Measuring nitrogen quadrupolar coupling with ^{13}C detected wide-line ^{14}N NMR under magic-angle spinning

Zhehong Gan*

A ^{13}C-detected ^{14}N wide-line NMR experiment is described for measuring nitrogen quadrupolar coupling under magic-angle spinning.

Nitrogen is one of the most important elements in nearly all branches of chemistry. While ^{15}N NMR has been widely used with the favorable NMR properties of a spin-1/2, the highly abundant ^{14}N has been rarely studied. ^{14}N NMR is capable of measuring quadrupolar coupling, which provides unique information on nitrogen electric-field-gradients (EFG) inaccessible through the spin-1/2 ^{15}N.[1–7] ^{14}N quadrupolar coupling has been mostly measured in the past by NQR at zero magnetic fields. The measurements can benefit greatly from high magnetic fields to enhance spectral resolution and sensitivity. Nevertheless, the ^{14}N energy levels of a spin-1 lack a central transition that half-integer quadrupolar nuclei have with zero first-order quadrupolar broadening. As a result, ^{14}N spectra at high magnetic fields are often over several MHz wide making direct NMR observation and resolution among multiple nitrogen sites very difficult. Ingenious NMR techniques using overtone transitions [8–13] have been developed to avoid the first-order quadrupolar broadening at a trade off of low signal intensity and excitation efficiency of the $1 \leftrightarrow -1$ double-quantum transition. This communication introduces an indirect detection technique that overcomes the sensitivity and resolution limitations of ^{14}N wide-line NMR for measuring nitrogen quadrupolar coupling under the high resolution magic-angle spinning (MAS) condition.

The proposed experiment extends the recent development of HMQC type experiment for ^{14}N–^{13}C or ^{14}N–^{1}H correlation using a combination of J, second-order quadrupolar–dipolar and direct dipolar couplings.[14–20] It replaces the pair of ^{14}N frequency-encoding pulses by a long pulse and measures the ^{13}C signal response as a function of scanning ^{14}N radio-frequency (rf) over the range of the first-order quadrupolar coupling. This type of wide-line experiment was briefly discussed in the development of the ^{13}C–^{14}N distance measurement technique, the rotational echo adiabatic passage double resonance (REAPDOR), but was not explored further.[21] It will be shown here that by choosing the ^{14}N pulse length and rf field properly such an indirect continuous wave experiment yields wide-line ^{14}N spectra from which both the quadrupolar

coupling constant and the asymmetry factor can be precisely determined.

Fig. 1a shows the indirect ^{14}N experiment through ^{13}C detection. The pulse sequence does not have the conventional evolution time t_1 of a two-dimensional (2D) experiment. Instead, wide-line ^{14}N spectra are acquired point by point with a scanning ^{14}N frequency υ_N. The ^{13}C part of the pulse sequence consists of ^{13}C–^{14}N dipolar recoupling with a spin-echo after the cross-polarization (CP). We use here rotary resonance for recoupling because it is less susceptible to spinning frequency fluctuation than other multiple π-pulse recoupling sequences.[22–24] With a rf field matching the spinning frequency $\upsilon_1 = \upsilon_r$, rotary resonance reintroduces both the ^{13}C–^{14}N dipolar interaction and ^{13}C chemical shift anisotropy under MAS. The rotor-synchronized spin-echo segment in the middle refocuses the ^{13}C CSA part. Any ^{14}N spin state change induced by the ^{14}N pulse results in incomplete refocusing of the ^{13}C–^{14}N dipolar interaction.[25,26] Therefore this experiment maps out the frequency response of ^{14}N spin population $\langle S_z \rangle$ to the ^{14}N pulse through the ^{13}C signal intensity of the rotary resonance echo.

Simulations in Fig. 1b show that the $\langle S_z \rangle$ frequency response under MAS and the static ^{14}N powder lineshape are very similar. The most obvious feature is the two edges near υ_{zz} and υ_{xx}. This feature can lead to the determination of all three principal components of the EFG tensor by utilizing $\upsilon_{xx} + \upsilon_{yy} + \upsilon_{zz} = 0$. The indirect wide-line experiment is demonstrated here with a naturally abundant tripeptide Ala-Gly-Gly in Fig. 1c. The ^{14}N spectra were obtained by measuring the difference ratio $(S_0 - S)/S_0$ where S and S_0 are the ^{13}C signal intensities with and without the ^{14}N pulse, respectively, in a manner similar to the rotational echo double resonance (REDOR) experiment.[27] The dipolar evolution time τ was fixed at 800 µs for one-bond ^{13}C–^{14}N dipolar coupling between an amide nitrogen and nearby CO. The resulting spectra shows clearly the two expected edges near υ_{zz} and υ_{xx} and a fitting of this feature yields quadrupolar coupling parameters $C_q = 3.48$ MHz, $\eta = 0.368$ for Ala1 and $C_q = 3.35$ MHz, $\eta = 0.373$ for Gly2. The results have a higher precision than the ones obtained from second-order effects and are in good agreement with previous studies in ref. 14 and 29.

The main advantage of this frequency-sweep method using a long pulse is the high efficiency over the HMQC-type experiment.[14–20] Magic-angle spinning modulates the first-order quadrupolar coupling and brings the ^{14}N rf irradiation on-resonance during the long pulse. The rf action during the level crossing is brief but effective and it covers all spins despite that

National High Magnetic Field Laboratory, 1800 East Paul Dirac Drive, Tallahassee, FL 32310, USA. E-mail: gan@magnet.fsu.edu; Fax: +01-850-644-1366; Tel: +01-850-64-4662
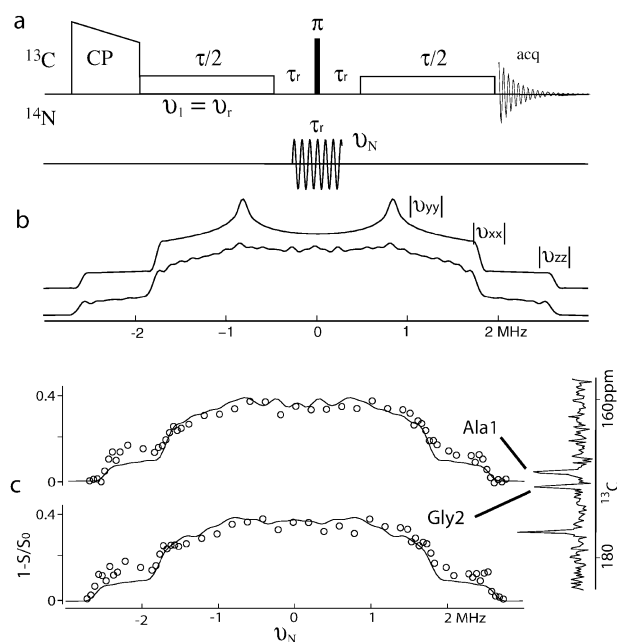
Fig. 1 (a) Pulse sequence for ^{13}C detected first-order ^{14}N quadrupolar experiment under MAS, (b) simulations of ^{14}N response to scanning frequency pulse under MAS (lower curve) and static first-order quadrupolar lineshape (upper curve), (c) first-order ^{14}N quadrupolar spectra (circles) and simulations (solid line) of two amide nitrogen sites in the natural abundant Ala-Gly-Gly detected *via* nearby CO. The wide-line spectra were acquired from the ^{13}C signal intensity by stepping the ^{14}N rf frequency v_N. Experimental parameters are: 14.1 TB_0, 600 MHz Bruker-DRX console, 4 mm triple-resonance probe with 10 kHz MAS, 100 μs ^{14}N pulse with 40 kHz v_1, 800 μs τ, 256 scans with 4 s delay for each point, SPINAL-64[28] ^1H decoupling with 85 kHz v_1 during the dipolar evolution and acquisition period.

their quadrupolar frequencies are distributed over two orders of magnitude larger than the rf field strength. More important, the overall effect on population $\langle S_z \rangle$ for the large number of spins in a powder sample is constructive. In contrary, any measurement of coherence for example by HMQC is subject to rapid dephasing due to the strong quadrupolar interaction. As a result, the signal intensity $(S_0 - S)/S_0$ in Fig. 1c ranges from near 20% at the outer edges to about 40% at the center.

It is interesting to note that the orientational distribution of level crossings under MAS resembles the powder average of a static sample. The simulations in Fig. 1b shows that the lineshape is very similar to the pattern of static ^{14}N first-order quadrupolar lineshape from which the v_{zz} and v_{xx} edges can be picked out easily. Fitting the first-order quadrupolar lineshape can yield quadrupolar coupling parameters more precisely than other methods relying on much smaller second-order effects especially in the presence of line broadening and other experimental imperfections like magic-angle offset, third-order quadrupolar effect and rf lineshape distortion. Wide-line NMR spectroscopy by direct detection are usually associated with low sensitivity and artifacts like baseline roll arising from probe and receiver dead-time. These limitation are absent in the indirect detection. The ^{13}C spectra under MAS provide both the resolution and sensitivity for acquiring and resolving
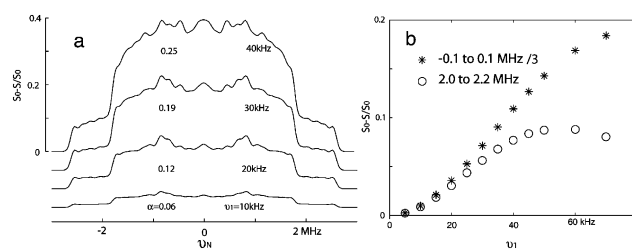

Fig. 2 (a) Simulations of ^{14}N lineshape with various rf field strength v_1 and adiabaticity parameter α and (b) average spectral intensities *versus* ^{14}N v_1 at the outer wings (circles) and at the center (stars) of the lineshape. The vertical axis for the stars was scaled by $\frac{1}{3}$. Quadrupolar coupling parameters $C_q = 3.48$ MHz $\eta = 0.368$ and 10 kHz MAS were used in the simulations.

ultra wide ^{14}N spectra of multiple nitrogen sites as illustrated in Fig. 1c.

The ^{14}N pulse is the central component of this experiment. For a pulse longer than one rotor period, level crossing occurs repeatedly. The accumulative effect from subsequent level crossings can cause v_r modulation with respect to the isotropic ^{14}N frequency. Therefore, a ^{14}N pulse longer than one rotor period should be avoided. The ^{14}N rf field needs be sufficiently strong to induce significant spin state change during the very brief level-crossing. An adiabaticity parameter $\alpha = v_1/(v_r v_q)^{1/2}$ has been introduced by Vega[30] to describe the level-crossing spin dynamics under rf irradiation. Fig. 2 shows simulations with various α. The spectral intensity increases quadratically with the rf field to about $\alpha \approx 0.1$ then approximately linearly until $\alpha \approx 0.25$ which corresponds to about 40 kHz ν_1 for typical 10 kHz MAS frequency and 3.5 MHz C_q of an amide nitrogen. The spectral intensity then starts to saturate when further increasing the rf field especially at the outer regions of quadrupolar lineshape. The lineshape in the middle also starts to distort more severely from the static quadrupolar lineshape. Nevertheless, the position of the sharp edges remains unchanged at higher rf fields. As the level-crossing mechanism depends solely on the adiabaticity parameter α, a slight adjustment on the ^{14}N rf field can compensate for larger quadrupolar couplings. This feature makes the wide-line method particularly attractive over the HMQC-type experiment on the efficiency of measuring large ^{14}N quadrupolar couplings.[14–20]

The discrete ^{14}N frequency sampling affects the digital resolution and the experiment time required for the scanning frequency measurement. In order to shorten the time, it is often feasible to measure just the regions near the v_{zz} and v_{xx} edges (Fig. 1b) for obtaining the main spectral feature which determines the quadrupolar coupling parameters. A quick and coarse run or prior knowledge of the range of quadrupolar coupling parameters helps in the selection of the sampling range for the ^{14}N frequency. In a technical aspect, the first-order ^{14}N quadrupolar spectra usually exceeds the probe bandwidth. For the high-Q probe used for the measurement in Fig. 1, the ^{14}N channel was retuned for every ~0.3 MHz frequency change. Therefore, narrowing the frequency range also helps to minimize the repeated tuning of the ^{14}N channel.

The indirect frequency scanning experiment described here is designed for insensitive nuclei with wide spectral lines such

as ^{14}N which lack any single-quantum transition without the first-order quadrupolar broadening. The method can be applied in principle to any nuclei with their NMR spectra too broad for direct excitation or pulsed frequency-encoding. The indirect detection can also be carried out using J and second-order quadrupolar–dipolar coupling. These couplings remain under MAS therefore can be used for the indirect detection with the need of dipolar recoupling.[14,15] Nevertheless, lengthening T_2' by applying strong and optimized proton decoupling becomes important in order to utilize these small couplings.

In conclusion, it has been shown that the first-order ^{14}N quadrupolar spectra can be acquired with ^{13}C detection under MAS. The ^{14}N level-crossing mechanism induced by MAS makes this experiment efficient and suitable for measuring large ^{14}N quadrupolar couplings without the requirements of strong rf and fast MAS. The high resolution and sensitivity of ^{13}C detection under MAS make this method applicable to large molecules for probing nitrogen electric-field-gradients not accessible from conventional ^{15}N NMR.

Notes and references

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