



Communication

3D ^1H - ^{13}C - ^{14}N correlation solid-state NMR spectrumRenée Siegel^a, Julien Trébosc^a, Jean-Paul Amoureux^{a,*}, Zhehong Gan^b^a UCCS (CNRS-8181), University of Lille-1, Fr-59652 Villeneuve d'Ascq, France^b NHMFL, Tallahassee, FL 32310, USA

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ABSTRACT

Nitrogen-14 (spin $I = 1$) has always been a nucleus difficult to observe in solid-state NMR and until recently its observation was restricted to one-dimensional (1D) spectra. We present here the first 3D ^1H - ^{13}C - ^{14}N NMR correlation spectrum. This spectrum was acquired on a test sample L-histidine-HCl-H₂O using a recently developed technique, which consists in indirectly observing ^{14}N nuclei via dipolar recoupling with an HMQC-type experiment.

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1. Introduction

Nitrogen is a nucleus of considerable chemical and biological importance. However, despite its high isotopic abundance (99.63%), ^{14}N has always been a nucleus difficult to observe in NMR. Thus, nitrogen NMR has been mostly limited to the much less abundant isotope ^{15}N , which then requires isotopic enrichment. The main drawback for the observation of ^{14}N is its often large quadrupole interaction, which results in broad NMR spectra in both liquid and solid states. In liquid-state, the broadening comes from the very fast quadrupolar relaxation. In solid-state, the large quadrupolar moment for this spin-1 nucleus often results in NMR spectra of several MHz wide, with powder samples, due to the first-order quadrupole interaction. The study of ^{14}N MAS spectra was thus reduced to samples with quadrupolar coupling of $C_Q \sim 1$ MHz or less [1–5]. In 1986, Tycko et al. [6–8], inspired by the work of Bloom and Legros [9], showed that using overtone NMR, the spectra would only be influenced by the much narrower second-order quadrupole interaction. However, this transition being only weakly allowed, the overtone technique requires very large radio-frequency (rf) field and thus often results in noisy NMR spectra. Recently, ^{14}N NMR has been the subject of more intensified research and, in 2006, Gan [10] as well as Cavadini et al. [11,12] have showed that one could indirectly detect ^{14}N in HMQC sequences, via a combination of scalar J -coupling and residual dipolar splitting (RDS). By synchronizing the indirect acquisition window on MAS, thus folding all first order sidebands, they

obtained 2D NMR spectra where only the second and higher orders of the quadrupole interaction are observed resulting in ^{14}N NMR signals of only a few kHz broad. More recently, Gan used the HMQC sequence and the dipolar rotary resonance recoupling (R^3) (instead of the previously used interactions), to excite multiple quantum coherences involving ^{14}N , leading to the D -HMQC experiment [13]. The dipolar coupling being more important than the J -coupling and RDS in solid-state, Gan et al. obtained a 2D ^1H - ^{14}N correlation NMR spectrum in just a few minutes using the R^3 D -HMQC experiment [14].

R^3 is one within many hetero-nuclear dipolar recoupling techniques. It was first introduced in 1988 by Levitt et al. [15,16] and recovers various anisotropic interactions while not others, depending on the R^3 parameter n correlating rf field to spinning speed: $n = \nu_{\text{rf}}/\nu_R$. The $n = 1/2$ condition, also known as HORROR [17], only recouples the homo-nuclear dipolar coupling. With $n = 1$, the recoupling occurs for CSA and both homo- and hetero-nuclear dipolar interactions. For $n = 2$, rotary resonance only reintroduces CSA and hetero-nuclear dipolar interaction.

The first purpose of this communication is to show that previous 2D hetero-nuclear dipolar methods (^1H - ^{13}C CP-MAS, and ^1H - ^{14}N and ^{13}C - ^{14}N D -HMQC) can naturally be combined to obtain a 3D ^1H - ^{13}C - ^{14}N through-space HETCOR spectrum. The second purpose is to show that the sensitivity of the R^3 dipolar transfer can be enhanced by using symmetry-based methods.

2. Experimental

All experiments were performed on a narrow-bore 18.8 T Avance II Bruker spectrometer using a 3.2 mm triple resonance

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probe and a sample volume of 35 μl . The chemical shifts were referenced using H_2O (^1H), NH_4Cl (^{14}N) and adamantane (^{13}C), which were also used to calibrate the rf power levels.

Our test sample, *L*-histidine-HCl· H_2O (98% ^{13}C enriched) was purchased from Cortecnet and used without further purification. It has three different nitrogen sites (Fig. 1) which are all connected to at least one ^1H and one ^{13}C . Nitrogen N_{11} connects with three ^1H and one carbon (C_2), while N_{12} and N_{13} each connects to one ^1H and two carbons: C_4/C_6 , and C_5/C_6 , respectively.

Fig. 2 describes *D*-HMQC sequence with R^3 ($n = 2$) hetero-nuclear recoupling used for 2D ^1H - ^{14}N correlation spectrum [14]. Delays between R^3 pulses need to be rotor-synchronized as well as t_1 evolution time on ^{14}N channel.

Fig. 5 describes triple resonance experiment involving ^1H , ^{13}C and ^{14}N that was used for 2D ^{13}C - ^{14}N correlation (Fig. 3) as well as for 3D ^1H - ^{13}C - ^{14}N correlation spectrum (Fig. 6). First, ^1H magnetization is excited and may evolve (t_1) in case of 3D experiment under chemical shift interaction. It is then transferred by cross

polarization (CP) to ^{13}C nuclei from which one starts *D*-HMQC between ^{13}C and ^{14}N applying rotor-synchronized R^3 pulses on ^{13}C channel. t_2 corresponds to ^{14}N indirect dimension evolution and is rotor-synchronized, while ^{13}C polarization is directly observed during t_3 . Hetero-nuclear dipolar ^1H decoupling is performed during the whole *D*-HMQC section. 2D ^{13}C - ^{14}N spectrum shown in Fig. 3 was acquired using this sequence without t_1 evolution.

All experimental parameters can be found in figure captions.

3. Results and discussion

As expected, we have observed with the 2D ^1H - ^{14}N - ^1H R^3 *D*-HMQC sequence all three ^{14}N sites connected to three different ^1H (Fig. 2). The ^{14}N quadrupolar coupling constant and asymmetry parameter values of histidine-HCl· H_2O have been previously determined by NQR [18], as well as the ^{15}N isotropic shifts [19]. Their values (corrected for NH_4Cl) are: $\nu_{\text{iso}} = 4.0/146.6/133.4$ ppm; $C_Q = 1.26/1.46/1.29$ MHz, and $\eta_Q = 0.36/0.30/0.97$, for N_{11} , N_{12} , and N_{13} , respectively. The simulated ^{14}N spectra (Fig. 2), although at the correct shifts, show much narrower peaks than the experimental ones. This broadening can *a priori* be attributed to several effects: the ^1H - ^1H flip-flop terms [14], the magic angle being slightly off, or some motion. This broadening is not observed on the 2D ^{13}C - ^{14}N - ^{13}C *D*-HMQC spectrum recorded just after the ^1H - ^{14}N - ^1H one (see Fig. 3), and is thus attributed to the proton-proton flip-flop terms. This problem could now be partly reduced by using a *D*-HSQC type of experiment as it was very recently proposed by Antonijevic et al. [20] and Cavadini et al. [21]

The 2D ^{13}C - ^{14}N - ^{13}C R^3 *D*-HMQC spectrum (Fig. 3) has been acquired at a slower spinning speed ($\nu_R = 12.5$ kHz) due to a 'conflict' between the spinning speed, the R^3 irradiation and the proton decoupling rf-field. Indeed, our triple-resonance probe can only achieve a ^1H decoupling rf amplitude of $\nu_{\text{rf-}^1\text{H}} = 80$ kHz, and we noticed that a minimum ratio $\nu_{\text{rf-}^1\text{H}}/\nu_R \approx 5$ is necessary to prevent ^1H - ^{13}C coherence built up during R^3 irradiation that leads to signal

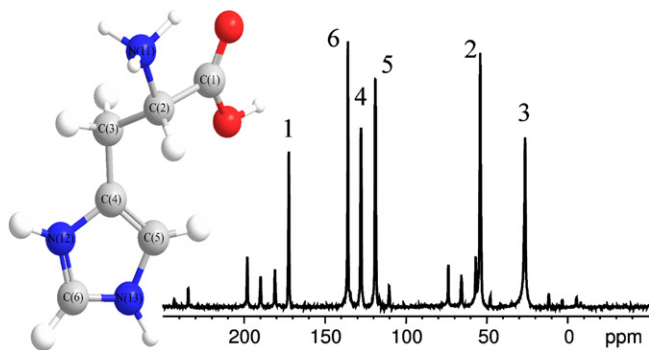


Fig. 1. Histidine-HCl· H_2O molecule along with the corresponding ^{13}C CP-MAS spectrum. $B_0 = 9.4$ T, $\nu_R = 7$ kHz. The small unlabeled peaks are spinning sidebands.

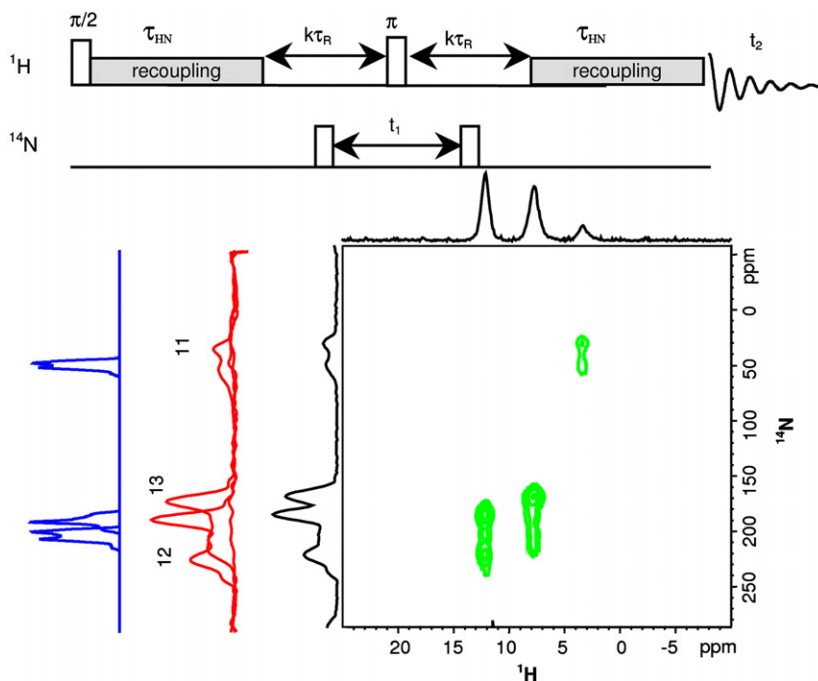


Fig. 2. Pulse sequence and ^1H - ^{14}N - ^1H R^3 *D*-HMQC spectrum of histidine-HCl· H_2O at $B_0 = 18.8$ T with $\nu_R = 20$ kHz, along with the projection of the different ^{14}N sites with their simulation calculated with ideal pulses on the left. The t_1 increment must be rotor synchronized and the magic angle set accurately in order to average completely the ^{14}N first-order quadrupole interaction. The coherence transfer time τ_{HN} (R^3) was $100 \mu\text{s}$ ($2\tau_R$) with $\nu_{\text{rf-}^1\text{H}} = 40$ kHz. Fifty rotor-synchronized t_1 increments ($\Delta t_1 = 50 \mu\text{s}$) of 8 scans each were collected. The ^{14}N pulses were $25 \mu\text{s}$ long with $\nu_{\text{rf-}^{14}\text{N}} = 50$ kHz. The recycle time was of 4 s, and the total experimental time of 27 min.

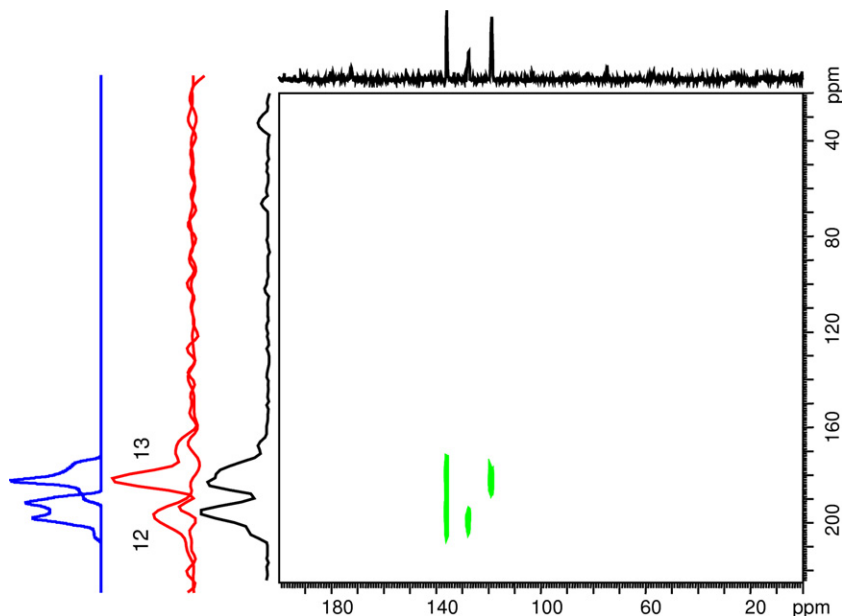


Fig. 3. 2D ^{13}C - ^{14}N - ^{13}C D-HMQC spectrum of histidine-HCl-H₂O at $B_0 = 18.8$ T with $\nu_R = 12.5$ kHz, along with the projection of the different ^{14}N sites with their simulation calculated with ideal pulses on the left. The coherence transfer time $\tau_{\text{CN}}(R^3)$ was $240 \mu\text{s}$ ($3\tau_R$) with $\nu_{\text{rf-}^{13}\text{C}} = 12.5$ kHz. Fifty rotor-synchronized t_1 increments of 16 scans each were collected. The ^{14}N pulses were $30 \mu\text{s}$ long with $\nu_{\text{rf-}^{14}\text{N}} = 40$ kHz. The recycle time was of 4 s, and the total experimental time of 55 min. ^1H - ^{13}C CP-MAS: contact time = 3 ms, $\nu_{\text{rf-}^1\text{H}} = 62.5$ kHz, $\nu_{\text{rf-}^{13}\text{C}} = 50$ kHz. ^1H decoupling: $\nu_{\text{rf-}^1\text{H}} = 80$ kHz. ^{13}C π and $\pi/2$ pulses: $\nu_{\text{rf-}^{13}\text{C}} = 50$ kHz.

decrease through spin diffusion on the proton side, in addition to lower resolution on ^{13}C spectrum. The N_{11} correlation peak, already small in the 2D ^1H - ^{14}N spectrum, is hardly observed in the 2D ^{13}C - ^{14}N one. The weakness of this peak could come from some experimental parameters ($\tau_{\text{CN}}(R^3)$ or CP contact time) which would necessitate being different from those of the other sites.

As stated before, many other recoupling techniques exist. To improve the 2D signal, we therefore tried a symmetry based one [22], the $R4_1^2(\pi_{90}\pi_{-90}\pi_{90}\pi_{-90})$ to which we added a consecutive $R4_1^{-2}(\pi_{-90}\pi_{90}\pi_{-90}\pi_{90})$. The $R4_1^2R4_1^{-2}$ sequence, which was used within a slightly more sophisticated super-cycle by Brinkmann et al. [23] lasts two rotor periods. Like R^3 ($n = 2$), $R4_1^2R4_1^{-2}$ reintroduces hetero-nuclear dipolar couplings, and decouples homo-nuclear dipolar interactions, which is of great importance for ^1H NMR. $R4_1^2R4_1^{-2}$ has several advantages over R^3 ($n = 2$) for the ^1H - ^{14}N dipolar recoupling. First, in Fig. 4, we observe a significant signal intensity increase. Depending on the ^{14}N site, improvement reaches from 1.5 to 2.1. One can explain the differences in enhancement by the fact that R^3 matching curves have a breadth

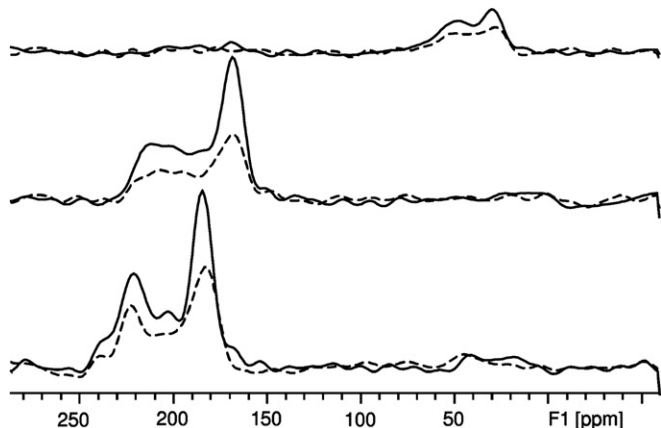


Fig. 4. Comparison of the $R4_1^2R4_1^{-2}$ (—) and R^3 (---) recoupling techniques on the different slices from the 2D ^1H - ^{14}N experiment.

that depends on CSA and hetero-nuclear dipolar strength. If the matching curve is narrow, one loses signal from end parts of the rotor experiencing lower B_1 field out of matching condition. $R4_1^2R4_1^{-2}$ is more robust due to a larger matching breadth always covering most of B_1 distribution along the rotor [24]. Second, compared to other symmetry based methods, such as the recently used $R20_5^9$ [25], the basic cycle of this technique only lasts two rotor periods, which allows short transfer times of 100–200 μs , often the optimum for ^1H - ^{14}N coupling of directly bonded NH.

The 3D pulse sequence (Fig. 5) consists first in a proton evolution time t_1 , followed by a $^1\text{H} \rightarrow ^{13}\text{C}$ CP transfer and then a ^{13}C - ^{14}N - ^{13}C D-HMQC dipolar correlation. The 3D experiment (Fig. 6) mainly shows two ^{14}N sites: N_{12} and N_{13} . Again the N_{11} correlation peak, which was already small in the 2D ^1H - ^{14}N spectrum and barely observed in the 2D ^{13}C - ^{14}N one, is hardly observed in the 3D ^1H - ^{13}C - ^{14}N spectrum as well. The reduced resolution along the ^1H dimension is due to the slower spinning speed ($\nu_R = 12.5$ kHz) we had again to use as explained previously. Although it was not necessary here, the 3D experiment can help us to clearly separate and assign all the ^{14}N sites in the molecule. This will be particularly useful for larger bio-molecules.

In conclusion, we have shown on a simple molecule, histidine-HCl-H₂O, that the acquisition of 3D solid state NMR through-space correlation spectra of ^1H - ^{13}C - ^{14}N is possible. This application opens the possibility to study many samples without the need for

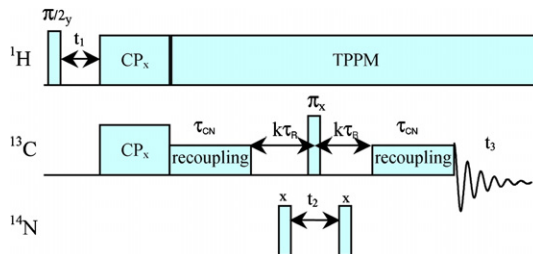


Fig. 5. Pulse sequence (---) used for the 3D experiment. The increment t_2 must be rotor synchronized.

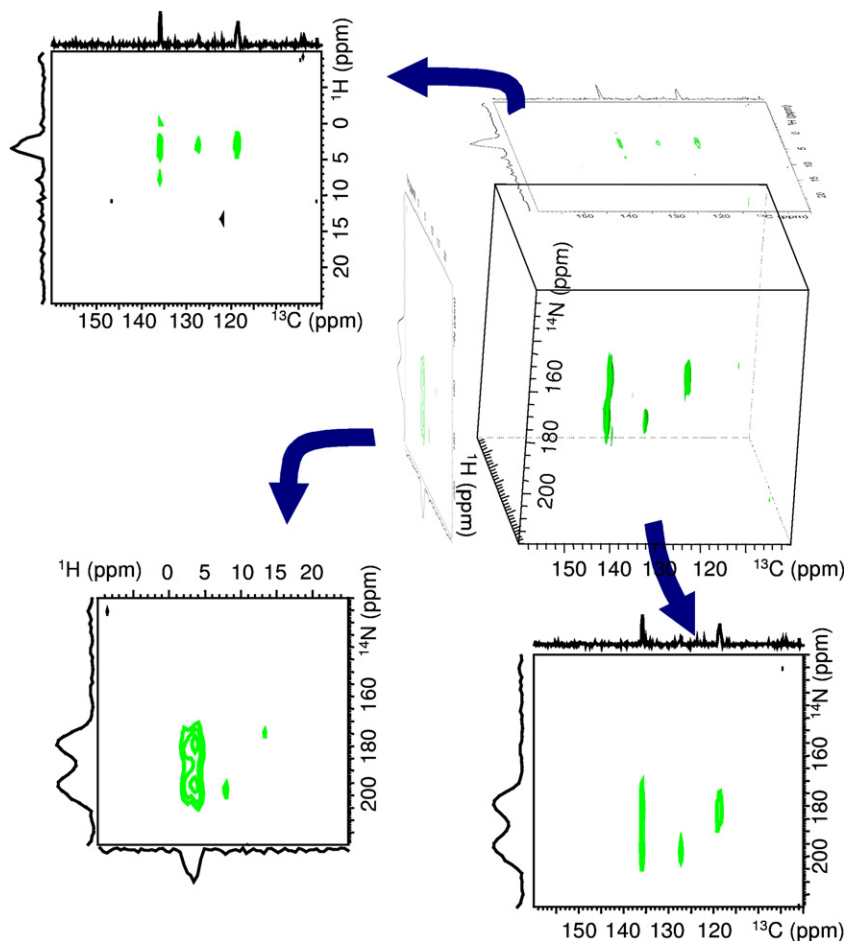


Fig. 6. 3D ^1H - ^{13}C - ^{14}N spectrum of histidine-HCl-H₂O at $B_0 = 18.8$ T with $\nu_R = 12.5$ kHz, according to pulse sequence in Fig. 5. The CP contact time was 3 ms and the coherence transfer time $\tau_{\text{CN}}(R^3) = 240$ μs ($3\tau_R$). 72 t_1 and 32 t_2 increments, of 20 and 80 μs , respectively, of 32 scans each were collected. The ^{14}N pulses were 30 μs long with $\nu_{\text{rf-}^{14}\text{N}} = 40$ kHz. The recycle time was of 4 s and the total experimental time of 85 h. Note. The 3D experiment was acquired before the improvement made using $R4_1^2R4_1^2$. Other experimental parameters as in Fig. 3.

any isotopic enrichment. Many improvements are still possible in terms of resolution and sensitivity thanks to ultra-fast MAS probes now commercially available. Rotor-synchronized indirect dimension spectral width benefits from large ν_R values to avoid the folding of ^{14}N resonances, especially with large magnetic fields. Fast spinning-speeds also lead to better proton decoupling that can be obtained with weak ^1H rf-field, thus avoiding the decoupling problem we have encountered with ^{13}C - ^{14}N and ^1H - ^{13}C - ^{14}N experiments. It also lengthens the T_2 constants [26], which is often critical in HMQC experiments. Additionally, smaller coil diameters allow for stronger rf-fields, which are especially important to manipulate efficiently broad powder sample spectra such as that of ^{14}N nucleus [27]. Moreover, more efficient hetero-nuclear dipolar recoupling techniques, such as SFAM₂ may be used, especially at fast or ultra-fast spinning speed [24]. In addition, ^1H resolution may be enhanced by a homo-nuclear ^1H decoupling scheme such as FSLG [28], PMLG [29], eDUMBO [30], or RN_n^v [31] at moderate ν_R or SAM [32] at fast or ultra-fast spinning speeds. Finally, the continuous-wave CP may be advantageously replaced by a multi-pulse CP at ultra-fast MAS ($\nu_R > 40$ kHz), as shown recently [33].

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