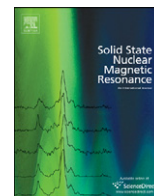




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Solid State Nuclear Magnetic Resonance

journal homepage: www.elsevier.com/locate/ssnmrProbing amide bond nitrogens in solids using ^{14}N NMR spectroscopySasa Antonijevic^{a,b,*}, Nicholas Halpern-Manners^a^a Department of Chemistry, University of California, Berkeley, 1 Cyclotron Road Building 11-D64, Berkeley, CA 94720, USA^b Materials Sciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road Building 11-D64, Berkeley, CA 94720, USA

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ABSTRACT

A novel two-dimensional nuclear magnetic resonance (NMR) experiment is proposed for indirect observation of ^{14}N nuclei in various types of nitrogen-containing solids. In a method somewhat similar to the heteronuclear single-quantum correlation (HSQC) experiment widely used for protein structure determination in solutions, this technique correlates spin $S = 1/2$ nuclei, e.g., ^1H , ^{13}C , with the ^{14}N spin $I = 1$ nucleus in solids. The present experiment, however, transfers coherence from neighboring ^1H or ^{13}C nuclei to ^{14}N via a combination of J -couplings and residual dipolar splittings (RDS). Projections of the two-dimensional NMR spectra onto the ^{14}N dimension yield powder patterns that reflect the ^{14}N quadrupolar interaction, which can be used to study molecular structure and dynamics. Indirect detection of amide nitrogen-14 via ^1H and ^{13}C is shown experimentally on a model compound of *N*-acetyl-glycine.

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Despite its presence in a wide range of materials and a natural abundance of 99.64%, nitrogen-14 remains relatively unexplored by nuclear magnetic resonance (NMR) techniques. The main reason for this is the large quadrupolar coupling which results in very broad ^{14}N resonances in both liquids and solids. Broadening in liquids arises from very rapid quadrupolar relaxation that cannot be suppressed, while in solids the large anisotropic broadening can be reduced. There have been a number of techniques that utilize both direct and indirect detection to study ^{14}N nuclei in solids which include nuclear quadrupole resonance (NQR), overtone and NMR spectroscopy applied to study single crystals or powders combined with magic angle spinning (MAS), and dynamic angle spinning or double sample rotation [1–13]. The quadrupolar tensor parameters, which reflect the local electronic charge distribution, can be used to determine structural parameters of the studied material [7,14]. Indirect detection of ^{14}N (spin $I = 1$) by NMR spectroscopy of solids via spin $S = 1/2$ nuclei has been demonstrated recently [15–24]. The experiment known as nitrogen excitation via residual dipolar splittings (NERDS) is analogous to the heteronuclear multiple-quantum correlation (HMQC) [25] experiment widely used to study molecules in solution. One of the principal differences is that the coherence transfer between the two different nuclei is conducted not only by J -couplings, J_{IS} , but also by second-order quadrupole-dipole cross-

terms, also known as residual dipolar splittings (RDS), D_{RDS} [26–31]. These cross-terms arise because the axis of the quantization of ^{14}N nuclei is subject to the large quadrupolar interaction and is thus tilted from the direction of the static magnetic field, thereby preventing complete averaging of the dipolar interactions between ^{14}N nuclei and neighboring S spins under MAS. As a result, the S resonances are split into 1:2 doublets where each component features a powder pattern, and the separation between the centers of mass of these two powder patterns is referred to here as the residual dipolar splitting, D_{RDS} . The residual dipolar splitting is a purely through-space interaction with strength that depends upon the quadrupolar coupling constant (C_Q), internuclear distance (r_{SI}), relative orientation of the internuclear vector, the largest component of the electric field gradient tensor, and the static magnetic field strength, B_0 . In this work we present an experiment that provides a counterpart to the heteronuclear single-quantum correlation (HSQC) [32] experiment (where single-quantum (SQ) refers to the evolution of SQ coherences of one type of nucleus during the t_1 interval). In our experiment it is only the ^{14}N coherences which evolve, but one can choose to observe either the SQ or double-quantum (DQ) ^{14}N coherences. The acronym sNERDS seems appropriate for coherences of a single (s) type of nucleus (^{14}N) during t_1 interval; likewise the acronym mNERDS seems appropriate for coherences of multiple (m) type nuclei. One application of this experiment probes amide bond nitrogen atoms in powdered solids, which is demonstrated on the model compound of *N*-acetyl-glycine (NAG).

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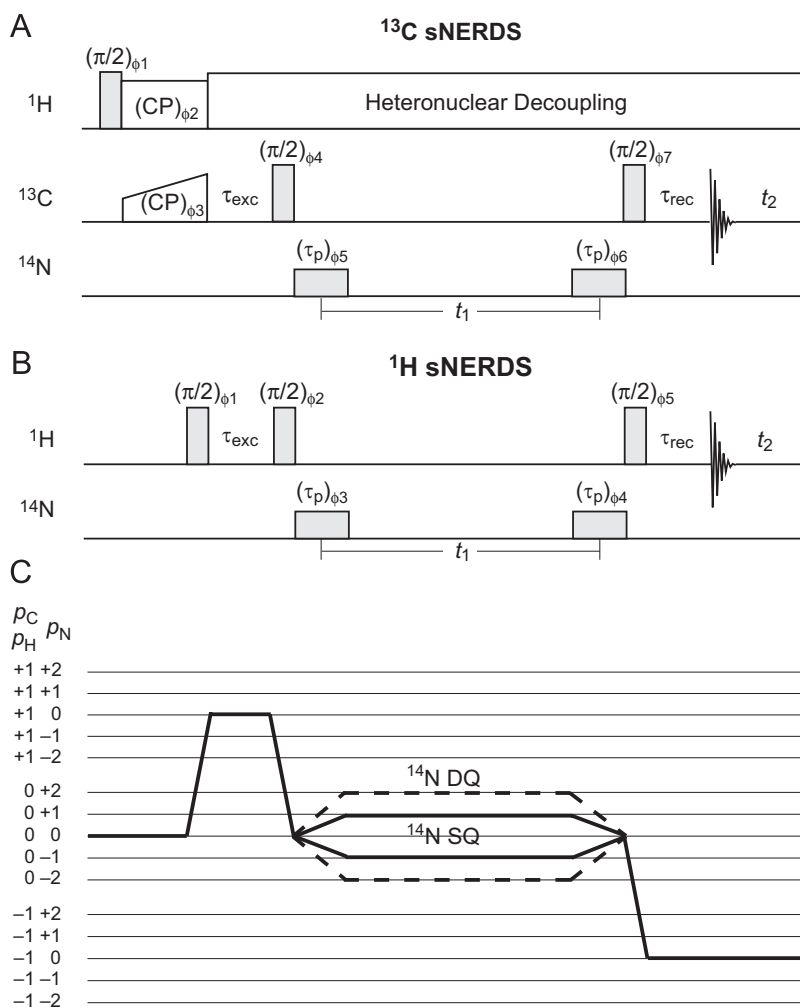


Fig. 1. Pulse sequences (A and B) and coherence transfer pathway diagrams (C) for the indirect detection of ^{14}N single- (solid lines) and double-quantum (dashed lines) spectra by coherence transfer from (A) ^{13}C or (B) ^1H to ^{14}N and back. Two separate experiments, utilizing the appropriate phase cycling, as indicated above with solid and dashed lines, can be used to observe ^{14}N SQ and DQ spectra. Alternatively these spectra can be obtained in a single experiment using multiplex phase cycling, provided one settles on a compromise for the pulse width τ_p .

demonstration of a novel technique to study peptide bond nitrogen-14 nuclei, which are subjected to quadrupolar coupling constants nearly three times larger than nitrogen-14 nuclei found in aminoacids studied by Cavadini et al. [23]. Peptide bond nitrogens have direct relevance to the major applications of these new techniques for studying biological solids. In addition, we propose to irradiate spy (spin $S = 1/2$) nuclei in a time-resolved fashion (in comparison to nitrogen-14 nuclei); by doing so, it is possible to extend further our experiment to include sophisticated steps such as the introduction of pulsed fields gradients in order to improve performance. The final difference is in the approach taken to quantify the efficiency of the experiment. This work was originally submitted as a manuscript to another journal and a preprint was provided to Prof Geoffrey Bodenhausen who kindly acknowledges this in Cavadini et al. [23].

Pulse sequences and coherence transfer pathway diagrams for the indirect observation of ^{14}N (spin $I = 1$) via ^{13}C or ^1H (spin $S = 1/2$) under MAS are shown in Fig. 1, while the phase cycles that can be used to obtain desired coherence selections are given in Table 1. Excitation of transverse magnetization of S spins, represented by a tensor operator $T_{1,\pm 1}^S$ [33], is achieved by means

of cross-polarization from ^1H to ^{13}C (when ^{13}C is used as the “spy” nucleus) or by single radiofrequency pulse irradiation of ^1H nuclei. After a delay $\tau_{\text{exc}} \approx 1/(2D_{\text{RDS}})$, the system reaches a state that can be loosely described by an antiphase operator $T_{1,\pm 1}^S T_{2,0}^N$. Since the coupling $D_{\text{RDS}}(^{14}\text{N-S})$ is anisotropic, and since the S nuclei magnetization often decays rapidly, the delay τ_{exc} must be optimized empirically. A radiofrequency pulse with flip angle $\pi/2$ applied to the S spins followed by a pulse of duration τ_p applied near the center of the ^{14}N spectrum leads to a partial conversion into $T_{1,0}^S T_{1,\pm 1}^N$, $T_{1,0}^S T_{2,\pm 1}^N$ and $T_{1,0}^S T_{2,\pm 2}^N$ which contain ^{14}N SQ ($T_{1,\pm 1}^N$ and $T_{2,\pm 1}^N$) and DQ ($T_{2,\pm 2}^N$) coherences only (unlike ordinary HSQC experiments with spin $1/2$ nuclei where only SQ coherences are involved). These are allowed to evolve freely during an evolution interval t_1 (where the increments $\Delta t_1 = 1/\nu_{\text{rot}}$ are synchronized with the spinning period), prior to reconversion into observable S coherence. 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 S nuclei spectrum in the ω_2 dimension. The SQ and DQ signal intensities can be optimized by choosing the intervals $\tau_{\text{exc}} = \tau_{\text{rec}}$ and the pulse width τ_p . These signals can be separated by phase cycling. It is also possible to obtain both SQ and DQ spectra in a single

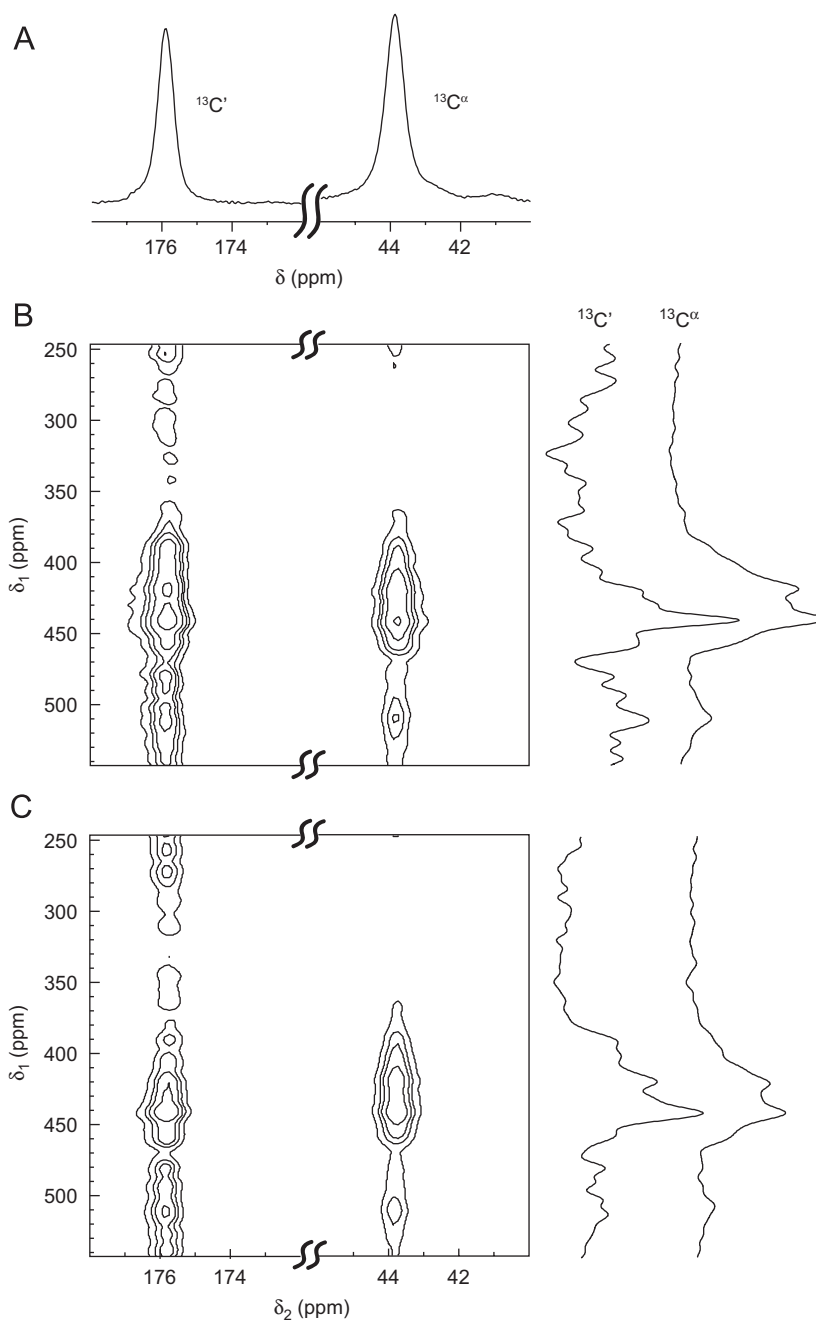


Fig. 2. (A) ^{13}C MAS NMR and two-dimensional correlation ^{13}C SQ NERDS NMR spectra of polycrystalline *N*-acetyl-glycine, ^{13}C enriched in both C' and acetyl carboxyl carbon C^α , recorded using a pulse sequence that selects (B) pure SQ ^{14}N coherences (sNERDS) and (C) ^{13}C and SQ ^{14}N coherences (mNERDS) during the t_1 evolution interval. The projections onto the ω_1 axis reveal quadrupolar powder patterns for the same ^{14}N indirectly observed by the two different carbons. Spectra are the result of averaging 128 transients for each of 64 t_1 increments with $\Delta t_1 = 1/\nu_{\text{rot}} = 66.6 \mu\text{s}$, with a relaxation interval of 10 s. The excitation and reconversion intervals $\tau_{\text{exc}} = \tau_{\text{rec}}$ were 1.2 ms, while the lengths of the two ^{14}N pulses were $\tau_p = 50 \mu\text{s}$.

amplifier, the ^{14}N pulses had a modest amplitude of $\nu_{\text{RF}}^{\text{N}} = 25 \text{ kHz}$, calibrated by direct detection of NH_4Cl . The chemical shifts of ^1H , ^{13}C and ^{14}N are referenced relative to external standards of TMS and NH_3 (liquid, 25°). Typically 150 mg of material is used to obtain spectra shown in this work.

In conclusion, we have demonstrated a NMR experiment for indirect detection of nitrogen-14 in solids, which is analogous to the HSQC experiment widely used for protein structure determination in solutions. The advantage of this sNERDS experiment over the mNERDS experiment introduced recently lies in the fact

that the indirect dimension is free from any broadening introduced from decoherence processes of the spy nuclei, as only ^{14}N coherences are allowed to evolve during the t_1 interval. This may be especially important when protons are used as spy nuclei due to their large refocused line widths. More generally, ^{14}N spectra offer a greater wealth of information in comparison to ^{15}N spectra due to quadrupolar broadening that reflects the local charge distribution. It has been shown that these studies could provide more insight into the geometry of peptide bonds [7,14].

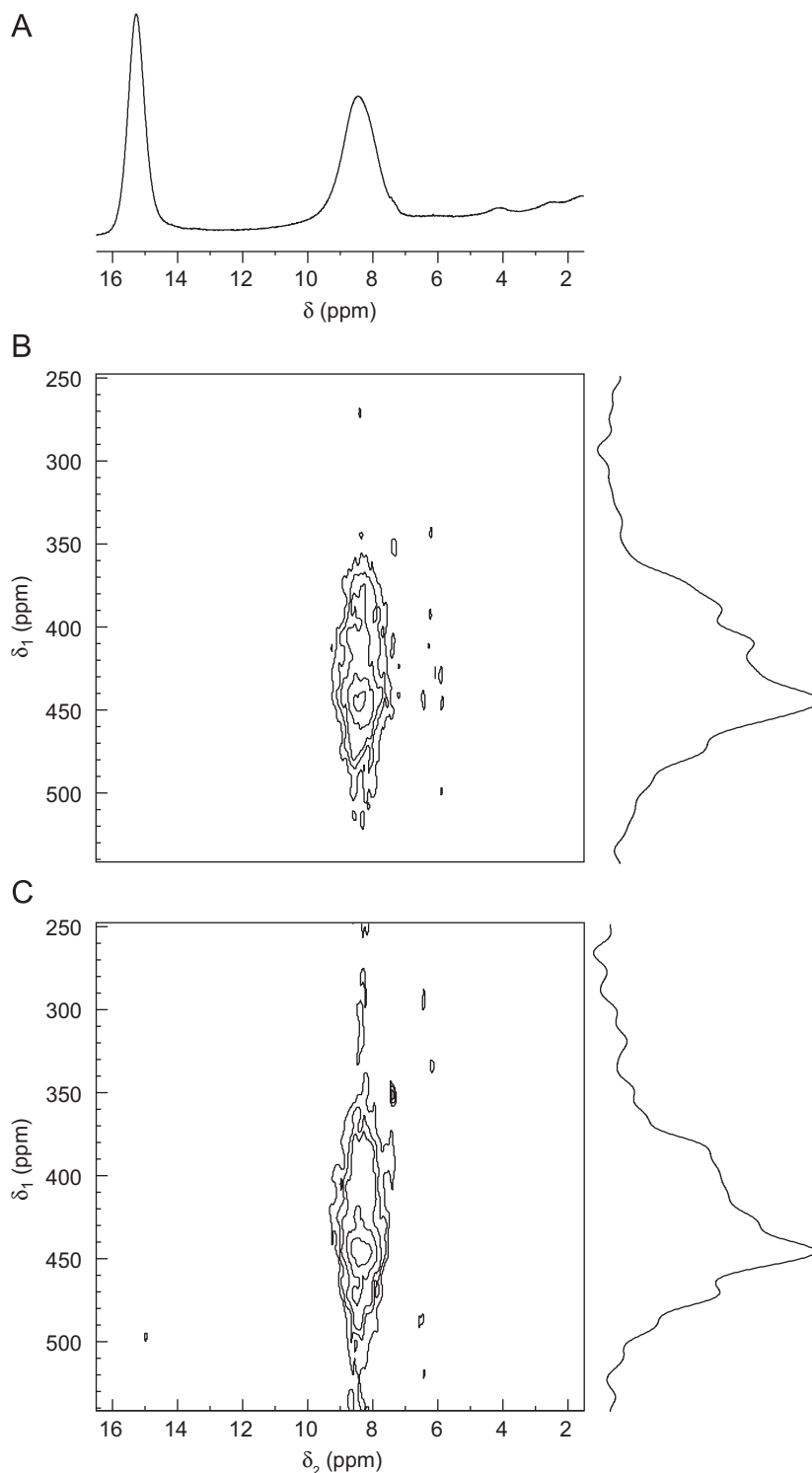


Fig. 3. (A) ^1H MAS NMR and two-dimensional correlation ^1H SQ NERDS NMR spectra of polycrystalline *N*-acetyl-glycine (selectively deuterated so that the amide and carboxyl groups remain protonated), recorded using a pulse sequence that selects (B) pure SQ ^{14}N coherences (sNERDS) and (C) ^1H and SQ ^{14}N coherences (mNERDS) during the t_1 evolution interval. Spectra are the result of averaging 64 transients for each of 64 t_1 increments with $\Delta t_1 = 1/\nu_{\text{rot}} = 67.2 \mu\text{s}$, with a relaxation interval of 10 s. The excitation and reconversion intervals $\tau_{\text{exc}} = \tau_{\text{rec}}$ were 0.8 ms, while the lengths of the two ^{14}N pulses were $\tau_p = 50 \mu\text{s}$.

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