

Compensating for pulse imperfections in REDOR

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Received 7 April 2003; revised 13 August 2003

Abstract

Rotational-echo double resonance (REDOR) is a magic-angle spinning technique for measuring heteronuclear dipolar couplings. Rotor-synchronized π pulses recouple the dipolar interaction. The accuracy of a REDOR determination of distance or orientation depends totally on the quality of the dephased (recoupled) and full-echo spectra. We present a scheme for measuring and compensating for the effects of pulse imperfections in REDOR experiments. No assumptions are made about the quality of the π pulses, and no pulses are added or taken away in implementing the compensation for incomplete REDOR dephasing by imperfect π pulses. © 2003 Elsevier Inc. All rights reserved.

1. Introduction

Rotational-echo double resonance (REDOR) NMR [1] has become a popular tool for measuring heteronuclear dipolar couplings [2–7]. In a REDOR experiment, static dipolar interactions, which are otherwise averaged to zero by magic-angle spinning, are reintroduced during an evolution period by rotor-synchronized π pulses. Two versions of the experiment are usually performed, one with and one without π pulses applied to the unobserved spin during the evolution time. In the latter, no dipolar evolution occurs and a reference signal (S_0) of full intensity is acquired. In the former, the π pulses reintroduce a non-vanishing dipolar coupling which results in a dephased signal (S) with a reduced amplitude. The ratio of the difference between the signals ($S_0 - S$) to the full signal, $\Delta S/S_0$, is a direct measure of the dipolar coupling [1].

REDOR distance and orientation information depend directly on the accuracy of the S and S_0 signal amplitudes. A number of factors affect the quality of these signal amplitudes including finite pulse lengths, radio-frequency inhomogeneity, radio-frequency amplitude drift, and resonance offsets. The deleterious effects of resonance offsets were identified as a serious problem in early experiments [8,9]. For example, the $^{13}\text{C}\{^{15}\text{N}\}$ dephasing for a recrystallized equimolar

mixture of L-[1- ^{13}C]alanine and L-[2- ^{13}C , ^{15}N]alanine showed that the carbonyl-carbon $\Delta S/S_0$ was sensitive to even a few hundred hertz shift of the ^{15}N carrier frequency [8]. Fortunately, relatively simple phase-cycling schemes like xy-8 [10] applied to both radio-frequency channels removed the offset problem [8], as well as the effects of other pulse imperfections, even when hundreds of π pulses were used [11]. However, in the presence of significant homonuclear coupling between the observed spins, the use of multiple π pulses on the observe channel in an xy-8 phase cycling scheme may not be an option because of the introduction of unacceptable frequency-dependent phase shifts in the resulting spectrum [12,13].

Chan and Eckert [14], and subsequently Salwächter and Fischbach [15], conducted numerical computer simulations and experiments to investigate the influence of pulse imperfections on the quality of REDOR results when there is only a single π pulse on the observe channel. Chan and Eckert examined an SI_2 spin system and assessed the effects on REDOR dephasing of I–I homonuclear dipolar coupling, and of experimental imperfections such as finite pulse lengths and misadjustments of the (non-observe) I-spin π pulses [14]. The results of their simulations and experiments showed that for the initial dipolar evolution ($\Delta S/S_0 \leq 0.3$), the effects of the pulse misadjustments can be compensated using a modified sequence having an additional I-spin π pulse exactly in the middle of the dipolar evolution time, coincident

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with the single S-spin refocusing π pulse. In this situation, if all the pulses are assumed to be perfect, the two central pulses effectively cancel, and the I-spin π pulses in the second half of the experiment undo the recoupling of those in the first half. A full echo is observed. If only the S-spin pulse is assumed to be perfect, then the difference ($\Delta S'$) between signal intensities of S_0 and S' (the latter is S obtained with the additional I-spin π pulse) is a measure of the effect of I-spin pulse imperfections on the dephasing. A corrected dephasing was taken as the sum of ΔS and $\Delta S'$ [14].

The Chan and Eckert scheme provides a useful but only approximate estimate of the error in REDOR dephasing because of imperfect pulses. The limitation is the assumption of an ideal S-spin π pulse. The S-spin pulse is itself likely to be flawed which will result in an incomplete cancellation of the coincident I-spin pulse and the introduction of errors not present from the use of the original sequence. In this paper, we present a scheme that measures and compensates for incomplete REDOR dephasing due to experimental imperfections in both the observe and dephase channels. No assumptions are made about the quality of either I- or S-spin π pulses, and no pulses are added or taken away in implementing the compensation for incomplete REDOR dephasing, which is effective up to and including $\Delta S/S_0 \approx 1$. We illustrate our correction scheme using $^{13}\text{C}\{^{19}\text{F}\}$ REDOR. We selected this particular spin pair for two reasons: first, the demand made on the ^{19}F dephasing pulses by the large ^{19}F chemical-shift tensor suggests that incomplete dephasing may result; and second, the interest in using ^{19}F for accurate distance measurements involving many dephasing pulses [7] requires calibration standards and procedures that so far have not been reported.

2. Experimental details

2.1. Sample and spectrometer

$^{13}\text{C}\{^{19}\text{F}\}$ REDOR NMR experiments were performed on a natural-abundance ^{13}C fluoro-polycarbonate [16]. The polymer has a low concentration of fluorine and is a useful calibration sample because it provides a reasonably isolated ^{13}C – ^{19}F pair with a two-bond dipolar coupling of 2 kHz (cf, below). Spectra were obtained using a spectrometer built around an Oxford 6-inch horizontal-bore superconducting solenoid operating at 2.35 T, and equipped with a triple-resonance (HFC) transmission-line probe [17]. The ^1H , ^{19}F , and ^{13}C operating frequencies were 100.856, 94.888, and 25.363 MHz, respectively. The ^{13}C and ^{19}F π -pulse lengths were 10 and 6.2 μs , respectively, and the ^1H decoupling amplitude was 100 kHz. Proton-carbon mat-

ched cross-polarization transfers were made at 50 kHz in 1.5 ms with a recycle delay of 1 s. Chemagnetics-Varian 9.5-mm outside-diameter ceramic rotors holding 700 mg samples (which filled about 90% of the effective coil volume) were spun at 3125 Hz.

2.2. Pulse sequences

The two-bond dipolar interaction between ^{19}F and the nearest non-protonated aromatic carbon in fluoro-polycarbonate is well approximated as a spin-1/2, isolated-pair coupling [16]. This interaction is suitable for a test of the quality of the REDOR pulses because the aromatic-carbon peak is easily identified and the expected dephasing can be calculated by established methods [1]. However, the ^{13}C – ^{19}F interaction is strong, and so a reduced-coupling version of REDOR [16,18] is required to study dipolar evolution over sufficiently long times that many pulses can be used. This was accomplished by changing the spacing between the ^{19}F dephasing pulses from 160 to 30 μs (Fig. 1, top). The resulting pulse sequence, which has a single ^{13}C refocusing π pulse, produces the normal REDOR S signal, and the same sequence without the ^{19}F pulses provides the full-echo S_0 signal.

The signal that we call S' is produced from a sequence that has the same number of pulses and phase cycling as that used to obtain S , but with the ^{19}F π pulses centered around $nT_r/2$ (Fig. 1, bottom). If all the pulses are perfect, this sequence results in a full-echo S_0 signal. If the pulses are not perfect, S' is less than S_0 .

REDOR S , S' , and S_0 signals were also generated using xy-8 phase cycling on both ^{13}C and ^{19}F channels using the pulse sequences of Fig. 2.

3. Results and discussion

3.1. Deconvolution

Fig. 3 shows $^{13}\text{C}\{^{19}\text{F}\}$ REDOR S spectra of fluoro-polycarbonate after 8, 16, and 24 rotor periods of dipolar evolution using the scaled-coupling sequence of Fig. 1 (top, gray and black ^{19}F pulses, 30- μs pulse spacings). The 137-ppm aromatic-carbon peak of fluoro-polycarbonate appears to be completely dephased after 24 rotor cycles, but perhaps not quite completely dephased after 16 rotor cycles. A more quantitative determination requires an estimate of contributions to the 137-ppm peak intensity from the strong signals to low and high field. Deconvolution using Gaussian or Lorentzian lineshapes could not adequately fit the inhomogeneously broadened S and S_0 spectra (not shown). We found that a better approach was to subtract a T_2 -scaled S spectrum (obtained after 24 rotor cycles) from the normal REDOR S and S_0 spectra for

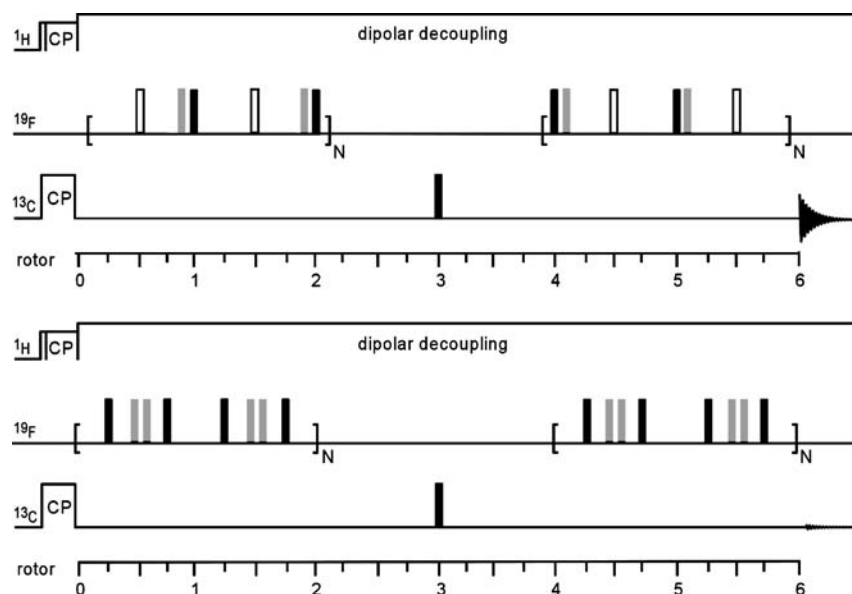


Fig. 1. (Top) $^{13}\text{C}\{^{19}\text{F}\}$ REDOR pulse sequence using a single refocusing ^{13}C π pulse. The signal observed with the dephasing ^{19}F π pulses is S , and without the ^{19}F pulses is S_0 . The REDOR difference is $S = S_0 - S$. Full dephasing is achieved using the ^{19}F π pulses shown in gray and black rectangles, and reduced dephasing (scaled coupling) using the ^{19}F π pulses shown in gray and black. Scaled coupling allows multiple values of $\Delta S/S_0 < 1$ as a function of evolution time to be obtained for strong C–F interactions. The xy-8 phase-cycling scheme is used for the ^{19}F pulses. (Bottom) $^{13}\text{C}\{^{19}\text{F}\}$ REDOR correction sequence using a single refocusing ^{13}C π pulse. The ^{19}F dephasing pulses are centered about $nT_r/2$ in both full-coupling (black) and scaled-coupling (gray) sequences. The spectrum obtained with ^{13}C and ^{19}F pulses is S' . For perfect pulses in the bottom sequence, $S' = S_0$; for imperfect pulses, $S' < S_0$. The same number of pulses is used for both top and bottom sequences.

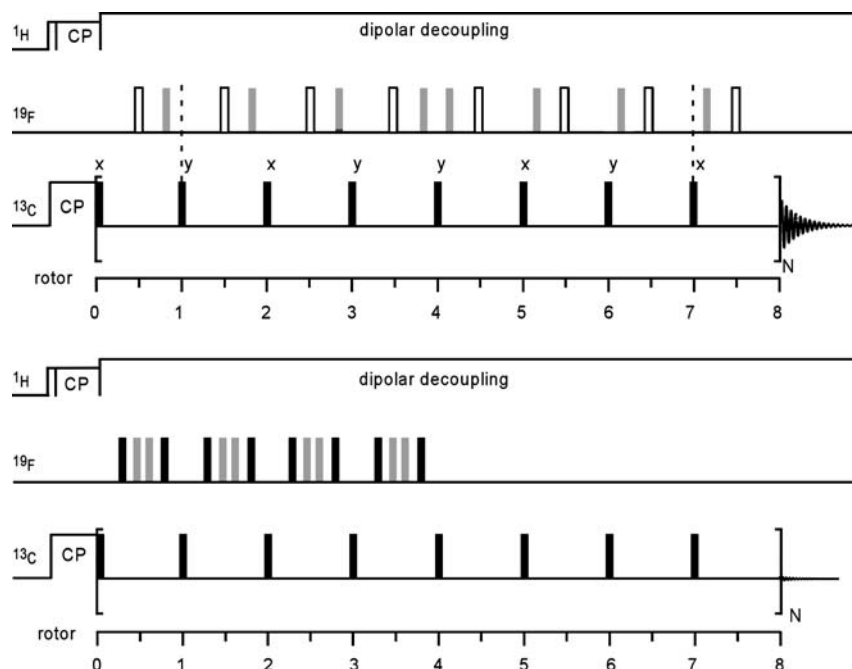


Fig. 2. $^{13}\text{C}\{^{19}\text{F}\}$ dephasing (top) and correction (bottom) sequences for full-coupling (open, top, and black, bottom, ^{19}F pulses) and scaled-coupling (gray, top and bottom, ^{19}F pulses) REDOR, using xy-8 phase cycling for both ^{13}C refocusing and ^{19}F dephasing π pulses. The pairs of ^{19}F pulses in the correction sequence are shown centered about $nT_r/2$ for $n = 1, 2, 3,$ and 4 , but other choices of n are equivalent. The same number of pulses is used for both top and bottom sequences.

shorter evolution periods. As suggested in Fig. 3 (and confirmed by calculation), the 137-ppm peak is completely dephased at $24 T_r$ [16,18]. Thus, the subtraction

has no effect on the 137-ppm S and S_0 peak intensities at 8 and $16 T_r$, but greatly reduces the intensities of the interfering peaks at 128 and 150 ppm (Fig. 4).

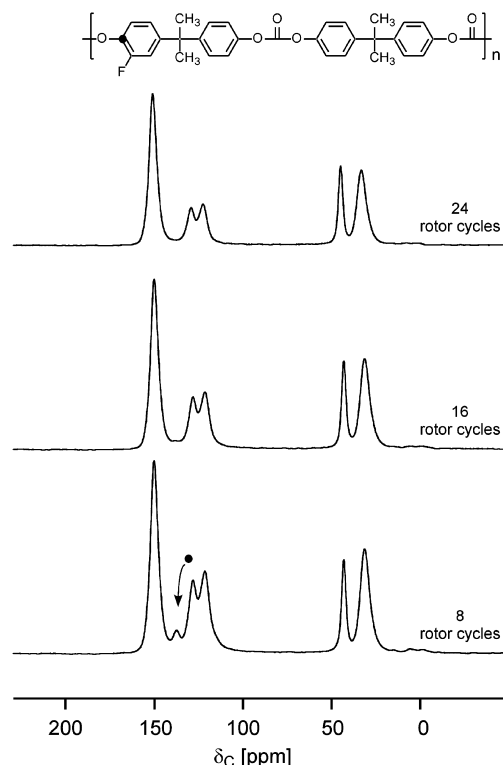


Fig. 3. 25-MHz $^{13}\text{C}\{^{19}\text{F}\}$ REDOR dephased-echo (S) spectra for fluoro-polycarbonate (inset) as a function of dipolar evolution. The spectra were obtained using the scaled-coupling version of the pulse sequence of Fig. 1 (top), with a dephasing pulse separation of 30 μs . The peak at 137 ppm (arrow) arises from the non-protonated aromatic carbon (solid circle) two bonds distant from the ^{19}F label. This peak is totally dephased after $24T_r$. Each spectrum was the result of the accumulation of 4096 scans. Magic-angle spinning was at 3125 Hz.

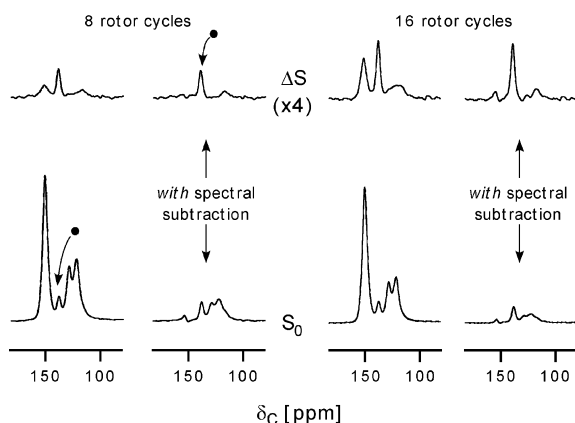


Fig. 4. 25-MHz $^{13}\text{C}\{^{19}\text{F}\}$ REDOR spectra for fluoro-polycarbonate as a function of dipolar evolution. The scaled-coupling sequence of Fig. 1 (top) was used with the separation of the ^{19}F pulses equal to 30 μs . Full-echo spectra are shown at the bottom of the figure and REDOR differences at the top. Spectral overlap of the 137-ppm peak (circle and arrow) with its neighbors at 128 and 150 ppm was reduced for both ΔS and S_0 spectra (second and fourth columns) by subtraction of a T_2 -scaled, $24T_r$ dephased-echo spectrum (see Fig. 3, top). Each spectrum was the result of the accumulation of 4096 scans. Magic-angle spinning was at 3125 Hz.

3.2. Incomplete REDOR dephasing

REDOR dephasing for the deconvoluted 137-ppm peak is shown in Fig. 5 (left, open circles) and is less than the calculated dephasing (solid line). Both experiment and calculation were for the scaled-coupling version of the pulse sequence of Fig. 1 (top). The origin of the discrepancy can be traced to imperfections in the π pulses, as measured by comparison of S' and S_0 spectra. These were collected using the pulse sequence of Fig. 1 (bottom, black) with ^{19}F pulses (S') and without ^{19}F pulses (S_0). The total dephasing time was either two rotor cycles using xy-4 phase cycling, or four rotor cycles, which allowed xy-8 phase cycling for the ^{19}F pulses [10]. The non-zero values of the REDOR correction differences ($\Delta S' = S_0 - S'$) in Fig. 6 clearly indicate the presence of pulse imperfections in one or both RF channels.

3.3. Correction to single-pulse-observe REDOR

The 137-ppm peak dephases completely in $2T_r$ in the full-coupling version of REDOR with magic-angle spinning at 3125 Hz because of the 2-kHz C–F coupling [16,18]. The combination of these values corresponds to $nT_r D_{CF} = 1.28$. Thus, we estimate that the $2T_r \Delta S'/S_0$ of 8% (Fig. 6, left) is the extent of the diminution of full dephasing due to pulse imperfections. It is possible to correct an observed $\Delta S/S_0$ by simply adding $\Delta S'/S_0$, as suggested by Chan and Eckert [14]. However, this requires making a determination of the correction for each value of the dephasing, which doubles the total time of the experiment. In addition, the experimentally determined value of $\Delta S'/S_0$ should not be used for an evolution time such that $nT_r D_{CF} > 1.5$. For long evolution times, a direct addition of the correction would overcompensate for pulse imperfections because $\Delta S'/S_0$ continues to increase for increasing dipolar evolution time well after $\Delta S/S_0$ has reached its maximum and is oscillatory. For example, if we add the $4T_r$ correction (Fig. 6, right) to the observed $\Delta S/S_0$ which is close to 1, the resulting corrected dephasing is substantially larger than the theoretical limit. The correction strategy that we describe below avoids this problem. It is just as simple as the Chan and Eckert approach, but takes much less time to implement and is, in general, applicable for all values of the evolution time.

An accurate determination of the $4T_r$ REDOR correction ($nT_r D_{CF} = 2.56$) was obtained by measuring $\Delta S'/S_0$ as a function of an intentional mis-setting of the ^{13}C π pulse width (Fig. 7). The relatively broad minimum of $\Delta S'/S_0 = 16\%$ is just double the full-coupling $2T_r$ correction of 8% (Fig. 6, left), indicating a linear dependence of $\Delta S'/S_0$ on evolution time (or total number of dephasing pulses), at least for short times. In fact, $\Delta S'/S_0$ is linear for evolution times as long as 30 ms

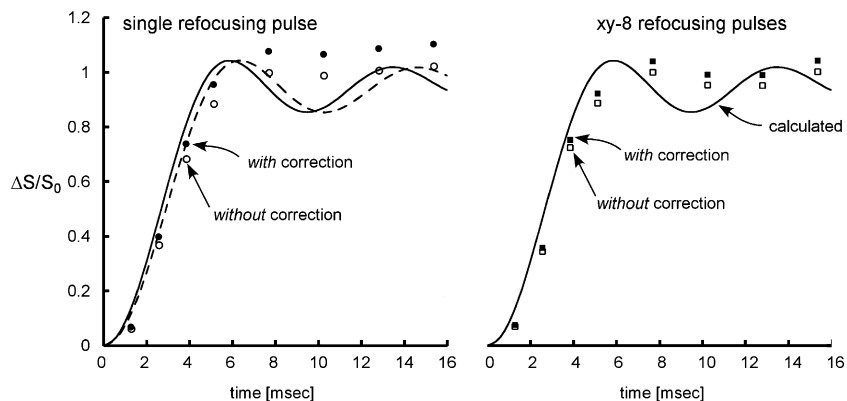


Fig. 5. $^{13}\text{C}\{^{19}\text{F}\}$ REDOR dephasing ($\Delta S/S_0$) for the 137-ppm peak of fluoro-polycarbonate using a single refocusing ^{13}C pulse (left) and xy-8 phase-cycled refocusing ^{13}C pulses (right). The open symbols show the observed dephasing and the solid symbols, the corrected dephasing. The scaled-coupling dephasing and correction sequences of Fig. 1 were used (with 30- μs pulse spacings) for data represented by the circles, and that of Fig. 2 for the data represented by the squares. The solid and dotted lines show the dephasing calculated for an isolated $^{13}\text{C}\text{-}^{19}\text{F}$ pair with dipolar couplings of 2068 and 1906 Hz, respectively, under magic-angle spinning at 3125 Hz. The scatter in the data is within the width of the symbols which indicates the experimental error of the measurements.

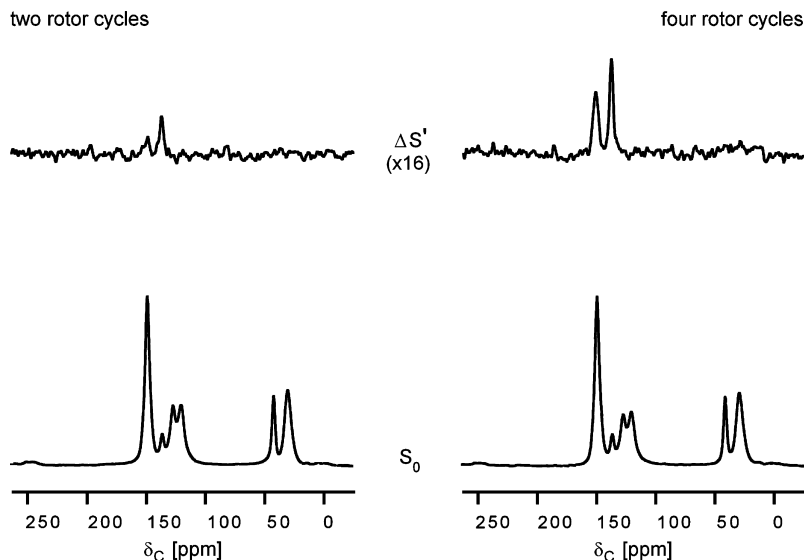


Fig. 6. 25-MHz $^{13}\text{C}\{^{19}\text{F}\}$ REDOR full-echo (bottom) and correction (top) spectra obtained using the full-coupling sequence of Fig. 1 (bottom) after two rotor cycles (left) and four rotor cycles (right) of dipolar evolution with magic-angle spinning at 3125 Hz. Each spectrum was the result of the accumulation of 8192 scans.

(Fig. 8). This means that we can add the *same* 8% correction to all the values of $\Delta S/S_0$ as a function of evolution time (Fig. 5, solid circles). This correction takes into account the effects of imperfections in the dephasing (I) as well as the observed (S) pulses. An I-spin π pulse that is imperfect because of phase transients or frequency offsets will not exactly invert the sign of the non-observable I_2S_x bilinear coherence generated during REDOR evolution, and this will reduce ΔS . The same imperfect I-spin pulse will also result in coherences like I_xS_x that no longer evolve under dipolar coupling and do not refocus in the REDOR correction experiment. This leads to a non-zero $\Delta S'$. Any reduction in the REDOR

ΔS due to an imperfect I-spin π pulse is matched by an increase in the correction $\Delta S'$.

The corrected dephasing in Fig. 5 is still slightly less than the calculated dephasing for evolution times less than 6 ms. This discrepancy is probably due to an underestimate of motional averaging of the C–F dipolar coupling. The corresponding crystal-structure C–F distance in fluorobenzenes is 2.355 Å [19], which means a rigid-lattice dipolar coupling of 2177 Hz. Calculation of the dephasing in Fig. 5 (solid line) assumed a coupling of 2068 Hz, a reduction of 5% of the rigid-lattice limit. This reduction arises from GHz-regime isotropic lattice motions and is a commonly used correction (see Fig. 10 in

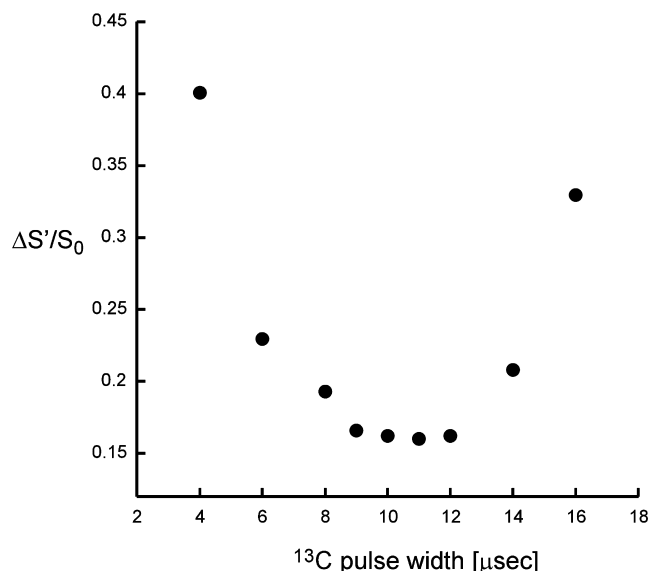


Fig. 7. $^{13}\text{C}\{^{19}\text{F}\}$ REDOR correction ($\Delta S'/S_0$) for the 137-ppm peak of fluoro-polycarbonate using a single refocusing ^{13}C pulse of variable width and the full-coupling sequence of Fig. 1 (top, black) after 4 rotor cycles of dipolar evolution with magic-angle spinning at 3125 Hz.

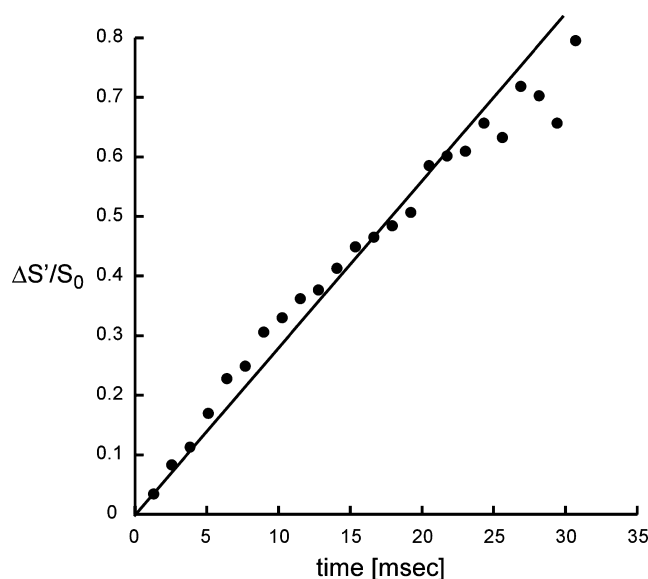


Fig. 8. $^{13}\text{C}\{^{19}\text{F}\}$ REDOR correction ($\Delta S'/S_0$) for the 137-ppm peak of fluoro-polycarbonate using a single refocusing ^{13}C pulse and the scaled-coupling sequence of Fig. 1 (bottom, gray, 30- μs pulse spacings) as a function of dipolar evolution with magic-angle spinning at 3125 Hz.

reference [1], and associated text). However, kHz–MHz regime small-amplitude oscillations about the ring C_2 axis are also likely to be present [20]. The 2-bond C–F coupling in fluoro-polycarbonate has been estimated as 1800 Hz using a constant-time 8- T_r version of $^{13}\text{C}\{^{19}\text{F}\}$ REDOR [21]. The best fit to the first four dephasing values of Fig. 5 (left, dotted line) indicates a coupling of 1906 Hz, which we consider a more accurate value. The

corrected dephasing is slightly greater than the calculated dephasing for evolution times greater than 8 ms and is not strongly oscillatory, a combination which suggests the possibility of contributions to dephasing from next-nearest-neighbor fluorines.

3.4. REDOR with xy -8 on both channels

With xy -8 phase cycling for both ^{13}C and ^{19}F π pulses, the REDOR dephasing ($\Delta S/S_0$) increases and the REDOR correction ($\Delta S'/S_0$) decreases (Fig. 5, right), relative to those for single-pulse-detected REDOR. The correction is reduced from the single-pulse-detected value of 8% to 4%; this indicates that both ^{13}C and ^{19}F pulses are imperfect and that xy -8 phase cycling for $^{13}\text{C}\{^{19}\text{F}\}$ experiments is apparently not capable of completely removing the effects of the imperfections. The corrected dephasing values are in reasonable agreement with calculation, at least to within the uncertainty about the extent of motional averaging of the C–F coupling (see above).

3.5. Correction strategy

Either of the REDOR pulse sequences of Figs. 1 and 2 is suitable for the determination of long-range ^{13}C – ^{19}F distances using dipolar evolution times as long as 30 ms and possibly longer. Underestimates of dephasing resulting from pulse imperfections in such experiments are not negligible, but they are not particularly large either. Furthermore, simple correction schemes that require no assumptions about the nature of the pulses have now been successfully tested for both single-pulse-detected REDOR and more elaborate versions of REDOR. Of course, the corrections are certain to be probe (and tuning) dependent, and are likely to be larger for high-field spectrometers, where the demands on π pulses to flip spins whose resonance frequencies span a wider range are greater. In fact, the single-pulse-detected $^{13}\text{C}\{^{19}\text{F}\}$ REDOR correction for our 500-MHz spectrometer [22] using 5- μs π pulses is approximately double the 8% value reported here for the 100-MHz spectrometer.

In practice, we envision correcting the experimentally determined REDOR dephasing by a determination of $\Delta S'/S_0$ on an analytical sample for one or two values of the dephasing time. Then the *same* percentage correction will be made for all values of $\Delta S/S_0$ obtained at the same spinning frequency. It will therefore not be necessary in general to spend as much spectrometer time on the correction as on the analytical dephasing. If probe tuning and sample volume are not substantively changed for analytical and calibration samples, a $\Delta S'/S_0$ determination for the analytical sample may not even be necessary. The correction schemes have been illustrated here for $^{13}\text{C}\{^{19}\text{F}\}$ REDOR because of the interest in this

experiment for long-range distance measurements with long evolution times and many pulses [7], but the general strategy should be applicable to other types of I–S coupled pairs as well.

Acknowledgments

This work was supported by NSF grant DMR-0097202. The fluoro-polycarbonate was synthesized by Jin H. Wu in the laboratory of Albert F. Yee (University of Michigan).

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