COMMUNICATIONS

Measuring ^{13}C–^{2}D Dipolar Couplings with a Universal REDOR Dephasing Curve

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A ^{13}C-observe REDOR experiment is described which allows ^{13}C–^{2}D dipolar couplings to be obtained by a universal dipolar dephasing curve. Previous ^{13}C-observe REDOR experiments on ^{13}C–^{2}D spin pairs generally relied on numerical simulations to obtain the dipolar coupling. The REDOR experiment described in this article is based on a deuterium composite pulse, and the data analysis eliminates the need for numerical simulations and is the same as the traditional REDOR analysis performed on pairs of spin-$\frac{1}{2}$ nuclei. © 2000 Academic Press

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An attractive feature of the REDOR experiment applied to a pair of spin-$\frac{1}{2}$ nuclei is the dipolar dephasing described by a universal dipolar dephasing curve (*1, 2*). The universal dipolar dephasing curve provides a simple way to obtain dipolar couplings since it is governed solely by the dipolar interaction. However, the measurement of ^{13}C–^{2}D dipolar interactions by ^{13}C-observe REDOR can be hampered by the large deuterium quadrupolar interaction. Typical radiofrequency power levels on multiply-tuned NMR probes are insufficient to fully excite the entire spectral range associated with the broad deuterium powder pattern. This is especially true when there is no fast motional averaging of the quadrupolar interaction. While ^{13}C–^{2}D REDOR experiments have been performed on samples with large deuterium quadrupolar coupling constants, data analysis required numerical simulations since the ^{13}C magnetization decay did not obey a universal dephasing curve (*3–8*). Hence, it is desirable to have a ^{13}C–^{2}D REDOR experiment where the ^{13}C magnetization dephasing follows a universal dipolar dephasing curve. Such a curve would depend only on the ^{13}C–^{2}D dipolar interaction, would be independent of the chemical shift anisotropies and the deuterium quadrupolar interaction, and would provide a straightforward method for obtaining dipolar couplings. A ^{13}C–^{2}D REDOR experiment, based on a deuterium composite pulse, that generates data obeying a universal dephasing curve is described in this Communication.

A ^{13}C–^{2}D REDOR experiment based on a deuterium $\pi/2$ dipolar recoupling pulse was described in a previous publication (*8*) and is shown in Fig. 1. Protons are used for cross-polarization (CP) and are subsequently decoupled by a strong RF field. A train of rotor-synchronized xy-4 phased π pulses (*9*) lasting for N_c rotor cycles is applied to the ^{13}C channel during the dipolar evolution period (*10*). The time between adjacent π pulses is one-half of a rotor period. The deuterium channel consists of a single nominal $\pi/2$ pulse. During the dipolar evolution time, the ^{13}C signal will decay from T_2 processes and will also dipolar dephase if the deuterium pulse is applied. The T_2 decay can be taken into account by performing an additional experiment which omits the deuterium pulse. Hence, for each value of the evolution time, two experiments are performed: one with the deuterium pulse and one without the deuterium pulse. The ratio of these two signals is called S_d and the *ideal* evolution of this normalized signal is due solely to the dipolar interaction.

For the $\pi/2$-based REDOR experiment, the dipolar-dephased ^{13}C magnetization, s_d, for a specific orientation of the ^{13}C–^{2}D dipolar vector obeys (*8*)

$$s_d(\tau) = \frac{1}{6}(1 + 4\cos\bar{\omega}_d\tau + \cos 2\bar{\omega}_d\tau). \qquad [1]$$

The dipolar evolution time, τ, is the product of the number of rotor cycles, N_c, and the rotor period, T_r. The average dipolar frequency, $\bar{\omega}_d$, depends on the spatial orientation of the spin pair and on the dipolar coupling, D (in units of Hz). The dipolar coupling D is equal to $d/2\pi$, where $d = \mu_0\gamma_D\gamma_C\hbar/4\pi r^3$. The average dipolar frequency for a given orientation of a ^{13}C–^{2}D internuclear vector is $\bar{\omega}_d = 2\sqrt{2}D\sin 2\beta\sin\alpha$, where α and β are the azimuthal and polar angles of the internuclear vector with respect to the rotor frame, respectively (*1, 2*). A normalized powder average of Eq. [1] provides the desired universal dipolar dephased signal, S_d. The solid curve in Fig. 2 shows S_d as a function of λ ($=N_cT_rD$).
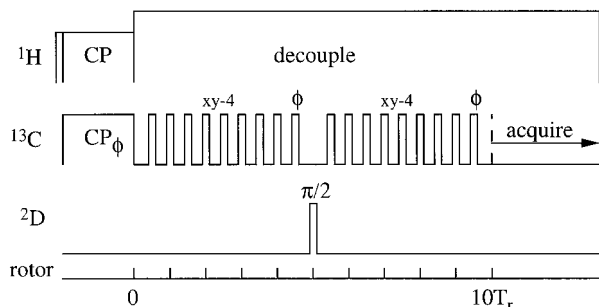
FIG. 1. The REDOR pulse sequence. The pulse sequence is shown with a ten rotor cycle dipolar evolution period. The nominal deuterium pulse flip angle is $\theta = \pi/2$. All ^{13}C pulses following the cross-polarization (CP) pulse are xy-4 phased π pulses except for the two specifically marked with the CP phase ϕ. Experiments were performed with RF field amplitudes of 50 kHz for ^{1}H–^{13}C cross-polarization, 110 kHz for ^{1}H decoupling, and 48.5 kHz for the ^{2}D pulse. The spinning rate was set to 3125 Hz and controlled to within 0.2 Hz. The experiments were performed on a homebuilt spectrometer operating at a ^{1}H NMR frequency of 151 MHz and based on a Tecmag Libra pulse programmer.

Experiments were performed on an alanine sample prepared by recrystallizing 20.6 mg of [3-^{13}C, 1-^{2}D]DL-alanine (99 at.% ^{13}C; 98 at.% ^{2}D; Isotec, Inc.) and 406.8 mg natural-abundance DL-alanine (Aldrich) from water. The quadrupolar coupling constant, QCC, is equal to 167 kHz for the methine deuteron of alanine (11). X-ray diffraction methods give a 2.17 Å separation between the ^{13}C and ^{2}D labels of alanine (12). The methyl ^{13}C resonance will contain contributions from ^{13}C spins coupled to ^{2}D spins and from ^{13}C spins that have no deuterium neighbor because of the dilution of the labeled alanine in natural-abundance alanine. Consequently, S_d is not what is obtained directly from analysis of the experimental spectra. Instead, the measured ratio is S_d^m. Two effects cause S_d and S_d^m to differ. First, because of the spin dilution only a fraction ρ of ^{13}C spins have a ^{2}D neighbor; this fraction can be calculated from the quantities of labeled and natural-abundance alanine used to make the sample. Second, the CP dynamics for ^{13}C spins on deuterated alanine may differ from the CP dynamics for ^{13}C spins on natural-abundance alanine. The differences in CP properties are not easy to calculate but will be noted by an empirical parameter ε. A simple model described elsewhere shows that S_d and S_d^m are related by (13)

$$S_d = 1 - (1 - S_d^m)/\alpha, \qquad [2]$$

where $\alpha = \rho\varepsilon$. Previous work showed that $\alpha = 0.70$ for this alanine sample (8).

REDOR experiments were performed for a series of dipolar evolution times on the alanine sample by applying a conventional rectangular $\pi/2$ pulse to the deuterium channel. The raw data, S_d^m, were corrected according to Eq. [2] and the corresponding values of S_d are shown as open circles in Fig. 2. It is clear that the REDOR data taken with a simple deuterium $\pi/2$ pulse do not follow the universal dipolar dephasing curve predicted by Eq. [1]. A problem with this data is that the 48.5

kHz amplitude deuterium pulse cannot cover the broad deuterium powder pattern characterized by a quadrupolar coupling constant of 167 kHz.

Broadband excitation and inversion deuterium pulses based on composite pulses have been described in the literature (14–18). The composite pulses have a much greater bandwidth than simple single-phase pulses and have found use in generating undistorted deuterium quadrupolar echo spectra. Based upon the success of such pulses, it seemed reasonable that the ^{13}C–^{2}D REDOR experiment would benefit from a composite deuterium $\pi/2$ pulse. There are many spin-1 composite pulses described in the literature. The composite $\pi/2$ pulse, $\theta_{cp}(\pi/2)_x$, that is particularly attractive is $\theta_{cp}(\pi/2)_x = 17_{\bar{x}}62_x99_{\bar{x}}144_x$, where the nominal on-resonance flip angles are given in degrees (16, 18). This windowless sequence of pulses was introduced by Shaka (18) and developed by Raleigh, Olejniczak, and Griffin as a building block for a spin-1 broadband inversion pulse for solids. The relatively short duration of this composite pulse is beneficial for our magic-angle spinning experiment.

REDOR experiments were performed with the aforementioned composite deuterium pulse. The raw data were corrected as described above and the corresponding values of S_d are shown as closed circles in Fig. 2. The data generated with $\theta_{cp}(\pi/2)_x$ obey the universal dephasing curve if the ^{13}C–^{2}D dipolar coupling is set to 431 Hz; this dipolar coupling corresponds to a methyl carbon to methine deuteron distance of 2.20 Å. X-ray diffraction measurements give a distance of 2.17 Å between the two spin labeled sites. The REDOR-measured distance is 1.4% longer than that found by X-ray diffraction, and this small difference is consistent with that typically found in REDOR measurements between pairs of spin-$\frac{1}{2}$ nuclei. Hence, the ^{13}C–^{2}D REDOR experiment based on the composite deuterium $\pi/2$ pulse performs very well.

The $\theta_{cp}(\pi/2)_x$ pulse that we have used for our REDOR application is known to perform poorly as a broadband exci-
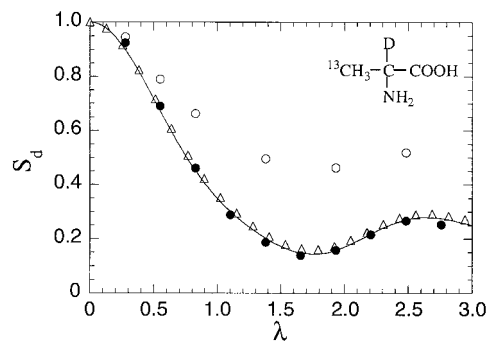

FIG. 2. The ideal behavior of the dipolar dephased ^{13}C signal on λ for isolated ^{13}C–^{2}D spin pairs is shown as the solid curve. The open circles represent the corrected data taken with a conventional deuterium $\pi/2$ pulse. The closed circles represent the corrected data taken with a composite deuterium $\pi/2$ pulse. Both sets of experimental data are plotted using a ^{13}C–^{2}D dipolar coupling of 431 Hz. Numerical simulations described in the text are shown as the open triangles. The inset shows the labeled alanine.

tation pulse for a ^{2}D-observe experiment since it results in significant phase distortion of the resulting deuterium spectrum (*16*). Hence, the good performance of $\theta_{cp}(\pi/2)_x$ as a REDOR pulse warrants further examination. We have performed numerical integration of the density matrix for the ^{13}C–^{2}D REDOR experiment with the $\theta_{cp}(\pi/2)_x = 17_{\bar{x}}62_x99_{\bar{x}}144_x$ deuterium pulse. A QCC of 167 kHz, a spinning rate of 3125 Hz, and a ^{2}D RF field strength of 50 kHz were used in the calculations. The numerical results (open triangles in Fig. 2) closely follow the ideal universal dephasing curve described by Eq. [1]. The good agreement between the universal dephasing curve and the numerical simulations provides further support that experimental data can be analyzed by using the universal dephasing curve without introducing much error in the measurement of the dipolar coupling. A more extensive examination of the nature of the deuterium composite pulse in REDOR applications is in progress.

It is desirable to also have a ^{13}C–^{2}D REDOR experiment based on a deuterium π pulse since the ideal dipolar dephasing is stronger than that generated by the REDOR experiment based on a deuterium $\pi/2$ pulse (*8*). Experimentally it is found that the REDOR experiment based on a simple π pulse performs poorly (*5, 7, 8*). Sack *et al.* have performed REDOR experiments by applying a train of deuterium composite π pulses. Their deuterium composite π pulses, based on hard $\pi/2$ and π pulses, were $90_x90_y90_x$ and $90_x180_y90_x$. Although they achieved substantially better dephasing of the ^{13}C magnetization with their composite pulses, they found that the ^{13}C magnetization did not decay according to the universal dephasing curve. Following their lead, we replaced the deuterium pulse in Fig. 1 with a composite deuterium π pulse based on $\theta_{cp}(\pi/2)_x\ \theta_{cp}(\pi/2)_y\ \theta_{cp}(\pi/2)_x$ and applied the experiment to the alanine sample. We obtained the best results with $\theta_{cp}(\pi/2)_x = 79_x86_x171_x73_{\bar{x}}41_x42_{\bar{x}}$ (*16*). The dipolar dephasing (data not shown) for the alanine sample caused by using this composite deuterium π pulse comes very close to obeying the predicted universal dephasing curve for a REDOR experiment based on a π pulse; however, the agreement between the experimental results and the universal dephasing curve is not good enough at this time to justify using the experiment. The results are encouraging enough, however, to continue pursuing a good REDOR experiment based on a composite deuterium π pulse.

In conclusion, we have demonstrated a ^{13}C-observe REDOR experiment that provides a means to measure ^{13}C–^{2}D dipolar couplings via a universal dephasing curve. Such a REDOR methodology allows ^{13}C–^{2}D distances to be measured in a straightforward and model-free way without the need to perform numerical simulations. Also, if the data set is large enough, then the REDOR transform (*19*) could be applied to the data to extract the dipolar coupling (the transform would generate three peaks: one at 0 Hz, one at D, and one at 2D).

REFERENCES

1. T. Gullion and J. Schaefer, Rotational-echo double-resonance NMR, *J. Magn. Reson.* **81**, 196–200 (1989).

2. T. Gullion and J. Schaefer, Detection of weak heteronuclear dipolar couplings by rotational-echo, double-resonance nuclear magnetic resonance, *in* "Advances in Magnetic Resonance, Vol. 13" (W. S. Warren, Ed.), pp. 57–83, Academic Press, New York (1989).

3. A. Schmidt, R. A. Mackay, and J. Schaefer, Internuclear distance measurement between deuterium ($I = 1$) and a spin-$\frac{1}{2}$ nucleus in rotating solids, *J. Magn. Reson.* **96**, 644–650 (1992).

4. D. Sandstrom, M. Hong, and K. Schmidt-Rohr, Identification and mobility of deuterated residues in peptides and proteins by ^{2}D–^{13}C solid-state NMR, *Chem. Phys. Lett.* **300**, 213–220 (1999).

5. I. Sack, A. Goldbourt, S. Vega, and G. Buntkowsky, Deuterium REDOR: Principles and applications for distance measurements, *J. Magn. Reson.* **138**, 54–65 (1999).

6. A. Schmidt, T. Kowalewski, and J. Schaefer, Local packing in glassy polycarbonates by carbon-deuterium REDOR NMR, *Macromolecules* **26**, 1729–1733 (1993).

7. I. Sack and S. Vega, Efficient deuterium-carbon REDOR NMR spectroscopy, *J. Magn. Reson.,* in press.

8. T. Gullion, A comparison between REDOR and θ-REDOR for measuring ^{13}C-^{2}D dipolar interactions in solids, *J. Magn. Reson.* **139**, 402–407 (1999).

9. T. Gullion, D. B. Baker, and M. S. Conradi, New compensated Carr-Purcell sequences, *J. Magn. Reson.* **89**, 479–484 (1990).

10. J. R. Garbow and T. Gullion, Improvements in REDOR NMR spectroscopy: Minimizing resonance-offset effects, *J. Magn. Reson.* **95**, 442–445 (1991).

11. D. T. Edmonds, Nuclear quadrupole double resonance, *Phys. Reports* **29**, 233–290 (1977).

12. M. S. Lehmann, T. F. Koetzle, and W. C. Hamilton, Precision neutron diffraction structure determination of protein and nucleic acid components. I. The crystal and molecular structure of the amino acid L-alanine, *J. Am. Chem. Soc.* **94**, 2657–2660 (1972).

13. R. C. Anderson, T. Gullion, J. M. Joers, M. Shapiro, E. B. Villhauer, and H. P. Weber, Conformation of [1-13C, 15N]acetyl-L-carnitine. Rotational-echo, double-resonance nuclear magnetic resonance spectroscopy, *J. Am. Chem. Soc.* **117**, 10546–10550 (1995).

14. M. H. Levitt, D. Suter, and R. R. Ernst, Composite pulse excitation in three-level systems, *J. Chem. Phys.* **81**, 3064–3068 (1984).

15. D. J. Siminovitch, D. P. Raleigh, E. T. Olejniczak, and R. G. Griffin, Composite pulse excitation in quadrupole echo spectroscopy, *J. Chem. Phys.* **84**, 2556–2565 (1986).

16. D. P. Raleigh, E. T. Olejniczak, and R. G. Griffin, Broadband pulses for excitation and inversion of $I = 1$ systems, *J. Magn. Reson.* **81**, 455–463 (1989).

17. D. Lu, S. Ali, and D. J. Simiinovitch, A quaternion analysis of time symmetry in spin-1 excitation, *J. Magn. Reson.* **122**, 192–203 (1996).

18. A. J. Shaka, Composite pulses for ultra-broadband spin inversion, *Chem. Phys. Lett.* **120**, 201–205 (1985).

19. K. T. Mueller, T. P. Jarvie, D. J. Aurentz, and B. W. Roberts, The REDOR transform: Direct calculation of internuclear couplings from dipolar-dephasing NMR data, *Chem. Phys. Lett.* **242**, 535–542 (1995).