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Nitrogen-14 NMR Spectroscopy Using Residual Dipolar Splittings in Solids

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Although nitrogen is of universal importance in virtually all branches of chemistry, material science, and biology and although ^{14}N is very abundant (99.64%), nitrogen-14 NMR has enjoyed relatively little popularity so far. In liquids, ^{14}N line widths are very broad because of rapid quadrupolar relaxation, except in rapidly tumbling molecules or in highly symmetrical systems.[1] In solids, the environment of ^{14}N nuclei often results in very large quadrupolar interactions. So far, ^{14}N nuclei were characterized by nuclear quadrupole resonance (NQR)[2,3] and by NMR of single crystals[4−7] or powders spinning at the magic angle (MAS).[8,9] It is possible to detect ^{14}N double-quantum (DQ) transitions, which are not affected by first-order quadrupole interactions, either indirectly[10] or directly by overtone spectroscopy,[11,12] possibly in combination with dynamic-angle spinning or double sample rotation.[13,14] Indirect detection of ^{14}N under MAS can also be achieved by recoupling heteronuclear dipolar interactions with a suitable spin S, such as ^{13}C.[15−18]

The two-dimensional NMR correlation experiments described herein,[19] which are closely related to those of Gan,[20] exploit second-order quadrupole−dipole cross terms between ^{14}N ($I = 1$) and "spy" nuclei, such as ^{13}C ($S = {}^1/_2$), also known as *residual dipolar splittings* (RDS),[21−24] not to be confused with *residual dipolar couplings* (RDC) observed in weakly aligned solutions.[25] The angular dependence of the RDS interaction cannot be averaged out completely by spinning at the magic angle. As a result, the S resonances are split into 1:2 doublets, each component having a powder pattern structure, with average frequencies separated by an effective splitting, D_{RDS}. These splittings are often masked by inhomogeneous broadening, which may be due to slight errors in the adjustment of the magic angle,[24] temperature gradients,[24] structural disorder, or magnetic susceptibility effects, but none of these factors represent obstacles to the success of our experiments. The splittings, which decrease in inverse proportion to the static magnetic field strength, B_0, are on the order of $D_{RDS}(^{13}C,^{14}N) \approx 30$ Hz for NH_3^+ groups in amino acids in zwitterionic form at $B_0 = 9.4$ T, while $^1J(^{13}C,^{14}N)$-couplings are only about 4 Hz.[23,26] The time constants T_2' of spin−echo decays[27] for $^{13}C^{\alpha}$ and $^{13}C'$ can be as long as 75 and 450 ms, respectively, corresponding to "refocusable line widths" that are as narrow as 4.2 and 0.7 Hz.[24] Thus, residual dipolar splittings, $D_{RDS}(^{13}C,^{14}N) \approx 30$ Hz, are sufficient to transfer coherence between ^{14}N and ^{13}C.

After exciting ^{13}C single-quantum (SQ) coherence S_x (C_x) in the usual manner by cross-polarization from ^{1}H to ^{13}C (Figure 1), a delay $\tau_{exc} \approx 1/(2D_{RDS})$ (see Figure 1) leads to an antiphase coherence $2S_xI_z^2$ ($2C_xN_z^2$). A radio frequency (RF) pulse applied in the center of the ^{14}N spectrum leads to a partial conversion into heteronuclear multiple-quantum coherences $2S_xI_x^2$ and $2S_xI_xI_z$ ($2C_xN_x^2$ and $2C_xN_xN_z$) that involve, apart from ^{13}C SQ coherence, ^{14}N SQ and DQ coherences. These evolve during an interval t_1 in the manner of two-dimensional spectroscopy, prior to reconversion into observable ^{13}C coherence S_x (C_x). The SQ experiment is reminiscent of heteronuclear multiple-quantum correlation (HMQC),[28] which has been applied to solids with $S = {}^1$H and $I = {}^{13}$C using $J(^1H,^{13}C)$,[29] with $S = {}^{27}$Al and $I = {}^{31}$P using $J(^{27}Al,^{31}P)$,[30] and with $S = {}^{27}$Al and $I = {}^{17}$O using $J(^{27}Al,^{17}O)$-couplings.[31] Chemical shifts and inhomogeneous decay of ^{13}C are eliminated by a π pulse in the middle of the t_1 period. The delays $\tau_{exc} = \tau_{rec} = n/\nu_{rot}$ and increments $\Delta t_1 = 1/\nu_{rot}$ must be synchronized with the spinning period. A two-dimensional (2D) Fourier transformation yields a correlation spec-

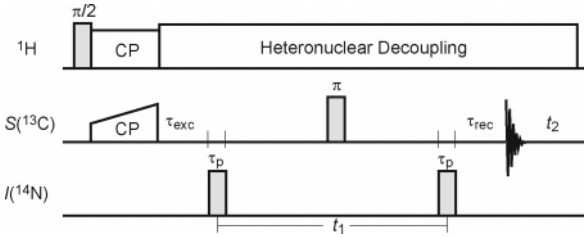

Figure 1. Pulse sequence for the excitation of ^{14}N single- or double-quantum coherences in two-dimensional correlation experiments for solids rotating at the magic angle.

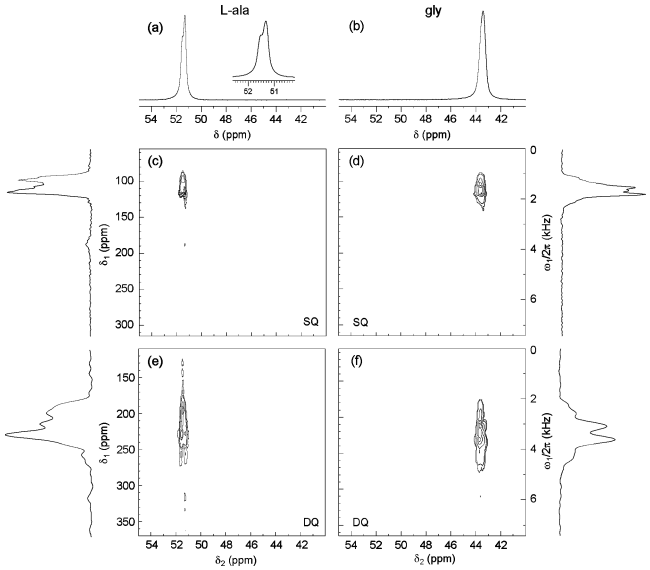

Figure 2. (a, b) ^{13}C CPMAS spectra and two-dimensional SQ and DQ spectra showing ^{14}N (c, d) single- and (e, f) double-quantum signals along the vertical ω_1 axis. (a) The ^{13}C CPMAS spectrum of L-alanine reveals an ill-resolved residual dipolar splitting; (b) in glycine, there is no visible splitting. Nevertheless, the ^{14}N single- and double-quantum coherences can be excited efficiently, and the projections onto the ω_1 axis (parts per million scale with respect to NH_4Cl; only 25% of the full spectral width is shown) reveal characteristic second-order quadrupolar powder patterns. The two-dimensional spectra result from averaging (c, d) 32 and (e, f) 96 transients for each of (c, d) 512 and (e, f) 170 t_1 increments $\Delta t_1 = 1/\nu_{rot} = 33.33$ μs, with a relaxation interval of 3 s. The intervals $\tau_{exc} = \tau_{rec}$ were 16 ms, while τ_p was (c, d) 11 and (e, f) 15 μs. The CP contact times for L-alanine and glycine were 0.5 and 1 ms, respectively.

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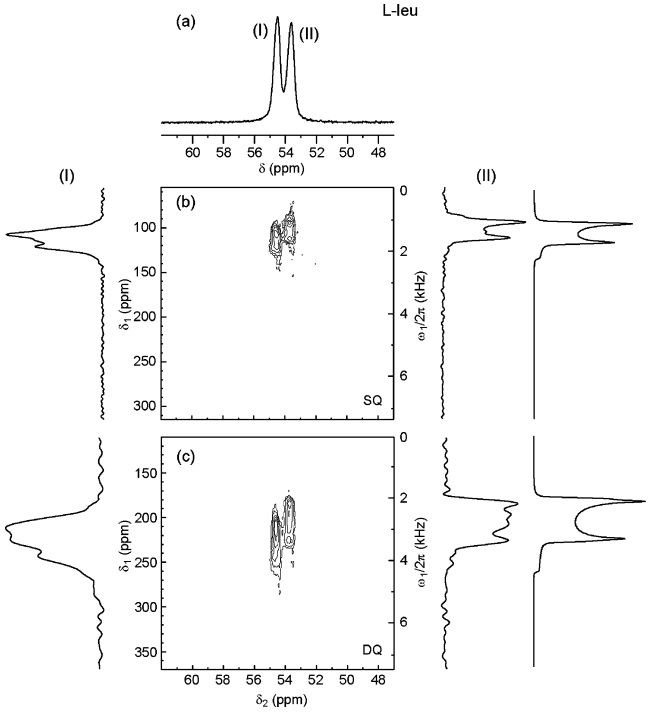

Figure 3. (a) ^{13}C CPMAS and SQ and DQ spectra of L-leucine that has two magnetically inequivalent sites I and II for ^{14}N and ^{13}C$^{\alpha}$. The experimental projections of site II on the right side can be compared with simulations of the second-order quadrupolar powder patterns calculated assuming uniform excitation for all crystallites. The 2D spectra result from averaging (b) 32 and (c) 96 transients for each of (b) 400 and (c) 190 t_1 increments of $\Delta t_1 = 1/\nu_{rot} = 33.33$ μs, with a relaxation interval of 3 s. The intervals $\tau_{exc} = \tau_{rec}$ were 16 ms, while the ^{14}N pulse lengths τ_p were (b) 11 and (c) 23 μs. The CP contact time was 0.6 ms.

trum with either ^{14}N SQ or DQ signals in the ω_1 domain and the conventional ^{13}C spectrum in the ω_2 domain. To obtain DQ spectra, the phase of one of the ^{14}N pulses must be stepped in increments of 90°, while the receiver phase is alternated; for SQ spectra, the phases of one RF pulse and the receiver must be alternated together.

In the experimental ^{14}N SQ and DQ spectra of L-alanine and glycine in Figure 2, the projections onto the vertical axes have line widths on the order of a few kilohertz. Since the magic angle is set very accurately,[25] the SQ spectra are twice as narrow as the DQ spectra. By fitting the line shapes, both of which reflect the isotropic shift and the second-order quadrupolar interaction, while the SQ signals are also affected by a third-order term, we estimate the ^{14}N quadrupolar parameters to be $C_Q = 1.13$ MHz, $\eta_Q = 0.28$ for L-alanine, and $C_Q = 1.18$ MHz, $\eta_Q = 0.50$ for glycine. The two magnetically inequivalent ^{13}C sites I and II in powdered L-leucine (Figure 3) correlate with two nondegenerate ^{14}N tensors with quadrupole couplings and asymmetry parameters $C_Q^{I} = 1.15$ MHz, $\eta_Q^{I} = 0.38$, $C_Q^{II} = 1.13$ MHz, $\eta_Q^{II} = 0.08$.

The sensitivity of these experiments is largely determined by the quantum yield of two-way coherence transfer from ^{13}C to ^{14}N and back. The efficiency for SQ and DQ transfers can be estimated by comparing the ^{13}C signal $S(t_1 = 0, \omega_2)$ with a ^{13}C spectrum obtained after a spin−echo with the same $\tau_{def} = \tau_{ref}$ but without ^{14}N pulses. The quantum yields in L-alanine are about 16 and 8% for SQ and DQ, respectively, in agreement with numerical calculations. In principle, the experiment can work with any "spy" nuclei, such as ^{31}P, ^{15}N, ^{29}Si, and even ^{1}H,[32] provided that the T_2' of the spy nuclei are long enough compared to $1/D_{RDS}$. It is possible to achieve indirect detection of nuclei with $S = {}^3/_2$, $^5/_2$, etc., such as ^{35}Cl, ^{17}O, etc., provided that one can exploit residual dipolar splittings with suitable spy nuclei.

The samples were packed in 2.5 mm ZrO_2 rotors and spun at 30 kHz in a Bruker triple resonance CPMAS probe at 9.4 T (^{13}C and ^{14}N Larmor frequencies of 100.6 and 28.9 MHz). Cross-polarization (CP) was used with $\nu_{RF}^{H} = 85$ kHz, while ν_{RF}^{C} was ramped. Two-pulse phase-modulation (TPPM) proton decoupling was used with $\nu_{RF}^{H} = 100$ kHz, pulse widths of 3.9 μs, and a phase difference between two successive pulses of 35°. With a modest 500 W amplifier, the ^{14}N pulses had an amplitude $\nu_{RF}^{N} = 50$ kHz, calibrated by direct detection of $^{14}NH_4NO_3$. The samples of L-alanine, glycine, and L-leucine, all enriched in the ^{13}C$^{\alpha}$ positions, were purchased from Cambridge Isotope Laboratories and used without further purification.

To summarize, we have shown that single- and double-quantum transitions of ^{14}N nuclei can be detected indirectly in solid powdered samples. The quadrupole tensor parameters, which reflect the local electronic charge distribution, can be obtained by analysis of the line shapes. Nitrogen-14 Excitation via Residual Dipolar Splittings (NERDS) could become a useful tool for chemistry, material science, and biology.

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