characteristic relaxation times of the system. From the above equation it is possible to extract M_0. M_0 is directly proportional to the number of spins, and consequently, it can be used for quantitative analysis. Unfortunately, this parameter can never be measured directly and the values M_0, T_{CH}, and $T_{1\rho H}$ were computed by fitting the experimental intensities M recorded at different contact times t_{cp} with the above analytical expression.

Therefore, for a given contact time, the correction factors to be applied on the different signal intensities and their corresponding integrals are easily calculated. It appears that a spin lock time of 1 ms is optimal for reaching the maximal polarization of all the wood carbons and that the maximum expected errors for the signal intensities are below 5%. The correction factors can be even be lower if one considers longer t_{cp} such as 3−5 ms, but the price to pay is a significant decrease in signal-to-noise ratio. Therefore, a 1-ms contact time was considered as a good compromise and no correction was applied to the measured integrals in the present case.

RESULTS AND DISCUSSION

Wood is a complex natural composite material, which contains three major components: cellulose (40−45%), a poly-β(1−4)-D-glucopyranosyl linear polymer; hemicelluloses (20−30%) in which, for beech, the poly-β(1−4)-D-xylopyranosyl polymer substituted by O-acetyl groups, is the major compound; and lignin (20−30%) a polyaromatic three-dimensional amorphous polymer made of phenylpropane units linked through ether bonds (Figure 1). The phenyl rings usually have methoxy substituents. Cellulose represents the crystalline part (fibrils) of wood, which is held together by the hemicelluloses and the lignins. The main function of
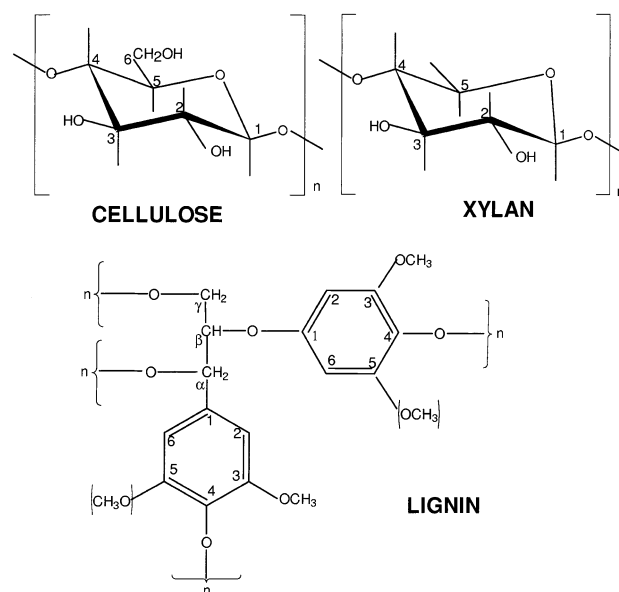
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Figure 1. Formulas of the main components of wood.

Table 1. Resonance Assignment of ^{13}C CPMAS Spectrum of Modern Beech

resonance number	chemical shift (ppm)	assignments[a]
1	172	carbohydrate: -COO−R, CH$_3$-COO-
2	152.6	lignin: S3(e), S5(e)
3	147	lignins: S3(ne), S5(ne), G1, G4
4	136	lignins: S1(e), S4(e), G1(e)
5	134.3	lignins: S1(ne), S4(ne), G1(e)
6	121	lignin: G6
7	114−106	lignins: G5,G6,S2,S6
8	104.8	carbohydrates: C1
9	88.7	carbohydrates: C4
10	83.8	lignins: Cβ. carbohydrates: C4
11	74.75	lignins: Cα. carbohydrates: C2,3,5
12	72.17	carbohydrates: C2,3,5
13	64.7	carbohydrates: C6
14	61.58	lignin;s Cγ
15	55.65	lignins: OCH$_3$
16	21	carbohydrates: CH$_3$−COO-

[a] Abbreviations: S, syringyl (aromatic unit with two methoxy groups); G, guaiacyl (aromatic unit with only one methoxy); ne, in nonetherified arylglycerol β-aryl ethers; e, in etherified arylglycerol β-aryl ethers.

hemicelluloses and lignins is to buttress the fibrils. Degradation of any of these wood constituents results in a decrease in the strength of the material.

Qualitative Analyses of the ^{13}C NMR Spectra. The ^{13}C CPMAS spectrum of both modern and archaeological beech woods are given in Figure 2.

(a) Modern Wood. The modern wood leads to record spectrum A, which presents the expected signals for such lignocellulosic materials, and it can be completely assigned on the basis of data reported in the literature data.[5,9,11,12] Table 1 summarizes the general assignments of the ^{13}C CPMAS spectrum of the main signals for modern beech wood. The main features of such spectra are the following:

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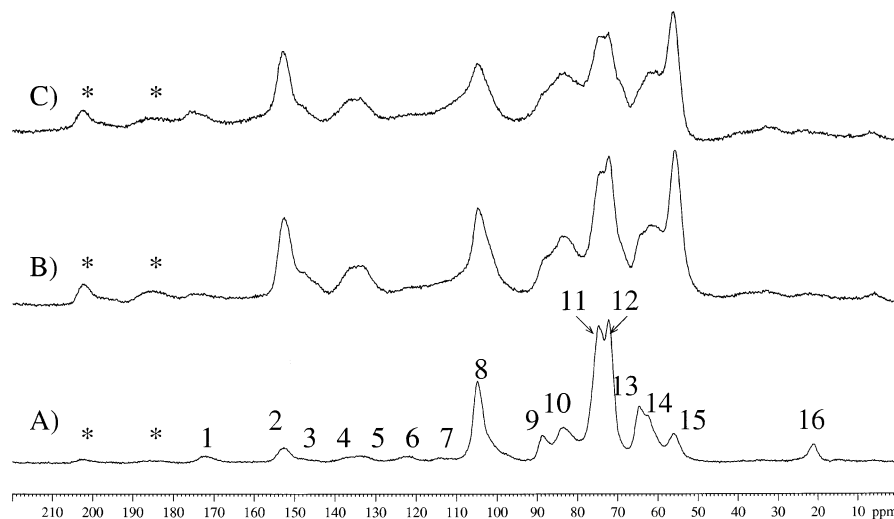
Figure 2. 100-MHz CP/MAS ^{13}C high-resolution solid-state NMR spectra of beech: moderm wood (A) and archaeological samples with water contents of 82% (B) and 91% (C), respectively.
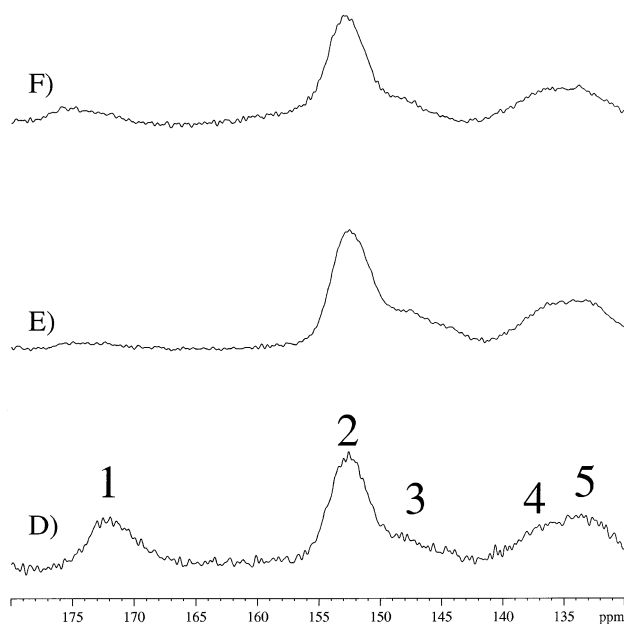

Figure 3. 100-MHz CP/MAS ^{13}C high-resolution solid-state NMR spectra of beech, enlargement of the region from 125 to 180 ppm: modern wood (D) and archaeological samples with water contents of 82% (E) and 91% (F), respectively.

Signal 16 at 21 ppm is assigned to the CH_3 carbon of the acetyl group in hemicelluloses. The region between 50 and 105 ppm is dominated by strong signals, 8−14, which are assigned mostly to the different carbons of celluloses. Hemicelluloses also give signals in this region. An interesting point is the presence of two signals 9 and 10 at 88.7 and 83.8 ppm assigned to the C-4 of cellulose allomorphs. The signal at 83.8 ppm is indicative of amorphous cellulose or less ordered surface cellulose molecules. These signals overlap those belonging to the different aliphatic carbons belonging to lignins. In this region, the only signal that can be mainly assigned to lignin is resonance 15 at 55.65 ppm belonging to the methoxy group of aromatic moieties. The presence of methoxy on sugars is known but should not be a significant contribution.

The region at a field lower than 105 ppm and below 160 ppm is specific for the aromatic carbons of lignins. The following assignments are generally proposed and are supported by numerous works: signal 2 at 152.4 ppm to C-3 and C-5 in syringyl involved in β-O-4 structures.

On the low-frequency side of the previous signal, the shoulder 3 is mainly assigned to the same carbons in nonetherified structures. The relative intensities of these two broad signals lead one to evaluate the depletion of the β-O-4 linkages inside the lignin polymers.

At 172 ppm appears signal 1 of carbonyls in acetoxy groups of hemicelluloses; this signal could also be due to acid groups present in wood.

On this spectrum, the two first-order spinning sidebands, indicated by the asterisk, of signals 2−5 clearly appear.. They are separated from their respective isotropic signals by the frequency of the spinning rate (5000 Hz). They are easily identified since their position on the spectra will vary with the spinning rates. Such signals have to be carefully assigned in order not to be assigned to isotropic chemical shifts. In the present case, the low-frequency spinning sidebands overlap with signals 8−10 respectively assigned to C-1 and C-4 of cellulose. Therefore, they contribute to increasing the integrals of the corresponding signals. This point must be taken into consideration to carry out quantitative treatments of these spectra.

(b) Archaeological Wood. The corresponding spectra B and C of the two representative samples of waterlogged wood are shown in Figure 2. The drastic differences in the signal intensities of these spectra relative to the spectra of modern wood are easily noted.

Signal 16 at 21 ppm assigned to the CH_3 of hemicelluloses completely disappears in both archaeological samples. However, signal 1 at 172 ppm, assigned mainly to the carbonyl of the acetoxy group in hemicelluloses, does not completely disappear. This indicates that it also originates from other types of carbonyls. Concerning the relative amounts of carbohydrates and lignins, if one uses signal 8 at 104.8 ppm assigned to the C-1 of cellulose as a reference, it clearly appears that the signals of lignins have much
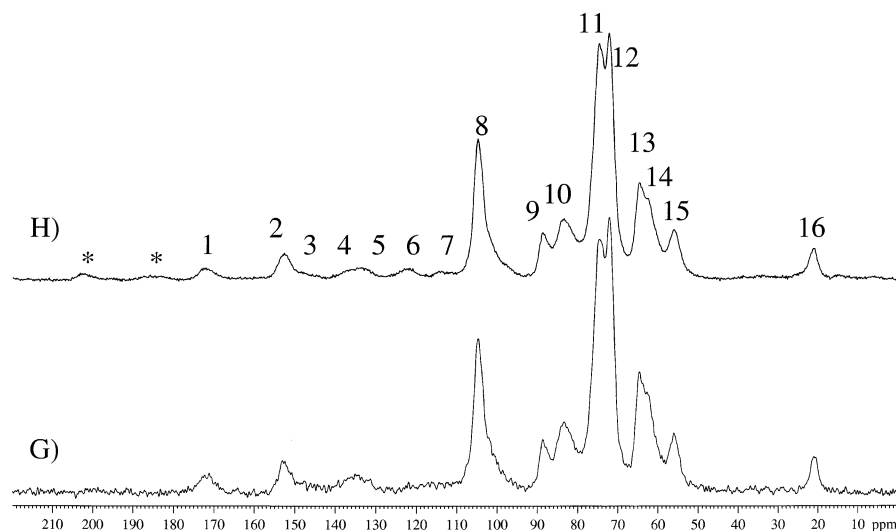
Figure 4. 100-MHz ^{13}C high-resolution solid-state NMR spectra of modern beech: classical 7-mm probe with 340-μL rotors and spinning rate of 5000 Hz (G) and 2.5-mm CPMAS probe with a 11-μL rotor and a spinning rate of 26 000 Hz (H). A total of 1600 and 8000 transients were respectively collected.

Table 2. Quantitative Treatment of CPMAS Spectra of Modern and Archaelogical Beech Woods with Water Contents of 82% and 91%, Respectively

sample of wood	integrals (in %)		carbons (in %)		weight (in %)	
	A_{high}	A_{low}	lignins	carbohydrates	lignins	carbohydrates
modern	14	86	23.8	76.2	18.5	81.5
arch. (82%)[a]	34.5	65.5	60.5	39.5	52.7	47.3
arch. (91%)[a]	34.3	65.7	60.3	39.7	52.5	47.5

[a] Water content based on wet sample.

higher intensities than in the modern wood. These spectra demonstrate the depletion of carbohydrates under these aging conditions.

The largest decrease is observed for the most degraded sample (Figure 1C) if one referred to its water content (91%); nevertheless, the differences between the two archaeological samples are not very large. Concerning the lignin moieties, some interesting observations can be done by comparing for the three samples the enlargements, shown in Figure 3, of the region ranging from 125 to 180 ppm. Signal 2 at 152.6 ppm and its low-frequency shoulder 3 respectively assigned to syringyl moieties in etherified and nonetherified structures (β-O-4) remains almost identical in the three samples. It demonstrates that these main interunit linkages were not degraded during aging.

Very surprisingly, although we observe very strong changes in the intensities of signals of carbohydrates relative to lignins, the line widths of their different signals do not change significantly. This is a strong indication that while the aging conditions induced strong depletion of the carbohydrates, no chemical rearrangements inside the biopolymers network occur. If such chemical rearrangements had occurred, they would have given new chemical species with specific NMR signals and important line broadening of the signals, due to chemical shift dispersion.[13,15] Such

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behaviors are widely observed in CPMAS spectra of lignocellulosic materials after chemical treatments and molecular rearrangements occurring in pulping processes.

Results Obtained with the 2.5-mm CPMAS Probe. The major drawback of qualitative analyses based on solid-state NMR is that up to now it has required 300–500 mg of material to fill the 340-μL rotors in order to record spectra with a reasonable signal-to-noise ratio. It can be definitively a problem if the amount of material available is a limiting factor. For the last five years, CPMAS probes have been commercially available that required much smaller amounts of material since the volume of rotor to be filled is 11 μL. Figure 4 shows the quality of the spectra that can be recorded with such probe. The price to pay is longer acquisition time: 3 h for the spectrum recorded with the 340-μL probe whereas with the 11-μL probe the spectrum was recorded during 16 h. Nevertheless, the signal-to-noise ratio obtained with the 11-μL probe is much higher than could be expected on the basis of theoretical considerations: signal-to-noise ratio is directly proportional to the amount of material, but it also is proportional to the square root of the number of coadded transients. The excellent sensitivity of such probes is mainly due to the fact that the coils of the probes are designed to work with small volumes and that the smaller sizeed rotors allow use of very high spinning rates, up to 32 000 Hz. Note that the spinning sidebands have completely disappeared, allowing a direct interpretation of the NMR signals. However, with such probes, quantitative treatments remain a problem since the polarization of the carbons not only

depends on the type of carbons but also on the spinning rates, making the magnetization buildup difficult to compute with simple analytical functions. Therefore, for routine quantitative analyses, the classical probes still have to be employed.

Quantitative Data. Quantitative treatment of the spectra can be carried out provided that the following precautions are taken. As mentioned previously, the spinning sidebands of aromatic carbons have to be considered in integrals. The spinning rate used to record the spectra was set to 5000 Hz; therefore, the spinning sidebands of the aromatic carbons, mostly signals 2 and 3, appear at high frequency in a region without any other signals that facilitate their integration. On the other hand, the low-frequency spinning sidebands appear in the area assigned to carbohydrates and will contribute to the enhancement of the area of this region. Therefore, for quantitative treatment, the integrals of the two high-frequency spinning sidebands were added twice to the area assigned to lignins and subtracted once from the area of carbohydrates; the corrected integrals are noted A_{high} and A_{low}, respectively.

The measured integrals can be either directly compared or used in the formulas proposed by Haw et al.[16] that allow one to calculate the percentages either in carbons or in weight by taking into account the average empirical formula of lignins and sugars. In our case, we used the formula $C_9H_{7.72}O_{2.75}(OMe)_{01.53}$ proposed for hardwood lignins with a molecular mass of 207 g mol^{-1} and for sugars the formula $C_6H_{10}O_5$ was assumed with a molecular mass of 183 g mol^{-1}.

Quantitative results obtained from the different spectra are reported in Table 2.

On one hand, they confirm the qualitative analyses performed previously which indicated the drastic depletion of carbohydrates during the water immersion. On the other hand, the quantitative analyses show that the chemical compositions of two types of archaeological wood, based on their water content, are not significantly different. This point is interesting to note since it illustrates that one has to be careful when inferring structural or chemical features of archaeological woods on only their water content. It is consistent with the results reported by De Jong[17] and Hedges[18] that show a large dispersion of data when the contents of lignins and celluloses are respectively correlated with the water contents of the wood samples. Quantitative analyses carried out from NMR spectra rely on integration of NMR signals recorded under appropriate conditions. The weak point of the strategy when working with complex material, such as modified wood, is that any errors in the assignment of signals will lead one to overestimate or to underestimate the corresponding component. It is certainly the main reason to explain the differences, on the order of 5%, which are generally observed between analyses established with NMR and with chemical methods. Nevertheless, as NMR measurements are very reproducible, the method is a powerful tool to compare structural features of samples analyzed under similar conditions. Moreover, chemical methods as mentioned in the introduction are not at all free of errors, especially when working on a strongly modified wood sample.

CONCLUSIONS

These results clearly show that under the aging conditions probably in a saturated water environment the degradation of cellulose and other sugars was enhanced. This is particularly evident for hemicelluloses since its characteristic signal at 21 ppm is no longer visible in the corresponding spectra. On the other hand, the overall structure of lignins does not change; the main β-O-4 interlinks are still present even in the sample that has be at the margin of the object. It is an indication that the storage conditions lead to strong degradation of celluloses and other sugars but do not affect lignin moieties. As no meaningful changes in the overall aspect of the NMR spectra of lignins was detected, especially in their line widths, the possible formation of lignin-like compounds arising from the degradation of sugars can be definitively ruled out.

Due to the high solubility of monosaccharides and their oligomers, the presence of water all around the wood certainly favored their regular leaching from the wood in the environment. From a technical point of view, this paper shows that quantitative analyses by solid-state ^{13}C NMR should become a classical tool to characterize archaeological lignocellulosic materials and should bring complementary data to those obtained with conventional chemical analyses. Finally, a very promising aspect was illustrated by recording high-quality spectra with the new commercially available 2.5-mm probes, which require only 6−10-mg amounts of wood. It makes this technique competitive for work on precious archaeological artifacts.

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