ACADEMIC
PRESS

Communication

Spin-state selection in solid-state NMR

Luminita Duma, Sabine Hediger, Anne Lesage, and Lyndon Emsley*

*Laboratoire de Chimie (UMR-5532 CNRS/ENS), Laboratoire de Recherche Conventionné du CEA (23V),
Ecole Normale Supérieure de Lyon, 46 Allée d'Italie, 69364 Lyon, France*

Abstract

Spin-state selection in solid-state NMR is demonstrated, using similar pulse sequences as used in liquid-state NMR. The different transitions of all three carbon resonances in fully ^{13}C-labeled L-alanine are separated in different spectra. By selecting spin-states, the contribution of the J-coupling to the linewidth is removed, leading to a considerable enhancement in resolution. The spin-state-selective technique is demonstrated for magic-angle spinning frequencies from 6 to 35 kHz. Other experimental conditions affecting the sensitivity of the experiments are discussed. Sensitivity losses due to the introduction of the spin-state-selective filter are shown to be acceptable. Finally, spin-state selection was used to experimentally confirm the differential broadening expected for the two transitions of the CH_3 resonance.
© 2003 Elsevier Science (USA). All rights reserved.

1. Introduction

During the last decade much progress has been made in the NMR spectroscopy of fully isotopically enriched materials (notably carbon-13 and nitrogen-15). This progress has been largely motivated by applications to peptides and proteins, culminating recently to the complete or partial assignment of different biomolecules [1–9] and to the first complete protein structure by solid-state NMR [10].

One of the major problems to extend the range of systems that could be studied remains the resolution of the spectra. To this end significant efforts have been made to improve sample preparation [11], to increase the quality of heteronuclear decoupling [12,13], or to develop partial labeling schemes. The dilution of spins has the additional advantage of reducing dipolar truncation effects that interfere with the determination of long-range distances necessary for structure determination [10,14,15].

In fully labeled carbon-13 compounds such as proteins, the homonuclear ^{13}C–^{13}C J-coupling represents a

significant contribution to the linewidth in standard magic-angle spinning (MAS) methods [1,16,17]. By analogy with widely used techniques in liquid-state NMR, Straus et al. [16] proposed in 1996 to use selective pulses for homonuclear J-decoupling in solid-state MAS NMR to improve resolution. This idea was recently taken up by Igumenova and McDermott [16,18]. However, homonuclear J-decoupling is limited to indirect dimension(s). In this article we demonstrate the possibility of *spin-state selection* in solids, which results in the removal of the broadening due to the J-coupling in both the direct and indirect detection dimensions, through selecting only one component of the multiplet.

Spin-state-selective experiments are well known in liquid-state NMR spectroscopy, and are used mainly for the measurement of scalar and residual dipolar coupling constants [19–25] or in the context of transverse relaxation optimized spectroscopy (TROSY), where the selection of the slowly relaxing doublet component leads to improved resolution and sensitivity in heteronuclear correlation experiments of biomolecules [26]. Techniques for spin-state selection can basically be separated into two categories. In the first, a direct-selective excitation of a single spin state is obtained, either through single-quantum coherences (SQC) in the S3E (Spin-State-Selective

* Corresponding author. Fax: +33-472-72-84-83.
E-mail address: lyndon.emsley@ens-lyon.fr (L. Emsley).

Excitation) experiment [20], or through zero- or double-quantum coherences (ZQC or DQC) as in S3CT (Spin-State-Selective Coherence Transfer) [22]. In the second category, spin-state separation is achieved by linear combination of experiments containing in-phase or anti-phase signals. These sequences are known as IPAP (for In-Phase-Anti-Phase) [23,24] and DIPSAP (for Double-In-Phase-Single-Anti-Phase) [25]. All these sequences were developed in liquid-state NMR for heteronuclear spin pairs using non-selective pulses.

The INADEQUATE-CR (Composite Refocusing) experiment introduced in 1995 by Nielsen et al. [27] has been applied previously in solids, where it was shown to work for two-spin systems [28]. This sequence was developed to enhance the sensitivity of the basic INADEQUATE experiment [29–33]. Tailored for homonuclear spin pairs, this sequence uses non-selective pulses to excite and efficiently reconvert the double-quantum coherences to only one component of the J doublet. It is however limited specifically to the homonuclear double-quantum–single-quantum correlation experiment.

In this article, we show that spin-state selection can be achieved in solid-state NMR in a general manner, even when the J-coupling is not resolved in the spectrum. Using selective pulses we measure all the different homonuclear spin-states of all three carbon resonances in fully ^{13}C-labeled L-alanine using a sequence based on IPAP. The resulting resolution and sensitivity obtained with the spin-state-selection filter are discussed. Besides the obvious enhancement in resolution of the carbon spectra obtained with the technique, it allowed us to further investigate the experimental carbon lineshapes of fully labeled compounds, and verify some recently made predictions concerning differential broadening in solids [17].

2. The IPAP filter in solid-state NMR experiments

The pulse sequence we used for spin-state-selective excitation is given in Fig. 1a. The filter for spin-state selection is based on the IPAP sequence [23,24], adapted for a homonuclear spin pair by the use of semi-selective pulses. The sequence including the IPAP spin-state-selective filter is adapted to make it compatible with conventional solid-state NMR building blocks allowing polarization transfer, magic-angle spinning, and heteronuclear dipolar decoupling. We consider the evolution of two J-coupled carbon-13 nuclei, denoted I and S. The carbon magnetization of all spins is first enhanced by conventional cross-polarization from the protons, using simultaneous radio-frequency fields on both the proton and carbon channels [34–37]. The carbon polarization is then stored along the static magnetic field direction by a non-selective ^{13}C $\pi/2$ pulse. The following z-filter of
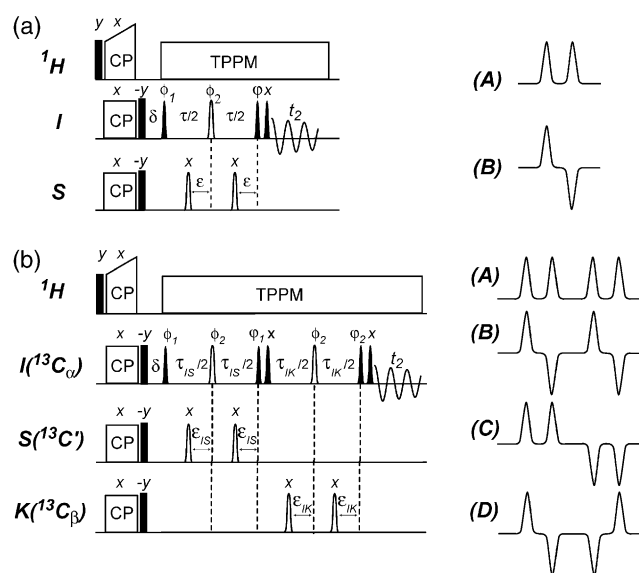

Fig. 1. (a) Pulse sequence using the IPAP spin-state-selection filter. 90° and 180° RF pulses are represented by filled and open bars for hard pulses and shapes for semi-selective pulses, respectively. For optimal performance of the IPAP spin-state-selection filter, the delay τ has to be adjusted to $(2^1 J_{IS})^{-1}$. Two data sets (A) and (B) are recorded using the following settings: (A) $\epsilon = \tau/4$, $\phi = x$ and (B) $\epsilon = 0$, $\phi = y$ [23,24]. The resulting spectra are schematically given next to the pulse sequence. The sum and difference of the (A) and (B) experiments yield two spectra, each of them containing only one of the doublet components of the I-spin. (b) Double IPAP experiment to separately measure the four spin-states of an I-spin coupled to two spins, S and K. As for the IPAP experiment in (a), the delays τ_{IS} and τ_{IK} of the consecutive IPAP block filters are set to $(2^1 J_{IS})^{-1}$ and $(2^1 J_{IK})^{-1}$, respectively, in order to ensure an optimal spin-state selection. Four experiments are recorded with the parameters ϵ_{IS}, ϵ_{IK}, ϕ_1, and ϕ_2 set as described in Table 1. The obtained I-spin resonances are again schematically depicted close to the pulse sequence. The phase cycle for both sequences was: $\phi_1 = x{-}x$; $\phi_2 = xxyy{-}x{-}x{-}y{-}y$; $\phi_{rec} = x{-}x{-}xx$. The pulse programs used in this work are available from the authors upon request, or from our website [56].

duration δ removes all residual transverse coherences. The spin-state-selection filter follows, based simply on a selective $\tau/2 - \pi - \tau/2$ block, with $\tau = (2 J_{IS})^{-1}$. As long as rotational-resonance conditions [38–40] are avoided, the spin system only evolves under the J-coupling during this block. All other interactions are removed either by MAS (heteronuclear ^{13}C–^{1}H and homonuclear ^{13}C–^{13}C dipolar couplings, and chemical shift anisotropy), by heteronuclear decoupling (heteronuclear dipolar and scalar couplings), or by the refocusing π pulses (isotropic chemical shifts). The selection of one of the I-spin transitions is based on the combination of two separately recorded experiments, denoted here (A) and (B), which only differ in the timing of the two π-pulses on the S-spin and the phase ϕ of the subsequent $\pi/2$ pulse on I-spin. In the first experiment (A), the homonuclear I–S scalar coupling is removed by applying the π-pulses on the S-spin at the middle of each $\tau/2$ evolution period

($\varepsilon = \tau/4$). No net evolution occurs during the IPAP filter (except for relaxation) and an in-phase signal (the splitting being caused by the *J*-coupling to the *S*-spin) is observed for the *I*-spin (after an additional *z*-filter) in the detection period t_2. In the second experiment (B), ε is set to zero. The net evolution under the *J*-coupling during the total delay τ leads after the final *z*-filter to an anti-phase signal of *I*-spin with respect to *S*-spin during the detection period t_2. Addition and difference of these two experiments yield spectra containing only one of the doublet components of the *I*-spin, corresponding to *I* magnetization with the coupled *S*-spin being either in the $\alpha = |-1/2\rangle$ or $\beta = |+1/2\rangle$ state.

In order to achieve optimum separation of the spin states in the combined spectra, it is essential that both the (A) and (B) experiments have exactly the same duration and the same number and type of pulses. This ensures that "relaxation effects" will occur in the same way in both experiments. For this reason, the two

"simultaneous" soft π-pulses in the (B) experiment where $\varepsilon = 0$ are for example performed sequentially. In the same experiment, the second π-pulse on the *S*-spin is maintained for the same reason. It additionally refocuses Bloch–Siegert type phase shifts [41–43] effects potentially introduced on the *I*-spin coherence by the first π-pulse. In addition, we noticed experimentally that a complete phase cycle of the π-pulse on the *I*-spin was mandatory for a good selection of the spin-state transitions.

Fig. 2 demonstrates the IPAP spin-state selection on a powdered sample of fully ^{13}C-labeled L-alanine. The sequence of Fig. 1a was applied to all three carbon resonances of L-alanine at different MAS spinning rates (6, 7, 12, 18, 28, and 35 kHz). As all three resonances are well resolved and only one resonance at one time is concerned by each soft pulse of the sequence, we used simple Gaussian shaped 90° and 180° pulses [44,45] as semi-selective pulses. The amplitude of the Gaussian
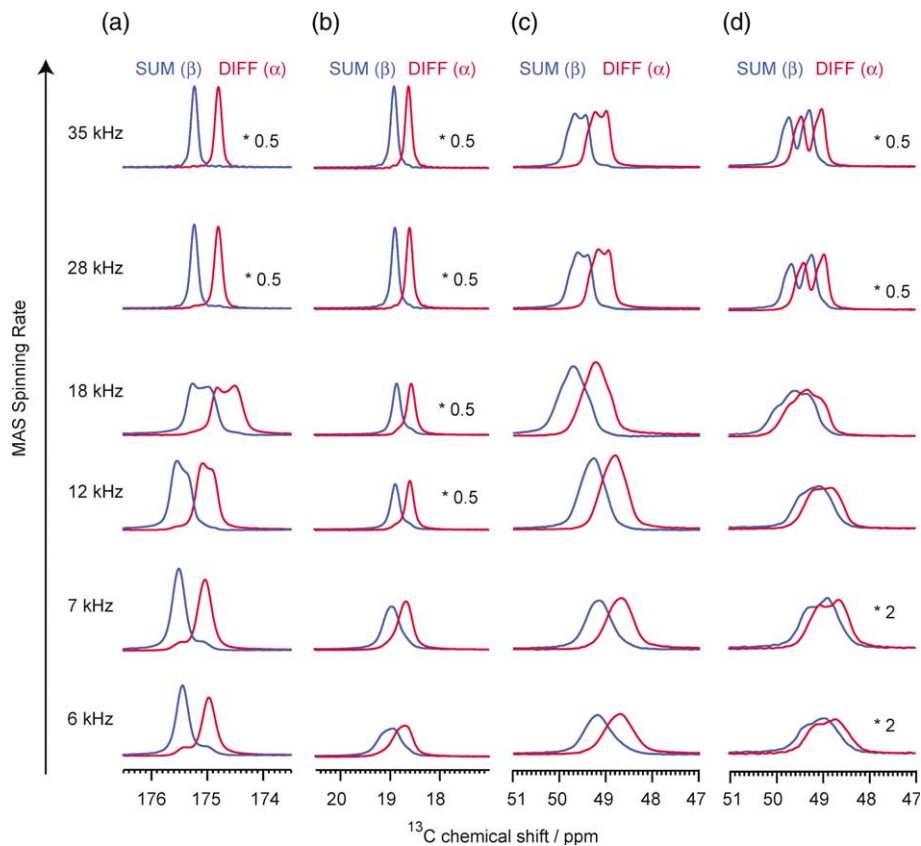

Fig. 2. Experimental α and β transitions for ^{13}C′ (a), ^{13}C$_\beta$ (b), and ^{13}C$_\alpha$ (c and d) resonances of fully ^{13}C-labeled L-alanine (purchased from Eurisotop and used without further purification) obtained with the sequence of Fig. 1a at different MAS spinning frequencies. The last two columns correspond to the ^{13}C$_\alpha$ resonance with the ^{13}C′ (Fig. 2c) or the ^{13}C$_\beta$ (Fig. 2d) being in α or the β state, respectively. All the experiments were performed on a Bruker 500 Avance spectrometer (^{1}H frequency at 500.13 MHz) equipped with a Bruker 2.5 mm double-resonance CP/MAS probe head. A ramped spin-lock on the ^{1}H channel was used to broaden the Hartmann–Hahn condition [34–37]. The CP contact time was set to 1 ms. The field strength of the TPPM decoupling [12] was 156 kHz. A delay δ of 5 ms was chosen to remove any residual transversal magnetization. The semi-selective pulses were all Gaussian pulses truncated at 1%. Their length was 1 ms for the experiments at 6 and 7 kHz MAS, 200 μs for the ^{13}C′, 800 μs for ^{13}C$_\alpha$ and ^{13}C$_\beta$ resonances at higher spinning speeds. The delay τ was set to 9.26 ms for the ^{13}C′–^{13}C$_\alpha$ spin pair (*J*-coupling of 54 Hz) and 14.706 ms for ^{13}C$_\alpha - ^{13}$C$_\beta$ spin pair (*J*-coupling of 34 Hz). To ensure the best resolution, the acquisition time was set to 60 ms ensuring complete decay of the signal, which was then processed without any apodization function.

pulses was calibrated using the carbon-13 magnetization with the sequence CP-$\pi/2$ (hard)–delay (5 ms)–$\pi/2$ (soft) or π (soft) with ^{1}H-decoupling (TPPM) during the detection period. At low spinning speeds, it was necessary that the Gaussian pulses were selective enough to not affect the other resonances over their whole range of frequencies, including their chemical shift anisotropy (i.e., spinning sidebands of other resonances were avoided). At higher spinning speeds we found it only necessary to avoid the isotropic resonances of other spins. We therefore used 1 ms Gaussian pulses in the slow spinning regime (6 and 7 kHz), and 200 and 800 μs pulses for the ^{13}C$'$ and ^{13}C$_\alpha$/^{13}C$_\beta$ resonances, respectively, at higher spinning speeds.

Spin-state selection is illustrated in Fig. 2 for all three carbon resonances with respect to each of the coupling partners by applying the sequence of Fig. 1a to each of the four J-coupled spin pairs in L-alanine, e.g., $\{^{13}C'-^{13}C_\alpha\}$, $\{^{13}C_\beta-^{13}C_\alpha\}$, $\{^{13}C_\alpha-^{13}C'\}$, $\{^{13}C_\alpha-^{13}C_\beta\}$. The two transitions corresponding to the components of the I-spin, with the coupling partner (S-spin) being in α or β state, are given on the top of each column. The τ-delay was experimentally set to mach $(2J_{IS})^{-1}$, e.g., 9.26 ms for the ^{13}C$'$–^{13}C$_\alpha$ spin pair (J-coupling of 54 Hz), and 14.706 ms for the ^{13}C$_\alpha$–^{13}C$_\beta$ spin pair (J-coupling of 34 Hz). For all resonances and spin pairs, the spin-state selection is remarkably good. In the case of the carboxyl resonance (^{13}C$'$), the MAS range covers a slow spinning regime (6 and 7 kHz) where the doublet due to the J-coupling to the ^{13}C$_\alpha$ is relatively well resolved in the CP/MAS spectrum, an intermediate regime (12 and 18 kHz) where off-rotational-resonance effects broaden the transitions leading to unresolved resonances in the carbon spectrum [17,38–40], and a fast spinning regime (28 and 35 kHz), well above any rotational-resonance conditions, where the doublet is very well resolved. In all these conditions, the separation of the spin-state is very good, and is perfect at high-spinning speeds. At the lowest spinning speeds, a small contribution from the other spin-state leaks through the experiment (having a maximum intensity of 12% of the selected spin-state). Synchronization of the refocusing delay τ with the MAS period was found not to be necessary. For the methyl resonance (^{13}C$_\beta$), the resolution of the doublet structure in a CP/MAS spectrum starts to be visible only at about 9 kHz MAS. Independently of the apparent resolution of the J-coupling with the ^{13}C$_\alpha$, the spin-state selection is again very good at all the spinning speeds used here.

The ^{13}C$_\alpha$ spin in L-alanine has two J-coupled partners, ^{13}C$'$ and ^{13}C$_\beta$. When rotational-resonance conditions are avoided, the CH resonance contains four components, corresponding to the carboxyl and the methyl carbons being each in either the α or the β state. This multiplet structure (doublet of a doublet) is not resolved at all in the CP/MAS spectrum, even at the highest MAS spin-

ning frequency considered here (35 kHz). A broad (linewidth of more than 100 Hz) and featureless lineshape is obtained for this resonance at all spinning speeds. Nevertheless, excellent spin-state selection was again obtained with the IPAP filter. Since the CH carbon has two coupled spins, the experiment of Fig. 1a was carried out once for the C$'$C$_\alpha$ pair with the delay τ matched to $^1J_{C'C_\alpha} = 54$ Hz (results in Fig. 2c) and once for the C$_\alpha$C$_\beta$ pair matched to $^1J_{C_\alpha C_\beta} = 34$ Hz (results in Fig. 2d). Depending on the chosen pair, the spin-state selection is performed only with respect to one of the coupled spins (carboxyl or methyl). Thus, each selected CH spin state therefore still contains two transitions separated by the J-coupling to the third spin. The doublet structure of the separated spin-states of the ^{13}C$_\alpha$ spin is very clear at high MAS spinning speed in Figs. 2c and d.

In Fig. 3, we show that it is possible to separate all four spin states of the ^{13}C$_\alpha$ resonance by applying two sequential IPAP filters. This "double IPAP" filter is implemented in the pulse sequence of Fig. 1b. In a homonuclear three-spin system $\{I-S-K\}$, e.g., $\{^{13}C_\alpha-^{13}C'-^{13}C_\beta\}$, with the I-spin being coupled to
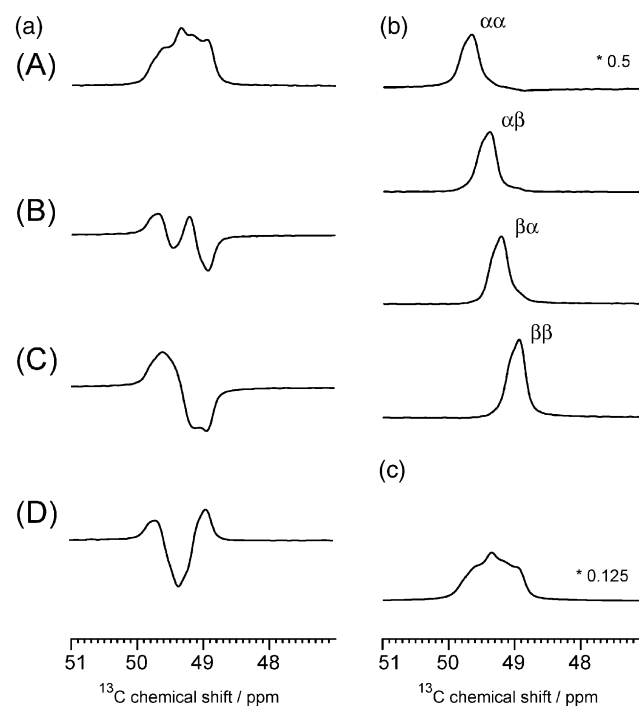

Fig. 3. Double-IPAP spectra (pulse sequence of Fig. 1b) of the ^{13}C$_\alpha$ resonance of fully ^{13}C-labeled L-alanine. (a) ^{13}C$_\alpha$ signal obtained in the four experiments (A)–(D) needed to separate the spin-states. The MAS frequency was set to 28 kHz. Gaussian pulses of 800 μs were used for all semi-selective pulses. The delays τ_{IS} and τ_{IK} were 9.26 and 14.706 ms, respectively. All other experimental conditions are given in the figure caption of Fig. 2. (b) Linear combinations of the four spectra of Fig. 3a leading to the four separated $\alpha\alpha$, $\alpha\beta$, $\beta\alpha$, and $\beta\beta$ spin-states of ^{13}C$_\alpha$ resonance. The exact linear combinations are given in the text. (c) ^{13}C$_\alpha$ resonance obtained in a standard CP/MAS experiment using the same experimental conditions as the double IPAP experiment.

Table 1
Experimental parameters for the successive IPAP filters of the "double IPAP" experiment ($\tau_{IS} = (2J_{IS})^{-1}$ and $\tau_{IK} = (2J_{IK})^{-1}$)

A (in-phase/in-phase)	$\varepsilon_{IS} = \tau_{IS}/4$	$\varepsilon_{IK} = \tau_{IK}/4$	$\varphi_1 = x$	$\varphi_2 = x$
B (in-phase/anti-phase)	$\varepsilon_{IS} = \tau_{IS}/4$	$\varepsilon_{IK} = 0$	$\varphi_1 = x$	$\varphi_2 = y$
C (anti-phase/in-phase)	$\varepsilon_{IS} = 0$	$\varepsilon_{IK} = \tau_{IK}/4$	$\varphi_1 = y$	$\varphi_2 = x$
D (anti-phase/anti-phase)	$\varepsilon_{IS} = 0$	$\varepsilon_{IK} = 0$	$\varphi_1 = y$	$\varphi_2 = y$

both S- and K-spins, the first IPAP filter applied to the I- and S-spins, with τ matched to $(2J_{IS})^{-1}$, delivers the I-spin states separated with respect to the S-spin. Applying a second IPAP filter to the I- and K-spins with τ matched to $(2J_{IK})^{-1}$, the spin states of I-spin separated with respect to both S- and K-spins are obtained. In practice, four datasets are recorded using the parameters summarized in Table 1 for the successive IPAP filters. In terms of product operators [46], these four experiments lead to the following signals:

$(A) : \sigma_{(A)} \propto I_y$,

$(B) : \sigma_{(B)} \propto 2I_y K_z$,

$(C) : \sigma_{(C)} \propto 2I_y S_z$,

$(D) : \sigma_{(D)} \propto 4I_y S_z K_z$.

Schematic spectra illustrating this outcome are drawn next to the pulse sequence in Fig. 1b. The following linear combinations of these spectra yield the four different resonance components:

$A + B + C + D : I_y S^\alpha K^\alpha$ (e.g., $\alpha\alpha$),

$A - B + C - D : I_y S^\alpha K^\beta$ (e.g., $\alpha\beta$),

$A + B - C - D : I_y S^\beta K^\alpha$ (e.g., $\beta\alpha$),

$A - B - C + D : I_y S^\beta K^\beta$ (e.g., $\beta\beta$).

Fig. 3 shows double IPAP spectra for the $^{13}C_\alpha$ resonance line of fully ^{13}C-labeled L-alanine at 28 kHz MAS. It is interesting to note that all four spin states isolated in the difference spectra of Fig. 3b have the same linewidth at half-height (within the limit of uncertainty of the measurement), but noticeably different intensities. These intensity differences are not an artifact of the experiment. Indeed, the sum of the four transitions of Fig. 3b, which corresponds actually to the experiment (A) in Fig. 3a, has essentially the same lineshape as the one obtained for a standard CP/MAS experiment (given in Fig. 3c) performed with the same experimental conditions. These differential intensities could result from the interference of the ^{14}N quadrupole interaction with the $^{13}C_\alpha$–^{14}N dipolar interaction [47].

At first sight, it may appear strange than we can separate transitions that are not resolved in the carbon spectrum, since the delays in the sequence are of the order of $\tau = (2J)^{-1}$. Similar to the case of the refocused INADEQUATE experiment [33,48], it is in fact not the

apparent linewidth, $1/(\pi T_2^*)$ which is relevant for the sensitivity of the experiment, but the non-refocused linewidth given by $1/(\pi T_2')$ measured in a spin-echo experiment [49]. The time constant T_2' contains all non-refocusable interactions in addition to the spin–spin relaxation time T_2. It was shown [33,49,50] that in most organic solids the carbon-13 dephasing time T_2^* is dominated by refocusable interactions. In both crystalline and disordered solids, the non-refocused carbon linewidth was found to be much narrower than the apparent linewidth, and generally smaller than the one bond homonuclear J-couplings. Since the spin-state-selective filters we use here are based on $\tau - \pi - \tau$ evolution periods, it is the non-refocusable linewidth, $1/(\pi T_2')$, that is pertinent for the preparation of the selective spin states.

3. Lineshape analysis

Recently, we have studied the unusual details of carbon lineshapes in fully labeled compounds at different MAS spinning rates [17]. By comparing experimental lineshapes of L-alanine with numerical simulations, we showed that the difference in intensity observed for the doublet components of the carboxyl resonance at some spinning speeds arises from coherent cross-correlation effects between the carboxyl CSA (chemical shift anisotropy) and the homonuclear dipolar coupling to the $^{13}C_\alpha$. Due to the smaller difference of chemical shifts between the methyl and $^{13}C_\alpha$ resonances, we predicted in the same work that the cross-correlation effect between these two nuclei may lead to a *differential broadening* of the two transitions of the methyl resonance. Some experimental indications of this effect were inferred from deconvolution of the methyl carbon lineshapes observed between 7 and 12 kHz MAS. However, since the doublet structure was not well resolved, the results of the deconvolution had to be taken with caution.

Using the spin-state-selection technique, we can now verify differential broadening of the methyl resonance doublet at low spinning speeds. Indeed, the pulse sequence of Fig. 1a allows the measurement of both doublet components separately (see Fig. 2b). The experimentally measured linewidths for each component are given in Table 2. The difference in linewidth is plotted as a function of the spinning speed in Fig. 4a. The largest difference in width between the two transitions is obtained at slow spinning speeds (as expected).

Table 2
Full linewidths at half height (Δ) of the two methyl resonance components obtained with the IPAP experiment at different spinning speeds

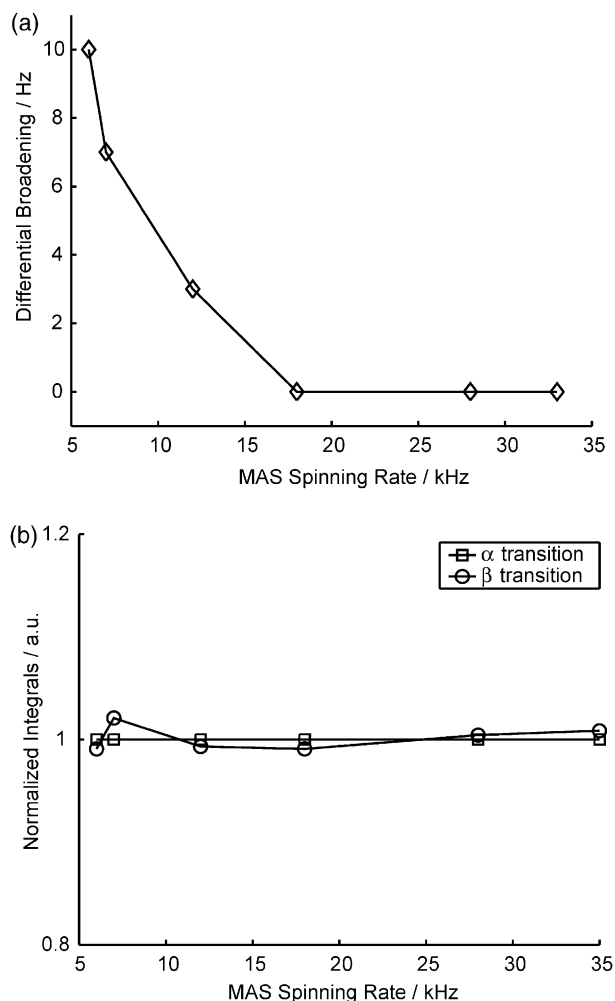| MAS	6 kHz	7 kHz	12 kHz	18 kHz	28 kHz	35 kHz
Δ_α (Hz)	58	43	24	24	20	19
Δ_β (Hz)	68	50	27	24	20	19

Fig. 4. Difference in linewidth (a) and normalized integral intensity (b) for both components of the CH_3 resonance in fully ^{13}C enriched L-alanine measured with the IPAP sequence of Fig. 1a as a function of the MAS spinning rate.

For example, a difference of 10 Hz is observed at 6 kHz MAS. The measurement of the differential broadening at even lower spinning speeds is difficult due to the appearance of off-rotational effects which lead to broadening of the resonances. By increasing the spinning speed, the difference in linewidth of the two components decreases and disappears at about 18 kHz MAS. The values measured here for the differential broadening are in very good agreement with the ones predicted in [17]

from the deconvolution of the CP/MAS lineshapes. For example, we measured with the spin-state-selective technique linewidth differences of 7 Hz at 7 kHz MAS, and 3 Hz at 12 kHz MAS. In [17], differential broadenings of 6 and 3 Hz were found at 7 and 12 kHz MAS, respectively.

This experiment confirms unambiguously that coherent CSA-DD cross-correlation effects can lead either to a differential broadening, in cases where the involved spins behave homogeneously [51], or to a differential intensity for inhomogeneous spin pairs [17]. For the carboxyl lineshape in L-alanine, only a difference in intensity was found in [17], showing that the chemical shift difference to the C_α is big enough for the homonuclear spin pair to behave inhomogeneously. We see here that a completely opposite situation is found for the CH_3 in L-alanine. Indeed, the integrals of each doublet component are the same over the whole range of MAS rates (see Fig. 4b). The coherent cross-correlation effect between the C_α and C_β is only characterized by a differential broadening of the CH_3 doublet, indicating a homogeneous coupled spin pair, as actually expected for a homonuclear spin system.

4. Discussion

It seems obvious from the spectra of Fig. 2 that the selection of spin states should be very useful for improving the resolution of spectra. In cases where the J-coupling is resolved, like for the carboxyl site of L-alanine, spin-state selection reduces by a factor of 2 the number of peaks. More importantly, for resonances where a relatively large J-coupling is not resolved, like for example for $^{13}C_\alpha$'s, the selection of single spin states greatly reduces the linewidth. In the case of the $^{13}C_\alpha$ resonance of L-alanine (see Fig. 2c) we obtained reductions of the linewidth (FWHH) from 30 to 40% (depending on the rotor speed) compared to the one found in CP/MAS spectra measured in the same conditions. The linewidths of the CH resonances are given in Table 3 for the CP/MAS spectra and the two separated spin states obtained with respect to the α or β carboxyl spin states at different MAS spinning speeds. Using the

Table 3
Full linewidths at half height (Δ) of CH resonance in CP/MAS spectra and for both CH spin-state selected transitions with respect to the carboxyl at different MAS rates

MAS	$\Delta_{CP/MAS}$ (Hz)	Δ_α (Hz)	Δ_β (Hz)
6 kHz	128	90	87
7 kHz	124	84	82
12 kHz	120	80	79
18 kHz	126	86	93
28 kHz	110	68	70
35 kHz	110	67	68

"double IPAP" pulse sequence, a 66% resolution enhancement is achieved for the CH resonance of L-alanine.

In order to efficiently implement spin-state selection in solid-state NMR of real systems, like proteins, it is mandatory that the sensitivity loss introduced by the selection filter be reasonable. Several factors are to be taken into account to optimize the sensitivity of the filter, such as the total length of the sequence (as short as possible), or the choice and length of the semi-selective pulses (as short as possible). Particularly, we find that the quality of the decoupling sequence used during the refocusing period of the selection filter is crucial. Very recent work has shown [52] that decoupling sequences performing with about the same efficiency in the direct dimension can behave quite differently during a refocusing period. In Fig. 5 we therefore compare the sensitivity of spin-state selected spectra of L-alanine obtained with the sequence of Fig. 1a using different decoupling schemes (CW [53], TPPM [12], and eDROOPY [54]). For the carboxyl resonance, the sensitivity loss due to the introduction of the IPAP filter is quite low (0–20%) when eDROOPY is used. The SPINAL [55] decoupling sequence (data not shown) gave comparable performance to eDROOPY. For the methyl resonance, intensity losses range from 30 to 70% depending on the rotor speed. For both resonances the sensitivity of the spin-state selected spectra improves with increasing spinning speed, and eDROOPY is seen to provide significantly better sensitivity than TPPM (or CW) decoupling.

In this contribution we have investigated only one spin-state-selective filter, based on the IPAP sequence [23,24]. The other known (non-selective) sequences, the S3E-family [20] and DIPSAP [25], work with the same type of evolution periods, and are expected to work generally in the same way for solids. Two main aspects should be taken into account for the choice of the filter sequence: its length and its sensitivity to variation of the *J*-couplings. In systems where the refocused relaxation time is short, the S3E sequence could be of advantage, being the shortest (a factor two shorter than IPAP). For the cases where the spin-state separation is desired for several spin-pairs with somewhat different *J*-couplings, the DIPSAP filter could be useful. IPAP presents a good compromise between the filter length and the tolerance to *J*-coupling variations, which is why we chose it here. We are currently investigating other spin-state-selective techniques in more detail.

Finally, we note that the same kind of sequences as discussed here should work on heteronuclear spin pairs (e.g., ^{13}C–^{15}N), as is the most common use in liquid-state NMR. However, as heteronuclear spins can be (relatively) easily decoupled from each other, the utility of spin-state-selective techniques for resolution enhancement is not as clear as for homonuclear systems.
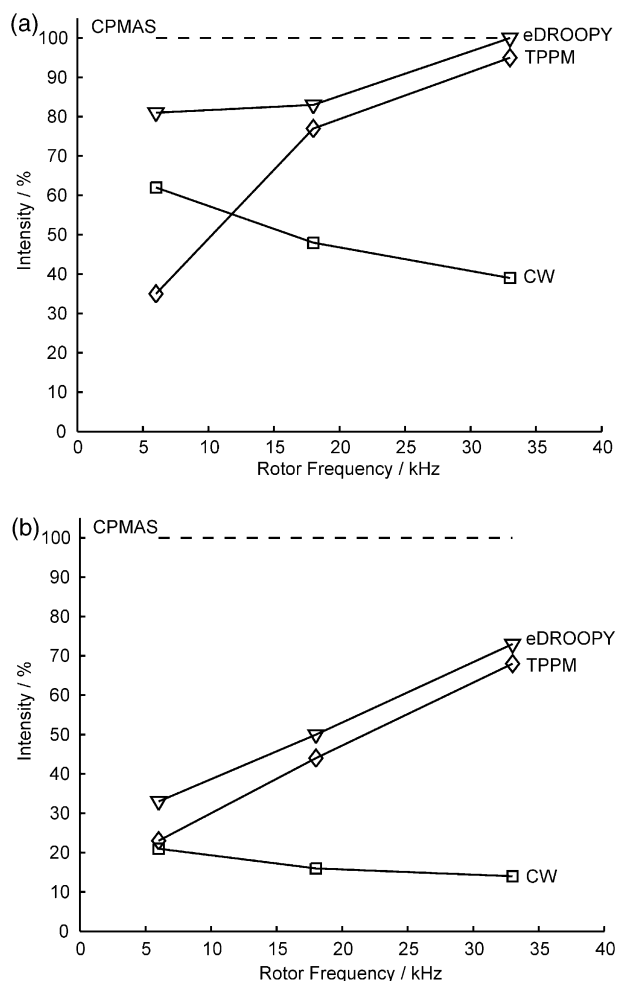

Fig. 5. Sensitivity of the IPAP sequence using different decoupling sequences compared to standard CP/MAS experiment recorded with TPPM decoupling. The comparison is made for the $^{13}C'$ (a) and $^{13}CH_3$ (b) resonances for different MAS spinning frequencies. For this comparison, the IPAP and the CP/MAS spectra were recorded with the same total number of transients. For each resonance and spinning rate, the signal intensities were calibrated such to give 100 for the CP/MAS experiment. The different decoupling sequences tested were continuous wave (CW) decoupling [53], TPPM [12], and eDROOPY [54]. They were all applied with a decoupling filed strength of 156 kHz. All the other experimental conditions are given in Fig. 2.

They could be useful for the measurement of *J*-couplings.

5. Conclusions

We have shown that the spin-state-selective excitation is possible in solid-state NMR. By adapting the heteronuclear liquid-state NMR selection filter IPAP [23,24], we were able to observe separately all the transitions of the three carbon resonances in fully ^{13}C-labeled L-alanine. The separation of the homonuclear spin-states was shown to be very good over a large range of MAS frequencies, as long as rotational-resonance conditions are

avoided. Thus, a significant resolution enhancement is obtained in the carbon spectrum of fully labeled compounds, even for resonances where the multiplet structure is not resolved. The different conditions relevant for the sensitivity of the experiment were discussed, and we have shown that the intensity losses due to the introduction of the spin-state-selective filter were acceptable, and can be minimized notably by the use of appropriate heteronuclear decoupling schemes.

Acknowledgments

References

[1] S.K. Straus, T. Bremi, R.R. Ernst, Experiments and strategies for the assignment of fully C-13/N-15-labelled polypeptides by solid-state NMR, J. Biomol. NMR 12 (1998) 39–50.

[2] A. McDermott, T. Polenova, A. Böckmann, K.W. Zilm, E.K. Paulsen, R.W. Martin, G.T. Montelione, Partial NMR assignments for uniformly (C-13, N-15)-enriched BPTI in the solid state, J. Biomol. NMR 16 (2000) 209–219.

[3] M. Hong, Solid-state NMR determination of C-13 alpha chemical shift anisotropies for the identification of protein secondary structure, J. Am. Chem. Soc. 122 (2000) 3762–3770.

[4] J. Pauli, M. Baldus, B. van Rossum, H. de Groot, H. Oschkinat, Backbone and side-chain C-13 and N-15 signal assignments of the alpha-spectrin SH3 domain by magic angle spinning solid-state NMR at 17.6 T, Chem. BioChem. 2 (2001) 272–281.

[5] B.J. van Rossum, F. Castellani, K. Rehbein, J. Pauli, H. Oschkinat, Assignment of the nonexchanging protons of the alpha-spectrin SH3 domain by two- and three-dimensional H-1–C-13 solid-state magic-angle spinning NMR and comparison of solution and solid-state proton chemical shifts, Chem. BioChem. 2 (2001) 906–914.

[6] Y. Ishii, C-13–C-13 dipolar recoupling under very fast magic angle spinning in solid-state nuclear magnetic resonance: applications to distance measurements, spectral assignments, and high-throughput secondary-structure determination, J. Chem. Phys. 114 (2001) 8473–8483.

[7] X.L. Yao, M. Hong, Dipolar filtered H-1–C-13 heteronuclear correlation spectroscopy for resonance assignment of proteins, J. Biomol. NMR 20 (2001) 263–274.

[8] M. Hong, R.A. McMillan, V.P. Conticello, Measurement of conformational constraints in an elastin-mimetic protein by residue-pair selected solid-state NMR, J. Biomol. NMR 22 (2002) 175–179.

[9] A. Böckmann, A. Lange, A. Galinier, S. Luca, N. Giraud, M. Juy, H. Heise, R. Montserret, F. Penin, M. Baldus, Solid-state NMR sequential resonance assignments and conformational analysis of the 2 × 10.4 kDa dimeric form of the Bacillus subtilis protein, Crh, J. Biomol. NMR (2003) (accepted).

[10] F. Castellani, B. van Rossum, A. Diehl, M. Schubert, K. Rehbein, H. Oschkinat, Structure of a protein determined by solid-state magic-angle-spinning NMR spectroscopy, Nature 420 (2002) 98–102.

[11] J. Pauli, B. van Rossum, H. Förster, H.J.M. de Groot, H. Oschkinat, Sample optimization and identification of signal patterns of amino acid side chains in 2D RFDR spectra of the alpha-spectrin SH3 domain, J. Magn. Reson. 143 (2000) 411–416.

[12] A.E. Bennett, C.M. Rienstra, M. Auger, K.V. Lakshmi, R.G. Griffin, Heteronuclear decoupling in rotating solids, J. Chem. Phys. 103 (1995) 6951–6958.

[13] E. Vinogradov, P.K. Madhu, S. Vega, High-resolution proton solid-state NMR spectroscopy by phase-modulated Lee–Goldburg experiment, Chem. Phys. Lett. 314 (1999) 443–450.

[14] P. Hodgkinson, L. Emsley, The accuracy of distance measurements in solid-state NMR, J. Magn. Reson. 139 (1999) 46–59.

[15] M. Hong, Determination of multiple phi-torsion angles in proteins by selective and extensive C-13 labeling and two-dimensional solid-state NMR, J. Magn. Reson. 139 (1999) 389–401.

[16] S.K. Straus, T. Bremi, R.R. Ernst, Resolution enhancement by homonuclear J decoupling in solid-state MAS NMR, Chem. Phys. Lett. 262 (1996) 709–715.

[17] L. Duma, S. Hediger, A. Lesage, D. Sakellariou, L. Emsley, Carbon-13 lineshapes in solid-state NMR of labeled compounds. Effects of coherent CSA-dipolar cross-correlation, J. Magn. Reson. 162 (2003) 90–101.

[18] T.I. Igumenova, A. McDermott, Experimental Nuclear Magnetic Resonance Conference, Santa Fe, USA, 2002.

[19] T. Fäcke, S. Berger, alpha/beta-ge-SELINCOR-TOCSY, a new method for the determination of H,C coupling constants, J. Magn. Reson. A 119 (1996) 260–263.

[20] A. Meissner, J.O. Duus, O.W. Sørensen, Spin-state-selective excitation. Application for E.COSY-type measurement of J(HH) coupling constants, J. Magn. Reson. 128 (1997) 92–97.

[21] A. Meissner, J.O. Duus, O.W. Sørensen, Integration of spin-state-selective excitation into 2D NMR correlation experiments with heteronuclear ZQ/2Q pi rotations for (1)J(XH)-resolved E.COSY-type measurement of heteronuclear coupling constants in proteins, J. Biomol. NMR 10 (1997) 89–94.

[22] M.D. Sørensen, A. Meissner, O.W. Sørensen, Spin-state-selective coherence transfer via intermediate states of two-spin coherence in IS spin systems: application to E.COSY-type measurement of J coupling constants, J. Biomol. NMR 10 (1997) 181–186.

[23] M. Ottiger, F. Delaglio, J.L. Marquardt, N. Tjandra, A. Bax, Measurement of dipolar couplings for methylene and methyl sites in weakly oriented macromolecules and their use in structure determination, J. Magn. Reson. 134 (1998) 365–369.

[24] P. Andersson, J. Weigelt, G. Otting, Spin-state selection filters for the measurement of heteronuclear one-bond coupling constants, J. Biomol. NMR 12 (1998) 435–441.

[25] B. Brutscher, Accurate measurement of small spin–spin couplings in partially aligned molecules using a novel J-mismatch compensated spin-state-selection filter, J. Magn. Reson. 151 (2001) 332–338.

[26] K. Pervushin, R. Riek, G. Wider, K. Wüthrich, Attenuated T-2 relaxation by mutual cancellation of dipole–dipole coupling and chemical shift anisotropy indicates an avenue to NMR structures of very large biological macromolecules in solution, Proc. Natl. Acad. Sci. USA 94 (1997) 12366–12371.

[27] N.C. Nielsen, H. Thogersen, O.W. Sørensen, Doubling the sensitivity of INADEQUATE for tracing out the carbon skeleton of molecules by NMR, J. Am. Chem. Soc. 117 (1995) 11365–11366.

[28] R. Verel, J.D. van Beek, B.H. Meier, INADEQUATE-CR experiments in the solid state, J. Magn. Reson. 140 (1999) 300–303.

[29] A. Bax, R. Freeman, S.P. Kempsell, Natural abundance C-13–C-13 coupling observed via double-quantum coherence, J. Am. Chem. Soc. 102 (1980) 4849–4851.

[30] A. Bax, R. Freeman, T.A. Frenkiel, M.H. Levitt, Assignment of C-13 NMR-spectra via double-quantum coherence, J. Magn. Reson. 43 (1981) 478–483.

[31] A. Bax, R. Freeman, T.A. Frenkiel, An NMR technique for tracing out the carbon skeleton of an organic-molecule, J. Am. Chem. Soc. 103 (1981) 2102–2104.

[32] A. Lesage, C. Auger, S. Caldarelli, L. Emsley, Determination of through-bond carbon–carbon connectivities in solid-state NMR using the INADEQUATE experiment, J. Am. Chem. Soc. 119 (1997) 7867–7868.

[33] A. Lesage, M. Bardet, L. Emsley, Through-bond carbon–carbon connectivities in disordered solids by NMR, J. Am. Chem. Soc. 121 (1999) 10987–10993.

[34] S.R. Hartmann, E.L. Hahn, Nuclear double resonance in the rotating frame, Phys. Rev. 128 (1962) 2042–2053.

[35] A. Pines, M.G. Gibby, J.S. Waugh, Proton-enhanced NMR of dilute spins in solids, J. Chem. Phys. 59 (1973) 569–590.

[36] O.B. Peersen, X.L. Wu, S.O. Smith, Enhancement of CP-MAS signals by variable-amplitude cross-polarization—compensation for inhomogeneous B-1 fields, J. Magn. Reson. A 106 (1994) 127–131.

[37] S. Hediger, B.H. Meier, R.R. Ernst, Adiabatic passage Hartmann–Hahn cross-polarization in NMR under magic-angle sample-spinning, Chem. Phys. Lett. 240 (1995) 449–456.

[38] D.P. Raleigh, M.H. Levitt, R.G. Griffin, Rotational resonance in solid-state NMR, Chem. Phys. Lett. 146 (1988) 71–76.

[39] M.H. Levitt, T.G. Oas, R.G. Griffin, Rotary resonance recoupling in heteronuclear spin pair systems, Isr. J. Chem. 28 (1988) 271–282.

[40] T. Nakai, C.A. McDowell, Calculation of rotational resonance NMR spectra using Floquet theory combined with perturbation treatment, Mol. Phys. 88 (1996) 1263–1275.

[41] F. Bloch, A. Siegert, Magnetic resonance for non rotating fields, Phys. Rev. 57 (1940) 522–527.

[42] N.F. Ramsey, Resonance transitions induced by perturbations at 2 or more different frequencies, Phys. Rev. 100 (1955) 1191–1194.

[43] L. Emsley, G. Bodenhausen, Phase-shifts induced by transient Bloch–Siegert effects in NMR, Chem. Phys. Lett. 168 (1990) 297–303.

[44] C. Bauer, R. Freeman, T. Frenkiel, J. Keeler, A.J. Shaka, Gaussian pulses, J. Magn. Reson. 58 (1984) 442–457.

[45] L. Emsley, in: D.M. Grant, R.K. Harris (Eds.), Encyclopedia of Nuclear Magnetic Resonance, Wiley, Chichester, 1996, pp. 4228–4236.

[46] O.W. Sørensen, G.W. Eich, M.H. Levitt, G. Bodenhausen, R.R. Ernst, Product operator-formalism for the description of NMR pulse experiments, Prog. Nucl. Magn. Reson. Spectrosc. 16 (1983) 163–192.

[47] A. Naito, S. Ganapathy, K. Akasaka, C.A. McDowell, Chemical shielding tensor and C-13–N-14 dipolar splitting in single-crystals of L-alanine, J. Chem. Phys. 74 (1981) 3190–3197.

[48] D. Sakellariou, S.P. Brown, A. Lesage, S. Hediger, M. Bardet, C.A. Meriles, A. Pines, L. Emsley, High-resolution NMR spectra of disordered solids, J. Am. Chem. Soc. (2003) 4376–4380.

[49] S.P. Brown, M. Perez-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) 1852–1853.

[50] B.A. Cowans, J.B. Grutzner, Examination of homogeneous broadening in solids via rotationally synchronized spin-echo NMR-spectroscopy, J. Magn. Reson. A 105 (1993) 10–18.

[51] M.M. Maricq, J.S. Waugh, NMR in rotating solids, J. Chem. Phys. 70 (1979) 3300–3316.

[52] G. De Paëpe, A. Lesage, L. Emsley, The performance of phase modulated heteronuclear dipolar decoupling schemes in fast magic angle spinning NMR experiments, J. Chem. Phys. (2003) (accepted).

[53] F. Bloch, Theory of line narrowing by double-frequency irradiation, Phys. Rev. 111 (1958) 841–853.

[54] G. De Paëpe, P. Hodgkinson, L. Emsley, Improved heteronuclear decoupling schemes for solid-state magic angle spinning NMR by direct spectral optimization, Chem. Phys. Lett. (2003) (accepted).

[55] B.M. Fung, A.K. Khitrin, K. Ermolaev, An improved broadband decoupling sequence for liquid crystals and solids, J. Magn. Reson. 142 (2000) 97–101.

[56] Available from http://www.ens-lyon.fr/STIM/NMR.