

Communication

Indirect detection of nitrogen-14 in solids via protons by nuclear magnetic resonance spectroscopy

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Abstract

This Communication describes the indirect detection of ^{14}N nuclei (spin $I = 1$) in solids by nuclear magnetic resonance (NMR) spectroscopy. The two-dimensional correlation method used here is closely related to the heteronuclear multiple quantum correlation (HMQC) experiment introduced in 1979 to study molecules in liquids, which has recently been used to study solids spinning at the magic angle. The difference is that the coherence transfer from neighboring ^1H nuclei to ^{14}N is achieved via a combination of J couplings and residual dipolar splittings (RDS). Projections of the two-dimensional correlation spectra onto the ^{14}N dimension yield powder patterns which reflect the ^{14}N quadrupolar interaction. In contrast to the indirect detection of ^{14}N via ^{13}C nuclei that was recently demonstrated [Gan, *J. Am. Chem. Soc.* 128 (2006) 6040; Cavadini et al., *J. Am. Chem. Soc.*, 128 (2006) 7706], this approach may benefit from enhanced sensitivity, and does not require isotopic enrichment in ^{13}C , although the ^1H line-widths may have to be reduced upon selective deuteration.

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Although ^{14}N has an isotopic abundance of 99.64%, the large quadrupolar interaction associated with its spin $I = 1$ has so far prevented nitrogen-14 NMR from becoming popular. In liquids, ^{14}N line-widths tend to be very broad due to rapid quadrupolar relaxation, except in small molecules that tumble rapidly or have nearly tetrahedral symmetry [1]. NMR spectra of ^{14}N in powders spinning at the magic angle (MAS) can feature hundreds of spinning sidebands that may span several MHz [2]. Although various methods have been developed to observe double-quantum (DQ) transitions between the $|m = +1\rangle$ and $|m = -1\rangle$ levels [3–5], which are not affected by the first-order quadrupole interaction, none of these techniques have made it possible to study nitrogen-containing materials in a straightforward manner.

The two-dimensional correlation experiments described in this Communication exploit a combination of scalar J couplings and residual dipolar splittings (RDS) [6–8], also known as second-order quadrupole–dipole cross term, between nitrogen-14 and neighboring protons that fulfill the role of “spy” nuclei. The latter couplings should not be confused with residual dipolar couplings (RDC), which are observed in weakly aligned liquid crystalline media [9]. In spinning solids, heteronuclear dipolar interactions ^{14}N – I with $\text{I} = ^{13}\text{C}$, ^1H , or other “spy” nuclei with spin $I = 1/2$, cannot be averaged out completely by magic angle spinning, because the large quadrupole coupling of the I nucleus prevents its quantization along the direction of the static field. The I resonances feature 1:2 doublets, each of the two components having the appearance of a narrow powder pattern [6–8,10]. The effective residual dipolar splitting $D_{\text{RDS}}(^{14}\text{N}$ – $\text{I})$ can be defined as the separation between the centers of gravity of these patterns. The RDS is

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used in experiments recently described by Gan [11] and by our group [12,13] for indirect observation of ^{14}N nuclei using ^{13}C as a “spy” nucleus. It is shown that the strength of the RDS is critical for the efficiency of the two-dimensional correlation experiments. The advantage of ^1H nuclei arises from the ratio $D_{\text{RDS}}(^{14}\text{N}-^1\text{H})/D_{\text{RDS}}(^{14}\text{N}-^{13}\text{C}) = \gamma_{\text{H}}/\gamma_{\text{C}} [r(^{14}\text{N}-^{13}\text{C})/r(^{14}\text{N}-^1\text{H})]^3 \approx 13.5$ when $r(^{14}\text{N}-^1\text{H}) = 1.0 \text{ \AA}$ and $r(^{14}\text{N}-^{13}\text{C}) = 1.5 \text{ \AA}$ which are typical distances found in amino acids and proteins. In glycine $\text{NH}_3^+\text{CD}_2\text{COO}^-$, $D_{\text{RDS}}(^{14}\text{N}-^{13}\text{C}) \approx 23 \text{ Hz}$ at 9.4 T assuming colinearity of the dipolar and quadrupolar tensors. The $D_{\text{RDS}}(^{14}\text{N}-^{13}\text{C})$ is much larger than $J(^{14}\text{N}-^{13}\text{C}) \approx 4 \text{ Hz}$ so the magnetization transfer occurs mainly through RDS. On the other hand, it is known that $J(^{14}\text{N}-^1\text{H}) \approx 65 \text{ Hz}$, while $D_{\text{RDS}}(^{14}\text{N}-^1\text{H}) \approx 106 \text{ Hz}$, taking into account the rapid rotation of the NH_3^+ group about its threefold axis which coincides with the N–C axis. This rotation leads to a scaling of the static RDS interaction by a factor of $(3\cos^2\theta-1)/2$, where $\theta \approx 71^\circ$ is the angle subtended between the threefold axis and the instantaneous position of one of

the $r(^{14}\text{N}-^1\text{H})$ vectors [14]. Since the two effective dipole-dipole vectors $r(^{14}\text{N}-^1\text{H})$ and $r(^{14}\text{N}-^{13}\text{C})$ are parallel due to averaging about the threefold axis, they have the same orientation with respect to the electric field gradient tensor of the ^{14}N nucleus. It is evident that $J(^{14}\text{N}-^1\text{H})$ and $D_{\text{RDS}}(^{14}\text{N}-^1\text{H})$ are considerably larger than their $^{14}\text{N}-^{13}\text{C}$ counterparts.

The remarkably simple pulse sequence of Fig. 1 is reminiscent of heteronuclear multiple-quantum correlation (HMQC) spectroscopy that is commonly used in liquids [15], and that has recently been applied in solids with magic angle spinning (MAS), where the magnetization transfer is achieved via J couplings, under the name of MAS-J-HMQC [16–18]. It is also closely related to the work of Gan [11] and ourselves [12,13] using ^{13}C as a spy nucleus. Note that the RDS is a through-space interaction, while the J coupling is a through-bond interaction. As a consequence, it is possible to correlate neighboring pairs of nuclei using RDS even when the J couplings are negligible.

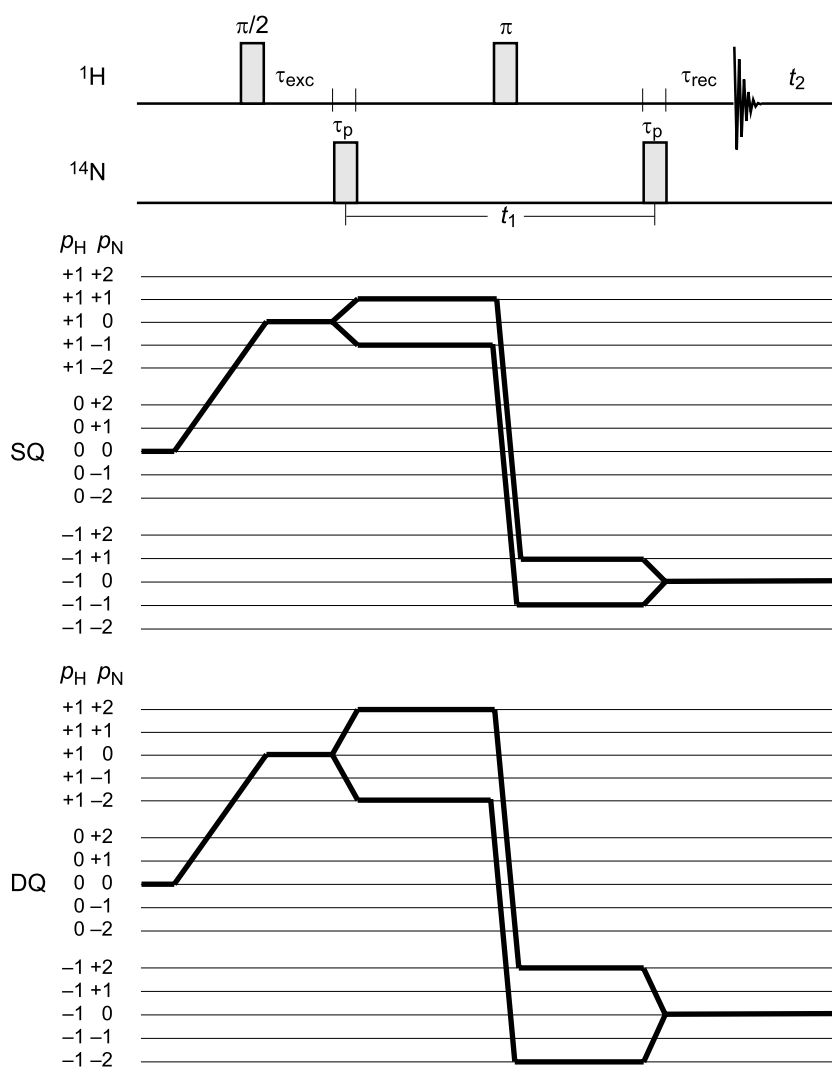


Fig. 1. Pulse sequence and coherence transfer pathway diagrams for the indirect detection of ^{14}N single- or double-quantum spectra by coherence transfer from ^1H to ^{14}N and back.

After exciting transverse proton magnetization represented by a tensor operator $T_{1,\pm 1}^H$ [19], a delay $\tau_{\text{exc}} \approx 1/(2D_{\text{RDS}})$ leads to a state that can be loosely described by a doubly-antiphase operator $T_{1,\pm 1}^H T_{2,0}^N$. Since the coupling D_{RDS} (^{14}N – ^1H) cannot be observed directly, and since the proton magnetization decays rapidly, the delay τ_{exc} must be optimized empirically. A radio-frequency pulse of duration τ_p applied near the centre of the ^{14}N spectrum leads to a partial conversion into $T_{1,\pm 1}^H T_{1,\pm 1}^N$, $T_{1,\pm 1}^H T_{2,\pm 1}^N$ and $T_{1,\pm 1}^H T_{2,\pm 2}^N$ which, apart from proton SQ coherence, also contains ^{14}N SQ ($T_{1,\pm 1}^N$ and $T_{2,\pm 1}^N$) and DQ ($T_{2,\pm 2}^N$) coherences. These are allowed to evolve freely during an evolution interval t_1 in the manner of two-dimensional spectroscopy, prior to reconversion into observable ^1H coherence by a second ^{14}N radio-frequency pulse again of duration τ_p . Proton chemical shifts and inhomogeneous contributions to the proton decay are eliminated by a π refocusing pulse in the middle of the t_1 period. The latter is defined as the interval between the centers of the two ^{14}N pulses. The initial duration (formally $t_1 = 0$) corresponds to an interval between the centers of the two ^{14}N pulses equal to the rotor period. The increments $\Delta t_1 = 1/\nu_{\text{rot}}$ are synchronized with the spinning period. A two-dimensional Fourier transformation yields a correlation spectrum with either ^{14}N SQ or DQ signals in the ω_1 dimension and the conventional proton spectrum in the ω_2 dimension. The SQ and DQ signal intensities can be optimized by the choice of the intervals $\tau_{\text{exc}} = \tau_{\text{rec}}$ and of the pulse width τ_p . These signals can be separated by phase-cycling. It is possible to obtain both SQ and DQ spectra in a single experiment using multiplex phase cycling [20–22], provided one settles on a compromise for the pulse width τ_p and the duration of the intervals τ_{exc} and τ_{rec} .

In spite of the magnitude of the residual dipolar splitting $D_{\text{RDS}}(^{14}\text{N}$ – $^1\text{H}) \approx 106$ Hz estimated for glycine and the large $J(^{14}\text{N}$ – $^1\text{H}) \approx 65$ Hz coupling, the ^1H spectrum at 400 MHz (Fig. 2a) of the powdered sample spinning at 30 kHz does not feature any resolved fine-structure. Both interactions are masked by homogeneous broadening due to proton–proton couplings, which occur in addition to the inhomogeneous broadening that also affects the ^{13}C spectra. The latter can be due to errors in the adjustment of the magic angle [10], to temperature gradients [10], structural disorder and magnetic susceptibility effects [23–25]. It is well known that dipolar interactions between protons lead to a rapid decay of the free induction decay, which is much faster than the predominantly inhomogeneous decay of carbon-13 coherences. To reduce the linewidths of the ^1H spectra, the sample has been deuterated except for the exchangeable protons that are attached to ^{14}N , the carboxylic group being deprotonated in the zwitterionic form of glycine $\text{NH}_3^+\text{CD}_2\text{COO}^-$. In a sample spinning at a frequency $\nu_{\text{rot}} = 30$ kHz in a static field of $B_0 = 9.4$ T, the proton line-width is ca. 755 Hz or 1.9 ppm, which may be compared with 1770 Hz for fully protonated glycine. The time constant T_2' of the proton spin-echo decays (measured with a single refocusing pulse) was found to be ca. 0.57 ms,

corresponding to a ‘refocusable’ line-width $1/(\pi T_2') \approx 560$ Hz. Despite this homogeneous broadening, it is nevertheless possible to transfer coherence from ^1H to ^{14}N and back, as evidenced by the two-dimensional correlation spectra in Fig. 2b and c. The life-time of proton free induction decays can be significantly extended by various homonuclear decoupling sequences in the manner of combined rotation and multiple pulse sequences (CRAMPS) [26], frequency-switched Lee–Goldburg (FSLG) decoupling [27,28] or decoupling using mind-boggling optimization (DUMBO) [29]. Depending on the T_2 's that can be achieved, experiments using proton detection may have significantly better sensitivity than those using carbon-13, since the former benefit from a favorable gyromagnetic ratio and a larger D_{RDS} .

In the experiments of Fig. 2b and c, the SQ spectra are, as expected, approximately half as wide as the DQ spectra. If the rotor axis is precisely aligned along the magic angle, the line-shapes are determined by second- and the third-order quadrupole interactions for the SQ spectra and by second-order terms for the DQ spectra. A deviation from the magic angle of $\Delta\theta = \theta_a - \theta_m$, where θ_a is the actual angle and θ_m is the magic angle, leads to a non-vanishing first-order quadrupolar powder pattern scaled by $(3\cos^2\theta_a - 1)/2$. Thus for $\Delta\theta = 0.005^\circ$, a first-order quadrupolar splitting of 1 MHz will be scaled down to ca. 123 Hz. The effects of the deviation from the magic angle will be illustrated elsewhere [30]. The sensitivity of these experiments is largely determined by the quantum yield of two-way coherence transfer from ^1H to ^{14}N and back, which depends on the choice of the intervals $\tau_{\text{exc}} = \tau_{\text{rec}}$ and of the pulse width τ_p . Experiments indicate an efficiency of 2% for SQ and 0.4% for DQ experiments compared to a proton spin echo experiment without ^{14}N pulses where $\tau_{\text{exc}} = \tau_{\text{rec}} = \tau_{\text{def}} = \tau_{\text{ref}}$. Using ^{13}C as a spy nucleus in glycine, the ^{14}N quadrupole coupling was found to be $C_Q = 1.18$ MHz and the asymmetry parameter $\eta_Q = 0.50$. The same should hold for partly deuterated glycine-d2. Indeed, the ^{14}N spectra in Fig. 2, which were obtained by detection through the neighboring protons, can be simulated with the same quadrupole tensor parameters as shown on the far right.

Deuterated glycine-d2 was purchased from Cambridge Isotope Laboratories and used without further purification. The sample was packed in a ZrO_2 rotor with an outer diameter of 2.5 mm (sample volume ca. 11 μl), and spun at 30 kHz in a Bruker triple resonance CPMAS probe in 9.4 T wide-bore magnet (^1H and ^{14}N Larmor frequencies of 400 and 28.9 MHz). The magic angle was adjusted within 0.005° , using the ^2H line-width of carboxyl deuterons in α -oxalic acid dihydrate-d6 as a criterion [10]. With a 500 W amplifier, the ^{14}N pulses had an amplitude $\nu_{\text{RF}} = 50$ kHz, calibrated by direct detection of NH_4NO_3 . The chemical shifts of ^1H and ^{14}N are referred to the external standards of TMS and $\text{NH}_4\text{Cl}(\text{s})$, respectively.

To summarize, we have shown that by transferring coherence from ^1H to ^{14}N and back in a solid powder

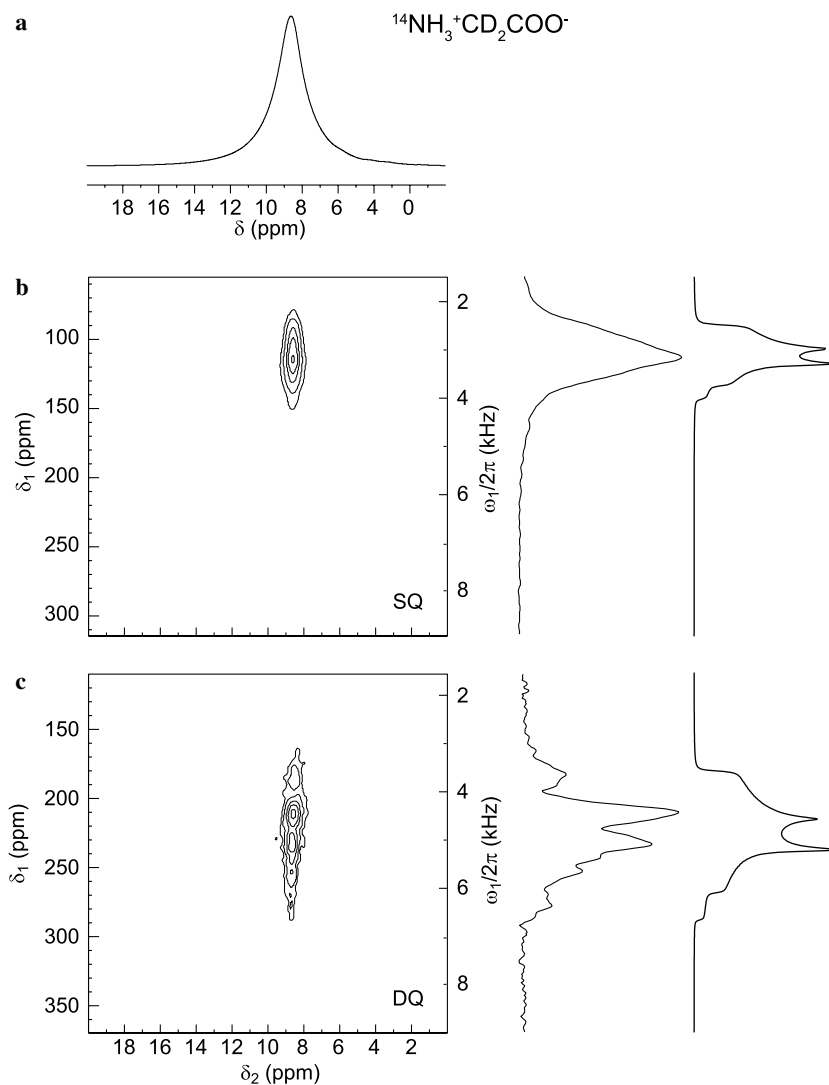


Fig. 2. (a) ^1H MAS NMR spectrum of polycrystalline glycine- d_2 $\text{NH}_3^+\text{CD}_2\text{COO}^-$ and (b and c) two-dimensional correlation (b) ^{14}N SQ- and (c) ^{14}N DQ-spectra. The projections onto the ω_1 axis reveal quadrupolar powder patterns that closely match simulated second-order patterns with $C_Q = 1.18$ MHz, $\eta_Q = 0.50$. The simulated spectra on the far right assume a uniform excitation of all crystallite orientations. The scale in ppm on the left is obtained by dividing the ω_1 frequency by the Larmor frequency (28.89 Hz/ppm at 9.4 T) for both SQ and DQ spectra. The spectrum (a) results from averaging 16 transients with a relaxation interval of 5 s. Each of the two-dimensional spectra (b and c) results from averaging 128 transients for each of 256 t_1 increments with $\Delta t_1 = 1/v_{\text{rot}} = 33.33$ μs , with a relaxation interval of 10 s. The excitation and reconversion intervals $\tau_{\text{exc}} = \tau_{\text{rec}}$ were 1 ms, while the lengths of the two ^{14}N pulses were (b) $\tau_p = 12$ μs for SQ and (c) $\tau_p = 35$ μs for DQ excitation.

spinning at the magic angle, one can indirectly detect the single- or double-quantum transitions of ^{14}N nuclei. Since quadrupole tensors are more straightforward to relate to the local environment than chemical shift tensors, the novel two-dimensional correlation nitrogen-14 NMR method could become an important tool for chemistry, biology and material science.

Acknowledgments

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