

Through-Bond Heteronuclear Single-Quantum Correlation Spectroscopy in Solid-State NMR, and Comparison to Other Through-Bond and Through-Space Experiments

Anne Lesage and Lyndon Emsley

Laboratoire de Stéréochimie et des Interactions Moléculaires, UMR-117 CNRS/ENS, Ecole Normale Supérieure de Lyon, 69364 Lyon, France

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A new through-bond carbon–proton correlation technique, the MAS-*J*-HSQC experiment, is described for solid-state NMR. This new pulse scheme is compared experimentally with the previously proposed MAS-*J*-HMQC experiment in terms of proton resolution on a model sample of powdered L-alanine. We show that for natural abundance compounds, the MAS-*J*-HMQC and MAS-*J*-HSQC experiments give about the same proton resolution, whereas, for ¹³C-labeled materials, narrower proton linewidths are obtained with the MAS-*J*-HSQC experiment. In addition we show that in scalar as well as in dipolar heteronuclear shift correlation experiments, when the proton chemical shift is encoded by the evolution of a single-quantum coherence, the proton resolution can be enhanced by simply adding a 180° carbon pulse in the middle of the *t*₁ evolution time. © 2001 Academic Press

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Two-dimensional carbon–proton isotropic chemical shift correlation spectroscopy (*J*) is an important tool for the characterization and structural investigation of solid-state compounds under magic angle spinning (MAS). The overlapping broad proton resonances are separated according to the chemical shift of the neighboring carbon nuclei, which are usually better resolved and spread over a larger frequency range, thereby providing useful additional information for the characterization of both the carbon and the proton MAS spectra. In particular, heteronuclear correlation techniques are some of the most powerful methods for the accurate determination of proton chemical shifts in solids. Such techniques have been numerously applied either with isotopic enrichment or at natural abundance for the structural investigation of organic molecules, polymers, or biological systems in the solid state (2–5).

Most of the heteronuclear correlation experiments (HETCOR) described so far in solid-state NMR used dipolar couplings to transfer the magnetization between protons and carbons. Various polarization transfer schemes have been proposed, like simple or modified Hartman–Hahn cross-polarization, WIM multiple-pulse sequences, or REDOR-type transfer (6–11). The resolution in the proton dimension is usually

improved by applying CRAMPS techniques (1, 12) or by using very fast magic angle spinning (11, 13).

Another way of transferring magnetization between carbons and protons is to use the heteronuclear scalar couplings, as is commonly the case in liquid-state NMR. The *J*_{CH} couplings constitute an interesting alternative to dipolar couplings, especially in terms of selectivity since, while the dipolar couplings act through-space, the heteronuclear scalar interactions provide unambiguous through-bond correlations. We recently demonstrated the feasibility of such a transfer in solid-state NMR and proposed a new heteronuclear correlation experiment dubbed MAS-*J*-HMQC (14). The sequence uses through-bond scalar couplings to generate heteronuclear *multiple quantum coherences* which provide isotropic chemical shift correlation between pairs of directly bonded (¹H, ¹³C) or (¹H, ¹⁵N) nuclei and was shown to be suitable for the complete characterization of the spectra of medium-size natural abundance organic compounds (15). In this Communication, we present a new scheme for obtaining through-bond correlations in solids, called MAS-*J*-HSQC, in which the proton chemical shift is encoded by allowing *single-quantum coherences* to evolve during the indirect detection time *t*₁. An experimental comparison between the two types of experiments is reported, mainly in terms of proton resolution, for samples of natural abundance and ¹³C-labeled L-alanine.

The pulse sequences for the MAS-*J*-HMQC and MAS-*J*-HSQC experiments are shown in Figs. 1a and 1b, respectively. After cross-polarization from protons (I spins) the magnetization of carbons (S spins) evolves during the delay τ under only the isotropic *J*_{CH} coupling Hamiltonian. Indeed, during this period a homonuclear decoupling sequence is applied on protons which removes the proton–proton dipolar couplings whereas the remaining inhomogeneous interactions, i.e., the chemical shift and the heteronuclear couplings, are averaged to their isotropic components by magic angle spinning. The FSLG homonuclear decoupling sequence (16–18) was used in the experiments presented here, but other schemes can be employed like the recently developed PMLG (19) or DUMBO sequences (20). Thus, for a pair of covalently bonded

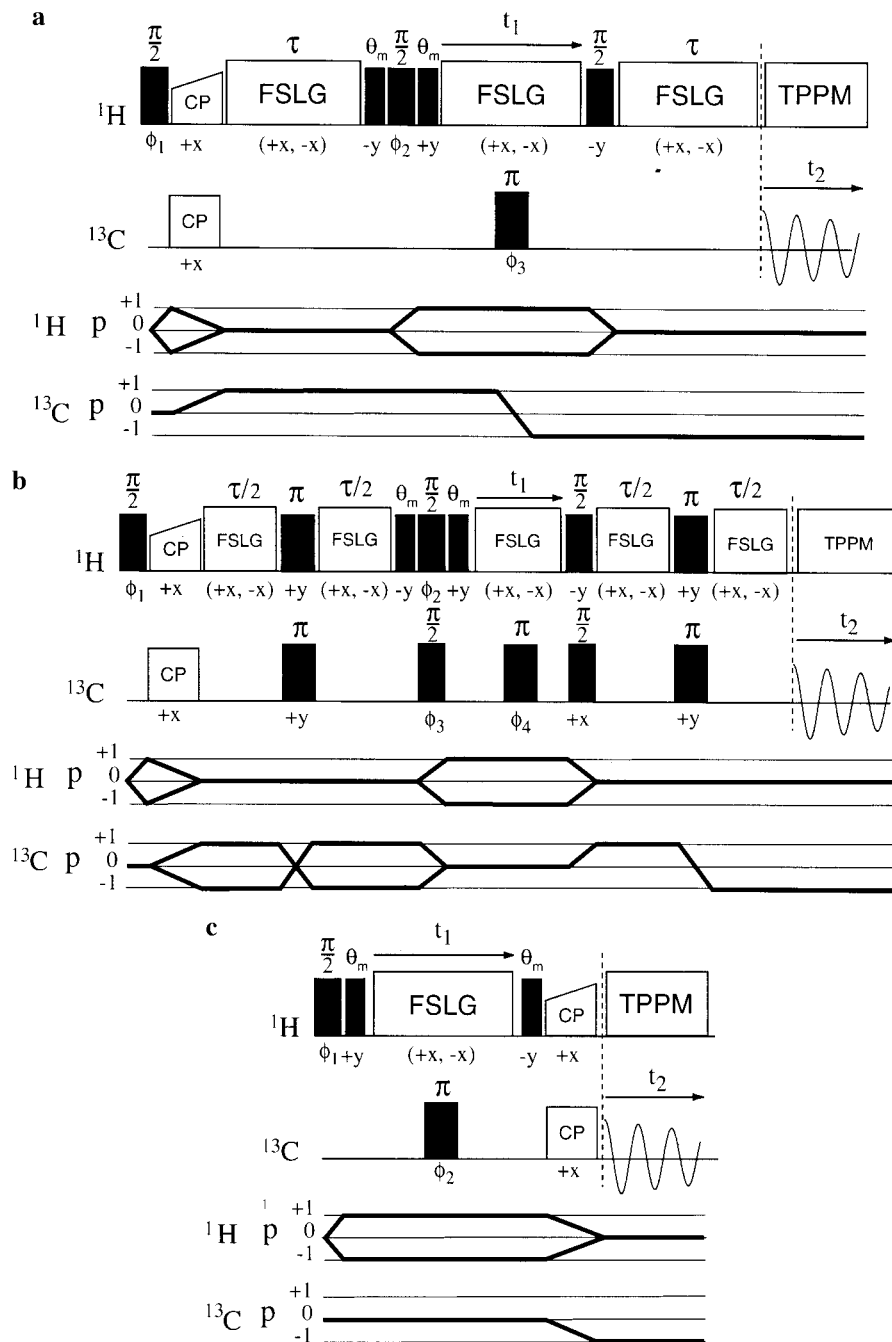


FIG. 1. Pulse sequences and coherence transfer pathways for the solid-state MAS-*J*-HMQC (a) and MAS-*J*-HSQC (b) experiments. A 16-step phase cycle was used for both experiments to select the coherence transfer pathways indicated (pulse programs are available on our web site (25) or by request to the authors). θ_m is a 54.7° pulse. Quadrature detection in ω_1 was achieved using the States method (26) by incrementing only the second 90° proton pulse. For the MAS-*J*-HMQC experiment, the following phase cycle was used: $\phi_1 = +y, -y$; $\phi_2 = +y, +y, -y, -y$; $\phi_3 = +x, +x, +x, +x, +y, +y, +y, +y, -x, -x, -x, -x, -y, -y, -y, -y$; receiver = 0, 2, 2, 0, 2, 0, 0, 2, and for the MAS-*J*-HSQC experiment: $\phi_1 = +y, -y$; $\phi_2 = +y, +y, -y, -y$; $\phi_3 = +x, +x, +x, +x, -x, -x, -x, -x, -x, -x, -x, -x, -x, -x, -x, -x, -x, -x, -x, -x$; receiver = 0, 2, 2, 0, 2, 0, 0, 2. (c) The pulse sequence used for the dipolar HETCOR experiments. The pulse sequence was adapted from Ref. (12). Quadrature detection in ω_1 was achieved using the TPPI method (27) by incrementing the first 90° proton pulse. A 4-step phase cycle was used: $\phi_1 = +y, -y$; $\phi_2 = +x, +x, -x, -x$; receiver = 0, 2.

^1H - ^{13}C spins, the carbon magnetization evolves from in-phase (S_x) into antiphase ($2I_zS_y$) coherence with respect to its attached proton. In the MAS-*J*-HMQC experiment (Fig. 1a), this

antiphase carbon coherence is converted into a heteronuclear multiple-quantum (MQ) coherence ($2I_xS_y$) by the 90° proton pulse, while in the MAS-*J*-HSQC experiment (Fig. 1b), two

simultaneous 90° pulses applied on both channels transform this antiphase carbon coherence into an antiphase heteronuclear single-quantum (SQ) proton coherence ($2I_xS_z$). The proton chemical shift is then encoded by allowing these MQ or SQ coherences to evolve during the indirect evolution time t_1 . Note that in the MAS-*J*-HMQC experiment, the carbon chemical shift evolution during t_1 (and during the periods τ) is refocused by the 180° ^{13}C pulse applied in the middle of the pulse sequence (a heteronuclear MQ coherence is insensitive to the direct coupling between the two spins involved), whereas in the MAS-*J*-HSQC experiment, the 180° ^{13}C pulse applied in t_1 serves only to remove the effects of the heteronuclear couplings (the scalar coupling as well as the possible residual dipolar broadening). In the MAS-*J*-HSQC experiment, the two 180° pulses applied simultaneously to protons and carbons in the middle of the fixed intervals τ are necessary to refocus the carbon chemical shift evolution while keeping the J_{CH} coupling active. At the end of the evolution time t_1 , the MQ and SQ coherences are converted back into antiphase carbon coherences by the 90° pulses, and these coherences evolve during the second period τ to become in-phase observable carbon coherences. A two-dimensional Fourier transform of the time domain signal gives chemical shift correlations between pairs of bonded protons in ω_1 and carbons in ω_2 . Note that the experiments presented here are quite analogous to certain versions of the liquid-state HMQC (21) and HSQC (22) experiments. However, unlike the liquid-state experiments which use inverse detection of the ^{13}C spectrum, in the MAS-*J*-HMQC and MAS-*J*-HSQC pulse schemes, the magnetization is transferred through the J_{CH} couplings, from carbons to protons, and the ^{13}C spectrum is detected directly in t_2 . Most importantly, these methods involve evolution of the relatively long-lived carbon transverse coherences during the fixed intervals τ , and there is no evolution of short-lived proton coherences during these periods. Currently, direct transposition of liquid-state sequences, including proton detection, is not straightforward since: (i) direct detection of the ^1H spectrum under CRAMPS decoupling is experimentally relatively difficult and is likely to introduce further artifacts into the spectrum; and (ii) magnetization transfer of proton transverse coherence to carbons through the J_{CH} couplings would be much less efficient due to the fast decay of proton magnetization under FSLG decoupling. At the current state of the art in homonuclear decoupling, this loss in signal-to-noise ratio is not compensated by direct proton detection. One should note, however, that this does not mean that inverse detection, in complete analogy to the currently most common liquid-state experiments, will not become feasible in the (not too distant) future for solids, for example, with the advent of improved CRAMPS techniques.

In both sequences the first magic pulse θ_m rotates the proton magnetization from the tilted z axis of the effective field under FSLG decoupling, i.e., from an axis inclined at 54.7° with respect to the B_0 field to the z axis of the rotating frame, while the second magic angle pulse θ_m applied after the 90° proton

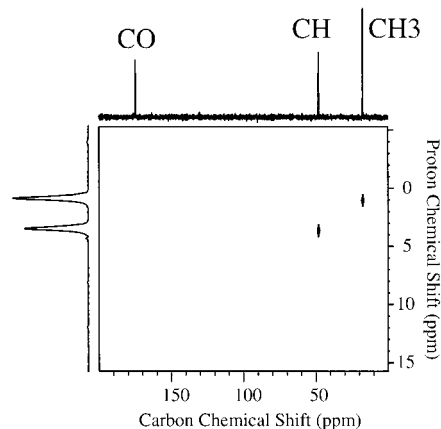


FIG. 2. Carbon-proton two-dimensional MAS-*J*-HMQC spectrum of a powder sample of natural abundance L-alanine. The sample was purchased from Sigma and used without further recrystallization. The experiment was performed on a Bruker DSX 500 spectrometer (proton frequency 500 MHz) using a 4-mm triple-resonance MAS probe. The sample volume was restricted to about $25\ \mu\text{l}$ in the center of the rotor to increase the radiofrequency field homogeneity. A total of 256 t_1 points with 32 scans each was collected. A spectral width of 13,530 Hz was used in the ω_1 dimension. The spinning frequency was 12.5 kHz and the delay τ equal to 2.4 ms. The proton RF field strength was set to 100 kHz during both the delays τ (FSLG decoupling) and acquisition (TPPM decoupling (28)). For the cross-polarization step, the contact time was set to 700 μs . The 1D CP-MAS ^{13}C spectrum is shown above the 2D spectrum, whereas the ^1H spectrum is the projection of the signal in ω_1 . No signal apodization was applied in the proton dimension. In all spectra reported in this paper, the proton chemical shift was referenced by setting the β resonance to 1 ppm.

pulse puts the proton transverse magnetization perpendicular to the effective field. This decomposition into three pulses is necessary to achieve quadrature detection in ω_1 (obtained by incrementing the phase of the second 90° proton pulse from one increment to another). At the end of the evolution time t_1 , no magic angle pulses are required: the second 90° proton pulse rotates the proton magnetization directly from the transverse tilted plane to the tilted z axis, so that it is aligned with the effective field during the second delay τ . (Note that for the MAS-*J*-HMQC experiment we had not proposed this simplification in our original paper.)

Figure 2 shows the two-dimensional MAS-*J*-HMQC spectrum of natural abundance L-alanine. The CH and CH_3 carbons are correlated with their attached protons whereas the carbonyl carbon gives no cross-peak in the ω_1 dimension. In theory, under FSLG decoupling, the proton chemical shift (as well as the heteronuclear couplings) is scaled by $1/\sqrt{3}$. The proton chemical shift scale has been corrected for this scaling in all of the spectra reported in this paper, and the proton linewidths have been measured on correctly scaled spectra. Figure 3a shows the proton traces extracted in the ω_1 dimension from this 2D MAS-*J*-HMQC spectrum. From these proton traces, it is clearly visible that, when using a short value for the fixed intervals τ (typically 2 ms), the experiment is highly selective for one-bond correlations, i.e., the α and β carbons are only

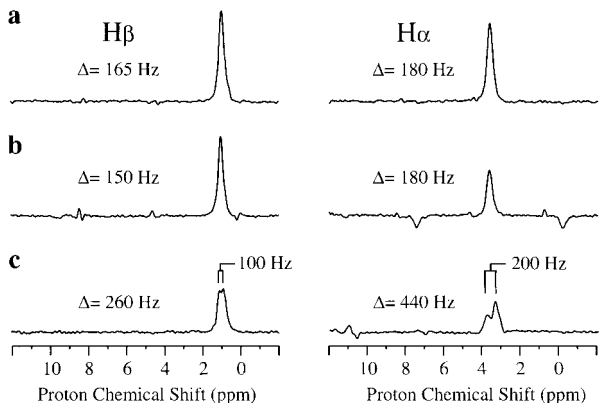


FIG. 3. Proton traces extracted from the ω_1 dimension from a two-dimensional MAS- J -HMQC (a) or MAS- J -HSQC (b) correlation spectrum of a powder sample of natural abundance L-alanine. The experimental conditions are the same as for Fig. 2. In (c), the proton traces are taken from a 2D MAS- J -HSQC spectrum recorded without a 180° carbon pulse during t_1 under the same experimental conditions as for (a) and (b). The proton full linewidth at half height Δ is indicated for each trace.

correlated with their directly attached protons (the choice of an optimal value for τ as well as a discussion on the selectivity of the MAS- J -HMQC experiment has already been reported in Ref. (14)). Proton linewidths of about 180 (0.36 ppm) and 165 Hz (0.33 ppm) were measured for the α and β protons, respectively. Note first that these linewidths are much narrower than those previously reported on the same sample but using other multiple-pulse decoupling techniques or fast MAS (23, 24). No real change in proton resolution is observed for natural abundance L-alanine when proton single-quantum coherence evolves during t_1 , as indicated by the ω_1 traces extracted from a 2D MAS- J -HSQC spectrum (Fig. 3b). We note, however, that in the MAS- J -HSQC experiment the signal-to-noise ratio is a little bit weaker for both the α and the β protons and that small artifactual peaks appear. This is probably simply related to the fact that the MAS- J -HSQC experiment has a larger number of pulses, each of which may introduce unwanted signals due to imperfections. In particular, to keep the phase cycle relatively short, the two 180° carbon pulses in the middle of the fixed intervals τ were not cycled.

As pointed out previously, the 180° carbon pulse applied during t_1 in the MAS- J -HSQC experiment is only necessary to remove the effects of the heteronuclear couplings. Indeed, when this π pulse is removed from the MAS- J -HSQC pulse sequence, a distinct splitting is observed for both the α and the β proton resonances, as shown in Fig. 3c. These splittings are caused by the J_{CH} scalar couplings. Thus, the resolution in the proton traces is currently sufficient to resolve the heteronuclear scalar couplings in the proton dimension, which, to our knowledge, has never been reported before for rigid solids. Splittings of 100 and 200 Hz are directly measured on the CH_3 and CH traces, respectively. While a doublet of 100 Hz corresponds well to what we can expect for a splitting due to a one-bond J_{CH}

coupling (for an aliphatic carbon, 130 Hz is a typical value for a one-bond J_{CH} coupling), a splitting of 200 Hz seems much too large. Numerical calculations are under investigation to explain this discrepancy.

The relative advantages of the multiple-quantum and single-quantum techniques were then investigated for a sample of fully ^{13}C -labeled L-alanine. The results obtained are presented in Figs. 4a and 4b, which show the ω_1 traces extracted from a MAS- J -HMQC and MAS- J -HSQC spectrum, respectively. The proton resolution is clearly enhanced when using the SQ correlation technique. We assume that the broadening observed in the MAS- J -HMQC proton traces (Fig. 4a) is due to the unresolved homonuclear carbon-carbon couplings which are active on the MQ coherences ($2I_xS_y$) created in the MAS- J -HMQC experiment, but which are not on the SQ coherences ($2I_xS_z$) present in the MAS- J -HSQC experiment. These couplings include notably the scalar J_{CC} couplings (typically 40 Hz between two bonded carbons) as well as the possible residual terms of the ^{13}C - ^{13}C dipolar interactions.

Finally we compared the proton resolution obtained in scalar and dipolar correlation techniques (a comparison in terms of selectivity or signal-to-noise ratio is not within the scope of this paper and has been already briefly discussed in Ref. (14)). For

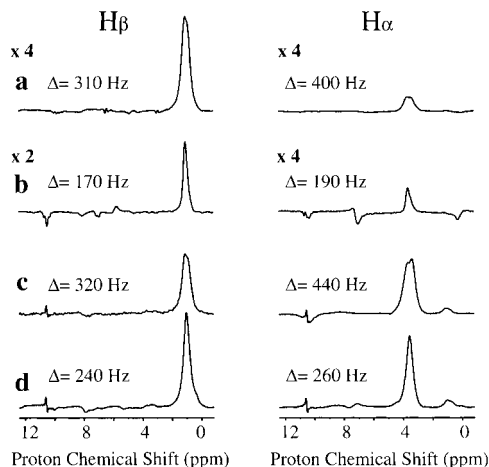


FIG. 4. Proton traces extracted in the ω_1 dimension from a two-dimensional MAS- J -HMQC (a) and MAS- J -HSQC (b) experiment recorded on a powder sample of fully ^{13}C -labeled L-alanine. The sample was purchased from Cambridge Isotope Laboratories and used without further recrystallization. The experiment was performed on a Bruker DSX 500 spectrometer (proton frequency 500 MHz). A total of 384 t_1 points with 16 scans each was collected. A spectral width of 13,364 Hz was used in the ω_1 dimension. The spinning frequency was 12.5 kHz and the delay τ equal to 2.4 ms. The contact time for the cross-polarization step was 700 μs . The proton RF field strength was set to 100 kHz during both the τ delays (FSLG decoupling) and during acquisition. (c) and (d) show the ω_1 traces extracted at the same carbon frequency from dipolar HETCOR experiments recorded (c) without and (d) with a 180° carbon pulse during t_1 . The contact time for cross-polarization in the HETCOR experiment was set to 100 μs . The other experimental conditions were the same as for the scalar correlations. In particular, the same FSLG decoupling conditions were used during t_1 . The linewidth at half height Δ is indicated for the CH and CH_3 proton resonances.

the dipolar HETCOR experiments we used the pulse sequence proposed by vanRossum *et al.* (12). It consists of a ($\pi/2 + \theta_m$) pulse on protons, directly followed by a proton chemical shift evolution time t_1 under FSLG decoupling, and subsequently a magnetization transfer to carbons using a normal cross-polarization step, after returning the proton magnetization to the xy plane by a θ_m pulse. The carbon magnetization is detected during t_2 under heteronuclear decoupling, and a two-dimensional Fourier transform of the time signal gives through-space correlations between pairs of nearby carbon and proton nuclei. The proton linewidths measured in the ω_1 traces of the HETCOR spectrum (Fig. 4c) are significantly larger than those observed in the MAS-*J*-HSQC traces (Fig. 4b). In the dipolar HETCOR experiment, the proton chemical shift is encoded by the evolution of a single quantum operator (I_x), on which the heteronuclear couplings are active (as is the case for the single-quantum operator $2I_xS_z$ created in the MAS-*J*-HSQC experiment) and may lead to a broadening of proton lines. Indeed, as expected, when we ran a HETCOR experiment with an additional 180° carbon pulse in the middle of t_1 (Fig. 1c), we observed a substantial proton line narrowing (Fig. 4d) since the net contribution of the heteronuclear couplings is removed. Thus, by slightly modifying the pulse sequence for the dipolar HETCOR experiments, the resolution of the proton spectra of crystalline samples can be significantly improved. The improvement in resolution has been demonstrated here on a ^{13}C -labeled sample, but a similar effect is observed for natural abundance compounds (data not shown). Note, however, that even with this refocusing pulse, the proton resonances in the dipolar HETCOR spectrum remain slightly broader than those obtained in the MAS-*J*-HSQC spectrum. This observation can be explained by the fact that, unlike the cross-polarization step, the magnetization transfer by scalar couplings favors carbon-proton pairs for which the residual couplings under the MAS and FSLG are weakest (the signal of more strongly coupled pairs undergoes comparatively greater attenuation through dephasing during the τ delays than that of the weakly coupled pairs).

In conclusion, the MAS-*J*-HMQC and MAS-*J*-HSQC experiments presented in this paper constitute a new class of heteronuclear correlation experiments for solid-state NMR, which use, like their liquid-state analogs, the scalar couplings to transfer magnetization between pairs of bonded heteronuclei. The MAS-*J*-HMQC experiment requires fewer pulses, seems more robust, and has a small advantage in terms of sensitivity and artifacts over the MAS-*J*-HSQC version for natural abundance samples. In these samples, we have shown that the proton resolution does not depend on the *J*-pulse scheme used. However, the SQ scheme provides a real advantage in terms of proton resolution when compared to the MQ scheme for fully labeled samples. In addition, our experiments indicate that the resolution in the proton dimension is significantly affected by the presence of the heteronuclear couplings when the proton chemical shift is encoded by the evolution of

a single-quantum operator. This effect, which is also observed for a dipolar correlation technique, can simply be removed by incorporating a 180° carbon refocusing pulse. The MAS-*J*-HMQC and MAS-*J*-HSQC spectra reported in this paper were carried out on a model sample, but they are applicable to a wide variety of crystalline and amorphous compounds and are expected to become very useful in the structural investigation of solid-state compounds.

REFERENCES

1. D. P. Burum, HETCOR in organic solids, in "The Encyclopedia of NMR" (D. M. Grant and R. K. Harris), pp. 2323–2329, Wiley, Chichester, 1997.
2. C. E. Bronnimann, C. F. Ridenour, D. R. Kinney, and G. E. Maciel, 2D ^1H - ^{13}C heteronuclear correlation spectra of representative organic solids, *J. Magn. Reson.* **97**, 522–534 (1992).
3. C. W. B. Lee and R. G. Griffin, Two-dimensional $^1\text{H}/^{13}\text{C}$ heteronuclear chemical shift correlation spectroscopy of lipid bilayers, *Biophys. J.* **55**, 355–358 (1989).
4. Z. Gu, C. F. Ridenour, C. E. Bronnimann, T. Iwashita, and A. McDermott, Hydrogen bonding and distance studies of amino acids and peptides using solid state 2D ^1H - ^{13}C heteronuclear correlation spectra, *J. Am. Chem. Soc.* **118**, 822–829 (1996).
5. B.-J. vanRossum, C. P. deGroot, V. Ladizhansky, S. Vega, and H. J. M. deGroot, A method for measuring heteronuclear (^1H - ^{13}C) distances in high speed MAS NMR, *J. Am. Chem. Soc.* **122**, 3465–3472 (2000).
6. P. Caravatti, G. Bodenhausen, and R. R. Ernst, Heteronuclear solid-state correlation spectroscopy, *Chem. Phys. Lett.* **89**, 363–367 (1982).
7. P. Caravatti, L. Braunschweiler, and R. R. Ernst, Heteronuclear correlation spectroscopy in rotating solids, *Chem. Phys. Lett.* **100**, 305–310 (1983).
8. J. E. Roberts, S. Vega, and R. G. Griffin, Two-dimensional heteronuclear chemical shift correlation spectroscopy in rotating solids, *J. Am. Chem. Soc.* **106**, 2506–2512 (1984).
9. D. P. Burum and A. Bielecki, An improved experiment for heteronuclear-correlation 2D NMR in solids, *J. Magn. Reson.* **94**, 645–652 (1991).
10. W. Sommer, J. Gottwald, D. E. Demco, and H. W. Spiess, Dipolar heteronuclear multiple-quantum NMR spectroscopy in rotating solids, *J. Magn. Reson.* **113**, 113–134 (1995).
11. K. Saalwächter, R. Graf, and H. W. Spiess, Recoupled polarization transfer heteronuclear ^1H - ^{13}C multiple-quantum correlation in solids under ultra-fast MAS, *J. Magn. Reson.* **140**, 471–476 (1999).
12. E. J. vanRossum, H. Förster, and H. J. M. deGroot, High-field and high speed CP-MAS ^{13}C NMR heteronuclear dipolar-correlation spectroscopy of solids with frequency-switched Lee-Goldburg homonuclear decoupling, *J. Magn. Reson.* **124**, 516–519 (1997).
13. E. J. vanRossum, G. J. Boender, and H. J. M. deGroot, High magnetic field for enhanced proton resolution in high-speed CP/MAS heteronuclear ^1H - ^{13}C dipolar-correlation spectroscopy, *J. Magn. Reson.* **120**, 274–277 (1996).
14. A. Lesage, D. Sakellariou, S. Steuernagel, and L. Emsley, Carbon-proton chemical shift correlation in solid-state NMR by through-bond multiple-quantum spectroscopy, *J. Am. Chem. Soc.* **120**, 13194–13201 (1998).
15. A. Lesage, P. Charmont, S. Steuernagel, and L. Emsley, Complete resonance assignment of a natural abundance solid peptide by

- through-bond heteronuclear correlation solid-state NMR, *J. Am. Chem. Soc.* **122**, 9739–9744 (2000).
16. A. Bielecki, A. C. Kolbert, H. J. M. deGroot, R. G. Griffin, and M. H. Levitt, Frequency-switched Lee–Goldburg sequences in solids, *Adv. Magn. Reson.* **14**, 111 (1989).
 17. A. Bielecki, A. C. Kolbert, and M. H. Levitt, Frequency-switched pulse sequence: Homonuclear decoupling and dilute spin NMR in solids, *Chem. Phys. Lett.* **155**, 341 (1989).
 18. M. H. Levitt, A. C. Kolbert, A. Bielecki, and D. J. Ruben, High-resolution ^1H NMR in solids with frequency-switched multiple-pulse sequences, *Solid State NMR* **2**, 151–163 (1993).
 19. E. Vinogradov, P. H. Madhu, and S. Vega, High-resolution proton solid state NMR spectroscopy by phase-modulated Lee–Goldburg experiment, *Chem. Phys. Lett.* **314**, 443–450 (1999).
 20. D. Sakellariou, A. Lesage, P. Hodgkinson, and L. Emsley, Homonuclear dipolar decoupling in solid-state NMR using continuous phase modulation, *Chem. Phys. Lett.* **319**, 253–260 (2000).
 21. A. Bax, R. H. Griffey, and B. L. Hawkins, Correlation of proton and nitrogen-15 chemical shifts by multiple quantum NMR, *J. Magn. Reson.* **55**, 301–315 (1983).
 22. G. Bodenhausen and D. J. Ruben, Natural abundance nitrogen-15 NMR by enhanced heteronuclear spectroscopy, *Chem. Phys. Lett.* **69**, 185 (1980).
 23. A. Naito, A. Root, and C. A. McDowell, CRAMPS proton spectra on amino acids, *J. Chem. Phys.* **95**, 3578 (1991).
 24. I. Schnell, A. Lupulescu, S. Hafner, D. E. Demco, and H. W. Spiess, Resolution enhancement in multiple-quantum MAS NMR spectroscopy, *J. Magn. Reson.* **133**, 61–69 (1998).
 25. <http://www.ens-lyon.fr/STIM/NMR>.
 26. D. J. States, R. A. Haberkorn, and D. J. Ruben, A two-dimensional nuclear Overhauser experiment with pure absorption phase in four quadrants, *J. Magn. Reson.* **48**, 286–292 (1982).
 27. D. Marion and K. Wüthrich, Application of phase sensitive two-dimensional correlated spectroscopy (COSY) for the measurements of ^1H – ^1H spin–spin coupling constants in proteins., *Biochem. Biophys. Res. Commun.* **113**, 967–974 (1983).
 28. A. E. Bennett, C. M. Rienstra, M. Auger, K. V. Lakshmi, and R. G. Griffin, Heteronuclear decoupling in rotating solids, *J. Chem. Phys.* **103**, 6951 (1995).