A MAS NMR Method for Measuring $^{13}C-^{17}O$ Distances

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Abstract: Results of ^{13}C,^{17}O-rotational-echo, double-resonance NMR (REDOR) and ^{13}C,^{17}O-rotational-echo, adiabatic-passage, double-resonance NMR (REAPDOR) experiments on asparagine monohydrate are reported. The measurement of carbon−oxygen distances in a solid sample with magic-angle spinning NMR and ^{17}O-enriched water is illustrated. These results show that determining the relative location of bound water in solids is possible and suggest that measuring distances between spin-$^1/_2$ nuclei and other chemical types of oxygen is feasible.

Introduction

Oxygen is important in many branches of materials science and characterization of the local oxygen environment is essential. Chemical and structural information about the oxygen site may be obtained through NMR. Unfortunately, the NMR active isotope of oxygen, ^{17}O, has very low natural abundance. Furthermore, magic-angle spinning (MAS) NMR studies of ^{17}O are difficult because second-order quadrupolar broadening of the central transition[1] severely limits improvements in resolution normally associated with MAS NMR. Even with these experimental difficulties, we will demonstrate that it is possible to obtain important structural information by using ^{17}O enrichment and MAS NMR. Specifically, we present results showing the potential of measuring $^{13}C-^{17}O$ internuclear distances in solids.

The measurement of internuclear distances is of fundamental importance in the chemical sciences. For solids, several high-resolution MAS NMR techniques exist for the accurate determination of distances between pairs of rare-spin spin-$^1/_2$ nuclei. These experiments include rotational resonance NMR,[2] which recovers the homonuclear dipolar interaction by setting the spinning frequency to a multiple of the difference between the isotropic chemical shifts of the two coupled spins. Other experiments, which use rotor-synchronized pulse trains, include C7,[3] DICSY,[4] DRAMA,[5,6] DRAWS,[7] REDOR,[8] RFDR,[9] MELODRAMA,[10] DEAR,[11] and TEDOR.[12] The measurement of distances between a spin-$^1/_2$ nucleus and a quadrupolar nucleus (e.g., a $^{13}C-^{17}O$ spin pair), however, is a more difficult problem even though there has been some recent progress in this area with the REAPDOR,[13,14] REDOR,[15,16] and TRAPDOR[17,18] experiments.

An example where the measurement of $^{13}C-^{17}O$ distances is important is in the determination of the location of bound water in proteins, carbohydrates, and synthetic and natural polymers.[19] Bound water (as well as mobile water) may play an important structural role in these materials. Consequently, it is desirable to determine the locations of bound water molecules. Frequently, the molecular system of interest is an amorphous solid or cannot be easily crystallized thereby restricting the use of diffraction techniques for structural determination. However, MAS NMR is not limited by the form of the solid. This paper demonstrates the feasibility of measuring distances between carbon nuclei and the oxygen nucleus of bound water by using $^{13}C-^{17}O$ rotational-echo, double-resonance NMR (REDOR) and rotational-echo, adiabatic-passage, double-resonance NMR (REAPDOR). These are the first results illustrating that carbon−oxygen distances in solids can be measured by MAS NMR.

Experimental Section

NMR experiments were performed on a sample of asparagine monohydrate with the water oxygen enriched to 20.8 atom % with the ^{17}O isotope (labeled water purchased from Isotec, Inc., Miamisburg, OH). The ^{13}C NMR results presented are for the methylene carbon of asparagine. The methylene carbon was chosen for analysis since the

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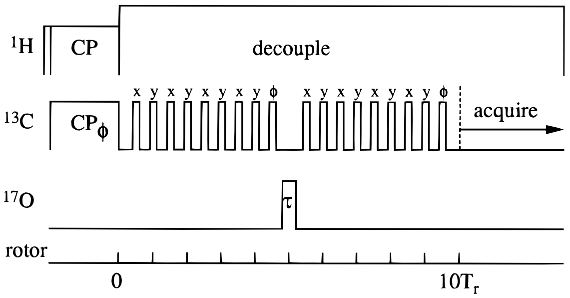

Figure 1. The ^{13}C-observed REDOR/REAPDOR pulse sequence. Carbon magnetization is enhanced through $^1H-^{13}C$ cross-polarization, and protons are decoupled throughout the remainder of the experiment. The dipolar evolution period (shown with $N_c = 10$) consists of rotor synchronized ^{13}C π pulses and a single ^{17}O pulse applied at the midpoint of the evolution period. The ^{13}C π pulses are spaced by one-half of a rotor period and are phased according to the *xy*-4 phase cycling scheme;[26] no ^{13}C π pulse is applied at the midpoint of the evolution period. For the REDOR experiment, the ^{17}O pulse length τ is set to $^1/_3$ the duration of a π pulse found for liquid water; hence the REDOR ^{17}O pulse is designated as $\pi/3$ in the text. For the REAPDOR experiment, the ^{17}O pulse length is $\tau = T_r/5$, where T_r is the rotor period.

^{13}C resonances of the carboxyl and amide carbons overlap and the amine carbon ^{13}C resonance is broadened by the residual dipolar interaction from its neighboring ^{14}N nucleus. There are four crystallographically inequivalent water sites per unit cell[20] and the twelve nearest (ranging from 3.20 to 8.10 Å) methylene−carbon to water−oxygen distances were used for data analysis. Previous REAPDOR experiments on this sample found the ^{17}O quadrupolar coupling constant to be 7.67 MHz,[21] which corresponds to a quadrupolar frequency, ν_Q, of 1.15 MHz. These values are similar to ^{17}O quadrupolar coupling parameters for water found in other systems.[22] We have not measured the asymmetry parameter, η, of the quadrupolar interaction, but $\eta \approx 0.9$ is a typical value for the water ^{17}O nucleus.[22]

Experiments were performed with a home-built spectrometer using a Tecmag pulse programmer and a 3.55 T magnet. Data were obtained with a home-built transmission-line triple-resonance probe using a Chemagnetics spinning assembly. The spinning speed, ν_R, was 1996 Hz and was stabilized to 1/5 Hz with a home-built spinning speed controller.[23] Excellent spinning speed control is a necessity for the REAPDOR experiment.[23,24] The ^{17}O resonance for liquid water was 20.523 MHz; the ^{17}O radio frequency field was applied at this frequency and at a frequency 20 kHz higher for the REDOR and REAPDOR experiments, respectively. While these REAPDOR data were taken with a 20 kHz offset, it is not necessary to perform the experiment with a resonance offset. Similar REAPDOR results were obtained with no resonance offset. All REAPDOR results were obtained with an adiabatic-passage pulse length of 1/5 of a rotor cycle. Radio frequency field strengths were 50 kHz for carbon, 59 kHz for oxygen, and 110 kHz for proton decoupling. Cross polarization radio frequency field strengths were 50 kHz. The delay between scans during signal averaging was 1 s.

Figure 1 shows the REDOR/REAPDOR pulse sequence used in this work.[13,25] The experiments are made of three parts: ^{13}C signal enhancement, an N_c rotor-cycle $^{13}C-^{17}O$ dipolar evolution period, and ^{13}C signal detection. The combination of the rotor-synchronized train of ^{13}C π pulses with the single ^{17}O pulse causes dipolar dephasing of ^{13}C spins coupled to ^{17}O nuclei; this produces a signal with intensity S_r. No net dipolar dephasing occurs when the ^{17}O pulse is omitted; this is the control signal with intensity S and accounts for T_2 decay. The difference signal is $\Delta S = S - S_r$. Dipolar dephasing of the ^{13}C magnetization is monitored through the ratio $\Delta S/S$. Analysis of the dependence of $\Delta S/S$ on the dipolar evolution time provides the dipolar couplings and, hence, internuclear separations.

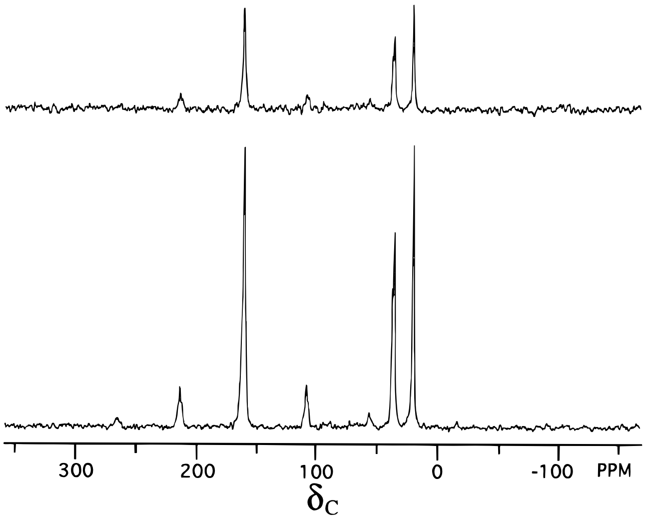

Figure 2. $^{13}C-^{17}O$ REAPDOR spectra of asparagine monohydrate.

The REAPDOR and REDOR simulations were performed by evaluating the time dependent matrix elements of the evolution operator of the $^{13}C-^{17}O$ spin pairs during the pulse sequence. The time-dependent spin Hamiltonian governing these elements contained the ^{17}O first- and second-order nuclear quadrupolar interaction, the ^{17}O off-resonance value, the chemical shift parameters of the ^{13}C nuclei, and the $^{13}C-^{17}O$ dipole−dipole interaction. During the ^{13}C and ^{17}O pulses, radio frequency terms were added to the Hamiltonian. A stepwise numerical integration, involving diagonalization of time-independent Hamiltonians during small time increments and a calculation of the expectation value of the ^{13}C transverse magnetization, was performed. In a typical calculation, the time increments during the delays between the pulses and during the ^{13}C pulses were 0.4 μs and the time increments during the ^{17}O adiabatic-passage pulse were 0.1 μs. The REAPDOR powder signals were evaluated by adding the signals from spin pairs with varying orientations in the rotor frame. The contributions to the dephasing signal, S_r, for ^{13}C nuclei with more than one neighboring ^{17}O spin were evaluated by calculating the dephasing signals of each of the different $^{13}C-^{17}O$ spin pairs in each crystallite and then multiplying these results together before powder integration. The control signal, S, was obtained in a similar fashion.

Results and Discussion

Typical $^{13}C-^{17}O$ REAPDOR spectra for the asparagine monohydrate sample are shown in Figure 2. These spectra were obtained with a dipolar evolution time lasting for 18 rotor cycles (9.02 ms). The bottom spectrum is the control spectrum and was obtained with the ^{17}O adiabatic-passage pulse omitted. The signal strength S is obtained from the control spectrum. The top spectrum is the difference spectrum; this spectrum provides ΔS. The difference spectrum was generated by subtracting the spectrum obtained with the ^{17}O adiabatic-passage pulse (not shown) from the control spectrum. The phasing of each of these spectra is very good because of the excellent spinning speed control. Poor spinning speed control produces spectra with phase distortions.[23]

Figure 3 shows REDOR and REAPDOR experimental data and numerical calculations for various dipolar evolution times for the asparagine monohydrate sample. The dipolar evolution times are N_c/ν_R. The REAPDOR data rise at a faster rate and reach larger values than the REDOR data. This shows that shorter dipolar evolution times can be used with REAPDOR

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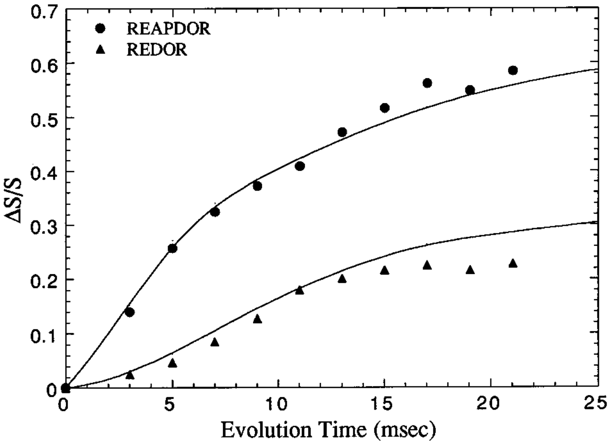

Figure 3. $^{13}C-^{17}O$ REDOR/REAPDOR data and simulations for asparagine monohydrate. The circles and triangles are the experimental REAPDOR and REDOR results, respectively. The upper and lower solid lines are the REAPDOR and REDOR numerical results, respectively.

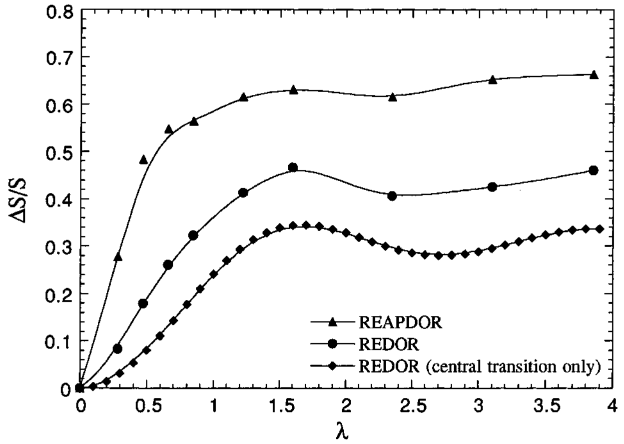

Figure 4. REDOR (circles) and REAPDOR (triangles) numerical calculations for a single $^{13}C-^{17}O$ spin pair. λ is the product of the dipolar coupling (in Hz) with the dipolar evolution time. The hypothetical central transition only REDOR experiment (diamonds) is shown for comparison. The solid lines are simple guides for the eye.

than with REDOR to obtain significant dipolar dephasing of the ^{13}C magnetization. Shorter evolution times are beneficial because the detected ^{13}C signals are larger since magnetization loss through T_2 processes is minimized. Simulations of the experiments were performed by numerical integration of the REDOR and REAPDOR experiments with a set of randomly oriented crystallites. The calculations assume a quadrupole coupling constant of 7.67 MHz, quadrupole asymmetry parameter of 0.9, second-order quadrupole shifts at an ^{17}O Larmor frequency of 20.5 MHz, dipolar couplings of the twelve aforementioned carbon−oxygen distances, and a 20.8% ^{17}O enrichment. With this enrichment, each methylene carbon may interact with multiple ^{17}O spins and such multiple couplings were taken into account. The possible ^{17}O occupancies of the twelve oxygen sites around each carbon were evaluated for up to five ^{17}O neighbors at a time, and all of the possible carbon−oxygen configurations were considered in the calculations. Clearly, the REAPDOR simulations and data are in good agreement, which illustrates that the potential of measuring $^{13}C-^{17}O$ distances is promising. However, the REDOR simulation required the input of a 1.80 μs pulse instead of the experimentally applied 2.84 μs pulse in order to obtain a reasonable fit to the experimental REDOR data. We believe that this is due to the high Q of our probe. For example, the rise and fall times of our ^{17}O pulses are over 1 μs long. Thus, our short applied ^{17}O $\pi/3$ pulse is far from an ideal pulse. In effect, the integrated intensity of the applied pulse is smaller than the desired value, which is reflected in the simulations by the need for a shortened pulse length.

The REAPDOR results presented in Figure 3 show that $^{13}C-^{17}O$ distances are measurable with this technique. For example, in typical REDOR experiments between a pair of spin-$^1/_2$ nuclei the measured REDOR $\Delta S/S$ values are fitted to the universal REDOR dephasing curve ($\Delta S/S$ vs λ, where λ is the product of the dipolar evolution time and the dipolar coupling).[8] The only fitting parameter for the data is the dipolar coupling between the coupled spin pair. Once a good fit is obtained, the distance between the pair of spins is obtained from the dipolar coupling. The REAPDOR $\Delta S/S$ values in Figure 3 are plotted against the dipolar evolution time instead of λ since there are multiple dipolar couplings. However, aside from the quadrupole coupling constant and asymmetry parameter, only the dipolar couplings between the various $^{13}C-^{17}O$ spin pairs were used to fit the data. This is in the same spirit of data analysis as used in the traditional REDOR experiment. If, for example, there was a single, isolated $^{13}C-^{17}O$ spin pair in our sample, then the REAPDOR analysis would have simply fitted the data for that single coupling and resulted directly in a $^{13}C-^{17}O$ distance, just like in the traditional REDOR approach. However, for REAPDOR data analysis to provide an internuclear distance requires, in principle, knowledge of the quadrupolar coupling constant, asymmetry parameter, and relative orientations between the quadrupolar, chemical shift, and dipolar tensors. We have performed numerical calculations that show that the same REAPDOR dipolar dephasing occurs when using quadrupolar coupling constants and asymmetry parameters varying by as much as 20% from their true values. Thus, REAPDOR does not require the exact values for these two parameters. Further simulations show that the uncertainty in the measured distance is less than 3% of the true value if the relative orientations between the three tensors is not known. The REAPDOR data in Figure 3 assumed all three tensors were collinear, which is not realistic. Yet, the data can be fitted very nicely by using the known dipolar couplings. REAPDOR is not overly sensitive to the relative orientations between the tensors, but does have a small inherent uncertainty in the distance. Thus, we conclude that $^{13}C-^{17}O$ distances can be measured by REAPDOR in a REDOR-like fashion, but with an inherent uncertainty of 3% in the distance. The results of the calculations just mentioned will be described in detail elsewhere.

A better understanding of the experimental results can be obtained by examining calculations for a single $^{13}C-^{17}O$ spin pair. Figure 4 shows numerical calculations of the REDOR (circles) and REAPDOR (triangles) experiments by using a single $^{13}C-^{17}O$ dipolar coupling of 94 Hz (all other spin parameters are the same as mentioned above). Again, the REAPDOR values of $\Delta S/S$ increase at a faster rate and reach a higher limiting value than the REDOR values. These results can be explained as follows. The REDOR experiment applies a simple $\pi/3$ pulse to the ^{17}O nuclei. For a sample with a large quadrupolar interaction one expects that only the populations of the center transition $|\pm 1/2\rangle$ are inverted by this pulse, leaving the populations of the $|\pm 3/2\rangle$ and $|\pm 5/2\rangle$ states unchanged. If so, only one-third of the ^{13}C nuclei would experience a full REDOR dephasing that would result in a maximum $\Delta S/S$ value of 1/3. The central-transition-only REDOR results in Figure 4 show such REDOR dephasing. That this picture of the REDOR experiment is oversimplified can be seen by the data generated by stepwise integration of the REDOR experiment shown in Figure 4. The calculated REDOR $\Delta S/S$ values (circles) for large λ exceed by a significant amount the value of 1/3, and the calculated REDOR curve shows a faster rise time than that for the central-transition-only REDOR curve (diamonds). A calculated ^{13}C $\Delta S/S$ value has six contributions due to the six possible ^{17}O spin states of the $I = {}^{5}/_{2}$ quadrupolar spin. A powder calculation providing a $\Delta S/S = 0.45$ value was found to contain contributions to $\Delta S/S$ equal to 0.31, 0.10, and 0.04 due to the two $|\pm 3/2\rangle$ states, the two $|\pm 3/2\rangle$ states, and the two $|\pm 5/2\rangle$ states of the ^{17}O spins, respectively. Thus, the REDOR $\pi/3$ pulse causes a change in spin state for nearly all of the ^{17}O spins initially in the $|\pm 1/2\rangle$ states and for nearly one-third of the ^{17}O spins initially in the $|\pm 3/2\rangle$ states. A small fraction of spins initially in the $|\pm 5/2\rangle$ states are also affected by the REDOR ^{17}O pulse. Because of the contributions to $\Delta S/S$ from the ^{13}C spins coupled to ^{17}O nuclei in the outer spin states, the REDOR $\Delta S/S$ exceeds the value 1/3 and reaches its limiting value quickly. For the adiabatic-passage pulse in the REAPDOR experiment, the $\Delta S/S$ contributions from the various I-spin states for a REAPDOR $\Delta S/S = 0.63$ value are 0.25, 0.18, and 0.20 for ^{17}O spins initially in the $|\pm 1/2\rangle$, $|\pm 3/2\rangle$, and $|\pm 5/2\rangle$ states, respectively. A large fraction of ^{17}O spins in any of the six spin states are affected by the REAPDOR pulse. The faster rise of the REAPDOR curve compared to the REDOR curves is due to the larger contributions to the REAPDOR ^{17}O from the $|\pm 3/2\rangle$ and $|\pm 5/2\rangle$ states.

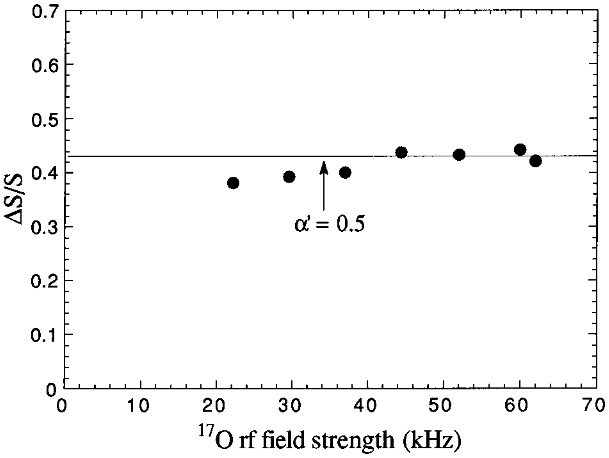

Figure 5. ^{17}O radio frequency field strength dependence of $\Delta S/S$ for the REAPDOR experiment. These data was obtained on a sample of asparagine monohydrate with the water enriched to 46.3 atomic % ^{17}O and a dipolar evolution time of 10 rotor cycles. The solid line is an eye guide and the arrow marks where $\alpha' = 0.5$.

An adiabaticity parameter, α', has been defined for a powder sample that provides a rough measure of the efficiency of the applied radio frequency field to cause transitions between the spin states of the quadrupolar nuclei.[17,18] Ideally, this parameter, $\alpha' = \nu_1^2/\nu_Q\nu_R$, should be >1 in order to have adiabatic passages. That the real process of inversion is more complicated can be seen in Figure 5, where it is shown that for α' values exceeding 0.5 the REAPDOR curve reaches an asymptotic limit. Even low radio frequency field strengths of 22 kHz, where $\alpha' \approx 0.2$, produce nearly maximum dephasing. Note that the data shown in Figure 3 were acquired with $\nu_1 = 59$ kHz, which corresponds to an α' value exceeding 1. Many different chemical types of ^{17}O have quadrupolar frequencies similar to that found for water. For example, the carbonyl oxygen of polyglycine[27] and other carbonyl oxygens have quadrupole coupling constants of 8.5 MHz,[22] and a quadrupole coupling constant of 5.96 MHz has been reported for phosphate oxygens.[22] These results suggest that ^{17}O-REAPDOR experiments will be applicable to a wide range of chemical compounds.

Conclusions

The good agreement between the experimental data and the calculations illustrates that the measurement of $^{13}C-^{17}O$ internuclear distances in solids is possible with MAS NMR. One such application will be determining the location of bound water in macromolecular systems. However, a broad range of applications of the ^{17}O nucleus are envisioned, including site-specific ^{17}O labeling of molecules or simple ^{17}O enrichment of inorganic systems (and measuring $^{29}Si-^{17}O$ distances, for example). REAPDOR and REDOR will be useful for a wide range of ^{17}O experiments, especially since the ^{17}O quadrupole coupling constants of many types of oxygens are similar to that found for bound water.

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