$^{13}C/^{14}N$ heteronuclear multiple-quantum correlation with rotary resonance and REDOR dipolar recoupling

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Abstract

A two-dimensional $^{13}C/^{14}N$ heteronuclear multiple quantum correlation (HMQC) experiment using dipolar recoupling under magic-angle spinning (MAS) is described. The experiment is an extension of the recent indirect ^{13}C detection scheme for measuring ^{14}N quadrupolar coupling under MAS. The recoupling allows the direct use of the much larger dipolar interaction instead of the small J and residual dipolar couplings for establishing $^{13}C/^{14}N$ correlations. Two recoupling methods are incorporated into the HMQC sequence, both applying rf only to the observed ^{13}C spin. The first one uses the REDOR sequence with two π-pulses per rotor cycle. The second one uses a cw rf field matching the spinning frequency, known as rotary resonance. The effects of CSA, T_2' signal loss, MAS frequency and stability and t_1-noise are compared and discussed.
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1. Introduction

With the importance of nitrogen in nearly all branches of chemistry, there has been a continuing interest in ^{14}N NMR for measuring the electric-field-gradient. However, the efforts have been hampered by the low gyromagnetic ratio and large quadrupolar coupling of the spin-1 nucleus. The lack of single-quantum transitions with no first-order quadrupolar broadening makes direct ^{14}N detection difficult. The detection of the overtone transition, both directly [1,2] and indirectly [3,4], can in principle avoid the first-order quadrupolar broadening but it suffers from the low second-order excitation and detection efficiencies of the double-quantum transition. Recently, an indirect approach for ^{14}N NMR using J and residual second-order quadrupolar–dipolar couplings under magic-angle spinning was introduced [5,6]. One-bond $^{13}C/^{14}N$ correlation can be observed with these couplings remained under MAS using

the heteronuclear multiple-quantum correlation (HMQC) pulse sequence [7]. The enhanced spectral resolution and sensitivity through indirect ^{13}C detection open up many opportunities of using the highly abundant ^{14}N (99.6%) and the quadrupolar spin interaction as additional probes not accessible by the traditional ^{15}N NMR.

The $^{13}C/^{14}N$ HMQC experiment using the J and residual quadrupolar–dipolar couplings requires a long coherence transfer time because these couplings are small. Significant signal losses can occur from ^{13}C T_2' relaxation during the long coherence transfer period. The residual dipolar Hamiltonian has a different form from the J and dipolar coupling, $I_z S_z^2$ instead of $I_z S_z$, and the resulting correlations are mixed with through-bond and through-space interactions. Furthermore, the second-order dipolar–quadrupolar coupling decreases with the magnetic field and it depends on many parameters including the quadrupolar coupling and the relative orientation with respect to the dipolar vector [8–15]. It is much more desirable to use the first-order $^{13}C/^{14}N$ dipolar coupling directly for the coherence transfer. This Communication presents the use

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of dipolar recoupling with the HMQC pulse sequence for $^{13}C/^{14}N$ correlation under MAS. For $^{13}C/^{14}N$ dipolar recoupling, it is important that the pulse sequences apply rf only to the observed ^{13}C spin because the ^{14}N quadrupolar coupling, typically in the order of MHz, is much larger than the rf. With this consideration, two recoupling methods are proposed here. The first one uses the well-known REDOR sequence with two π-pulses per rotor cycle [16–18]. The second one uses the rotary resonance with a cw rf field matching the spinning frequency [19–22]. It will be shown that ^{13}C chemical shift anisotropy (CSA) recovered under both sequences plays an important role for the $^{13}C/^{14}N$ HMQC experiment. Unstable and incomplete CSA refocusing of the observed ^{13}C spin can cause t_1-noise and signal losses, which must be especially avoided because of the low ^{14}N pulse efficiency of the difference experiment. In the following, the REDOR-HMQC (R-HMQC) method is described first. The importance of refocusing CSA, the requirement of precise MAS control and the effect on t_1-noise are discussed. The rotary resonance-HMQC (R2-HMQC) is presented next followed by demonstrations of t_1-noise reduction and sensitivity improvement for one-bond and long-range $^{13}C/^{14}N$ correlations.

2. REDOR-HMQC

The rotational echo double resonance (REDOR) sequence applies evenly spaced π-pulses (two per rotor cycle) for dipolar recoupling [16–18]. The π-pulses alternate the sign of dipolar coupling modulated under MAS

$$H_d(t) = 2\pi D[\sin^2\beta\cos2(\omega_r t + \alpha) - \sqrt{2}\sin2\beta$$
$$\cos(\omega_r t + \alpha)]I_z S_z \qquad (1)$$

Here $D = \gamma_I\gamma_S h/2\pi^2 r^3$ is the dipolar coupling frequency and (β, α) are the polar angles of the internuclear vector in the rotor frame. The sign changes prevent a complete average of the dipolar Hamiltonian by the MAS,

$$H_d^{REDOR} = \omega_d I_z S_z, \quad \omega_d = 4\sqrt{2}D\sin(2\beta)\sin(\alpha). \qquad (2)$$

The isotropic parts of the J and the residual dipolar couplings, which are not modulated by the MAS, are thus averaged to zero by the evenly spaced π-pulses. The anisotropic parts of the two interactions partially recoupled under REDOR are neglected here as they are much smaller as compared to the direct dipolar coupling.

The ^{13}C (I) coherence is transfer to two-spin coherence with ^{14}N (S): $I_x \rightarrow \sin(\omega_d\tau/2)2I_y S_z$ and then back: $2I_y S_z \rightarrow \sin(\omega_d\tau/2)I_x$ (τ is defined as the total time period for the two-step transfer). The coherence transfer leads to the HMQC signal intensity

$$s(\tau) = \sin^2(\omega_d\tau/2) \cdot R \cdot N, \qquad (3)$$

where R is the I-spin relaxation factor during the two transfer periods and N is the efficiency of the ^{14}N pulses. A powder average of Eq. (3) leads to approximately the expression for the total HMQC signal intensity

$$\langle s(\tau)\rangle = \frac{1}{2}[1 - S_d(D\tau)] \cdot R \cdot N, \qquad (4)$$

$$S_d(D\tau) = \frac{1}{4\pi}\int_0^\pi d\alpha \int_0^\pi \sin\beta\,d\beta\cos(\omega_d\tau), \qquad (5)$$

$1 - S_d(D\tau)$ is the REDOR dipolar dephasing known as the universal REDOR fraction $\Delta S/S_0$ [16–18]. This function rises from zero then flattens off at one with $\tau \gg 1/D$. The powder distribution of anisotropic dipolar coupling frequencies averages out the oscillatory behavior of isotropic J-transfer. However, the efficiency of dipolar transfer is a factor of 2 lower than the optimal J-transfer.

The ^{14}N pulse efficiency N depends on the quadrupolar coupling constant, the rf field strength and the spinning frequency. It is usually low due to the insufficient ^{14}N rf field as compared to the large quadrupolar frequencies. The low ^{14}N efficiencies make the difference experiment especially sensitive to t_1-noise.

Fig. 1 shows the $^{13}C/^{14}N$ REDOR-HMQC spectra of natural abundant glycine. One-bond $^{13}C/^{14}N$ correlation with the Cα can be established with $\tau = 3.2$ ms. Two-bond correlation with the CO can be observed by doubling the transfer time to 6.4 ms. However, the CO cross peak is mixed with a ridge of t_1-noise. The t_1-noise comes mainly from the ^{13}C CSA. Any heteronuclear dipolar recoupling sequence with rf applied only to the ^{13}C spin automatically reintroduces the ^{13}C CSA because of identical rotation properties under the rf and MAS of the two spin interactions. The CSA is refocused by the middle π-pulse of the HMQC pulse sequence. The refocusing occurs only at the very end and it requires an identical CSA evolution during the two coherence transfer periods. Unfortunately, the CSA recovered under REDOR depends critically on the rotor position with respect to the multiple π-pulse sequence. Small MAS frequency drifts and fluctuations accumulate timing error over the long transfer periods and consequently result in incomplete and unstable CSA refocusing. This problem increases with the CSA, the magnetic field, the coherence transfer time, the MAS frequency and the deviation of spinning frequency from rotor synchronization condition. It has been shown that a 0.1 Hz change of MAS frequency can distort peak phase and attenuate signal intensities [18,23]. Most commercial spectrometers are presently equipped with MAS controllers with only about ±1 Hz stability, which may not be sufficient for the $^{13}C/^{14}N$ HMQC experiment. Because of the low ^{14}N efficiencies of the difference experiment, small ^{13}C signal phase and intensity fluctuations can cause large t_1-noise especially for long-range $^{13}C/^{14}N$ correlation and sites with large ^{13}C CSA at high fields as seen here for the CO site of glycine.

Rotor synchronization is not only important for refocusing CSA but also for the ^{14}N spectra along the indirect dimension. The two ^{14}N pulses need be rotor-synchronized with precisely set magic-angle for the complete averaging of the first-order quadrupolar coupling, similarly to the satellite transition magic-angle spinning (STMAS) experiment [24,25]. Such settings yield ^{14}N spectra with only
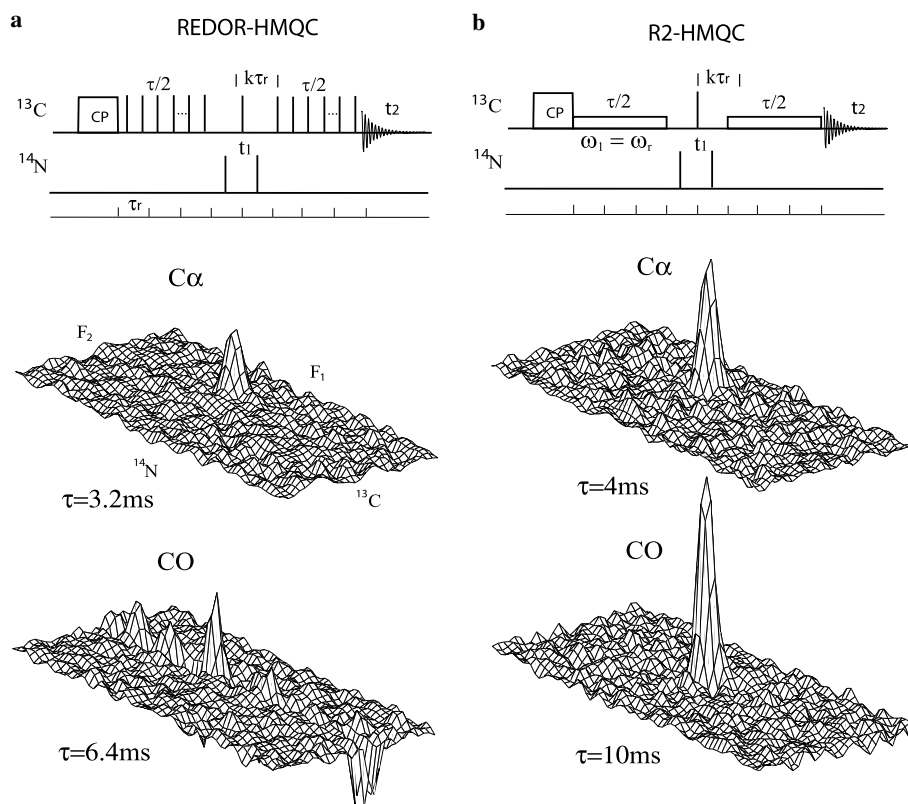
Fig. 1. ^{13}C/^{14}N HMQC with (a) REDOR recoupling and (b) rotary resonance. The experiments were performed at 14.1 T with a Bruker-DRX console using a 4 mm triple-resonance wide-bore MAS probe and a natural abundant glycine sample. The ^{13}C rf field was 66kHz during cross polarization and 10 kHz during rotary resonance matching the 10 kHz MAS frequency. The ^{14}N pulse lengths were 3 μ with 42 kHz rf. It is important to rotor-synchronize the t_1 period and to set the magic-angle precisely for complete averaging the ^{14}N first-order quadrupolar coupling. It is also very important to rotor-synchronize the spin-echo segment in the middle as shown in the pulse sequence. The total duration for the spin-echo starts from $2\tau_r$ and increases $2\tau_r$ a step for every two t_1 increments. Finite pulse length needs be included in the rotor synchronization conditions. For the REDOR recoupling, the two π-pulses in each rotor cycle were placed at $\tau_r/4$ and $3\tau_r/4$ with XY-8 phase cycling [28] (note the spin-echo segment is rotor-synchronized with respect the REDOR unit cycle, not the π-pulses, as illustrated in the pulse sequence). All four spectra were acquired with on-resonance rf and identical experimental setting, and are displayed with absolute intensity scale. A total of 32 rotor synchronized t_1 increments were collected in less than 9 min with 8 scans and 2 s recycle delay for each increment.

the chemical and the second-order quadrupolar shifts along the indirect dimension [5,6]. Details of rotor synchronization for the ^{13}C/^{14}N HMQC experiment can be found in Fig. 1 and the caption.

2.1. Rotary resonance HMQC

Heteronuclear dipolar coupling can also be recovered under MAS with rotary resonance. Rotary resonance applies a cw rf field matches the chemical shift and dipolar coupling modulation frequencies under MAS $\omega_1 = n\omega_r$ [19–22,26]. It can be considered as an extreme case of REDOR by making the π-pulses softer and longer until the multiple-pulse sequence becomes cw. The rotary resonance recoupling (R^3) designed for dipolar recoupling is not applicable here because it applies the $\omega_1 = n\omega_r$ rf to the coupling spin [21,22]. The rotary resonance scheme used here for ^{13}C/^{14}N HMQC is actually the one originally developed for recovering CSA [19,20] (it recovers the heteronuclear dipolar coupling though it was not mentioned). A

rotor-synchronized π-pulse can refocus the ^{13}C CSA leaving only the heteronuclear dipolar coupling for the ^{13}C/^{14}N HMQC experiment. The rotor-synchronization is very important for rotary resonance echo. Changing the gap between the two rotary resonance periods in Fig. 1b by a half rotor period can destroy the rotary resonance echo completely [26].

The $n = 1$ rotary resonance has a larger effect than $n = 2$ and is used here. The dipolar Hamiltonian under $n = 1$ rotary resonance is similar to that of REDOR, differing only by a scaling factor $\pi/4$ assuming that the ^{13}C/^{14}N dipolar coupling is smaller than the ^{13}C CSA [26,27].

Fig. 1b shows the ^{13}C/^{14}N correlation with rotary resonance for glycine. The coherence transfer time was increased slightly adjusted for the $\pi/4$ scaling factor (the transfer time for REDOR-HMQC of the CO sites was shortened because of the t_1-noise problem). The side-by-side comparison shows much higher peak intensities for the spectra using rotary resonance. Most important, the R2-HMQC spectra are free of t_1-noise. The higher peak

intensities are the results of long and stable rotary resonance echo. First, the ^{13}C rf field during REDOR sequence was about $\upsilon_1 \approx 66\,\text{kHz}$ that may interfere with the $\upsilon_1 \approx 100\,\text{kHz}$ proton decoupling, especially for the Cα site. The ^{13}C rf field for rotary resonance is much lower so the interference with the decoupling is negligible. Second, incomplete CSA refocusing due to MAS frequency fluctuation causes not only the t_1-noise but also signal attenuation as a result of signal average. Rotary resonance echo is more stable and has a longer T_2'. It has been observed that rotary resonance echo T_2' can become even longer than regular spin echo [26]. The long T_2' and stable rotary resonance echo make the R2-HMQC more efficient than the R-HMQC.

The long and stable rotary resonance echo comes at no surprise. After all, rotary resonance is a cw sequence, thus timing is not an issue. The only requirement for rotary resonance echo is to keep the short spin-echo segment in the middle rotor-synchronized. This segment starts from two rotor periods. Timing errors from MAS frequency deviations are much smaller than REDOR in which the timing errors accumulate over the long coherence transfer periods. This feature of rotary resonance makes R2-HMQC especially immune to spinning frequency fluctuation and t_1-noise, as demonstrated here by the experimental results.

The rotary resonance condition is a match between the rf field and the spinning frequency. One may expect that rf field homogeneity and MAS frequency control are critical. Indeed, the resonance condition needs be matched within the dipolar coupling if assuming no CSA. The condition would become very narrow for weak dipolar couplings and require very homogenous rf field and precise spinning frequency setting for rotary resonance. The presence of CSA changes this requirement completely. As both CSA and heteronuclear dipolar interactions are present, the resonance condition needs be matched within the larger of the two. ^{13}C CSA is usually in the kHz range or larger, therefore rotary resonance condition can be easily met regardless the ^{13}C/^{14}N distance. The broad resonance condition also makes R2-HMQC capable of covering several kHz in chemical shift bandwidth. Rotary resonance is a rare case where large CSA actually helps dipolar recoupling instead of being a nuisance.

3. Conclusions

It has been shown that ^{13}C/^{14}N HMQC spectra can be obtained efficiently under MAS with REDOR and rotary resonance dipolar recoupling. The REDOR recoupling is broadband but it requires very stable MAS control. The rotary resonance uses a weak cw rf, and therefore is band-selective. Nevertheless, the robust rotary resonance has the advantages of long T_2', little t_1-noise, and susceptible to spinning frequency fluctuation. The R2-HMQC also works better with large ^{13}C CSA, fast MAS and high magnetic field. Coherence transfer using direct dipolar coupling enhances the efficiency of the ^{13}C/^{14}N HMQC experiment for indirect observation of the important but difficult ^{14}N nucleus through ^{13}C detection.

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