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Indirect High-Resolution Observation of ^{14}N NMR in Rotating Solids

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Despite its importance and omnipresence in biomolecules, the nucleus of ^{14}N is not very popular in NMR. One reason for this unpopularity is the low sensitivity due to its small gyromagnetic ratio ($\gamma_{^{14}\mathrm{N}}/\gamma_{^{1}\mathrm{H}} \approx 0.072$), and the other reason is wide spectral distribution of ^{14}N due to the large quadrupole interaction. To overcome the spectral range problem, ^{14}N overtone NMR in solids has been proposed,[1,2] in which one observes an "overtone" $|+1\rangle \rightleftharpoons |-1\rangle$ transition. Since the overtone transition is not affected by the large first-order quadrupole interaction, it is possible to excite a whole ^{14}N overtone spectrum by cross polarization or a single pulse. The observed ^{14}N overtone spectrum is governed mainly by a second-order quadrupole interaction. So far, overtone ^{14}N NMR has been applied to determine quadrupole coupling constants and asymmetry parameters for powdered and single-crystal samples.[2−5]

For a powder sample, the orientational dependence of the second-order quadrupolar interaction brings spectral distribution ranging from 10 to a few hundreds kHz. Hence, when a molecule contains more than one ^{14}N nucleus, it becomes difficult to resolve ^{14}N overtone powder patterns. Feasibility of high-resolution ^{14}N overtone NMR in a powder sample by dynamic-angle spinnig (DAS)[6,7] and double rotation (DOR)[8] has been discussed;[9,10] however, no experimental spectra have yet been reported. This is because the sensitivity of overtone NMR is significantly reduced due to averaging of the orientationaly dependent transition moment by sample rotation. In this work, we developed a method for high-resolution ^{14}N overtone NMR by adopting indirect ^{13}C detection. High-resolution is achieved via highly resolved ^{13}C resonances under magic-angle spinning (MAS), whose line widths are affected by ^{13}C−^{14}N dipolar couplings recoupled by rf irradiation at ^{14}N overtone resonances. It is worthy to point out that the strength of a ^{13}C−^{14}N dipolar coupling is independent of the magnitude of ^{14}N polarization, and the low-sensitivity problem is thus overcome. An alternative indirect detection method has been proposed by Garroway and Miller,[11] in which ^{14}N overtone resonances are observed as a decrease of ^{1}H dipolar order due to the polarization transfer to ^{14}N. Since it is difficult to achieve high resolution for ^{1}H in solids, indirectly detected ^{14}N overtone resonances via ^{1}H inevitably overlap each other when several ^{14}N nuclei are involved.

At the first-order approximation, the ^{13}C−^{14}N dipolar interaction is averaged out to be zero by MAS. To recover the ^{13}C−^{14}N dipolar coupling under MAS, we adopted the modulatory-resonance recoupling (MORE) method.[12] It relies on a consequence of a secular average Hamiltonian theory,[13] that is, when there exist noncommutable two time-dependent interactions in the total Hamiltonian, the terms having common Fourier components can be coupled to lead a static cross term. MORE was originally demonstrated for recoupling of ^{13}C−^{15}N dipolar couplings under MAS. In that case, the ^{13}C−^{15}N dipolar interaction modulated by MAS is coupled to the Zeeman interaction between ^{15}N spins and a rf field applied to ^{15}N, whose intensity is modulated as

$$H_{\mathrm{rf}}(t) = H_1 \cos \omega_{\mathrm{AM}} t I_X \tag{1}$$

When the modulation frequency ω_{AM} is matched to the spinning frequency, the ^{13}C−^{15}N dipolar interaction is recovered. For ^{14}N overtone recoupling, a rf field is applied to the ^{14}N overtone transition to interfere the MAS modulation of the ^{13}C−^{14}N dipolar interaction, but it is not necessary to modulate its amplitude as shown in the following.

The ^{14}N quadrupole coupling ν_{Q} is typically an order of magnitude smaller than the Zeeman frequency ν_{N}; hence, the quadrupolar interaction is treated as a perturbation to the Zeeman interaction. The three Zeeman states ($|+1\rangle$, $|0\rangle$, $|-1\rangle$) are mixed with each other to give the three corresponding eigenfunctions ($|\phi_i\rangle$, $i = \pm1, 0$). For overtone irradiation, the two eigenfunctions ($|\phi_i\rangle$, $i = 1, -1$) are relevant, and we shall use a fictitious spin-1/2 operator I in the ($|\phi_1\rangle$, $|\phi_{-1}\rangle$) basis. At the first-order approximation, the interaction between the fictitious spin and a rf field with an intensity of ν_1 to an overtone transition can be represented as

$$H_{\mathrm{rf}}(t) = \frac{\nu_{\mathrm{Q}}}{\nu_{\mathrm{Z}}}\nu_1 \sum_{k=0,\pm1,\pm2} (R_k I_+ + R_k^* I_-) \exp(-2\pi k \nu_R t) \tag{2}$$

where R_k represents a geometrical part and ν_{R} is a spinning frequency. Note here that the intensity of the effective rf field is modulated by MAS and also is scaled by $\nu_{\mathrm{Q}}/\nu_{\mathrm{Z}}$. The modulation is brought about by the orientational dependence of the overtone transition probabilities. Analogous to the case of ^{13}C−^{15}N MORE,[12] the $k \neq 0$ terms contribute to ^{13}C−^{14}N MORE when $\nu_{\mathrm{Q}}\nu_1/\nu_{\mathrm{Z}} \approx \nu_{\mathrm{R}}$. The $k = 0$ term also works to recouple ^{13}C−^{14}N dipolar interactions via rotary resonance[14] when $\nu_{\mathrm{Q}}\nu_1/\nu_{\mathrm{Z}} \approx \nu_{\mathrm{R}}$. In contrast to ^{13}C−^{15}N MORE, no artitificial modulation is required for ^{13}C−^{14}N recoupling, and rf irradiation with a constant intensity and phase is usable for ^{14}N overtone resonances. Since the recoupling condition does not apparently include ν_1, the requirement for the intensity ν_1 is not very crucial.[12] However, for larger ν_1, the irradiation would work to decouple ^{13}C−^{14}N. In fact, overtone decoupling has been examined by using a stationary single crystal, and averaging of the ^{13}C dipolar-split peaks connected to the $|\pm1\rangle$ spin states of ^{14}N was observed.[15] For a single crystal, the decoupling becomes appreciable when $\nu_{\mathrm{Q}}\nu_1/\nu_{\mathrm{Z}} \geq d_{\mathrm{CN}}$, where d_{CN} is the strength of the ^{13}C−^{14}N dipolar interaction. For a rotating solid, however, decoupling occurs when $\nu_{\mathrm{Q}}\nu_1/\nu_{\mathrm{Z}} \gg \nu_{\mathrm{R}}$,[12] showing that a much larger rf power is required for rotaing solids. In the following, we show that a conventional rf power would lead to recoupling instead of decoupling.

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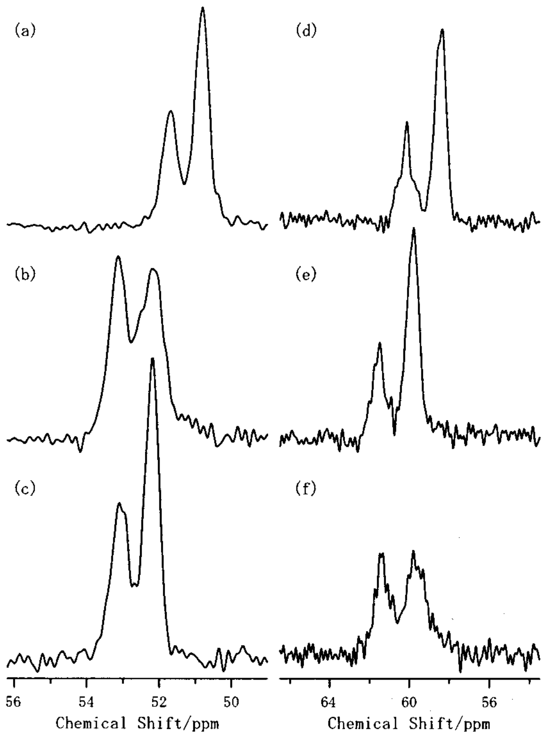

Figure 1. ^{13}C MAS spectra of the CH carbons in L-alanine (a−c) and *N*-acetyl-D,L-valine (d−f) without ^{14}N overtone irradiation (a,d) and under irradiation at (b,e) 28.875 and (c,f) 28.990 MHz. All spectra were collected on a home-built spectrometer operating at ca. 4.7 T. A triply tuned MAS probe was built based on a Doty 7ϕ MAS spinning module. The resonance frequencies are 199.8, 50.2, and 28.8 MHz, for ^{1}H, ^{13}C, and ^{14}N overtone, respectively. The rf-field strength for high-power ^{1}H decoupling was about 70 kHz, and ν_R was about 7 kHz. The rf-field strength for overtone irradiation ν_1 was calibrated to be ca. 100 kHz from an amount of the Bloch−Siegert shift[18] of ^{13}C peaks upon overtone irradiation.

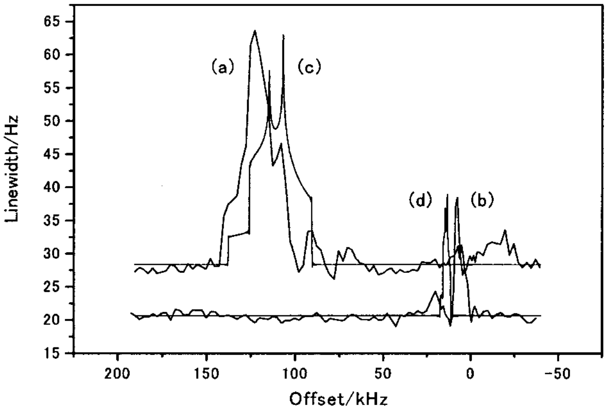

Figure 2. Observed dependence of the line width of the low-frequency peak in the asymmetric doublet on the ^{14}N overtone irrdiation frequency: (a) *N*-acetyl-D,L-valine and (b) L-alanine. The observed CH line shapes are least-squares fitted to a sum of two Lorentzian peaks to deduce the line width. The typical standard deviation (2.5σ) for the obtained line width is 0.7−1.1 Hz. Calculated ^{14}N overtone spectrum under MAS for (c) *N*-acetyl-D,L-valine with the quadrupole coupling constant and asymmetric parameter of $e^2qQ/h = 3.21$ MHz and $\eta = 0.31$, respectively, and (d) L-alanine with $e^2qQ/h = 1.148$ MHz and $\eta = 0.276$. The origin of the offset frequencies for both compounds were estimated from each ^{14}N NMR resonance in H_2O.

Figure 1 shows ^{13}C MAS spectra of the CH carbons in L-alanine (a−c) and *N*-acetyl-D,L-valine (d−f) without ^{14}N overtone irradiation (a,d) and with irradiation at different frequencies (b,c,e,f). These spectra were taken at a relatively low magnetic field of 4.7 T, so that the CH lineshapes without ^{14}N overtone irradiation (a,d) show a 1:2 asymmetric doublet characteristic for a ^{13}C spin directly bonded to ^{14}N. This asymmetric line shape is caused by residual ^{13}C−^{14}N dipolar interactions not averaged away by MAS.[16,17] The larger peak of the doublet comes from transitions $|^{13}\mathrm{C},^{14}\mathrm{N}\rangle = |^1/_2,\phi_{\pm1}\rangle \leftrightarrow |-^1/_2,\phi_{\pm1}\rangle$, thus being related to the overtone transition. On the other hand, the smaller peak is from $|^{13}\mathrm{C},^{14}\mathrm{N}\rangle = |^1/_2,\phi_0\rangle \leftrightarrow |-^1/_2,\phi_0\rangle$, thus being indifferent to the overtone irradiation. In fact, in addition to a Bloch−Siegert shift[18] of the whole ^{13}C resonance lines caused by the irradiation, when we apply the rf field on-resonant to the overtone transition, it induces line-broadening for the $|^{13}\mathrm{C},^{14}\mathrm{N}\rangle = |^1/_2,\phi_{\pm1}\rangle \leftrightarrow |-^1/_2,\phi_{\pm1}\rangle$ transition (Figure 1, b and f), which is ascribed to the recoupled ^{13}C−^{14}N dipolar interactions. On the other hand, for off-resonance irradiation (Figure 1,c and e), no appreciable effects are observed for either doublet peak.

In Figure 2, a and b, the line widths of the $|^{13}\mathrm{C},^{14}\mathrm{N}\rangle = |^1/_2,\phi_{\pm1}\rangle \leftrightarrow |-^1/_2,\phi_{\pm1}\rangle$ transition are plotted to the irradiation frequency for *N*-acetyl-D,L-valine (Figure 2a) and L-alanine (Figure 2b). Figure 2, c and d, show ^{14}N overtone line shapes simulated for *N*-acetyl-D,L-valine and L-alanine based on the theory given in ref 9. It is true that the simulated line shape does not directly represent the line width curve in Figure 2, a and b; however, the observed agreement between these line shapes indicates that the efficient recoupling occurs for on-resonance irradiation, indicating the possiblity of determining the quadrupole coupling constant and the asymmteric parameter from the indirect observation. The disagreement between the calculated and the observed line shape would be ascribed by the coarse approximation in the calculation[9,10] and errors in the estimation of the origin of the offset frequency. Development of the theory of ^{13}C MAS line shapes under ^{14}N overtone iradiation is currently underway to deduce ^{14}N quadrupole coupling constants. Further, applications of this approach for amide nitrogens in biomolecules are in progress to correlate quadrupolar couplings with a secondary structure of a peptide.

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