Normal Angle Spinning Dipolar Spectroscopy for Structural Studies by Solid-State Nuclear Magnetic Resonance

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An important recent development in solid-state nuclear magnetic resonance (NMR) spectroscopy has been the invention of techniques for extracting information about distances between nonbonded atoms from high-resolution, magic angle spinning (MAS) spectra of complex molecules.[1-12] This information is contained in homonuclear[1-8] and heteronuclear[9-12] magnetic dipole–dipole couplings, which are averaged to 0 in simple MAS measurements. We have shown that radio-frequency (rf) pulse sequences applied in synchrony with MAS can restore homonuclear couplings. We call such sequences DRAMA (dipolar recovery at the magic angle).[1-3] An important limitation of DRAMA sequences has been the degradation of their performance when the chemical shift anisotropies (CSA) and isotropic shift differences are large. While progress has been made,[3] sequences that are effective over the full range of ^{13}C chemical shifts in high field (≥20-kHz bandwidth) have not yet been reported. Others have exploited the R^2 (rotational resonance) effect,[4,5] which restores dipole–dipole couplings only between pairs of nuclei with isotropic shift differences that are multiples of the sample spinning frequency, and the use of rotor-synchronized π pulses (the SEDRA,[6] RFDR,[7] and DICSY[8] techniques), which restore couplings between pairs of like nuclei with large isotropic or anisotropic chemical shift differences but have little effect when the shift differences are small. Thus, the development of techniques for measuring and using dipole–dipole couplings over the full ^{13}C chemical shift range remains an important goal.

This communication presents a method for making use of homonuclear dipole–dipole couplings in MAS experiments over quite large ranges of chemical shifts and CSA, limited only by the excitation bandwidth of the applied rf pulses. The method uses rapid switching of the direction of the sample rotation axis, specified by the angle θ_s between this axis and the dc magnetic field direction, between the *normal angle* ($\theta_s = \pi/2$) and the *magic angle* ($\theta_s = \cos^{-1} 3^{-1/2} \equiv \theta_m$). Angle switching has been exploited previously in several contexts.[9,13-15] In the proposed method, NMR signals are acquired at $\theta_s = \theta_m$, so that the high resolution and sensitivity of MAS are preserved. In a separate period of the experiment, $\theta_s = \pi/2$ and π pulses are applied at the end of every odd-numbered rotor cycle. As shown below, nonzero average dipole–dipole couplings are created during this period. We refer to the application of rf pulses in synchrony with sample spinning at $\theta_s = \pi/2$, for the purpose of obtaining useful homonuclear dipole–dipole couplings, by the acronym NASDY (normal angle spinning dipolar spectroscopy). Below, we demonstrate the use of NASDY in double-quantum filtered ^{13}C MAS NMR.[2,3,16]

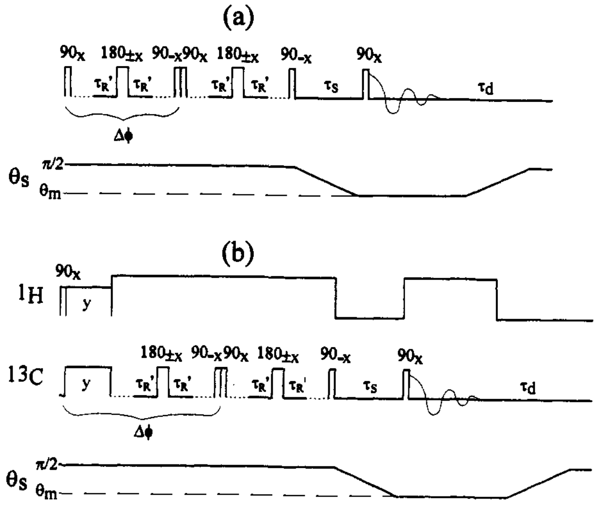

Figure 1. (a) Single-resonance pulse sequence for multiple-quantum filtering using NASDY. In the NASDY sequence, the sample rotates at angle $\theta_s = \pi/2$ with period τ_R and π pulses are applied at the end of every other rotor cycle. For multiple-quantum filtering, two NASDY sequences are employed, each sandwiched between a pair of $\pi/2$ pulses; overall phase shifts $\Delta\varphi$ are applied to the rf pulses in the first multiple-quantum excitation period as described in the text. After a period τ_s for switching to $\theta_s = \theta_m$ (the magic angle), NMR signals are detected following a $\pi/2$ pulse. τ_d is the delay between scans. $\tau_R' = \tau_R - t_p/2$, where t_p is the π pulse length. (b) Double-resonance version of the NASDY multiple-quantum filtering sequence, with cross-polarization and proton decoupling.

Under sample rotation with period τ_R and the application of ideal π pulses at times $(2n + 1)\tau_R$, the spin Hamiltonian for a pair of nuclei with angular momenta $\mathbf{I}_1$ and $\mathbf{I}_2$ can be written, in the rotating frame and in the interaction representation determined by the rf pulses,

$$H(t) = \delta_1(t)I_{z1} + \delta_2(t)I_{z2} + d(t)[{}^2/_3 I_{z1}I_{z2} - {}^1/_6(I_{+,1}I_{-,2} + I_{-,1}I_{+,2})] \quad (1)$$

where the chemical shifts $\delta_i(t)$ and the dipole–dipole coupling parameter $d(t)$ are time-dependent due to the sample rotation and the applied pulses. For $|d(t)|\tau_R \ll 1$, the spin evolution up to multiples of $2\tau_R$ is determined by an effective average Hamiltonian H_{eff}

$$H_{\text{eff}} = \frac{1}{2\tau_R}\int_0^{2\tau_R} \mathrm{d}t\, d(t) \times \left[\frac{2}{3}I_{z1}I_{z2} - {}^1/_6(I_{+,1}I_{-,2}e^{i\phi(t)} + I_{-,1}I_{+,2}e^{-i\phi(t)})\right] \quad (2)$$

where $\phi(t) = \int_0^t \mathrm{d}t'\,[\delta_1(t') - \delta_2(t')]$ and $\phi(2n\tau_R) = 0$. There are two important limits: (1) For small shift differences (i.e., $|\delta_1 - \delta_2|\tau_R \ll 1$), $\phi(t) \ll 1$ and $H_{\text{eff}} \approx \overline{d(t)}[{}^2/_3 I_{z1}I_{z2} - {}^1/_6(I_{+,1}I_{-,2} + I_{-,1}I_{+,2})]$. The average coupling $d(t)$ is generally nonzero for $\theta_s \neq \theta_m$. (2) For large shift differences (i.e., $|\delta_1 - \delta_2|\tau_R \gg 1$), $\phi(t)$ becomes large and strongly time-dependent. Then $H_{\text{eff}} \approx$

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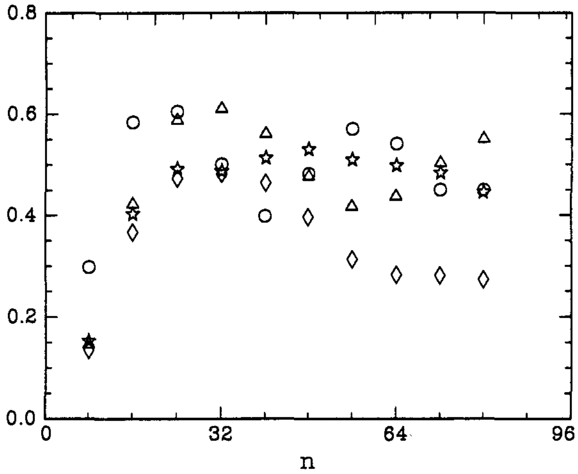

Figure 2. Simulations of the orientationally-averaged double-quantum filtering efficiency $\bar{E}(n)$ for application of the sequence in Figure 1a, with n rotor cycles in each NASDY period, to a system of randomly-oriented, dipole-coupled spin-$^1/_2$ nuclei with $d_{\max}$ = 0.5 kHz. π pulses have amplitude 50 kHz; τ_R = 225 μs; $\pi/2$ pulses are ideal; no spin relaxation is included. Circles: $\delta_1^{\rm iso} = \delta_2^{\rm iso} = 0$; $\delta_1^{\rm ani} = \delta_2^{\rm ani} = 0$. Triangles: $\delta_1^{\rm iso} = -\delta_2^{\rm iso}$ = 2.0 kHz; $\delta_1^{\rm ani}$ = 4.0 kHz; $\delta_2^{\rm ani}$ = −1.0 kHz. Stars: $\delta_1^{\rm iso} = -\delta_2^{\rm iso}$ = 4.0 kHz; $\delta_1^{\rm ani}$ = 8.0 kHz; $\delta_2^{\rm ani}$ = −2.0 kHz. Diamonds: $\delta_1^{\rm iso} = -\delta_2^{\rm iso}$ = 8.0 kHz; $\delta_1^{\rm ani}$ = 16.0 kHz; $\delta_2^{\rm ani}$ = −4.0 kHz.

$^2/_3 d(t) I_{z1} I_{z2}$. Between these two limits, $H_{\rm eff}$ depends on the details of the CSA tensors but does not become small. We choose $\theta_s = \pi/2$ to maximize both $|d(t)|$ and the transverse rf fields.

Figure 1a shows an rf pulse sequence based on NASDY for multiple-quantum filtered MAS NMR. For M-quantum filtering, the sequence is repeated a multiple of $2M$ times, with overall rf phase shifts $\Delta\phi = (\pi/M)(m - 1)$ in the mth repetition and with alternate addition and subtraction of the free induction decay (FID) signals.[16] Effects of finite rf pulse amplitudes and rf inhomogeneity are reduced by phase shifts of the π pulses in the NASDY multiple-quantum excitation periods according to the sequence $\{x, -x, -x, x\}$. Switching of θ_s occurs during the period τ_s. Figure 2 shows simulations of the orientationally-averaged double-quantum filtering efficiency $\bar{E}(n)$ for a pair of dipole-coupled spin-$^1/_2$ nuclei with maximum coupling $d_{\max}$ = 0.5 kHz (for ^{13}C nuclei separated by r, $d_{\max} = 22.6/r^3$ kHz Å^3) and for several values of the isotropic chemical shifts $\delta_i^{\rm iso}$ and the shift anisotropies $\delta_i^{\rm ani}$. $\bar{E}(n)$ is defined as in ref 3, with n being the number of rotor cycles in each NASDY period. The CSA tensors are taken to be axially symmetric, with their unique axes parallel to the internuclear vector ($\delta_i^{\rm ani} \equiv \delta_\parallel - \delta_\perp$, where $\delta_\parallel$ and $\delta_\perp$ are the parallel and perpendicular components of the CSA tensor). From Figure 2, it is clear that the condition $\bar{E}(n) \approx 0.5$, which is the ideal large-n limit,[3] can be attained for CSA values and isotropic shift differences that are typical of high-field ^{13}C NMR spectra of organic systems. The degradation of $\bar{E}(n)$ for large CSA and shift differences and large n is due solely to the finite rf pulse amplitudes; shorter π pulses or composite π pulses increase $\bar{E}(n)$.

Figure 3 shows experimental ^{13}C MAS spectra, obtained at 100.5 MHz (9.39 T), of a physical mixture of N-acetyl-L-valine (L-NAV), methionine hydrochloride (Met), glycine ethyl ester hydrochloride (GEE), and glycine methyl ester hydrochloride (GME) powders (80 mg total); *9.3% of the GME molecules are doubly-^{13}C-labeled, at the carbonyl and methyl sites* ($d_{\max} \approx$ 1.6 kHz; $r \approx$ 2.4 Å). These sites have CSA principal values {260, 141, 113} ppm and {85, 69, 13} ppm relative to TMS. The mixture was prepared so that the labeled GME sites and the natural-abundance L-NAV, Met, and GEE sites have comparable signal intensities in an unfiltered MAS spectrum. Such a spectrum, obtained with cross-polarization and storage of ^{13}C magnetization at $\theta_s = \pi/2$, switching to $\theta_s = \theta_m$ during a period τ_s, and detection of the ^{13}C FID after a $\pi/2$ pulse, is shown in Figure 3a. Figure 3b shows the NASDY double-quantum filtered spectrum, obtained with the sequence in Figure 1b; this sequence is the double-resonance analogue of the sequence in Figure 1a. *Only the signals from the labeled sites are apparent above the noise in Figure 3b*; *all natural-abundance signals are strongly suppressed.* The experimental filtering efficiency for the labeled sites, from a comparison of parts a and b of Figure 3, is 0.40 ± 0.03. MAS spin-echo measurements indicate that $T_2 \approx$ 25 ms for both labeled carbons, implying that spin relaxation reduces the experimental efficiency by roughly 15%.

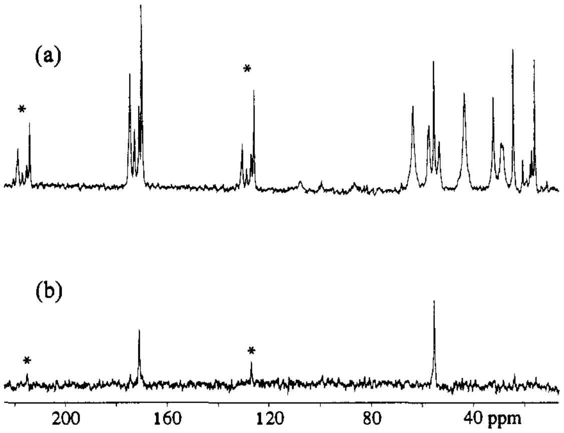

Figure 3. Unfiltered (a) and NASDY double-quantum filtered (b) ^{13}C MAS NMR spectra of a physical mixture of N-acetyl-L-valine, methionine hydrochloride, glycine ethyl ester hydrochloride, and glycine methyl ester hydrochloride (GME); 9.3% of the GME molecules are doubly-^{13}C-labeled. The unfiltered spectrum is the result of 64 scans. The double-quantum filtered spectrum, obtained with the pulse sequence in Figure 1b with eight rotor cycles in each NASDY period, is the result of 128 scans. The rf carrier is at 112.5 ppm. Asterisks indicate spinning sidebands.

Spectra in Figure 3 were obtained at room temperature, with τ_s = 500 ms, τ_R = 225 μs, and τ_d = 10 s. Proton decoupling fields of 80 kHz at $\theta_s = \pi/2$ and 73 kHz at $\theta_s = \theta_m$ were used. ^{13}C rf fields were 50 kHz at $\theta_s = \pi/2$. The home-built double-resonance probe, based on a Doty Scientific 5-mm spinner assembly, uses a simple pneumatic system to switch θ_s (details available on request).

Double-quantum filtering experiments of the type demonstrated in this communication can be used to obtain the natural-abundance ^{13}C MAS NMR spectrum of a spatially localized region around a singly-labeled site in a macromolecule, with a maximum radius greater than 5 Å.[2,3] Such a spectrum, which may show resolved resonances even when the unfiltered MAS spectrum does not, potentially contains information about the chemical composition (through the observed isotropic shifts), the structure (through the dependence of the intensities on n), and the dynamics (through spin relaxation rates and spinning sideband intensities) of the localized region. This region may be, for example, the active site of an enzyme or the binding site of a receptor. Proteins of molecular weight greater than 60000$\bar{E}(n)$ are amenable to study in this way in current spectrometers.[2,3] NASDY may also be incorporated into other solid-state structural techniques that are based on homonuclear dipole–dipole couplings, such as two-dimensional correlation spectroscopy and nuclear magnetization exchange.[4–8] The direct determination of internuclear distances from dipolar powder pattern line shapes in a two-dimensional NASDY/MAS experiment[1] is possible as well.