Cross Polarization and Dynamic-Angle Spinning of ^{17}O in L-Alanine

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I. Introduction

The study of biologically active and other organic compounds by solid-state NMR has for the most part been limited to spin-1/2 nuclei such as ^{1}H, ^{13}C, ^{15}N, ^{19}F, and ^{31}P. The study of ^{17}O, a quadrupolar nucleus (S = 5/2), in solid organic compounds has been limited due to its low natural abundance, low magnetogyric ratio, and strong second-order quadrupolar interactions. The first two difficulties can be alleviated to some extent through isotopic substitution, the use of high magnetic fields, and through cross polarization (CP) (1) from ^{1}H to the central $(1/2 \leftrightarrow -1/2)$ ^{17}O transition. For a static sample, it is theoretically possible to achieve a one-shot sensitivity enhancement of 7.3 (assuming a large excess of ^{1}H compared to ^{17}O). An alternative approach involving adiabatic slow passage to transfer magnetization from the satellite transitions to the central transition of ^{17}O could be used to generate an enhancement factor of 5.0 (2). However, when the sample is spun about an axis inclined with respect to the magnetic field, there can be a significant decrease in CP efficiency (3) because the time dependence of the first-order quadrupolar interaction interferes with Hartmann-Hahn matching. Cross polarization while spinning the sample at an angle of 0° (parallel) with respect to the magnetic field is tantamount to operating under static conditions and maximum CP efficiency can be achieved.

As shown recently (4,5), the effects of strong quadrupolar interactions can also be averaged coherently by spinning the sample about two axes. For the central transition of quadrupolar nuclei of half-integer spin, the chemical shift anisotropy and the second-order quadrupolar interactions are generally the dominant broadening mechanisms; the quadrupolar coupling constant, e^2qQ/h, of ^{17}O in organic molecules typically ranges from 5 to 12 MHz. Solid-state line narrowing techniques such as magic-angle spinning (MAS) do not fully average second-order interactions and, therefore, generally do not give sufficiently narrowed spectra, unless the coupling constant is less than about 0.5 MHz. However, in dynamic-angle spinning, the ef-

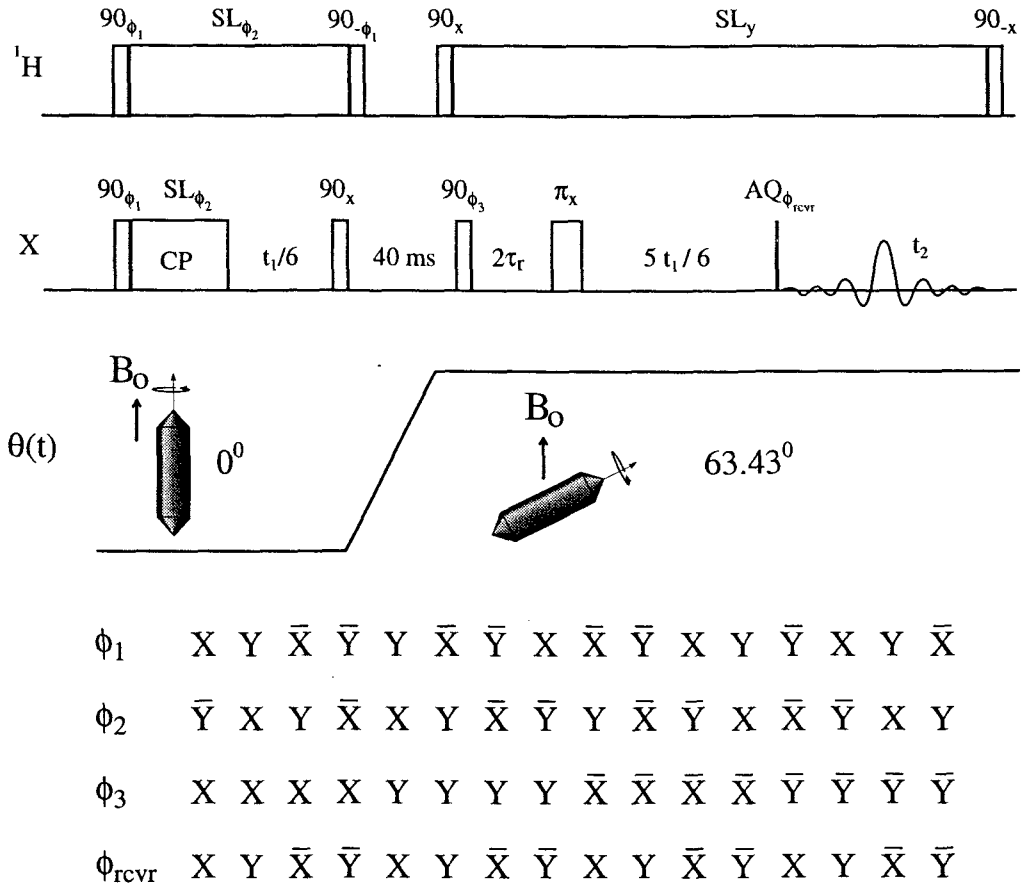

Figure 1: Pulse sequence and phase cycle for dynamic-angle spinning with cross-polarization (CP/DAS). SL signifies spinlock/decoupling and τ_r is the rotor period.

fects of second-order quadrupolar broadenings (as well as chemical shift anisotropy) are removed by allowing a spinning sample to evolve under icosahedral symmetry, resulting in a spectrum of narrow, isotropic peaks, correlated with powder lineshapes (6). There exists a continuum of DAS angle pairs, but an appropriate set of angles for combining cross polarization with DAS is the icosahedral pair 0° and 63.43°, which allows cross polarization to occur under static conditions (0°) while detection occurs at 63.43°. We have demonstrated that cross-polarized dynamic-angle spinning (CP/DAS) (7) can be performed using this method and, in this paper, we demonstrate an application to ^{17}O in solid L-alanine.

II. Experimental

A sample of L-alanine, enriched approximately to 20% in ^{17}O, was synthesized by acid-catalyzed exchange of oxygen in ^{17}O labeled water at 80°C, followed by neutralization with aniline and precipitation of the free amino acid. Powder x-ray diffraction was consistent with the known structure of L-alanine (8). A polycrystalline sample of approximately 200 mg was used for the following experiments. Experimental details of the DAS experiment have been published previously (9). Cross-polarization experiments were performed at a field of 7.04 T (301.2 MHz for the ^1H frequency and 40.832 MHz for the ^{17}O frequency) on a home-built spectrometer using a Tecmag acquisition system and a home-built DAS probe (10) spinning at 6 kHz, with the pulse sequence shown in Figure 1. The probe was equipped with a double-tuned rf-circuit with a 3/4" static coil based on a description by Doty *et al.* (11). A decoupling power level of 500 W on the ^1H channel produced a pulse width of approximately 7 μs. A 7 μs ^{17}O pulse selective to the central transition was also used to achieve the Hartmann-Hahn match condition. The cross-polarization contact time was 1 μs, which gave a

CP efficiency per scan (signal compared to a single pulse FID on oxygen with hydrogen spin decoupling) of approximately 200%. The theoretical maximum was not achieved because of short rotating frame relaxation times. T_1 relaxation times were 750 ms for ^1H and 2.5 s for ^{17}O.

DAS experiments at a field strength of 11.7 T (67.797 MHz) were performed on a CMX spectrometer using the single-tuned DAS probe described in ref. (10). No decoupling or cross-polarization was performed at this field.

III. Results and Discussion

The structure of this amino acid, shown in Figure 2, has been determined previously by x-ray crystallography and neutron diffraction (8,12) and indicates two inequivalent ^{17}O sites due to a difference in hydrogen bonding of the two oxygen atoms (12), so the spectrum should consist of two overlapping powder patterns. Figure 3 shows the ^{17}O MAS and DAS spectra of L-alanine taken at 11.7 T, both without spin decoupling. The MAS spectrum shows a broad powder pattern with a number of singularities. In addition, sidebands complicate the powder pattern, resulting in a spectrum that is difficult to simulate. In contrast, the DAS spectrum shows a separated isotropic peak and sideband pattern. The two sites in alanine are not clearly resolved in this spectrum and appear as one peak. The isotropic position is assigned to 200±7 ppm by comparison with a spectrum taken at a different spinning speed.

Figure 4 shows the 2D-CP/DAS spectrum of alanine, along with the projection of the isotropic shift dimension, recorded at 7.0 T. Spin decoupling of ^1H resulted in lines significantly narrower than that of the experiment without decoupling in Figure 3. The two sites are clearly resolved and are assigned to 51±4 and 80±4 ppm by comparison to a spectrum taken at a different spinning speed. The advantages of using cross polarization are, first of all, that the signal intensity per scan is approximately twice that seen in an experiment without cross polarization. Secondly, the recycle time is determined by the T_1 of ^1H rather than that of ^{17}O, resulting in an increase in the signal-to-noise ratio by a factor of two, giving an overall four-fold increase in the signal-to-noise ratio. As mentioned above, cross polarizing from ^1H to ^{17}O can result in an increase in

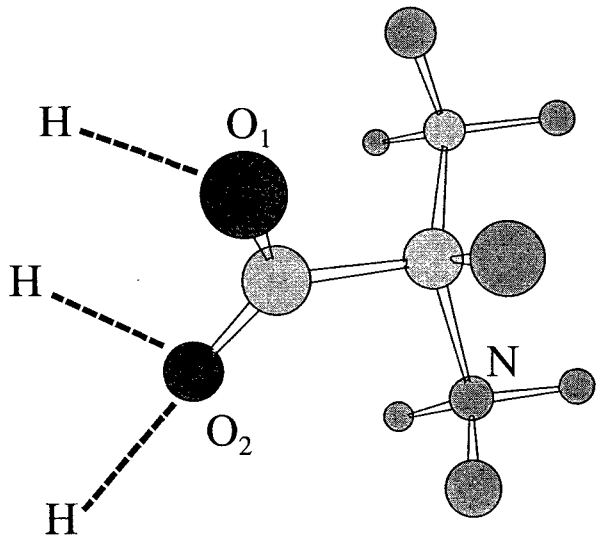

Figure 2: Structure of L-alanine showing differences in hydrogen bonding of the two oxygen sites.

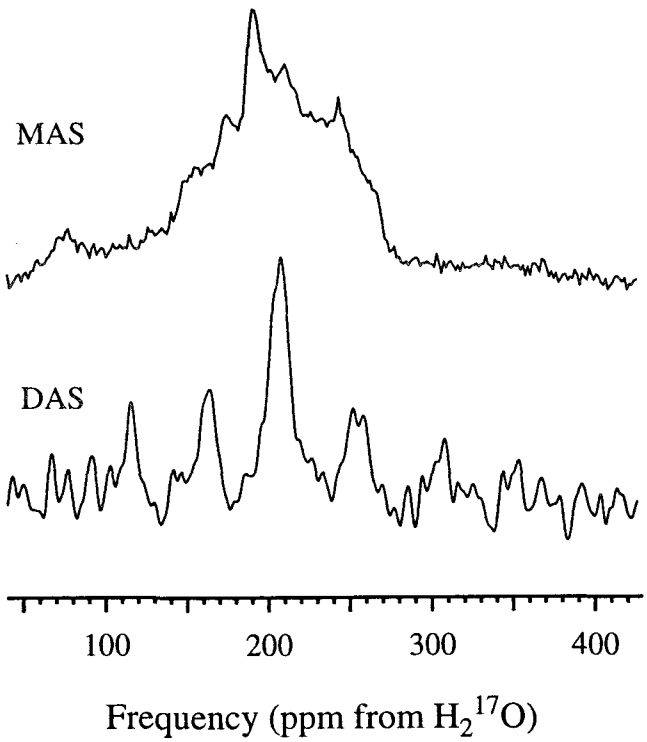

Figure 3: Magic-angle spinning (MAS) and dynamic-angle spinning (DAS) spectra of ^{17}O in L-alanine at 11.7 T (67.797 MHz), without proton spin decoupling. The spectra are referenced to ^{17}O labeled H_2O.

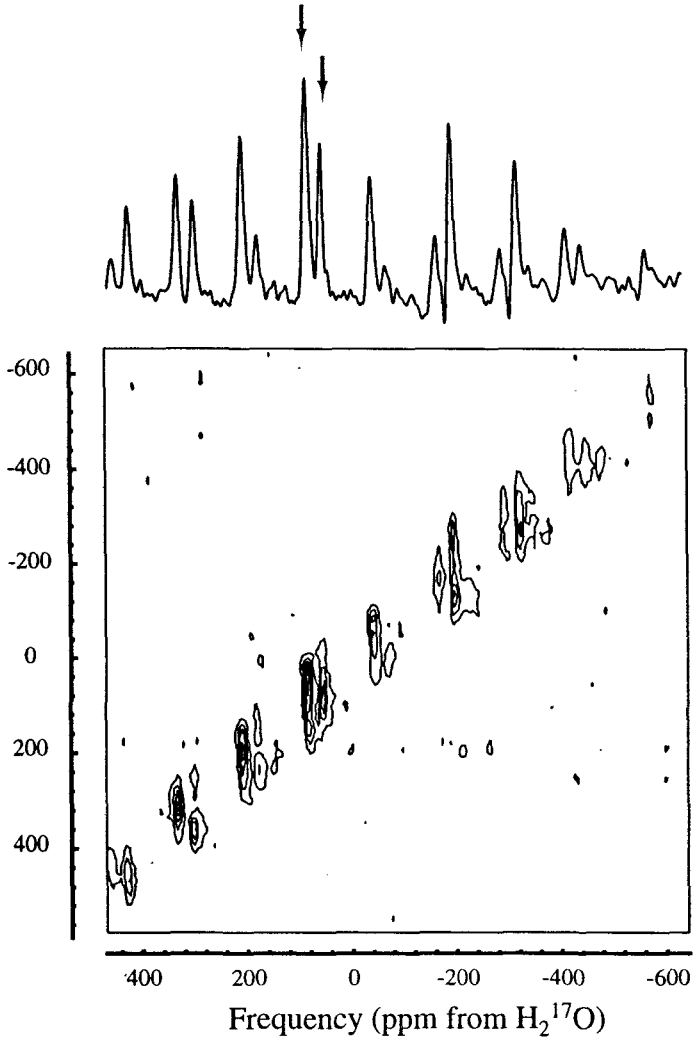

Figure 4: Two-dimensional DAS with cross polarization (CP/DAS) and proton spin decoupling spectrum of ^{17}O in L-alanine at 7.04 T. The projection of the isotropic shift dimension is shown at the top. The spectrum is referenced to ^{17}O labeled H_2O.

intensity by a factor of 7.3, so with favorable relaxation times the enhancement of the signal-to-noise ratio can be considerable and in fact could be crucial in rendering an experiment feasible.

Using the results of the experiments at the two different fields, the isotropic chemical shifts and quadrupolar coupling products can be calculated (13) by solving a system of simultaneous linear equations, with the results given in Table 1. The observed isotropic shift (in ppm), δ_{obs}, is related to the isotropic chemical shifts, $\delta_{iso,cs}$, and quadrupolar coupling product, P_Q, by

$$\delta_{obs} = \delta_{iso,cs} - \frac{3 \times 10^6}{160} \frac{4I(I+1) - 3}{\omega_0^2 I^2 (2I-1)^2} P_Q^2. \quad (1)$$

The quadrupolar coupling product, P_Q, is given by

$$P_Q = \frac{e^2 qQ}{h} \sqrt{1 + \frac{\eta_Q^2}{3}}, \quad (2)$$

where I is the spin, ω_0 is the Larmor frequency, and η_Q is the quadrupolar asymmetry parameter. The values for the quadrupolar coupling product are in good agreement with the quadrupolar coupling constant measured for the carboxyl oxygen atoms in similar compounds using NQR (14). Due to the similarities of the sites, it is not possible to assign the spectra to particular ^{17}O sites. However, further work on amino acids might reveal trends in isotropic chemical shift and quadrupolar coupling products which allow for the assignment of sites.

NMR of ^{17}O in L-alanine has been performed previously by Goc, *et al.* (15), in which the static lineshape of a polycrystalline sample was simulated. Their simulation assumed that there was only a single ^{17}O site, while our work and the crystal structure are consistent with two inequivalent sites. The reported values for e^2qQ/h of 6.6 MHz and for η_Q of 0.55, which were reported to be precise to 20% (15), give a P_Q from their data of 6.9 MHz, which agrees (to within 20%) with our calculations for either site.

Both Figures 3 and 4 show the disadvantages of insufficient spinning speeds. While the sidebands are clearly separated from the isotropic peaks in these spectra, in general, the large number of sidebands normally present in ^{17}O NMR of organic compounds can be a considerable problem. The types of compounds one would like to study with solid-state NMR, such as small peptides or carbohydrates, will typically have numerous inequivalent sites. However, fast spinning speeds are becoming easier to achieve in DAS experiments resulting in fewer sidebands. In addition, such techniques as dynamic-angle hopping (DAH) (16) can eliminate sidebands altogether in cases where adequate spinning speeds cannot be obtained.

IV. Acknowledgments

This work was supported by the Director of the Office of Energy Research, Office of Basic Energy Sciences, Materials Sciences Division of the U. S. Department of Energy under Contract No. DE-AC03-76SF00098. J.H.B. was supported by a NSF

Table 1: Isotropic shifts and quadrupolar coupling products for L-alanine.

Site	$\delta_{obs}^{7.04T}$	$\delta_{obs}^{11.7T}$	P_Q	$\delta_{iso,cs}$
1	51±4 ppm	200±7 ppm	8.1±0.3 MHz	285±8 ppm
2	80±4 ppm	200±7 ppm	7.2±0.3 MHz	268±8 ppm

graduate fellowship. E.W.W. was supported by a NIH postdoctoral fellowship.

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