Measuring multiple carbon–nitrogen distances in natural abundant solids using R-RESPDOR NMR†

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Multiple carbon–nitrogen distances can be measured in natural abundant solids using Rotary Resonance Echo Saturation Pulse Double Resonance (R-RESPDOR) solid state NMR experiment.

The capability of measuring internuclear distances makes solid-state NMR a powerful method for structural characterization in both ordered and disordered states. The heteronuclear distance measurement was demonstrated in the early days by spin-echo double resonance which measures weak dipolar couplings selectively in the presence of other large spin interactions. The principle has been extended to rotational echo double resonance (REDOR) under magic-angle spinning (MAS). Enhanced sensitivity and resolution of MAS has made REDOR a robust method widely used for structural determination of complex systems such as protein, amyloid fiber, cell wall, glass, zeolite etc. The REDOR distance measurement has also been extended to quadrupolar spin systems with TRAPDOR, REAPDOR, DEAR, and RIDER.

This Communication presents a robust solid state NMR method for simultaneously measuring multiple $^{13}$C/$^{14}$N distances in natural abundant solids. $^{14}$N is a highly abundant (99.6%) spin-1 quadrupolar nucleus. Its distances with surrounding carbons provide useful information for molecular structure and spectral assignment. At $\sim$1% $^{13}$C natural abundance, many $^{13}$C/$^{14}$N pairs can be treated as isolated spin systems allowing simultaneous measurement of multiple $^{13}$C/$^{14}$N distances without complications from $^{13}$C homonuclear dipolar coupling that often occur in uniformly $^{13}$C-labelled molecules. The rotary resonance echo saturation pulse double resonance (R-RESPDOR) method described in this Communication introduces two important changes to the rotational echo adiabatic passage double resonance (REAPDOR) previously developed for measuring distances between a spin-1/2 and a quadrupolar spin. First, REAPDOR applies a multiple-pulse recoupling sequence to the observed $^{13}$C spin and it requires an extremely stable MAS frequency ($\sim$0.1 Hz). R-RESPDOR uses the rotary resonance for dipolar recoupling, an analog of the rotary resonance recoupling by switching the recoupling rf to the observed $^{13}$C spin. The cw recoupling scheme is susceptible to spinning frequency fluctuation therefore applicable with most commercial NMR spectrometers. Second, R-RESPDOR uses $^{14}$N saturation instead of adiabatic-passage pulse for measuring $^{13}$C/$^{14}$N dipolar dephasing. A typical $^{14}$N adiabatic-passage pulse is restricted to about $\tau/3$ and requires a large adiabaticity parameter $\alpha = v^2/(\omega_0 \tau) > 0.25$ for efficient dipolar dephasing and data interpretation using a semi-empirical universal curve. This adiabatic condition may not be met under fast MAS with large $^{14}$N quadrupolar couplings and insufficient rf field for the low-$\gamma$ nucleus. In contrast, $^{14}$N saturation can be implemented easily with long enough pulses. The saturation not only achieves efficient dipolar dephasing but also smooths out the orientation and other parameter dependencies allowing simple data interpretation with a universal function.

Fig. 1a shows the R-RESPDOR pulse sequence for measuring $^{13}$C/$^{14}$N dipolar coupling $D = \gamma_{C/N}^2 h/4\pi^2 r^3 (\text{C})_{\text{N}}$ under MAS. The $^{13}$C/$^{14}$N dipolar interaction is recoupled when the cw rf field matches the MAS frequency $\omega_0 = \omega_c$. The $\omega_0 = 2\omega_c$ rotary resonance also can be used but it has a smaller recoupling effect. Isotropic chemical shift, $\delta$ and $^{14}$N residual dipolar couplings are averaged by the rf. The rotary resonance also recovers the chemical shift anisotropy (CSA) of the observed $^{15}$C spin. The CSA is refocused by the middle $\pi$-pulse in the pulse sequence forming a rotary resonance echo. A change of the $^{14}$N spin state by the saturation pulse interrupts the refocusing of the $^{13}$C/$^{14}$N dipolar interaction causing modulations of the rotary resonance echo. The dipolar dephasing can be measured by two experiments, one with (S) and the other without ($S_0$) the $^{14}$N pulse. The signal fraction $\Delta S/S_0 = (S_0 - S)/S_0$ is compared with the simulation for the determination of the dipolar coupling constant (Fig. 1b).

Rotor-synchronization and MAS frequency stability are important for the echo and recoupling experiments. In REAPDOR, the CSA interaction is recovered under the REDOR multiple-pulse recoupling sequence and it depends on the timing of rotor position with respect to the $\pi$-pulses. The whole evolution and refocusing periods need be rotor-synchronized for a complete refocusing of the CSA occurring at the very end. Small MAS frequency fluctuations can accumulate timing errors over the long periods making the experiment extremely sensitive to MAS frequency control. Rotary resonance uses cw recoupling and the CSA refocusing requires only the middle spin-echo segment rotor-synchronized. Therefore, R-RESPDOR is much less sensitive to spinning frequency fluctuations.

Dipolar modulation to the rotary resonance echo is the result of $^{14}$N spin state change induced by the $^{14}$N pulse. For a spin-1, the transitions can be of zero, single, or double quantum. The double-quantum $1 \leftrightarrow -1$ transition has a dipolar evolution twice as fast as the single-quantum, and no contribution takes place for the zero quantum part. Saturation of the three-level system implies 3:4:2 in probabilities for zero, single, and double-quantum transitions.
of natural abundant L-tryptophan. The $^{13}$C rf phase was kept constant and (c) MAS spectrum showing aromatic fragment and peak assignments and simulations using the listed dipolar coupling frequencies, dimensionless parameter $150\, \text{MHz}$ Bruker-DRX console, 10 kHz MAS with 4 mm triple-resonance (not shown) require separate experiments due to the chemical shift with $y$

The measurements for the CO, $C_\text{b}$, $C_\text{p}$ sites (not shown) require separate experiments due to the chemical shift offsets for the rotary resonance. The two carbons next to indole $^{14}$N (a and h) show dipolar oscillations from the one-bond $^{13}$C/$^{14}$N coupling before reaching the equilibrium value about 0.45. This value differs from $\Delta S/S_0 \to 2/3$ expected for ideal situations. The difference mainly comes from a constant component that is always observed in CSA and dipolar rotary resonance experiment.$^9$–$^{12,17}$ The constant component implies that a fraction of the signal does not contribute towards the dipolar evolution consequently lowering the equilibrium value. Incomplete $^{14}$N saturation also contributes towards the difference because of higher probabilities for the non-contributing zero-quantum part in eqn (1). To account for these effects, a factor of 0.45$\pm0.67$, estimated from the $\Delta S/S_0$ curves of one-bond distance (a and h), is multiplied with eqn (1) for all carbon sites. After this correction, Fig. 1b shows good agreements between the R-RESPDOR simulations and the $\Delta S/S_0$ results for all sites. The dipolar coupling frequencies used in the simulations were obtained from a separate $^{13}$C/$^{15}$N REDOR measurement of a selectively $^{15}$N-labeled sample.$^{17}$ The dipolar coupling frequency $D = \gamma_{^{13}}C/\gamma_{^{14}}N/^{13}C^{14}N$ can be used to extract carbon–nitrogen distances for one and two bond $^{13}$C/$^{14}$N pairs. For $^{13}$C/$^{14}$N pairs with three bonds and beyond (e, d, f), the determination of the distances must take the intermolecular contributions and the effect from the other $^{14}$N spin (NH$_3$) into accounts. These contributions are evident from the faster dephasing curves than the two-spin simulations using the distances from $^{13}$C/$^{15}$N REDOR measurement.

The R-RESPDOR method compliments with REAPDOR in cases of large CSA, fast MAS, and high magnetic fields. All three factors make REAPDOR experiments more difficult on MAS control, rf field requirement, and adiabatic-passage conditions. In contrast, these factors work favorably for R-RESPDOR. Large CSA and fast MAS drive the rotary resonance. The presence of a larger CSA makes the rotary resonance condition for heteronuclear recoupling wider and less dependent on rf field homogeneity. Furthermore, rotary resonance uses weak $^{13}$C rf, therefore the interference with proton decoupling is less of a problem especially for protonated carbons sites (at the expense of reduced bandwidth for chemical shift). On the $^{14}$N aspect, the saturation pulse length is not restricted by the spinning frequency and rapid quadrupolar frequency modulation helps to accelerate $^{14}$N saturation by a pulse over several rotor periods.

It has been shown that the rotary resonance and $^{14}$N saturation scheme make the R-RESPDOR a robust method for measuring $^{13}$C/$^{14}$N distances in solids. The method can be applied to other quadrupolar nuclei. The capability of measuring multiple $^{13}$C/$^{14}$N distances simultaneously at natural abundance makes R-RESPDOR a potential useful tool for spectral assignment and structural analysis of small to medium size molecules without the need of isotope labeling.

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**Notes and references**