Rotational-Echo Double-Resonance NMR

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Slichter and co-workers have measured $^{13}C-^{17}O$ dipolar coupling in spin-echo double-resonance (SEDOR) experiments involving double-labeled CO adsorbed on catalytically active surfaces (*1*). Slichter created a Carr–Purcell string of ^{17}O echoes at times $2\tau, 4\tau, 6\tau, \ldots$, whose intensity was modulated by the heteronuclear $^{13}C-^{17}O$ dipolar coupling. Slichter observed the central ^{17}O transition and inserted π pulses in the carbon channel at times $\tau, 3\tau, 5\tau, \ldots$. During each time interval, τ, the ^{17}O spins dephased and during the next interval, τ, they refocused, but not completely. The ^{13}C π pulses reversed the sign of the $^{13}C-^{17}O$ dipolar coupling during the second interval and prevented complete refocusing (*1, 2*). The difference between ^{17}O echo maxima with and without the ^{13}C pulses yielded the $^{13}C-^{17}O$ coupling directly.

We have performed an analogous experiment with magic-angle sample spinning which we call rotational-echo double-resonance (REDOR) NMR. We will illustrate REDOR in this communication by the results of experiments performed on ^{13}C- and ^{15}N-labeled alanines.

In a solid with $^{13}C-^{15}N$ dipolar coupling, the ^{13}C rotational spin echoes which form each rotor period following a $^{1}H-^{13}C$ cross-polarization transfer can be prevented from reaching full intensity by inserting two ^{15}N π pulses per period. One of the π pulses is synchronized with the completion of the rotor period, and the other with a time less than or equal to half the rotor period (Fig. 1). The difference between a ^{13}C NMR spectrum obtained under these conditions, and one obtained with no ^{15}N π pulses, measures the $^{13}C-^{15}N$ coupling. This use of the dephasing of ^{13}C rotational echoes by ^{15}N π pulses to measure dipolar coupling is similar to the use of the dephasing of ^{13}C rotational spin echoes by ^{13}C π pulses to measure chemical-shift anisotropy in high-speed magic-angle spinning experiments (*3, 4*).

The ^{13}C REDOR NMR spectrum of [^{15}N]alanine spinning at 1 kHz shows a difference signal for the methine carbon ($\delta_c = 40$) which is as large as the normal signal (Fig. 2, left). These spectra were obtained with the first ^{15}N π pulse positioned at half the rotor period ($P = 2$), and with dephasing accumulated over two rotor periods. The difference signals for the other two carbons of alanine are comparable to one another, but weaker than that of the carbon directly bonded to ^{15}N.

The ^{13}C NMR signal from an alanine co-crystallized from a mixture of singly ^{15}N- and ^{13}C-labeled alanines arises predominantly from the ^{13}C labels (Fig. 2, right). The REDOR difference signal therefore is due to the weak dipolar coupling of ^{13}C's in one molecule with ^{15}N's in another. The shortest intermolecular $^{13}C-^{15}N$ pair distances in
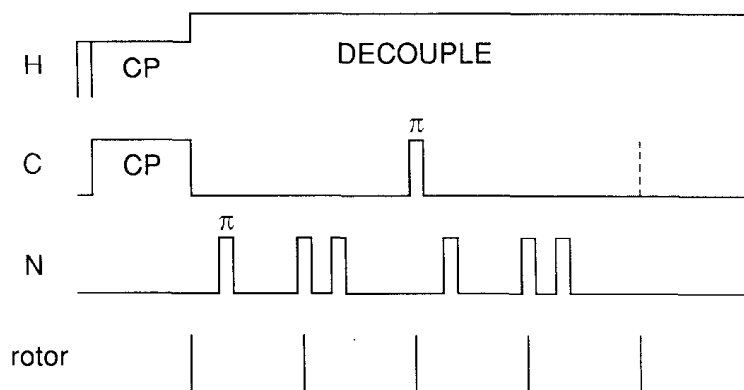
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FIG. 1. Pulse sequence for ^{13}C-^{15}N rotational-echo double-resonance (REDOR) NMR. Carbon magnetization is prepared by a cross-polarization transfer from protons and then evolves under proton decoupling and the influence of two ^{15}N π pulses per rotor period. The first ^{15}N π pulse is placed at one-half the rotor period ($P = 2$), or one-third ($P = 3$), or one-fourth ($P = 4$), and so on, including delays as short as one-seventh of the rotor period ($P = 7$). The second ^{15}N π pulse occurs at the completion of each rotor period. A single ^{13}C π pulse replaces the ^{15}N π pulse in the middle of the evolution period and refocuses isotropic chemical shifts. The illustration is for four rotor cycles with $P = 3$.
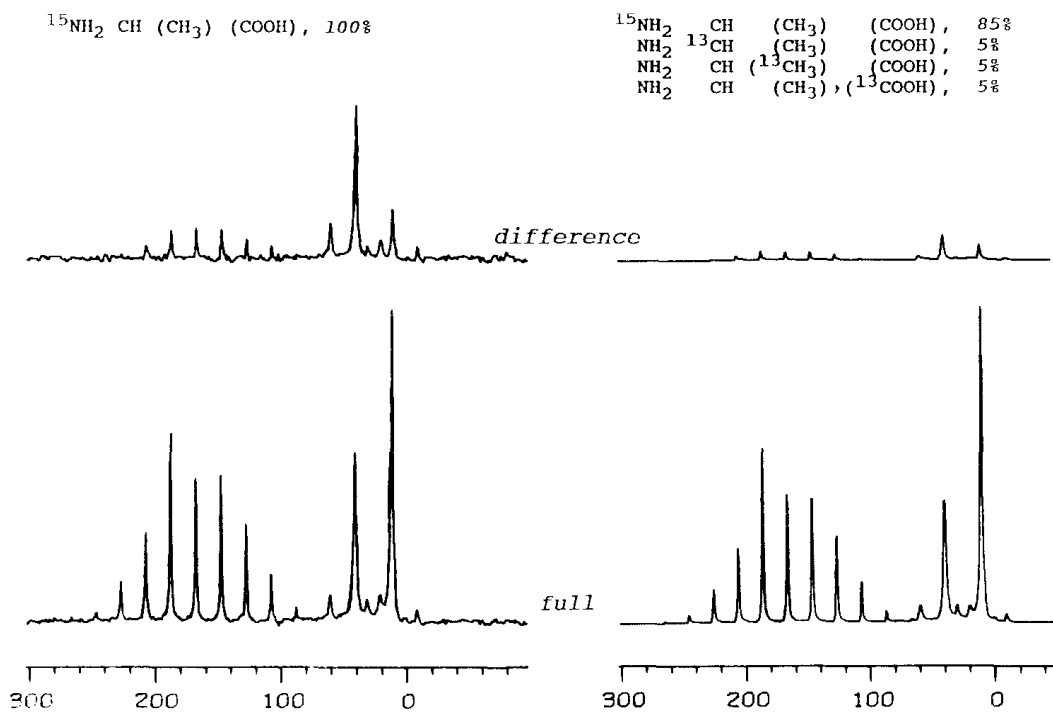

FIG. 2. The 50.3 MHz REDOR ^{13}C NMR difference signals (top) and standard rotational spin echoes (bottom) for [^{15}N]alanine (left) and an alanine recrystallized from a mixture of four specific, single-labeled ^{13}C and ^{15}N alanines (right). A ^{13}C REDOR difference signal is the difference between spectra obtained with and without ^{15}N π pulses. The pulse sequence of Fig. 1 was used over two rotor cycles with $P = 2$. Magic-angle spinning was at 1 kHz.

alanine are between molecules in adjacent unit cells (5). These C–N distances are of the order of 4–6 Å, or the equivalent of three to four bond lengths (5). With dephasing accumulated over eight rotor periods, REDOR difference signals are about 50% of the normal signal for the mixed-label alanine (Fig. 3, right). REDOR difference signals due to intramolecular ^{13}C–^{15}N couplings are as large as the normal rotational echoes for all the carbons of alanine after eight rotor cycles (Fig. 3, left).

Using the methods introduced by Griffin and co-workers to describe the behavior of rotational spin echoes under π pulses (6, 7), we have calculated the ratio of the REDOR difference signal intensity to the normal rotational echo signal intensity, $\Delta S/S$, under the condition that the first ^{15}N π pulse occurs either at half the rotor period ($P = 2$), or at one-seventh the rotor period ($P = 7$). The second π pulse in each rotor period always occurs at the end of the period (see Fig. 1). The calculations were performed for dephasing accumulated over two rotor cycles for the directly bonded ^{13}C–^{15}N pair of alanine, assuming a dipolar coupling of 895 Hz (8). More dephasing is calculated for $P = 2$ than for $P = 7$ under high-speed spinning, and the reverse under low-speed spinning. These predictions are in agreement with experiment (Fig. 4). In general, maximizing a REDOR difference signal for a given dipolar coupling requires selection of both pulse location and number of rotor cycles.
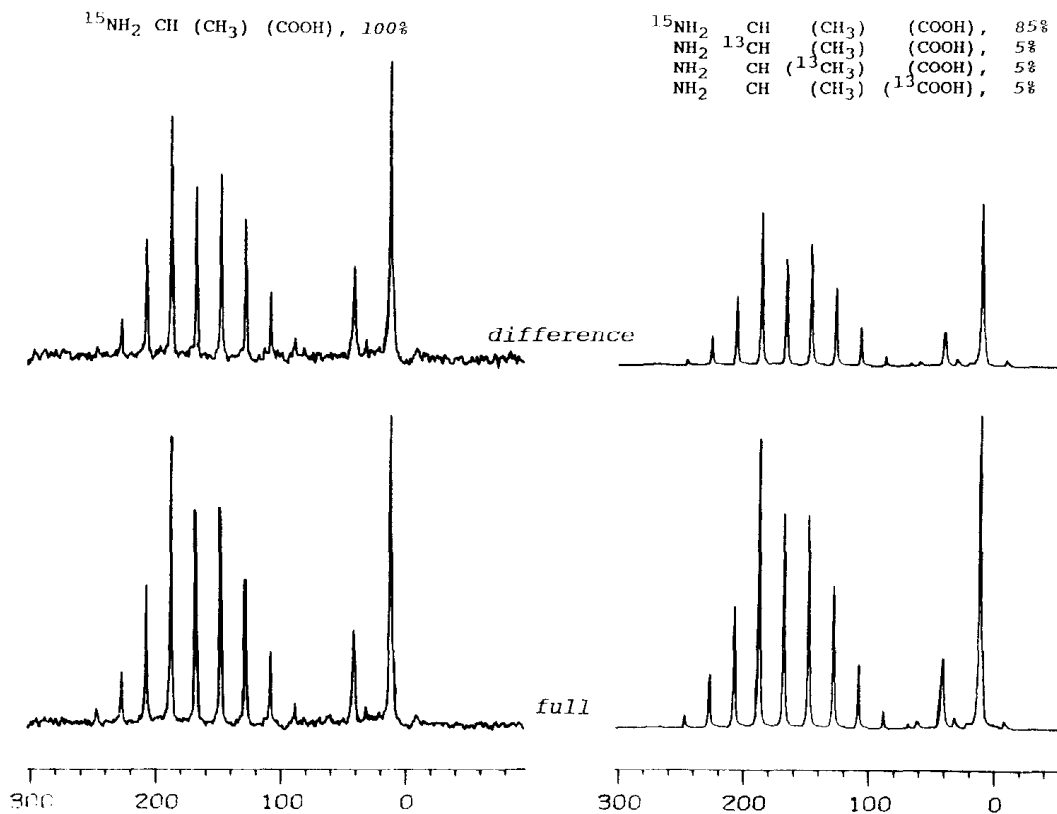

FIG. 3. The 50.3 MHz REDOR ^{13}C NMR difference signals (top) and standard rotational spin echoes (bottom) for [^{15}N]alanine (left) and an alanine recrystallized from a mixture of four specific, single-labeled ^{13}C and ^{15}N alanines (right). The pulse sequence of Fig. 1 was used over eight rotor cycles with $P = 2$. Magic-angle spinning was at 1 kHz.
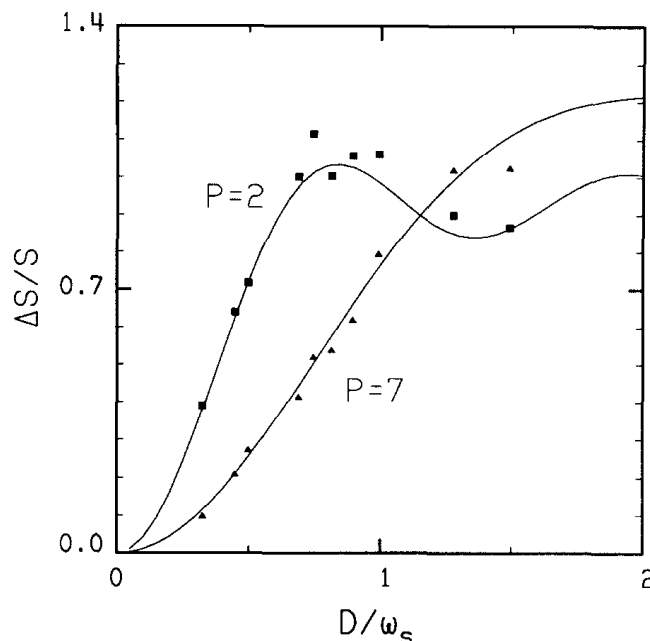
FIG. 4. REDOR difference signal intensity relative to normal rotational-echo intensity, $\Delta S/S$, of [2-^{13}C,^{15}N]alanine (recrystallized in the presence of 10 times as much natural-abundance alanine) for P = 2 (squares) and P = 7 (triangles) after two rotor cycles, as a function of spinning rate. The calculated solid lines agree with experiment, assuming the dipolar coupling, D, is equal to 895 Hz.

In the past, we have used double-cross-polarization magic-angle spinning (DCPMAS) to detect ^{13}C–^{15}N labels in studies of metabolism and structure of bacteria (9), plants (10), and insects (11). Some comparisons between DCPMAS and REDOR may be of interest. The mechanism of ^{13}C–^{15}N detection in DCPMAS is polarization transfer from one rare spin to another, while in REDOR the mechanism is the dephasing of magnetization of one spin in the presence of the local dipolar field of the other. The DCPMAS ^{13}C–^{15}N transfer rate depends on proton–proton spin flips and is difficult to interpret (12). REDOR dephasing arises from an isolated spin pair and is simple to interpret. Dipolar couplings and internuclear distances can be obtained directly from REDOR spectra of solids having several types of chemically different carbons and nitrogens.

Both DCPMAS and REDOR require control of the spinning speed to about 0.3%. DCPMAS also requires the accurate control of long spin-lock pulses for the Hartmann–Hahn match between rare-spin nuclei. This technically demanding condition, which in some experiments must be maintained for periods of the order of days, is eliminated in REDOR experiments. However, as in all echo-rephasing experiments involving long pulse trains, phase errors due to transients can accumulate in REDOR pulse sequences. We have found that phase cycling helps control the effect of transients on REDOR dephasing. Our experience is that REDOR difference signals are bigger than DCPMAS difference signals, sometimes by as much as an order of magnitude. Some of this sensitivity advantage is the result of little homogeneous dephasing in the systems we have examined so far (homogeneous dephasing decreases REDOR

sensitivity), combined with our instrumental limitation of low-γ H_1's of only 40 kHz (a weak spin-lock field decreases DCPMAS sensitivity).

Additional REDOR experiments, calculations, and applications, some of which involve pairs of rare spins other than ^{13}C and ^{15}N, are in progress.

ACKNOWLEDGMENTS

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