

Determination of Through-Bond Carbon–Carbon Connectivities in Solid-State NMR Using the INADEQUATE Experiment

Anne Lesage, Céline Auger, Stefano Caldarelli,[†] and Lyndon Emsley*

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

Received April 7, 1997

In powder samples, cross polarization, magic-angle spinning (MAS), and proton decoupling can yield high-resolution high-sensitivity NMR spectra of dilute spins such as carbon-13. Over the last 10 years a multitude of techniques based on magnetization transfer through the homonuclear dipolar coupling have been proposed to obtain *through-space* ¹³C chemical shift correlations, yielding detailed information about internuclear distances.^{1–10} However, virtually no techniques exist to determine *through-bond* carbon-13 connectivities. Nevertheless these through-bond connectivities are necessary to establish an unambiguous assignment of the NMR spectra, before through-space interactions can be used to determine structure. Very recently, it was shown that it is possible to exploit scalar couplings in solids in a total through-bond correlation experiment.¹¹ The experiment yields net polarization transfer and in-phase correlations. It requires high-power homonuclear proton decoupling and through-space interactions are difficult to suppress entirely. The INADEQUATE experiment is a well-known liquid-state technique to establish direct scalar connectivities in the ¹³C skeleton.¹² However it yields correlations in antiphase. Experiments yielding antiphase lineshapes have previously been considered as impractical in ordinary organic solids because of signal cancellation due to line width. Such experiments were shown to be practical, about 10 years ago, on plastic crystals with line widths of a few hertz¹³ and more recently on highly ordered crystals of nucleotides and sugars with narrow line widths and under conditions of high power decoupling and fast MAS.¹⁴ In this communication, we demonstrate the experimental feasibility of the INADEQUATE experiment on solid samples exhibiting line widths of several tens of hertz, such as ordinary powdered amino acids. We show that the INADEQUATE experiment leads to a sufficiently efficient excitation

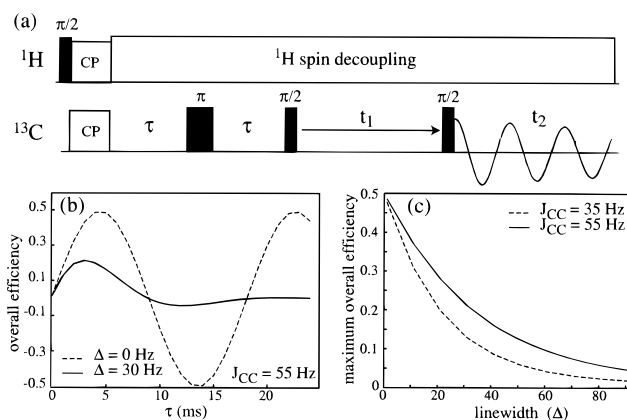


Figure 1. (a) The solid-state INADEQUATE pulse sequence. A composite π pulse of the form $(90^\circ)_x(239^\circ)_x(90^\circ)_y$ is applied in the middle of t_1 . A 32 step phase cycle is used (pulse program available on request). The resulting 2D spectrum yields a pair of crosspeaks for each pair of bonded carbons which appear as two antiphase doublets in ω_1 , and as singlets at $(\omega^A + \omega^B)$ in ω_2 . (b) The theoretical overall efficiency, calculated as the ratio of the maximum amplitude of the antiphase doublet in a 1D INADEQUATE experiment to the amplitude of the in-phase doublet in a single-pulse experiment is shown as a function of the delay τ . These numerical calculations take into account the efficiency of transfer into anti-phase coherence, the decay during τ due to the (Gaussian) line width, and the attenuation of the detected signal by cancellation effects. The calculations were performed for two values of the line width Δ , of 0 and 30 Hz. (Note that this is the full line width at half height for a single component and that the corresponding overall line width observed in the carbon spectrum of the enriched compound will be roughly twice this value.) The J coupling was fixed at 55 Hz. (c) The maximum overall efficiency calculated as a function of the line width Δ , using the corresponding optimal value of the delay τ for each value of Δ . Two values of the J coupling 55 and 35 Hz (corresponding to J couplings typical for the $C\alpha$ –CO and $C\alpha$ – $C\beta$ bonds respectively in amino acids) were considered. The overall efficiency tends to 0.5 in the absence of line broadening, since as usual in a double-quantum filtered experiment, the zero-quantum coherences created with the double quantum coherences are not detected.

of double quantum coherence and that the detection of antiphase components remains possible. The resulting experiment, which provides unambiguous identification of scalar connectivities, is particularly simple and robust. The method is shown to be practicable not only on ¹³C-labeled products but also on *natural abundance* compounds.

The pulse sequence for the experiment is shown in Figure 1a. Apart from the cross-polarization period, the experiment is completely analogous to the liquid-state version of the INADEQUATE experiment. After cross-polarization from ¹H, the ¹³C magnetization evolves during the delay 2τ under the isotropic homonuclear J coupling Hamiltonian. The C–C homonuclear dipolar couplings are removed by fast magic-angle spinning and the isotropic chemical shift is refocused by the 180° pulse. The double-quantum coherence created by the first carbon 90° pulse evolves during t_1 at the sum frequency of the two spins, $\omega_{DQ} = \omega_{SQ}^A + \omega_{SQ}^B$, and is converted back into an antiphase transverse coherence by the last 90° pulse. Note that unlike previously proposed correlation schemes, the INADEQUATE experiment does not require any rotor synchronization and, thus, can be performed at very high spinning frequencies. Ideally, the degree of conversion into double quantum coherence is maximum for $\tau = 1/4J_{CC}$ and is zero for $\tau = 1/2J_{CC}$. This optimum delay decreases with increasing line widths as illustrated by the simulations of Figure 1b. Figure 1c shows that although the overall efficiency of the INADEQUATE experiment decreases rapidly with increasing line width, the efficiency is still significant for $\Delta \approx J_{CC}$.

* To whom correspondence should be addressed.

[†] Present address: Institut de Recherches sur la Catalyse, 2, av. Albert Einstein, 69626 Villeurbanne, France.

(1) Menger, E. M.; Vega, S.; Griffin, R. G. *J. Am. Chem. Soc.* **1986**, *108*, 2215.

(2) Raleigh, D. P.; Levitt, M. H.; Griffin, R. G. *Chem. Phys. Lett.* **1988**, *146*, 71.

(3) Nielsen, N. C.; Creuzet, F.; Griffin, R. G.; Levitt, M. H. *J. Chem. Phys.* **1992**, *96*, 5668.

(4) Tycko, R.; Dabagh, G. *J. Am. Chem. Soc.* **1991**, *113*, 9444.

(5) Bennet, A. E.; Ok, J. H.; Griffin, R. G. *J. Chem. Phys.* **1992**, *96*, 8624.

(6) Nielsen, N. C.; Bildsoe, H.; Jakobsen, H. J.; Levitt, M. H. *J. Chem. Phys.* **1994**, *101*, 1805.

(7) Fujiwara, T.; Sugase, K.; Kainosho, M.; Ono, A.; Ono, A. M.; Akutsu, H. *J. Am. Chem. Soc.* **1995**, *117*, 11351.

(8) Sun, B. Q.; Costa, P. R.; Kocisko, D.; Lansbury, P. T.; Griffin, R. G. *J. Chem. Phys.* **1995**, *102*, 702.

(9) Lee, Y. K.; Kurur, N. D.; Helmle, M.; Johannesses, O. G.; Nielsen, N. C.; Levitt, M. H. *Chem. Phys. Lett.* **1995**, *242*, 304.

(10) Baldus, M.; Iulucci, R. J.; Meier, B. H. *J. Am. Chem. Soc.* **1996**, *119*, 1121.

(11) Baldus, M.; Meier, B. H. *J. Magn. Reson.* **1996**, *121*, 65.

(12) Bax, A.; Freeman, R.; Frenkiel, T. A. *J. Am. Chem. Soc.* **1981**, *103*, 2102.

(13) Early, T. A.; John, B. K.; Johnson, L. F. *J. Magn. Reson.* **1987**, *79*, 134. Benn, R.; Grondey, H.; Brevard, C.; Pagelot, A. *J. Chem. Soc., Chem. Com.* **1988**, 102. Gray, I. D.; Jones, C. H. W.; Sharma, R. D. *J. Magn. Reson.* **1991**, *91*, 186.

(14) Mehta, A.; Tounge, B. A.; Zilm, K. W. Abstract (p 335) and Wednesday Poster (no. 226) at the 37th ENC, Asilomar, 1996.

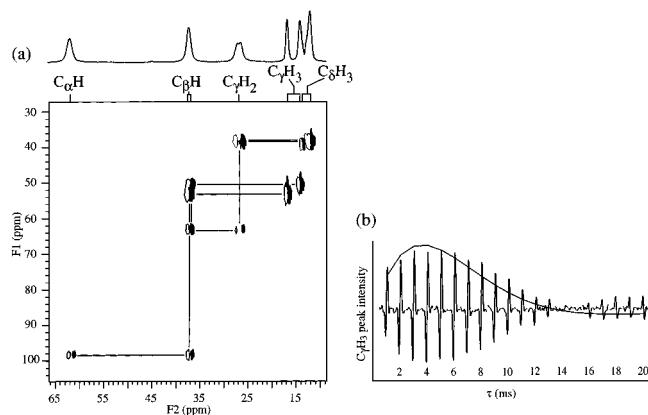


Figure 2. (a). Expansion of the aliphatic region of the 2D INADEQUATE spectrum of fully ^{13}C -labeled L-isoleucine. The spectrum was obtained at 125 MHz on a Varian Unity+ narrow-bore spectrometer using a 5 mm double-tuned MAS probe. The spinning speed was 8.2 kHz, and the proton decoupling field strength was $\omega_1 = 62$ kHz, using a TPPM scheme.¹⁶ A total of 256 t_1 increments with 32 scans each were collected. The delay τ was set to 3 ms, and the contact time for cross-polarization to 1 ms. Quadrature detection in ω_1 was achieved using the States method. Cross-peaks shown in black and in white correspond to positive and negative signals, respectively. Two directly bonded carbons share a common frequency in the F_1 dimension. The various connectivities between the bonded carbons are indicated by solid lines. The overall efficiency was found to be about 5%. Note that we used very modest experimental conditions (relatively low spinning rate and decoupling field). The 1D ^{13}C spectrum is shown above the 2D spectrum. The overall line widths range from 80 to 180 Hz in this 1D spectrum. (b) The intensity of the antiphase doublets as a function of the delay τ in a 1D INADEQUATE experiment ($t_1 = 0$). The evolution is shown for the $\text{C}_\gamma\text{H}_3$ resonance at 16.7 ppm. The solid curve is a simulation calculated with J coupling and line width values of both 35 Hz. The only adjustable parameter was the overall intensity. The line width, corresponding to that of one doublet component, was obtained from simulation of the normal 1D spectrum.

As in solution NMR, directly bonded carbon-13 resonances are identified by the fact that they generate a common double-quantum frequency. The aliphatic region of the 2D INADEQUATE spectrum of uniformly ^{13}C -enriched L-isoleucine is shown in Figure 2a. This spectrum allows the assignment of all the carbon resonances, which, in the case of isoleucine, arise from two distinct spin systems corresponding to two crystallographically different molecules in the unit cell.¹⁵ For example, the 2D spectrum allows us to determine which of the two $\text{C}_\gamma\text{H}_3$ is correlated with which of the two C_βH . The intensity of the antiphase doublets measured as a function of the delay τ , shown in Figure 2b, passes through zero for τ between 14 and 15 ms, which corresponds to $1/2J_{\text{CC}}$ for a J coupling of between 33 and 35 Hz. Subsequently, intensity changes sign as expected. The good agreement between the experimental and simulated data (solid curve) indicates without any doubt that the mechanism for transfer is solely governed by the scalar coupling and that the contribution of any residual dipolar coupling to the antiphase coherence transfer is negligible. Note that the poor overall efficiency of the experiment (about 5% in the case of isoleucine) does not constitute a real problem when dealing with enriched compounds. (In the example of Figure 2, the total acquisition time was limited by the number of steps in the phase cycle.) Note also that the efficiency would increase when using a higher decoupling power and a faster spinning frequency.

(15) Torii, K.; Iitaka, Y. *Acta Crystallogr.* **1971**, *27*, 2237.

(16) Bennett, A. E.; Rienstra, C. M.; Auger, M.; Lakshmi, K. V.; Griffin, R. G. *J. Chem. Phys.* **1995**, *103*, 6951.

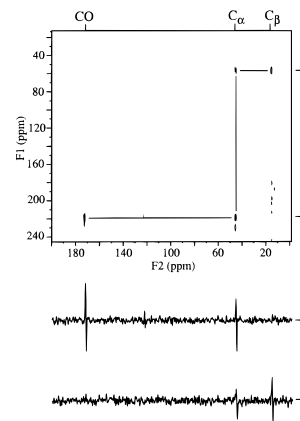


Figure 3. 2D INADEQUATE spectrum of a powder sample of natural abundance L-alanine. The spectrum was obtained at 100 MHz on a Bruker DSX400 wide-bore spectrometer using a 7 mm CP-MAS probe. The spinning rate was 5 kHz, and the proton decoupling field strength was $\omega_1 = 70$ kHz (continuous wave decoupling). A total of 256 t_1 increments with 800 scans each was collected. Quadrature detection in ω_1 was achieved using the TPPI method. The delay τ was set to 4 ms, and the contact time for cross-polarization to 0.5 ms. Traces extracted from the 2D spectrum in the F_2 dimension are shown under the 2D spectrum. The overall line widths in the 1D spectrum range between 30 and 60 Hz. The experimental overall efficiency was 12%.

In Figure 3 we show that the solid-state INADEQUATE experiment is also feasible at natural abundance on ordinary powdered amino acid samples, with the natural abundance 2D spectrum of L-alanine. This spectrum, which has relatively high resolution and good signal to noise ratio, was acquired in a quite reasonable experimental time (about a weekend) using modest experimental conditions. Note that, in this case, we observe the one per ten thousand molecules having two particular directly bonded carbon-13 atoms.

In conclusion, we have shown that the overall efficiency of the solid-state INADEQUATE experiment is sufficient for it to be performed on ordinary solid powder samples such as amino acids. (Note that since INADEQUATE is possible, other homonuclear J correlation experiments such as DQF-COSY also become possible, and we have successfully recorded DQF-COSY spectra on enriched compounds (not shown).) Coherence transfer is exclusively mediated by the scalar J interactions, which leads to the unambiguous identification of through-bond connectivities. For enriched compounds this extremely simple and robust experiment appears to be competitive with more complex in-phase transfer techniques to assign the carbon spectrum. The two different molecules in the unit cell of L-isoleucine represent one of the largest organic spin systems that has been assigned by direct correlation methods to date. The experiment should be particularly useful to elucidate spin systems in enriched solid peptides, thereby providing an essential step toward structure determination. Moreover, the method was sufficiently sensitive to allow us to record the first experimental solid-state correlation spectrum (of any type) of an amino acid at natural abundance. The efficiency of the experiment rapidly increases with decreasing line width. Thus, we can expect that with future technical and theoretical developments in sample rotation and proton decoupling, the technique will become practicable on solid peptides at natural abundance.

Acknowledgment. We thank Varian Associates and Dr. H. Foerster (Bruker, Karlsruhe) for the loan of equipment used in this work. S.C. was supported during this work by a Marie Curie Fellowship from the European Community (contract number ERBFMBICT960917).