

Measurement of Internuclear Distances by Switched Angle Spinning NMR

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Received: April 26, 1994; In Final Form: June 27, 1994*

Dipolar switched angle spinning, an NMR technique due to Terao et al. [Terao, T.; Miura H.; Saika, A. *J. Chem. Phys.* **1986**, *85*, 3816], has been used to measure ^{13}C - ^{13}C distances in the solid state. The experiment involves rotation of the sample at two different angles during different periods of a two-dimensional experiment. An evolution period with off-magic-angle spinning and chemical shift refocusing, followed by detection of the signal under magic angle spinning, yields scaled Pake patterns in ω_1 , correlated with their isotropic shifts in ω_2 , allowing the high-resolution measurement of dipolar couplings. We demonstrate this experiment on samples of doubly ^{13}C labeled zinc acetate and a 14 amino acid peptide, in which we show that under optimal conditions distances of up to 5 Å may be measured.

Introduction

The measurement of interatomic distances with solid state nuclear magnetic resonance (NMR) has recently attracted a great deal of attention,¹⁻⁵ largely because solid state NMR is one of the only methods of obtaining structural information on materials lacking long range order. Several techniques have been developed to extract distances between homonuclear coupled spin pairs. Yannoni et al. have developed nutation NMR which utilizes a strong radio-frequency (rf) field to average chemical shifts, while preserving the dipolar interaction, scaled by one-half.^{6,7} Griffin and co-workers have recently employed longitudinal magnetization exchange via rotational resonance to measure distances between ^{13}C -labeled spin pairs of up to 7 Å with a 0.5-Å resolution.^{1-3,8,9} The magnetization transfer curves thus obtained are sensitive to the dipolar coupling and therefore the internuclear distance. Several methods involving rotor synchronized homonuclear dipolar interaction during magic angle spinning,^{5,10,11} Terao and co-workers have previously investigated strong heteronuclear dipolar couplings by correlating magic angle spinning (MAS) spectra with static heteronuclear dipolar powder patterns,¹² accomplished by spinning off the magic angle during the evolution period of a two-dimensional experiment, switched angle spinning (SAS). The spectra obtained along the ω_1 axis are dependent only upon the dipolar coupling, allowing the straightforward extraction of the internuclear distance. As the dipolar couplings were large and involved proton spins, small rotor flip angles were used as well as proton multiple-pulse decoupling during the t_1 period. Recently, in our laboratory, instrumentation for dynamic angle spinning of quadrupolar nuclei has been developed which involves the rapid reorientation of the sample spinning axis by up to 63°.¹³ This has prompted us to investigate an extension of the method of Terao et al.¹² to the measurement of weak homonuclear dipolar couplings which require large deviations from the magic angle in ω_1 so as to avoid excessive scaling of the dipolar interaction. Tycko has recently reported a related application of switched angle spinning to the problem of multiple quantum filtering during MAS.¹⁴

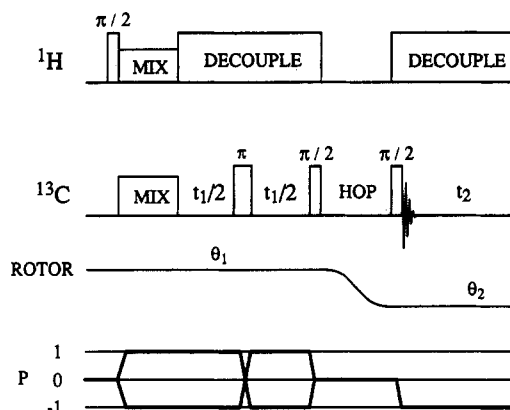


Figure 1. Pulse sequence for dipolar switched angle spinning NMR. The coherence transfer pathway is shown beneath.

Results and Discussion

The correlation of MAS spectra with dipolar patterns is implemented via the pulse sequence of Figure 1. Following cross-polarization, the coherence evolves for a time t_1 under the influence of the homonuclear dipolar interaction, the chemical shifts being refocused by a π pulse at $t_1/2$. The π pulse is asynchronous with the sample rotation and will result in spinning sidebands spaced at one-half of the spinning speed in ω_1 , as shown previously.¹⁵⁻¹⁷ During this evolution period the rotor is spinning at θ_1 , usually 0° or 90°, leading to a nonvanishing two-spin dipolar coupling, which varies as $P_2(\cos \theta_1)$. After the evolution period, the coherence is then stored along the z axis with a $\pi/2$ pulse, the rotor axis is hopped to θ_2 , typically the magic angle, the coherence is restored to the x - y plane, and the signal is detected. High-power proton decoupling is applied during the entire experiment, except during the axis hop period. Two-dimensional Fourier transformation of the resulting signal, acquired as a function of t_1 and t_2 , yields a high-resolution MAS spectrum correlated with a two-spin dipolar pattern scaled by $P_2(\cos \theta_1)$. This is demonstrated by the 2D dipolar SAS spectrum of di- ^{13}C -labeled zinc acetate as shown in Figure 2. The spectrum was acquired with $\theta_1 = 90^\circ$ rather than $\theta_1 = 0^\circ$, leading to a scaling factor of 0.5, due to the difficulty of performing ^1H decoupling with the sample spinning about the z axis. For small dipolar couplings, spinning about the z axis during the t_1 period may be desirable to overcome the scaling factor of 0.5.¹⁸ Well-resolved dipolar patterns are observed along the ω_1 axis, correlated with their MAS spectra along the ω_2 axis. The dipolar spectra are distorted

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• Abstract published in *Advance ACS Abstracts*, August 1, 1994.

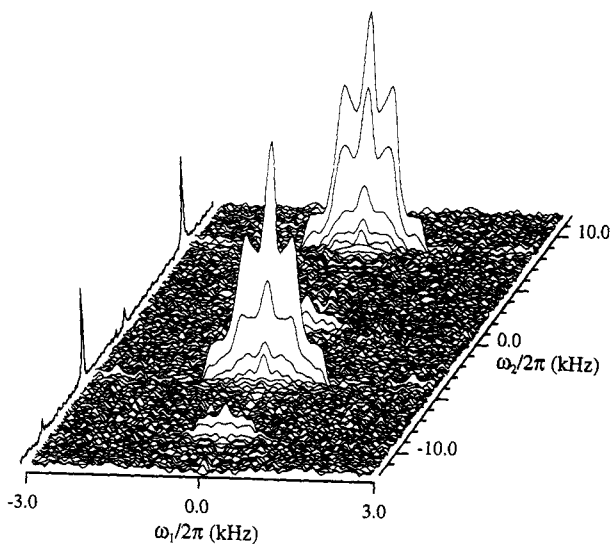


Figure 2. 2D Dipolar SAS spectrum of di- ^{13}C -labeled $\text{Zn}(\text{Ac})_2$ diluted 1:10 in natural abundance $\text{Zn}(\text{Ac})_2$. A total of 64 t_1 increments were acquired with 256 scans per FID and a recycle time of 1 s. The dwell times were 41.7 and 100 μs in t_2 and t_1 , respectively, and the spinning speed was 5110 Hz. The hop time was approximately 50 ms, and the angle of the rotor with respect to the magnetic field was 90° during t_1 .

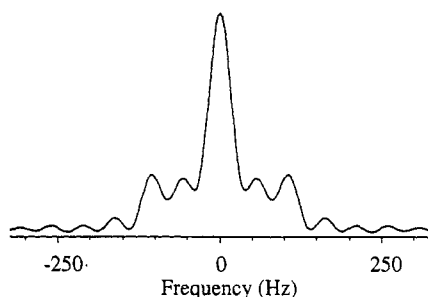


Figure 3. Slice taken from a 2D dipolar SAS spectrum parallel to the ω_1 axis at a point corresponding to the COO^- line center band in ω_2 . The rotor was at $\theta_1 = 52.7^\circ$ during t_1 and at the magic angle for detection. The scaling factor of the homonuclear dipolar coupling due to the off-angle spinning during t_1 is approximately 5%. Line narrowing of ~ 30 Hz has been applied to the FID in ω_1 , to enhance the singularities.

by an additional peak at zero frequency, presumably due to isolated single ^{13}C labels in the sample. The dipolar coupling of $b_{\text{IS}} = \gamma^2 \hbar / 2\pi r^3 = 2.00$ kHz, corresponding to a 1.5-Å distance, may easily be measured from either the parallel or perpendicular edges. Figure 3 shows a slice extracted from a 2D spectrum acquired as in Figure 2, with the difference that $\theta_1 = 52.7^\circ$, 2° away from the magic angle, yielding a scaling factor of 0.05. This appears to be close to the resolution limit for this experiment, and corresponds to a spin pair with a 4.2-Å distance, were the experiment performed with $\theta_1 = 0^\circ$. The spectra obtained in this manner are sensitive only to the dipolar coupling between sites and are unaffected by rf inhomogeneity.^{6,7} For dipolar couplings which are comparable to the chemical shift separation, second order effects may become important resulting in patterns that reflect the imperfect refocusing of the chemical shifts in ω_1 .¹⁹ For the examples reported here, the dipolar couplings are substantially smaller than the shift differences, and the Hamiltonian can be considered to be properly truncated at first order. In situations in which a nonideal pake pattern is observed, an exact calculation of the dipolar and two chemical shift tensor Hamiltonian is required to extract accurate distances from fits to the ω_1 projections.¹⁹

When the homogeneous line width becomes comparable to the dipolar splittings in the static powder pattern, the distance can no longer be measured directly from the singularities in ω_1 . Rather, the ω_1 spectra must be compared with broadened simulated pake

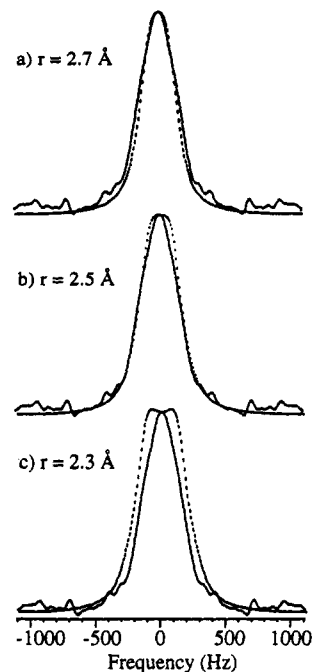


Figure 4. Slice taken from a 2D spectrum of the 14-mer, H1, on the position of the carbonyl carbon in ω_2 , overlaid with simulations of broadened dipolar spectra. A total of 32 t_1 slices were acquired with 1000 scans per FID and a recycle time of 1 s. The dwell time in t_1 was 400 μs , and the spinning rate was 5.2 kHz. The dipolar simulations were Lorentzian broadened by 173 Hz and are plotted with dotted lines.

patterns. As all interactions in ω_1 and ω_2 are identical, with the exception of the isotropic shift in ω_2 and the homonuclear dipolar interaction in ω_1 , the broadening function may be estimated from the MAS line width and has roughly the same influence upon fits to the data as the zero-quantum T_2 has in rotationally enhanced magnetization transfer curves.⁹ As an example, we present in Figure 4 slices taken from a 2D spectrum of a doubly ^{13}C enriched 14 amino acid peptide, H1, which plays a crucial role in the conformation of the prion protein (PrP) responsible for the development of scrapie in Syrian hamsters.²⁰ The 14-mer is labeled on the C-1 and C-2 carbons of Ala-115 and Ala-116, respectively, which are connected by a peptide bond. The experimental spectra are overlaid in (a)–(c) with simulated pake patterns, broadened by the carbonyl MAS line width of 173 Hz. The best fit to the data is spectrum b with $r = 2.5$ Å. Deviations of ± 0.2 Å from (b) yield a noticeably poorer fit. This result is in reasonable agreement with distances expected from typical peptide bond geometry, 2.45–2.49 Å.²¹ Future work will involve the measurement of critical distances within this and other PrP peptides, which can adopt either α -helix or β -sheet forms. We wish to determine whether there are unusual elements in the secondary structure that could explain the pathogenicity of PrP.

Experimental Section

The doubly ^{13}C labeled zinc acetate sample was prepared by refluxing zinc oxide with 99% doubly ^{13}C labeled acetic acid (Cambridge Isotopes, Woburn, MA). The sample was recrystallized 1:10 in natural abundance zinc acetate to reduce intermolecular dipolar couplings, and 3 mol % manganese acetate was added to shorten the proton relaxation time. All experiments were performed on a home built pulse spectrometer operating at a ^1H Larmor frequency of 300 MHz. The hopping probe was also home-built,¹³ utilized a commercial 7-mm spinning system (Doty Scientific, Columbia, SC), and could achieve large angle reorientations of the spinning axis in approximately 50 ms. In the fast spinning limit, the spectra were symmetric about $\omega_1 = 0$; therefore, quadrature detection in that dimension was not employed.

Conclusions

We have described an application of the 2D dipolar SAS technique¹² to the measurement of interatomic carbon-carbon distances in solids. Off magic angle spinning during the evolution period of a two-dimensional experiment, with chemical shift refocusing, has been shown to yield spectra in ω_1 which are sensitive to the distance between labeled spins. Fits to the experimental data are sensitive only to the distance for the case of strong couplings. For instances in which the homogeneous line width is not small compared to the dipolar coupling, the fits are convolutions of the MAS line width with the idealized peak pattern. Internuclear distances thus extracted are accurate to approximately 0.2 Å.

Acknowledgment. This work was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Materials Sciences Division of the U. S. Department of Energy, under Contract DE-AC03-76SF00098. M.A.B and S.B.P. acknowledge support from the National Institute of Health and the American Health Assistance Foundation. A.C.K. is a fellow of the Miller Institute for Basic Research in Science.

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