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REDOR with Adiabatic Dephasing Pulses

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The response of a spin $\frac{1}{2}$ ensemble, at thermal equilibrium and experiencing chemical shift anisotropy (CSA), to the application of adiabatic inversion pulses has been studied under magic-angle spinning (MAS). Numerical simulations and experimental studies on such systems, carried out under slow spinning conditions, show that the response to adiabatic inversion pulses has much more favorable characteristics than the response to conventional rectangular pulses. We have also explored the possibilities of employing adiabatic 180° pulses as dephasing pulses in rotational-echo double-resonance (REDOR) experiments. Our results show that it is indeed possible to employ such adiabatic inversion pulses conveniently in REDOR experiments to eliminate resonance offset and H_1 inhomogeneity effects which may arise from the usage of conventional rectangular 180° pulses.

Key Words: **REDOR; adiabatic pulses; chemical shift tensor; CSA; MAS; solid-state NMR.**

INTRODUCTION

Enhanced sensitivity and spectral resolution can be obtained in solid-state NMR studies of polycrystalline specimens by carrying out the experiments under magic-angle spinning (MAS) rather than under static conditions (*1*). Structural information contained in weak homo- and heteronuclear dipolar couplings is generally lost under MAS. However, a variety of dipolar recoupling procedures have been developed recently to inhibit the spatial averaging of these couplings (*2, 3*). These experiments have opened up the possibility for obtaining structural data, in terms of distances and torsion angles, in a variety of interesting systems. Rotational-echo double-resonance (REDOR) (*4, 5*) is a powerful dipolar recoupling technique that permits the measurement of distances between dipolar coupled heteronuclei such as ^{13}C and ^{15}N. REDOR is generally carried out as a spin-echo experiment, with the application of a single 180° pulse on the observed spins to refocus evolution due to isotropic chemical shifts. The heteronuclear dipolar coupling is reintroduced by the application of rotor-synchronized 180° dephasing pulses on the unobserved spins. However, in most of the REDOR studies that have been reported in the literature to date, only one chemically shifted observed spin has been considered. In such cases, it is possible to carry out the REDOR experiment without applying any 180° refocusing pulses on the observed spins, keeping the transmitter offset on resonance. In any case, the additional dephasing of the magnetization of the observed spins introduced by the 180° dephasing pulses gets reflected as a reduction in the integrated spin-echo intensity. The latter is then monitored as a function of the dipolar evolution time to extract the distance information.

Goetz and Schaefer (*6*) have shown recently that REDOR sideband intensities are sensitive to the Euler angles defining the orientation of the dipolar vector between the heteronuclei in the chemical shift (CS) tensor principal axes system of the observed spins. A knowledge of the CS tensor orientation in the molecular frame of nuclei such as the backbone amide nitrogens in peptides and proteins is required for a variety of solution and solid-state NMR investigations (*2, 3, 7–13*). By orienting the ^{15}N CS tensor with respect to the one-bond ^{15}N–^{13}C and ^{15}N–^{1}H dipolar vectors via ^{15}N–^{13}C REDOR and ^{15}N–^{1}H DIPSHIFT (*14, 15*) MAS experiments, respectively, we have shown recently the possibility of obtaining the orientation of ^{15}N CS tensors in the molecular frame (*16, 17*). It is seen from our studies that the sensitivity of the REDOR sideband intensities to the Euler angles is maximal when the REDOR dephasing is at its maximum (*17*). Since the ^{15}N chemical shift anisotropy (CSA) is small, one has to carry out the experiments at low spinning speeds so as to retain a sufficient number of spinning sidebands for a reliable extraction of the tensor information. This means that, for an isolated and directly coupled ^{15}N–^{13}C spin system with a heteronuclear dipolar coupling of ~1 kHz, one or two rotor periods of dipolar recoupling would be sufficient to achieve maximal REDOR dephasing at low spinning speeds (1–2 kHz).

Low spinning speeds coupled with the need to have maximal REDOR dephasing lead in general to poor signal-to-noise ratios (SNR) in these experiments. Collection of data with good SNR therefore necessarily involves the acquisition of a large number of transients, and even a simple 1D experiment becomes time consuming, especially when a large recycle time has to be employed. One of the simplest approaches for increasing the SNR would be the usage of a larger sample volume where possible. However, increasing the sample vol-

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ume usually results in an increased H_1 inhomogeneity within the sample volume. This would result in a nonuniform REDOR dephasing across the sample volume if conventional rectangular 180° dephasing pulses were employed, making the data analysis unreliable. To avoid H_1 inhomogeneity effects, one typically restricts the sample to a smaller central volume of the rotor (*18*). Coupled with this, if one tries to improve the SNR by using a rotor with a larger diameter, then for a fixed transmitter power available this will result in a significant reduction in the H_1 field strength. In situations where the dephasing spins have a large CSA, as in the case of carbonyl ^{13}C nuclei, a reduced H_1 field may result in a nonuniform inversion across the spectral range of interest, again making the data analysis difficult. It has been shown that carrying out the REDOR experiment with several rotor periods of dipolar recoupling and with a suitable application of phase-alternating 180° pulses can lead to the elimination of resonance offset effects arising from a distribution in the isotropic chemical shifts (*19*). However, such schemes will have limited utility when one is simultaneously dealing with H_1 inhomogeneity and resonance offsets arising both from CSA and from a distribution in isotropic chemical shifts. We have tried to address these problems by making use of the full hardware capabilities of the current generation of solid-state NMR spectrometers. We have performed initial numerical and experimental MAS NMR studies carried out in the slow-spinning speed regime, exploiting the potential of adiabatic inversion pulses (*20–23*). As seen from our results, it may be possible to solve effectively all the problems mentioned above by avoiding the usage of conventional rectangular 180° inversion pulses and making use of adiabatic pulses instead. In other areas of magnetic resonance, the latter have been already shown to generate uniform flip angles, despite H_1 inhomogeneities and resonance offsets, at or above a minimum threshold H_1 value where the adiabatic condition is satisfied. Application of adiabatic pulses has the additional advantage that they completely eliminate the need for time-consuming pulse-width calibrations. It is only required to have an initial estimate of the available H_1 field strength under the given experimental conditions.

ADIABATIC INVERSION PULSES

The basic principle behind the functioning of adiabatic inversion pulses is that during the time course of the RF pulse the frequency of the applied RF field is swept through the spectral range of interest. The sweep rate has to satisfy the adiabatic condition $|d\chi/dt| < \gamma H_{eff}$. Here, H_{eff} is the effective RF field strength, the resultant of γH_1 and the offset $\Delta\omega$, and χ is the angle of inclination of the effective field with respect to the $+X$ axis of the rotating frame which is rotating at the instantaneous frequency of the applied irradiation (*20–23*). If the effective field is sufficiently intense, then the magnetization remains essentially aligned along the effective field and the nuclear spin is perfectly inverted. When χ is arranged to vary from $+\pi/2$ to $-\pi/2$ during the adiabatic inversion pulse, a perfect inversion is achieved irrespective of the homogeneity of the RF field. The extent to which the adiabatic condition is fulfilled is generally expressed in terms of the adiabaticity parameter $Q = \omega_{eff}/|d\chi/dt|$. It has been noted that one obtains the desired performance characteristics from the adiabatic inversion pulses for Q values much greater than unity. Adiabatic inversions can in principle be achieved by keeping the amplitude of the RF field constant and sweeping only the frequency of irradiation. However, the need to ensure that the effective field starts at the $+Z$ and ends at the $-Z$ axis, for achieving good nuclear spin inversion, generally requires that the amplitude of the RF field be brought down smoothly to zero at the two extremes of the sweep (*21–23*). In this context a variety of amplitude and frequency modulation functions have been proposed for the implementation of adiabatic inversion pulses with varying performance characteristics (*22, 23*). It should be noted that for the time dependence of the resonance offset term $\Delta\omega(t)$ during an adiabatic inversion pulse, there exists only one difference between liquid and static solid-state NMR experiments with respect to MAS NMR experiments. In the latter, the time dependence in $\Delta\omega(t)$ arises not only due to the time variation in the irradiation frequency $\omega(t)$ but also due to the periodic modulations under MAS of the anisotropic interaction terms in the Hamiltonian, such as the CSA. Still, provided the conditions mentioned above are satisfied, it is expected that the nuclear spins can be inverted via the application of adiabatic pulses irrespective of whether the experiment is carried out under magic-angle spinning or not. The time needed to achieve nuclear spin inversion via adiabatic pulses is in general much longer than the corresponding time needed with rectangular pulses. However, it is seen from our studies that it is indeed possible to make use of adiabatic inversion pulses without any difficulty in MAS NMR experiments carried out in the slow spinning speed regime.

To assess the efficacy of adiabatic inversion pulses in magic-angle spinning experiments we have employed, as an example, one of the many adiabatic inversion sequences that can be implemented via the Varian shaped RF pulse generation software Pbox. The amplitude profile and the corresponding optimized (to achieve a constant adiabaticity factor over the sweep range) frequency-sweep function of the inversion sequence employed were (*23*), respectively,

$$\omega_1(t) = \omega_{1,max}\cos(\beta t)$$

and

$$\Delta\omega(t) = (1/2)\lambda[\beta t + \sin(\beta t)\cos(\beta t)],$$

where $\beta = \pi/2\tau$, $\lambda = \omega_{1,max}^2/\beta Q$ and 2τ is the total duration of the adiabatic pulse with $-\tau < t < +\tau$. The frequency sweep is

generally implemented in the spectrometer hardware as a phase modulation $\phi(t) = \int \Delta\omega(t)dt$. The shaped pulse is divided into a finite number (200 in our case) of time slices of equal duration, with each slice characterized by a proper amplitude and phase so as to mimic the required amplitude and frequency modulation. This information concerning amplitude and phase of the RF pulse in each slice corresponding to a given shaped pulse is available in a text file in the Varian spectrometer system and has been employed to assess the inversion profile of adiabatic pulses under MAS.

In our approach we consider an ensemble of spin $\frac{1}{2}$ nuclei, for which the MAS Hamiltonian in the usual rotating frame has been given before (*24*), and calculate the response of the spin system via standard density matrix calculations. In our calculations of the time evolution operator during the application of the adiabatic pulse we assume that the time dependence of the MAS Hamiltonian during the individual time slices of the RF pulse can be neglected. That is, the resonance offset variations due to magic-angle spinning is assumed to vary only from one time slice to another. The total time evolution operator for the entire adiabatic pulse is then calculated by a time-ordered product of the time evolution operators corresponding to the individual time slices. Typically we have considered ~33,000 crystallite orientations in our calculations. All numerical calculations were carried out using the software package "SPINME" (*17*) that is being developed in-house.

EXPERIMENTAL

All experiments were performed at room temperature on a 500 MHz wide-bore Varian $^{\text{UNITY}}$INOVA solid-state NMR spectrometer equipped with a 5-mm DOTY supersonic triple-resonance probe and a waveform generator for pulse shaping. Cross-polarization under Hartmann–Hahn matching conditions was employed, and the spectra were collected under high-power ^{1}H TPPM decoupling (*25*). The pulse sequence employed for experimentally assessing the performance characteristics of the adiabatic inversion sequence is given in Fig. 1A. Keeping the observed spins on-resonance, we have carried out a one-rotor-period REDOR experiment via the sequence given in Fig. 1B employing a single 180° dephasing pulse at the center of the rotor period. The ^{15}N spectra were referenced indirectly relative to DSS (*26*). Other relevant experimental parameters are given in the figure captions. A (^{13}C, ^{15}N) labeled acetanilide sample, prepared as described (*27*), was packed so as to fill the full rotor volume and was employed in our investigations. An adiabatic inversion sequence with the amplitude and frequency modulation functions given above and having a constant adiabaticity factor of 4 has been employed uniformly in our work.

RESULTS AND DISCUSSION

In Fig. 2 we give the simulated response of an ensemble of spin $\frac{1}{2}$ nuclei to the application of adiabatic inversion pulses.

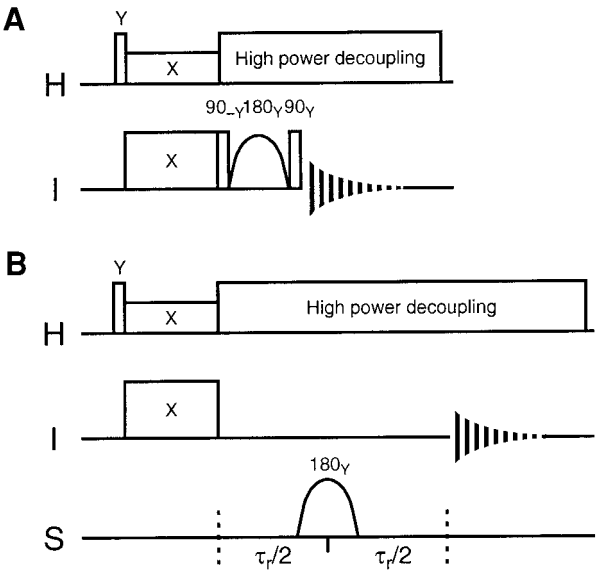

FIG. 1. (A) CPMAS sequence employed to assess the performance characteristics of adiabatic inversion pulses and (B) a one-rotor-period REDOR pulse sequence with a single adiabatic inversion pulse on the S channel at the middle of the rotor period.

Starting from a thermal equilibrium density matrix of $\rho(0) = I_z$, the inversion characteristics of the adiabatic pulses are monitored by applying an ideal 90° pulse following the inversion pulse. Unless indicated otherwise, the calculations were done at a spinning speed of 1.3 kHz, at a spectrometer frequency of 125 MHz, and employing the carbonyl carbon CS tensor principal elements of the amide fragment of acetanilide: $\sigma_{11} = 90$ ppm, $\sigma_{22} = 175$ ppm, and $\sigma_{33} = 248$ ppm (*27*). For comparison we have also given the response following a normal 180° RF pulse of 8-μs duration. The calculations were carried out for different values of RF field strength so as to assess the potential utility of adiabatic pulses under an inhomogeneous distribution of the H_1 field in magic-angle spinning experiments. The calculations with the adiabatic pulses were also carried out with some moderate variations in the spinning speed and pulse width so as to assess the performance of the adiabatic pulses with different ratios of the pulse duration to the rotor period. It is seen from numerical simulations (data not shown), carried out under static conditions and neglecting CSA, that a frequency sweep width of 80 kHz provides adequate inversion band width (greater than 95% inversion efficiency over an isotropic chemical shift range of +20 to −20 kHz). Hence, the sweep width of the adiabatic pulses was restricted in general to 80 kHz (+40 to −40 kHz). Figures 2A–2C represent, respectively, the plots obtained employing the rectangular 180° pulse, a 120-μs adiabatic pulse, and a 200-μs adiabatic pulse. Figure 2D shows the plots obtained at a spinning speed of 2000 Hz with a 120-μs adiabatic pulse. From the plots it is seen that, in comparison to the rectangular pulse, all the adiabatic pulses employed do provide satisfactory performance at or above the minimum threshold RF field

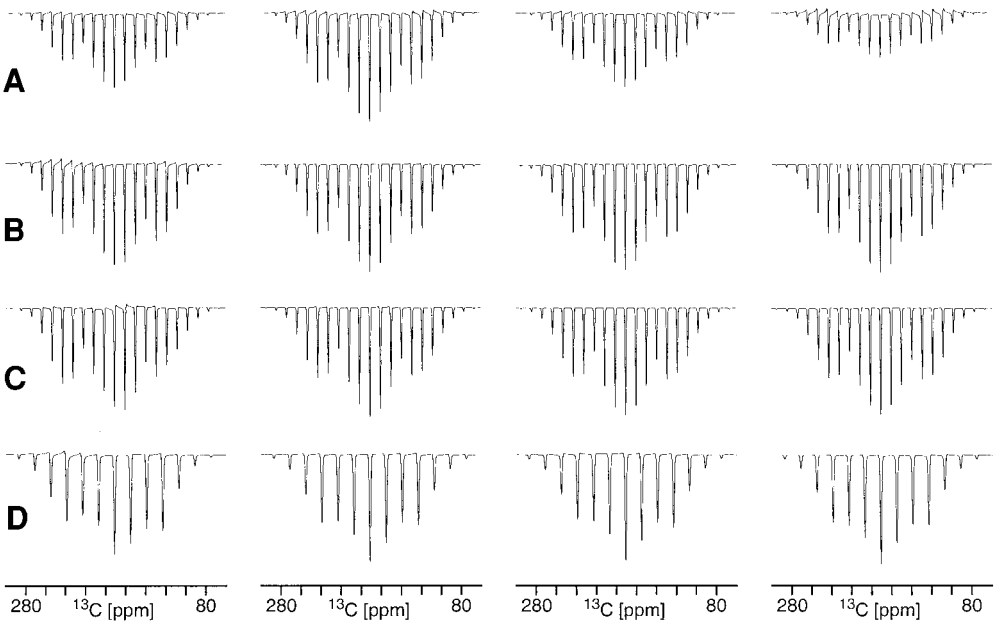

FIG. 2. Simulated plots (ν_r = 1.3 kHz (unless indicated otherwise), ω_0 = 125 MHz) of $\langle I_z \rangle$ following the application of different inversion pulses starting from a thermal equilibrium density matrix $\rho(0) = I_z$ and at different power level settings (left to right). CS tensor parameters of σ_{11} = 90 ppm, σ_{22} = 175 ppm, and σ_{33} = 248 ppm were employed in these simulations. The plots were generated for different values of RF field strengths (corresponding to the different relative values of the transmitter power levels indicated) employing: (A) a rectangular 180° pulse of 8 μs and with power levels of +2, 0 (ideal, 62.5 kHz γH_1), −2, and −4 dB; (B) a 120-μs adiabatic pulse of 80 kHz sweep width with power levels of −2, 0 (minimum threshold level, 31.3 kHz γH_1), +2, and +6 dB; and (C) a 200-μs adiabatic pulse of 80 kHz sweep width with power level settings of −2, 0 (minimum threshold level, 24.9 kHz γH_1), +2, and +6 dB. (D) Plots obtained at a spinning speed of 2000 Hz with a 120-μs adiabatic pulse of 80 kHz sweep width with power levels of −2, 0 (minimum threshold level, 31.3 kHz γH_1), +2, and +6 dB.

strength needed. Even at a spinning speed of 3 kHz, the 120-μs adiabatic pulse has the desired inversion profile (data not shown).

The experimental performance characteristics of the inversion pulses have been assessed via the pulse sequence given in Fig. 1A. The transverse magnetization following cross-polarization is flipped back to the Z axis via a 4-μs rectangular 90° pulse, and the I_z magnetization is then inverted by the different inversion pulses. The inversion characteristics of the 180° pulses employed are then assessed by applying a subsequent 90° pulse. Figures 3A–3D show the performance under varying RF field strengths of the 180° pulses employed. All spectra were recorded employing usual CP phase cycling schemes. From the spectral data shown, it is seen that the performance characteristics of the adiabatic pulses are superior to those of a rectangular 180° pulse, as expected from the plots given in Fig. 2.

In Fig. 4 we show, as a typical example, the experimental inversion profile as a function of the transmitter offset (+20 to −20 kHz) of the 120-μs adiabatic RF pulse which has the desired inversion profile. It should be noted that the inversion bandwidth of a simple rectangular pulse is limited by the RF power employed. However, it is possible to obtain, with the typical transmitter power available in solid-state NMR spectrometers, a larger inversion bandwidth with adiabatic pulses. This can be achieved by employing a larger range of sweep width that may be allowed without violating the adiabatic condition. Together with the data shown in Fig. 3, it is clear that the adiabatic inversion pulses do provide a possible means for eliminating imperfections that may arise from the usage of rectangular 180° inversion pulses.

We have assessed the quality of REDOR data obtained employing an adiabatic 180° pulse as dephasing pulse in an ^{15}N–^{13}C REDOR experiment. In these studies we have used an (^{15}N, ^{13}C) labeled sample of acetanilide, a system that has been recently studied by us via MAS NMR to obtain the orientation of the ^{15}N CS tensor in the molecular frame (*17*). Keeping the observed spins on resonance, we have carried out the experiment with one rotor period of dipolar recoupling by applying a single 180° dephasing pulse at the center of the rotor period (Fig. 1B). The experiments were carried out at a spinning speed of 1.3 kHz with different power levels of the 180° pulse, and the REDOR data thus obtained are shown in Fig. 5. For comparison we have also shown the performance of the REDOR sequence with a rectangular 180° RF pulse of 8-μs duration. The sideband intensities seen in the REDOR spectra obtained with the application of adiabatic inversion pulses, at and above the minimum threshold RF level, are consistent with the REDOR data obtained with a rectangular 180° pulse having the correct RF power level setting. These REDOR sideband intensities are consistent with the orientation of the ^{15}N–^{13}C dipolar vector in the ^{15}N CS tensor frame in acetanilide (*17*).

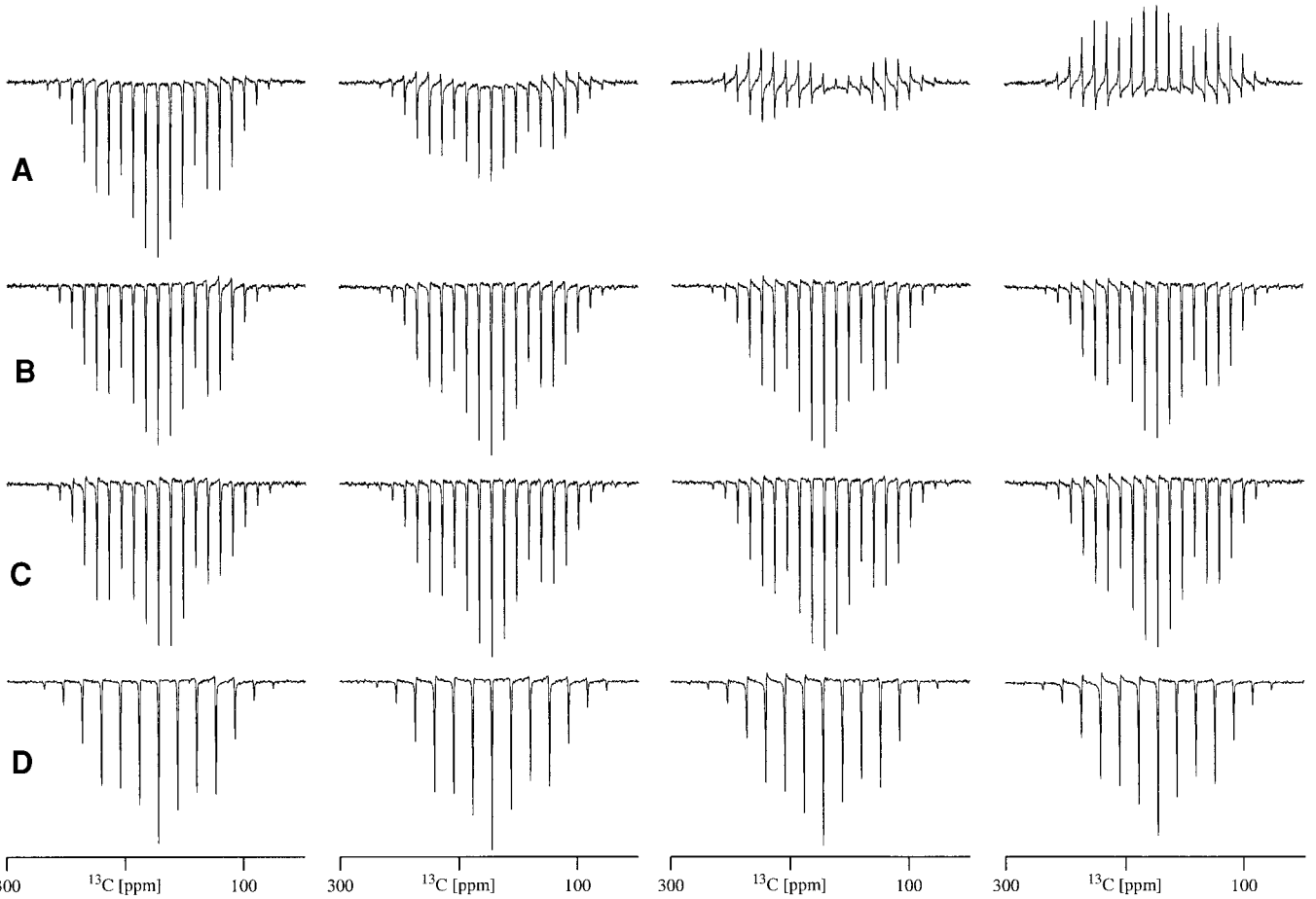

FIG. 3. ^{13}C CPMAS spectra of (^{13}C, ^{15}N) labeled acetanilide obtained at 1300 Hz spinning speed (unless indicated otherwise) following the application of the sequence given in Fig. 1A and employing different inversion pulses and power levels settings (left to right) as indicated. Spectra were obtained with: (A) a rectangular 8-μs 180° pulse and with power level settings of 0 (ideal, 62.5 kHz γH_1), -2, -4, and -6 dB; (B) a 120-μs adiabatic pulse having an 80-kHz sweep width with power levels of 0 (minimum threshold level, 31.3 kHz γH_1), $+2$, $+4$, and $+6$ dB; and (C) 200-μs adiabatic pulse having an 80-kHz sweep width with power level settings of 0 (minimum threshold level, 24.9 kHz γH_1), $+2$, $+4$, and $+6$ dB. (D) Spectra obtained at a spinning speed of 2000 Hz employing a 120-μs adiabatic pulse with an 80-kHz sweep width with power levels of 0 (minimum threshold level, 31.3 kHz γH_1), $+2$, $+4$, and $+6$ dB. All spectra were recorded with 16 scans, 64 s of recycle time, and 1 ms of CP.

However, the sideband intensities seen with inaccurate setting of the RF power level of the rectangular 180° pulse are not consistent with the sideband intensities seen with the correct setting of the power level. This clearly demonstrates the possibility of avoiding H_1 inhomogeneity effects for the accurate extraction of orientational, distance, and other information of interest from the REDOR experiment via the application of adiabatic dephasing pulses.

A prerequisite for the extraction of orientational information from the REDOR experiment is to have a knowledge of the relevant dipolar coupling strength. This information can be easily extracted from the REDOR experiment by monitoring, as a function of the dipolar recoupling time, the ratio $\Delta S/S_0$ of the REDOR difference intensity to the intensity observed without the application of a dephasing pulse. For acetanilide, we have used the sequence given in Fig. 1B with a 120-μs adiabatic dephasing pulse, employing spinning speeds of 1700, 1500, and 1300 Hz. The ratio $\Delta S/S_0$ was measured as a function of the dipolar recoupling time, and the data thus obtained, along with the corresponding spectra, are given in Fig. 6. An ^{15}N–^{13}C dipolar coupling strength of $\sim$1200 Hz is estimated from Fig. 6, which is consistent with the value that we obtained earlier employing a rectangular 180° dephasing RF pulse and a small sample volume (*17*). From the initial results presented here, the potential of adiabatic dephasing pulses in REDOR experiments is clearly seen.

Most of our numerical and experimental studies with the adiabatic inversion pulses were carried out with a sequence having a duration of 120 μs and a sweep width of 80 kHz. These parameter values were chosen taking into account not only the desired inversion bandwidth needed but also the available transmitter power and the power handling capabilities of the probe. It is worth pointing out that we have been able to reduce the adiabatic inversion pulse duration to values smaller

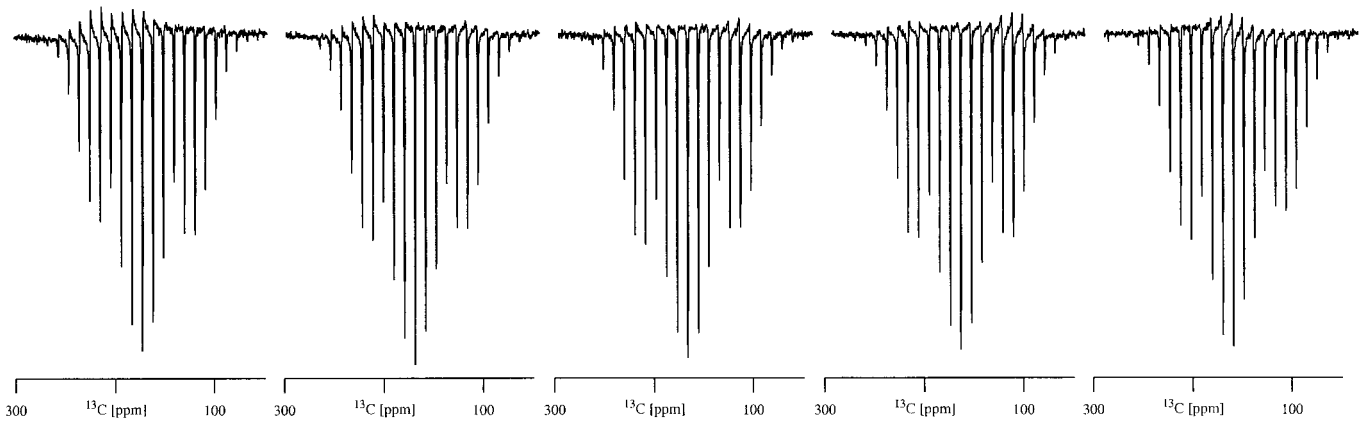

FIG. 4. ^{13}C CPMAS spectra of (^{13}C, ^{15}N) labeled acetanilide obtained employing the pulse sequence given in Fig. 1A with a 120-μs adiabatic inversion pulse and with an 80-kHz sweep width as a function of the frequency offset (left to right: -20, -10, 0, $+10$, $+20$ kHz) of the inversion pulse. All spectra were recorded with 16 scans, 64 s of recycle time, and 1 ms of CP.

than 120 μs and still obtain satisfactory inversion performance. However, the performance characteristics could be assessed only over a limited range of power levels above the critical threshold needed. The adiabatic sequence employed in this work is one of several sequences that can be employed in MAS NMR experiments. It is conceivable that sequences with short duration and with good performance characteristics can be designed specifically for MAS applications, and work in this direction is already in progress in our laboratory. The availability of such sequences with a short overall duration can

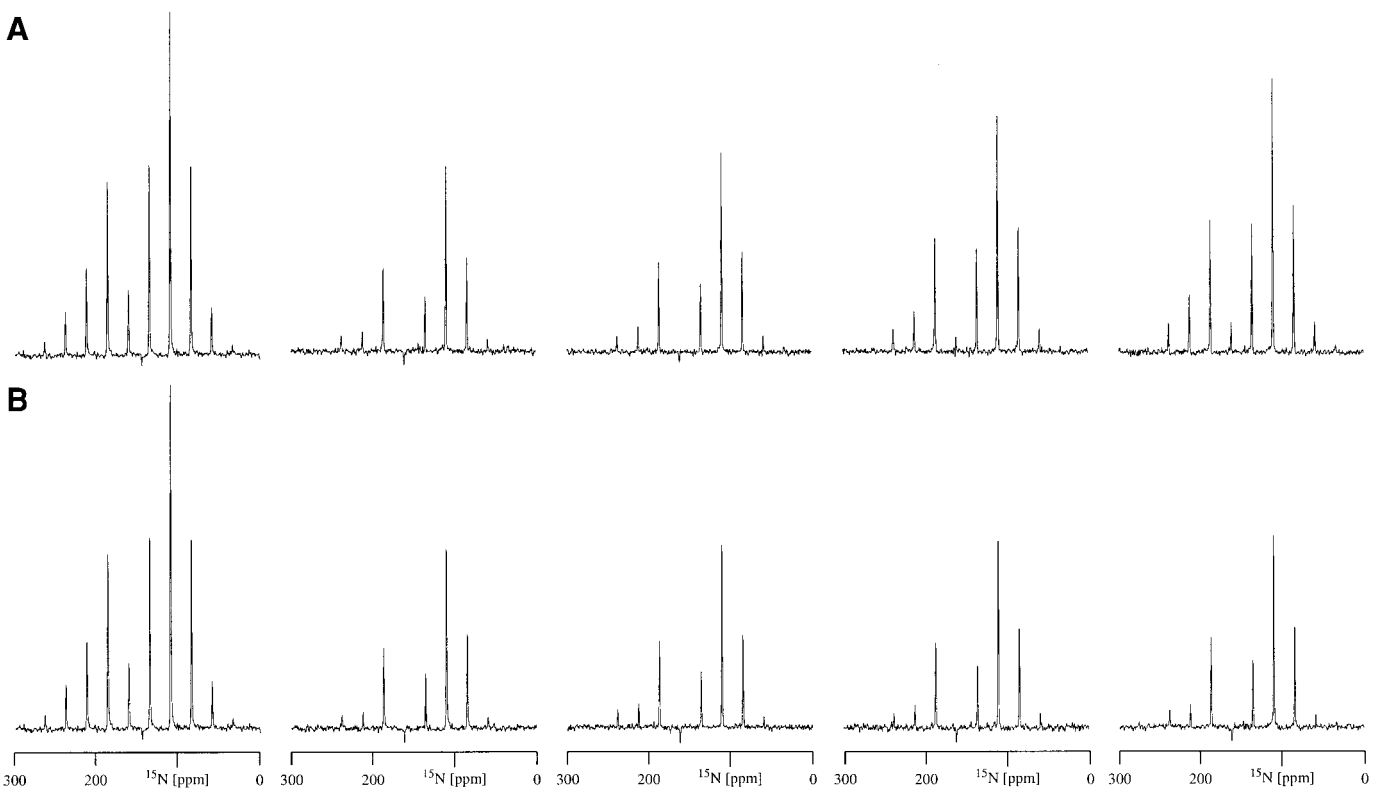

FIG. 5. Experimental ^{15}N–^{13}C REDOR spectra of acetanilide obtained employing the pulse sequence given in Fig. 1B at 1300 Hz spinning speed using (A) a rectangular 180° pulse of 8 μs duration and (B) a 120-μs adiabatic inversion pulse with an 80-kHz sweep width for recoupling, at different power levels. (left to right) Normal MAS spectrum (first column, without dephasing pulse) and recoupled spectra obtained with: (A) power-level settings of 0 (ideal, 62.5 kHz γH_1), -2, -4, and -6 dB; and (B) power levels of 0 (minimum threshold level, 31.3 kHz γH_1), $+2$, $+4$, and $+6$ dB. Experiments were carried out using 128 scans, 64 s of recycle time, and 1 ms of CP.

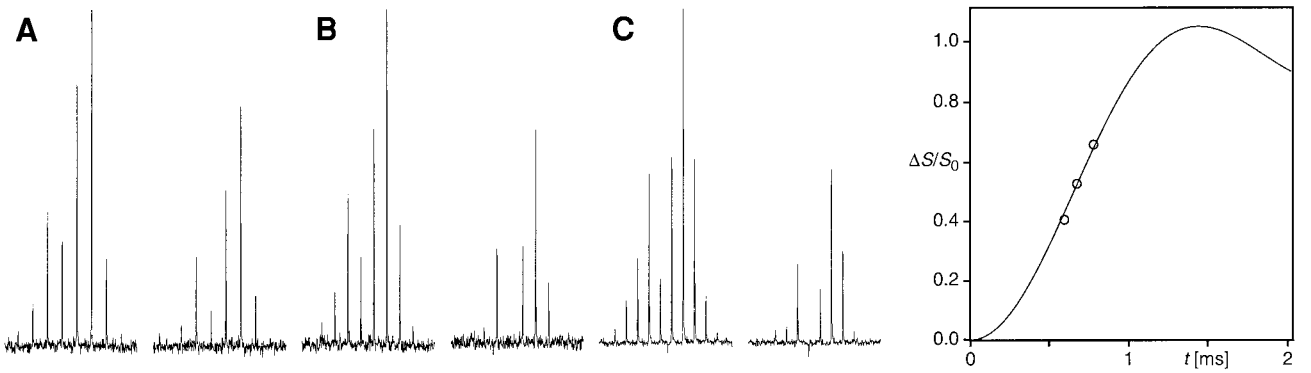

FIG. 6. Experimentally measured ratio of the REDOR difference to full echo intensity ($\Delta S/S_0$) as a function of the mixing time t obtained employing the one-rotor-cycle REDOR pulse sequence given in Fig. 1B. The continuous REDOR curve has been generated assuming a dipolar coupling of ~1200 Hz. The respective spectra (64 s of recycle time, 16 scans) without and with REDOR dephasing at ν_r = 1700 Hz (A), 1500 Hz (B), and 1300 Hz (C) are also shown.

facilitate the application of adiabatic pulses in MAS experiments carried out even at moderate spinning speeds. With the adiabatic pulse employed here we have already seen satisfactory inversion profiles even at a spinning speed of 3 kHz, as mentioned earlier. From our results it is seen that adiabatic pulses can be possibly employed successfully in situations where MAS experiments with large rotor volumes are possible and may be needed to improve the SNR and hence to reduce the data acquisition time. While we have explored earlier the potential of composite 180° pulses for MAS applications (*24*), the usage of adiabatic pulses may provide an alternative convenient approach to overcoming the problems associated with imperfections in rectangular 180° pulses. Adiabatic pulses occupy a much larger portion of the rotor period than a conventional high-power rectangular 180° pulse. However, the performance of the former is far superior to that of the latter. It therefore appears that it may be advantageous to use adiabatic pulses wherever possible, taking into account in the calculations, if needed, the relatively large duration of the adiabatic pulses. Although we have explored the potential of adiabatic inversion pulses in the context of REDOR experiments, applications are also conceivable in other situations where the information of interest is obtained from sideband intensities of MAS experiments carried out at low spinning speeds. Similar to the adiabatic inversion pulses employed here, one can also envisage the possibilities of using adiabatic refocusing pulses in MAS NMR experiments.

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