Available online at www.sciencedirect.com

SCIENCE DIRECT®

Journal of Magnetic Resonance 171 (2004) 43–47

JMR
Journal of Magnetic Resonance

www.elsevier.com/locate/jmr

Communication

The 2D MAS NMR spin-echo experiment: the determination of ^{13}C–^{13}C *J* couplings in a solid-state cellulose sample

Steven P. Brown[a,*], Lyndon Emsley[b]

[a] *Department of Physics, University of Warwick, Coventry CV4 7AL, UK*
[b] *Laboratoire de Chimie, UMR 5182 CNRS/ENS, Laboratoire de Recherche Conventionné du CEA (23V),*
Ecole Normale Supérieure de Lyon, 69364 Lyon, France

Received 13 April 2004; revised 26 July 2004
Available online 1 September 2004

Abstract

A simple ^{13}C MAS spin-echo experiment is applied to a partially ^{13}C-labelled cellulose sample extracted from wood. ^{13}C–^{13}C *J* couplings are determined even though considerable chemical disorder leads to observed linewidths in the normal 1D ^{13}C CP MAS spectrum which far exceed the *J* couplings. The fitting of the experimental data also allows the quantification of the degree of isotopic enrichment.

Keywords: MAS; Spin-echo; *J* couplings; Cellulose; ^{13}C

1. Introduction

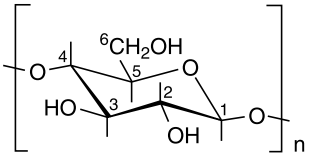
Solid-state NMR is well-suited to the investigation of disordered samples, e.g., natural or synthetic polymers, because of its applicability to systems lacking long-range order [1]. In solid-state NMR, disorder does, nevertheless, lead to a distribution of chemical shifts, which has the effect of often seriously reducing the resolution in a normal one-dimensional spectrum. In particular, splittings due to *J* couplings are usually obscured. In solution-state NMR, such *J* couplings provide valuable structural information about, for example, bonding types or conformations. In this communication, the application of a simple magic-angle spinning (MAS) spin-echo experiment to a partially ^{13}C labelled cellulose sample extracted from wood is described. It is shown that spectral broadening due to disorder in the normal ^{13}C cross-polarisation (CP) MAS spectrum does not preclude the determination of specific ^{13}C–^{13}C *J* couplings.

In the early 1980s, ^{13}C CP MAS NMR spectra contributed much to the understanding of the complex solid-state structure of cellulose [2–4]. Twenty years on, unanswered questions remain, and the investigation of cellulose structure is a subject of ongoing current research, both by solid-state NMR [5] and other methods such as X-ray and neutron fibre diffraction [6]. In this communication, we investigate a cellulose (**1**) sample extracted from a sample of wood, which was grown in 20% $^{13}CO_2$ atmosphere, such that the carbons sites are partially ^{13}C labelled [7]. It has been shown that the carbon–carbon connectivities in this sample can be mapped out in a two-dimensional ^{13}C refocused INADEQUATE experiment, whereby the correlations are established by means of the through-bond ^{13}C–^{13}C *J* couplings [8]. Specifically, we and co-workers have recently demonstrated that chains of correlated chemical shifts are revealed in a two-dimensional ^{13}C refocused INADEQUATE spectrum of the cellulose sample provided that a combination of a sufficiently fast MAS

---

[*] Corresponding author.
E-mail addresses: S.P.Brown@warwick.ac.uk (S.P. Brown), Lyndon.Emsley@ens-lyon.fr (L. Emsley).

1090-7807/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.jmr.2004.07.022



1

frequency and efficient heteronuclear ^{1}H decoupling is employed [9]. Similar two-dimensional ^{13}C refocused INADEQUATE spectra have also recently been obtained by Kono et al. [10] for *Cladophora* and tunicate celluloses at natural abundance.

Homonuclear *J* couplings can be measured in solid-state NMR using a simple MAS spin-echo experiment, and various applications to the determination of ^{31}P–^{31}P *J* couplings in inorganic and organometallic systems have been presented [11–14]. Moreover, it has been further shown that the ^{15}N MAS spin-echo method allows ^{15}N–^{15}N hydrogen-bond mediated *J* couplings to be measured with a very high precision such that differences in hydrogen-bonding strength can be identified [15]. Recently, we and co-workers have presented a detailed analysis of the MAS spin-echo experiment, which combines a theoretical treatment, numerical simulations and experimental data for model systems [16]. This study demonstrated, for the case of a homonuclear pair of dipole–dipole coupled nuclei, that the dominant spin-echo modulation frequency is exactly equal to the *J* coupling in nearly all cases, with the chemical shift anisotropies and dipole–dipole couplings actually stabilising rather than obscuring the *J*-modulation. The partial ^{13}C labelling of the cellulose sample means that the spin-pair treatment of [16] is valid here for nearly all subunits containing more than one ^{13}C spin.

A key feature of the spin-echo sequence is that it refocuses the evolution of all terms that appear as offsets, in particular those due to a distribution of chemical shifts. Importantly, in the solid state, the "non-refocusable" linewidth, $\Delta' = 1/\pi T'_2$, which is defined by the time constant, T'_2, measured in a spin-echo experiment, is usually significantly less than the "apparent" linewidth [8,17,18]. Thus, the incorporation of spin echoes into solid-state NMR experiments enables the detection, utilisation, and quantitative determination of *J* couplings, *even if no splitting can be observed in the normal spectrum*. The ability to extract ^{13}C–^{13}C *J* couplings for the cellulose sample studied here relies on this important fact.

2. Experimental details

The preparation of the cellulose sample is described in [7].

Solid-state NMR experiments were performed on a Bruker DSX 500 NMR spectrometer operating at ^{1}H and ^{13}C Larmor frequencies of 500.1 and 125.8 MHz, respectively. A 4 mm triple-resonance probe (operating in double-resonance mode) was used. The sample volume was restricted by flat spacers to the central third of the rotor. A MAS frequency of $\nu_r = 12.0$ kHz was employed. The ^{1}H and ^{13}C 90° pulse lengths were 2.5 and 4.0 μs, respectively. The pulse sequence employed consisted of ramped cross-polarisation [19,20] from ^{1}H to ^{13}C followed by a simple Carr–Purcell spin-echo (τ–π–τ) where the spin-echo delay τ is an integer number of rotor periods, with ^{1}H TPPM decoupling [21] at a rf field of $\nu_1 = 100$ kHz being applied during both the spin-echo and the acquisition time, t_2. The CP contact time was 1.5 ms. The recycle delay was 6 s. For each τ value, 32 transients were co-added. An 8-step phase cycle was employed to select $\Delta p = \pm 1$ (2 steps) and ± 2 (4 steps) on the ^{1}H $\pi/2$ and ^{13}C π pulses, respectively (Δp refers to the change in coherence order, p). The pulse program is available from our website [22] or upon request.

3. Results and discussion

A ^{13}C CP MAS spectrum of the cellulose sample recorded at a MAS frequency of $\nu_r = 12.5$ kHz is shown at the top of Fig. 1(see Fig. 2b of [18] for experimental details). Four broad resonances are observed which can be assigned to the C1 (100–110 ppm), C4 (80–90 ppm), C2 & C3 & C5 (70–80 ppm), and C6 (60–70 ppm) resonances, respectively. Fine structure is seen within each resonance; this indicates the presence of both amorphous (e.g., the broad resonances at 83 and 65 ppm) and crystalline regions (e.g., sharp peaks at 107.3, 105.1, 89.0, 87.7, 76.8, 75.1, 73.0, 63.2, and 62.6 ppm) within the sample. (Note that there is a referencing error in Fig. 3 of [9].) Two-dimensional ^{13}C spin-diffusion experiments have shown that the crystalline and amorphous regions are intimately mixed. [23].

Fig. 1 plots the dependence of the integrated frequency-domain signal, $S(\tau)$, on the spin-echo delay, τ, for the four broad resonances in the normal ^{13}C CP MAS spectrum. All $S(\tau)$ data sets have been normalised such that the first point is set equal to unity. Similar spin-echo curves obtained at the lower MAS frequency of 6 kHz were presented in Fig. 4a of [8]. In that case, however, the focus was on determining the non-refocusable linewidths which are characterised by the time constants, T'_2, such that the data were fitted to a simple exponential function. A close inspection of Fig. 4a of [8] reveals that there appears to be an additional modulation to that described solely by the simple exponential function. This modulation is even more marked in Fig. 1. This additional modulation is

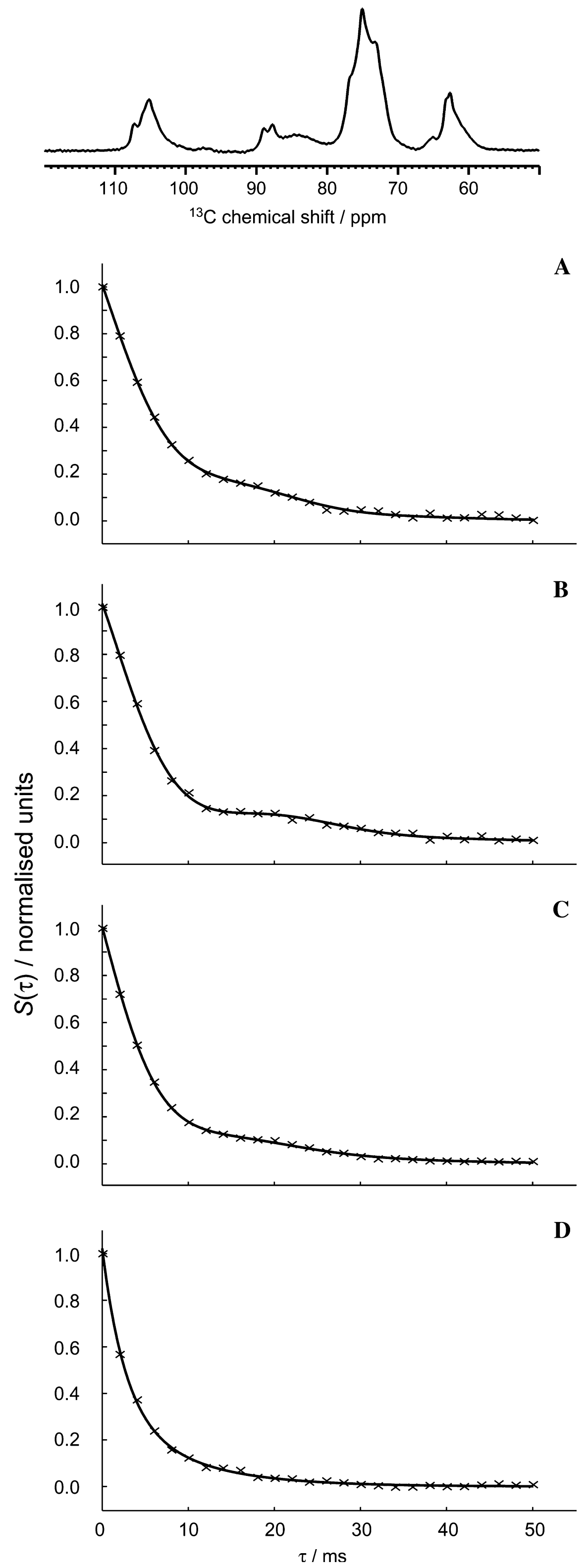


Fig. 1. Plots of $S(\tau)$ against τ for the (A) C1 (95–115 ppm), (B) C4 (80–95 ppm), (C) C2, C3, & C5 (67–80 ppm), and (D) C6 (55–67 ppm) resonances of cellulose, together with the best-fit analytical curves (see Table 1). Note that spectral noise corresponds to no more than 1% of the maximum integrated intensity. A ^{13}C CP MAS spectrum of the cellulose sample (ν_r = 12.5 kHz) is shown at the top (see Fig. 2b of [18] for experimental details).

a consequence of the partial ^{13}C labelling of the carbon sites in the cellulose sample.

For example, consider first the resonance peak in the ^{13}C spectrum due to C1, where the C1 carbon is directly bonded to only one other carbon, C2. Let us assume that all carbon sites are ^{13}C labelled to the same degree, such that a proportion p are NMR-active ^{13}C nuclei, while $(1 - p)$ are NMR-invisible ^{12}C nuclei. Considering the C1 ^{13}C nuclei, a proportion p will have a C2 ^{13}C neighbour, while a proportion $(1 - p)$ will have a C2 ^{12}C neighbour. Let us further assume that ^{13}C–^{13}C J-couplings over two- or more bonds are negligible and that the dephasing of $S(\tau)$ can be described by a mono-exponential function. It is then apparent that, while the spin-echo curve for the proportion $(1 - p)$ having a C2 ^{12}C neighbour is described by a simple exponential function, for the proportion p having a C2 ^{13}C neighbour, it is necessary to additionally consider the modulation due to the ^{13}C–^{13}C J coupling, namely $\cos(2\pi J\tau)$. The solid-line in Fig. 1A corresponds to the best-fit (see Table 1) to a five-parameter function based on the above arguments. It is evident that the experimental data are very well fit by this function.

Fig. 1B displays the spin-echo data for the C4 resonance. Even though the C4 carbon is directly bonded to two carbons, an excellent fit (see Table 1) of the experimental data to the same five-parameter function used before is again obtained. Such a fit only considers the C4 ^{13}C carbons having either no or one ^{13}C neighbour, i.e., it neglects the contribution of the small proportion of C4 ^{13}C carbons having two ^{13}C neighbours (at both C3 and C5 positions). It also does not take into account the possibility that the C3–C4 and C4–C5 ^{13}C–^{13}C J couplings are different. However, attempts to fit the data to more complex functions failed—it seems that five independent fit parameters is the limit. The spin-echo data in Fig. 1C for the overlapping resonances due to the C2, C3, and C5 carbons, which all have two carbon neighbours, is also well fit to the five-parameter function.

The fitted parameter values are given in Table 1 for the three cases discussed above. The fitted α values indicate that about 20% of all carbons are ^{13}C labelled—note that the α values for the carbons having two carbon neighbours (C4 and C2&C3&C5) are approximately double that for the case of carbon C1 which has only one carbon neighbour. This is what is predicted by basic probability theory: the proportion, α, of ^{13}C carbons having one ^{13}C neighbour is equal to p and $2p(1 - p)$ for a carbon bonded to one and two carbons, respectively. It is to be noted that the as-determined proportion of carbons which are ^{13}C labelled, namely 20%, corresponds to the 20% atmosphere ^{13}CO$_2$ in which the cellulose sample was prepared. It is, however, different to the previously stated overall ^{13}C enrichment of 11%. [7] In this respect, it is to be remembered that the solid-state NMR method here is measuring a conditional

Table 1
Parameters extracted from the fits to the spin-echo spectra

Site	α	T'_{2a} (ms)	T'_{2b} (ms)	^{1}J (Hz)	$[\varepsilon^2]$[a]
1[b]	0.18 ± 0.02	20 ± 1	16 ± 2	45.0 ± 1.9	0.0007
4[b]	0.38 ± 0.03	24 ± 1	16 ± 1	37.6 ± 1.1	0.0008
2&3&5[b]	0.43 ± 0.02	21 ± 1	11 ± 1	38.3 ± 1.3	0.0002
6[c]	0.67 ± 0.02	18 ± 2	5 ± 1	—	0.0008

[a] $\varepsilon^2 = \sum [I_{\text{fit}}(n) - I_{\text{exp}}(n)]^2 / \sum I_{\text{exp}}(n)^2$.
[b] Fit to $A[(1-\alpha)\exp(-2\tau/T'_{2a}) + \alpha\cos(2\pi J\tau)\exp(-2\tau/T'_{2b})]$.
[c] Fit to $A[(1-\alpha)\exp(-2\tau/T'_{2a}) + \alpha\exp(-2\tau/T'_{2b})]$.

probability, namely the proportion of carbons which are ^{13}C labelled for those subunits where there is at least one ^{13}C. Therefore, a possible explanation of the anomaly is that the wood sample contains regions at both natural abundance and at 20% ^{13}C enrichment. Further inspection of Table 1 reveals that the time constant characterising the spin-echo decay is shorter in all three cases for the proportion, α, of ^{13}C carbons having a directly bonded ^{13}C neighbour. This observation is consistent with the expectation that the presence of a ^{13}C–^{13}C dipole–dipole coupling leads to faster dephasing.

Consider now the fitted ^{13}C–^{13}C J couplings. From an analysis of a two-dimensional INADEQUATE ^{13}C spectrum of the cellulose sample in N,N-dimethylacetamide-d$_9$/LiCl solution, it was possible to determine ^{13}C–^{13}C J couplings of 44.5 and 36 Hz for the C1–C2 and C5–C6 pairs, respectively. [7] It is then evident that the fitted solid-state value for the C1 site in Table 1 is in excellent agreement with the solution-state data. While the other two extracted J couplings cannot be assigned to a specific pair of carbons, it is to be noted that the values are significantly different to that for the C1 site and are similar to the solution-state value for the C5–C6 pair. It may be possible to determine the separate J couplings by combining the spin-echo experiment with a double-quantum filter, such that only the signal due to molecules containing two or more neighbouring ^{13}C nuclei is observed. [24]

In Fig. 2, modified spin-echo data, $S'(\tau)$, obtained by subtracting the fitted contribution of ^{13}C nuclei having no ^{13}C neighbours from the experimental $S(\tau)$ data, is plotted against τ. In this way, the zero-crossings characteristic of the $\cos(2\pi J\tau)$ modulation are clearly revealed. The observation of well-defined J modulations for the C1 to C5 resonances suggests that the ring structure in the studied cellulose sample is rigid. However, it is to be noted that the employed spin-echo approach relies on an integration of the signal intensity—it is thus not possible to distinguish between the overlapping crystalline and amorphous peaks noted above. Therefore, it may be that there is a distribution of J couplings associated with conformational disorder in the amorphous regions, and this may simply be obscured by the dominant J modulation arising from the crystalline region.

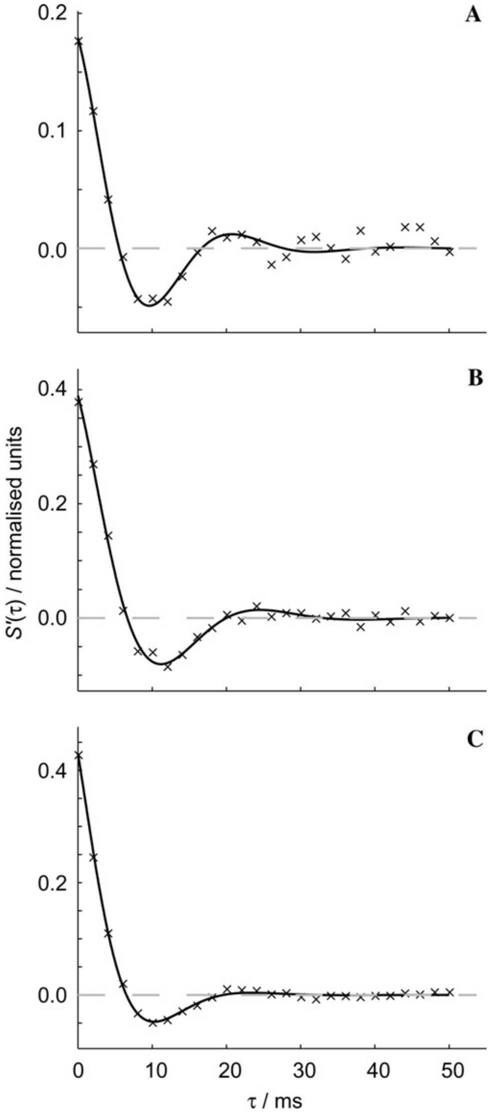


Fig. 2. The modulation by the ^{13}C–^{13}C J couplings is revealed by subtracting $A(1-\alpha)\exp(-2\tau/T'_{2a})$ (where A, α, and T'_{2a} are fitted parameters—see Table 1) from the experimental $S(\tau)$ data. Plots of this modified spin-echo data, $S'(\tau)$, against τ are shown for the (A) C1 (95–115 ppm), (B) C4 (80–95 pm), and (c) C2, C3, & C5 (67–80 ppm) resonances, together with the best-fit analytical curves corresponding to the $A\alpha\cos(2\pi J\tau)\exp(-2\tau/T'_{2b})$ term (see Table 1).

For the C6 carbon, it was not possible to determine the ^{13}C–^{13}C J coupling to the C5 carbon. Instead the fitting (see Table 1 and Fig. 1D) revealed the presence of two components exhibiting mono-exponential decay characterised by significantly different time constants.

The same observation has also been made by Earl and VanderHart for a cellulose sample at natural abundance in ^{13}C. [3] We tentatively assign the two components to the crystalline and amorphous regions. The failure to observe the ^{13}C–^{13}C J coupling to the C5 carbon could be due to conformational disorder (for the crystalline as well as the amorphous regions) associated with the orientation of the C6–OH linkage with respect to the ring. We are currently pursuing further experiments to explore this hypothesis, for example, a three-dimensional refocused INADEQUATE experiment, whereby the third time dimension corresponds to the incrementing of one of the spin-echo periods. In this way, we hope to observe different J couplings to C6 for the different chains of correlated chemical shifts observed in the two-dimensional refocused INADEQUATE spectrum in [9].

To conclude, this communication has shown that ^{13}C–^{13}C J couplings can be extracted using a simple MAS spin-echo approach for a solid-state cellulose sample exhibiting considerable spectral broadening due to the presence of both crystalline and amorphous environments. The method also allows the quantification of the degree of isotopic enrichment. This approach is applicable to other polymeric systems, be they natural or synthetic, with one potential application being the study of delocalisation in conjugated polymers.

Acknowledgments

S.P.B. was supported by a (E.U.) Marie Curie Fellowship (Contract Number HPMFCT-2000-00525). S.P.B. thanks the EPSRC for the award of his current advanced research fellowship. We are grateful to Michel Bardet (Grenoble) for providing us with the cellulose sample. We thank Paul Hodgkinson (Durham) for making available his MATLAB fitting routines. The two anonymous referees are thanked for their constructive and insightful comments on our original manuscript.

References

[1] K. Schmidt-Rohr, H.W. Spiess, Multidimensional Solid State NMR and Polymers, Academic Press, New York, 1994.

[2] R.H. Atalla, J.C. Gast, D.W. Sindorf, V.J. Bartuska, G.E. Maciel, ^{13}C NMR spectra of cellulose polymorphs, J. Am. Chem. Soc. 102 (1980) 3249.

[3] W.L. Earl, D.L. Vanderhart, High-resolution, magic angle sample spinning ^{13}C NMR of solid cellulose I, J. Am. Chem. Soc. 102 (1980) 3251.

[4] R.H. Atalla, D.L. Vanderhart, Native cellulose: a composite of two distinct crystalline forms, Science 223 (1984) 283.

[5] H. Kono, S. Yunoki, T. Shikano, M. Fujiwara, T. Erata, M. Takai, CP/MAS ^{13}C NMR study of cellulose and cellulose derivatives. 1. Complete assignment of the CP/MAS ^{13}C NMR spectrum of the native cellulose, J. Am. Chem. Soc. 124 (2002) 7506.

[6] Y. Nishiyama, P. Langan, H. Chanzy, Crystal structure and hydrogen-bonding system in cellulose Iβ from synchrotron X-ray and neutron fiber diffraction, J. Am. Chem. Soc. 124 (2002) 9074.

[7] M. Bardet, D. Gagnaire, R. Nardin, D. Robert, M. Vincendon, Use of ^{13}C enriched wood for structural NMR investigation of wood and wood components, cellulose and lignin, in solid and in solution, Holzforschung 40 (1986) 17.

[8] A. Lesage, M. Bardet, L. Emsley, Through-bond carbon–carbon connectivities in disordered solids by NMR, J. Am. Chem. Soc. 121 (1999) 10987.

[9] D. Sakellariou, S.P. Brown, A. Lesage, S. Hediger, M. Bardet, C.A. Meriles, A. Pines, L. Emsley, High-resolution NMR correlation spectra of disordered solids, J. Am. Chem. Soc. 125 (2003) 4376.

[10] H. Kono, T. Erata, M. Takai, Determination of the through-bond carbon-carbon and carbon-proton connectivities of the native celluloses in the solid state, Macromolecules 36 (2003) 5131.

[11] T. Allman, The observation of two-dimensional NMR-spectra of crystalline solids. The ^{31}P CP-MAS COSY and J-resolved spectra of a phosphine complex of Hg(II), J. Magn. Reson. 83 (1989) 637.

[12] A. Kubo, C.A. McDowell, One- and two-dimensional ^{31}P cross-polarization magic-angle-spinning nuclear magnetic resonance studies on two-spin systems with homonuclear dipolar coupling and J coupling, J. Chem. Phys. 92 (1990) 7156.

[13] G. Wu, R.E. Wasylishen, Homonuclear ^{31}P J-resolved 2D spectra of rhodium(I) phosphine complexes in the solid-state, Inorg. Chem. 31 (1992) 145.

[14] F. Fayon, I.J. King, R.K. Harris, R.K.B. Gover, J.S.O. Evans, D. Massiot, Characterization of the room-temperature structure of SnP_2O_7 by ^{31}P through-space and through-bond NMR Correlation Spectroscopy, Chem. Mater. 15 (2003) 2234.

[15] S.P. Brown, M. Pérez-Torralba, D. Sanz, R.M. Claramunt, L. Emsley, Determining hydrogen-bond strengths in the solid state by NMR: the quantitative measurement of homonuclear J couplings, Chem. Commun. (2002) 1852.

[16] L. Duma, W.C. Lai, M. Carravetta, L. Emsley, S.P. Brown, M.H. Levitt, Principles of spin-echo modulation by J-couplings in magic-angle-spinning solid-state NMR, Chem. Phys. Chem. 5 (2004) 815.

[17] D.L. VanderHart, W.L. Earl, A.N. Garroway, Resolution in ^{13}C NMR of organic solids using high-power proton decoupling and magic-angle sample spinning, J. Magn. Reson. 44 (1981) 361.

[18] G. De Paëpe, N. Girauld, A. Lesage, P. Hodgkinson, A. Böckmann, L. Emsley, Transverse dephasing optimized solid-state NMR spectroscopy, J. Am. Chem. Soc. 125 (2003) 13938.

[19] S. Hediger, B.H. Meier, N.D. Kurur, G. Bodenhausen, R.R. Ernst, NMR cross-polarization by adiabatic passage through the Hartmann–Hahn condition (APHH), Chem. Phys. Lett. 223 (1994) 283.

[20] G. Metz, X.L. Wu, S.O. Smith, Ramped-amplitude cross-polarization in magic-angle-spinning NMR, J. Magn. Reson. Ser. A 110 (1994) 219.

[21] A.E. Bennett, C.M. Rienstra, M. Auger, K.V. Lakshmi, R.G. Griffin, Heteronuclear decoupling in rotating solids, J. Chem. Phys. 103 (1995) 6951.

[22] http://www.ens-lyon.fr/STIM/NMR.

[23] M. Bardet, L. Emsley, M. Vincendon, Two-dimensional spin-exchange solid-state NMR studies of ^{13}C-enriched wood, Solid State Nucl. Magn. Reson. 8 (1997) 25.

[24] W.C. Lai, S.P. Brown, L. Duma, L. Emsley, M.H. Levitt, in: The 3rd Alpine Conference on Solid-State NMR: Chamonix-Mont Blanc, France, 2003, p.69.