Introduction to Rotational-Echo, Double-Resonance NMR

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ABSTRACT: Rotational-echo, double-resonance nuclear magnetic resonance (REDOR) is a magic-angle spinning experiment for measuring internuclear distances between heteronuclear spin pairs. The basic principles of the REDOR technique are presented. The experimental requirements necessary for quantitative REDOR measurements are also provided. © 1998 John Wiley & Sons, Inc. Concepts Magn Reson 10: 277–289, 1998

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INTRODUCTION

Measuring internuclear distances provides important structural information for all branches of the chemical sciences. Traditional techniques such as X ray and neutron scattering and solution-state nuclear magnetic resonance (NMR) have found the most use in solving structural problems. However, scattering techniques are of limited use for amorphous systems such as glassy polymers or inorganic glasses and for large macromolecular systems lacking high-quality crystals. Also, solution-state NMR studies are problematic for high-molecular-weight systems because of limited solubilities and spectral crowding. Solid-state NMR can provide important structural information without limits imposed by molecular weight and can be applied to crystalline, microcrystalline, and amorphous systems. Consequently, solid-state NMR may be useful when the more conventional techniques are impractical.

Several high-resolution magic-angle spinning (MAS) NMR techniques exist for the accurate determination of distances between pairs of rare-spin nuclei. Distances between nuclei can be obtained by MAS NMR by measuring the dipolar interaction between coupled spin pairs. The dipolar interaction is inversely proportional to the cube of the internuclear distance. Measuring the distance between a homonuclear spin pair (e.g., $^{13}$C–$^{13}$C) may be accomplished by rotational resonance NMR (1–3), which recovers the homonuclear dipolar interaction by matching an integral multiple of the spinning frequency to the isotropic chemical shift difference between the two coupled spins. Other homonuclear experiments, which use rotor-synchronized pulse trains, include synchronized radiofrequency pulse cycles combined in a sevenfold-symmetric phase shift scheme (C7) (4), dipolar correlation spectroscopy (DICSY) (5), dipolar recovery at the magic angle (DRAMA) (6–8), dipolar recovery with a window-less sequence (DRAWS) (9), radio frequency driven dipolar recoupling (RFDR) (10), simple excitation for the dephasing of rotational-echo amplitudes (SEDRA) (11), and melding of spin-locking and DRAMA (MELODRAMa) (12). The
measurement of the distance between a spin-$\frac{1}{2}$ heteronuclear spin pair (e.g., $^{13}$C-$^{15}$N) may be performed with the dipolar exchange-assisted recoupling (DEAR) ($I_d$), rotational-echo, double-resonance (REDOR) ($I_d$, $I_S$), and transfer-echo, double-resonance (TEDOR) ($I_d$, $I_S$) techniques.

Recent demonstrations have shown that the dipolar interaction between a spin-$\frac{1}{2}$ nucleus and a quadrupolar nucleus (e.g., a $^{13}$C-$^{17}$O spin pair) may also be measured. Internuclear distances between spin-$\frac{1}{2}$ and quadrupolar nuclei may be obtained using the rotational-echo, adiabatic-passage, double-resonance (REAPDOR) ($I_d$, $I_S$), REDOR ($I_d$, $I_S$), and transfer-of-populations-in-double-resonance (TRAPDOR) ($I_d$, $I_S$) experiments.

A description of REDOR NMR is presented in this article. REDOR is a versatile and straightforward experiment to implement. It has become the workhorse MAS experiment for recovering heteronuclear dipolar interactions. This MAS sample experiment uses simple rotor-synchronized $\pi$ pulses to recover the heteronuclear dipolar interaction. An important feature of the experiment is that dipolar couplings can be obtained in a simple manner without the need to know the chemical shift interaction or any other interaction; only the dipolar interaction is important in the evolution of the spin system. A semiclassical treatment of the REDOR experiment is provided below. A more formal density matrix treatment of the experiment can be found in Ref. 24. The experimental requirements for the REDOR technique are also provided.

**SEDOR AND REDOR FOR SPIN-$\frac{1}{2}$ NUCLEI**

Kaplan and Hahn introduced a basic double-resonance experiment in 1958, designed to show that two dissimilar nuclei were physically near one another ($25$). That experiment is spin-echo, double-resonance (SEDOR) NMR. The SEDOR experiment is applied to stationary samples. An important catalyst for the REDOR experiment was the descriptions and applications of the SEDOR experiment by Shore et al. ($26$) and Slichter ($27$). Because of the simplicity and elegance of the SEDOR experiment, it is worthwhile to examine how SEDOR works before proceeding to the REDOR experiment.

Spin-bearing nuclei generate magnetic dipole fields. These magnetic fields are generally very weak compared to the applied external field. Like any dipole field, the field strength of the nuclear magnetic dipole field is proportional to $1/r^3$, where $r$ is the distance from the nucleus. A nuclear spin $S$ placed in the vicinity of nuclear spin $I$ will experience the magnetic dipole field generated by the $I$ spin. This local field at the site of the $S$ spin has a $z$ component that is determined by the spin orientation of the $I$ spin. Figure 1 shows the relationship between the $S$ and $I$ spins and the $z$ component of the local field, $H_L$, at the site of the $S$ spin that is generated by the $I$ spin. The precession rate of the transverse component of the $S$-spin magnetization, $S_{+}$, is determined by the strength of the local field at the site of the $S$ spin. The equation of motion of $S_{+}$ in the rotating frame is (neglecting relaxation)

$$\frac{dS_{+}}{dt} = S_{+} \times \gamma_S H_L$$

where $\gamma_S$ is the magnetogyric ratio of the $S$ nucleus. This simplified Bloch equation describes a magnetization vector in the $x$-$y$ plane precessing about the $z$ axis. An important observation is that the sense of precession of the $S$ spin changes if the orientation of the local field is changed. A way to change the orientation of the local field at the site of the $S$ spin is to change the orientation of the $I$ spin. This can be done by applying a simple $\pi$ pulse to the $I$ spin as is shown in Fig. 1. We now have the foundations for the classical picture of the SEDOR experiment.

Figure 2(a) shows a spin-echo pulse sequence for two spin-$\frac{1}{2}$ nuclei $S$ and $I$. For simplicity, the only interaction present in this analysis is the dipolar coupling between the two nuclei. The spin-echo pulse sequence consists of $(\pi/2)$, and $(\pi)$ pulses applied to the $S$ spin. These two pulses are separated by a time $\tau$. No $I$-spin pulses are applied in this pulse sequence. Hence, the local field at the $S$ spin remains the same throughout the experiment as is shown in the plot.
of $H_L$ located under the pulse sequence. The corresponding trajectory of the $S$-spin magnetization for this pulse sequence is shown in Fig. 3(a). For the time between the two $S$-spin pulses, the transverse magnetization is shown precessing in a clockwise direction. Just prior to the application of the $S$-spin ($\pi$) pulse, the transverse magnetization has accumulated a phase $\phi$ with respect to the $x$ axis. The $S$-spin ($\pi$) pulse rotates the magnetization about the $x$ axis; now the magnetization makes an angle of $-\phi$ with respect to the $x$ axis. Since no pulses are applied to the $I$ spin, the sense of precession of the $S$-spin magnetization does not change, since the local field at the site of the $S$ spin does not change. Therefore, at a time $\tau$ after the ($\pi$) pulse, the magnetization is realigned with the $x$ axis. The net result is the formation of a spin echo, and the signal intensity is maximized at this point.

Now consider what happens when a $\pi$ pulse is applied to the $I$ spin simultaneously with the application of the $S$-spin $\pi$ pulse. This is the SEDOR experiment and is shown in Fig. 2(b). The local magnetic dipole field at the site of an $S$-spin changes orientation after the application of the $I$-spin $\pi$ pulse. The local field is also shown in Fig. 2(b), where the local field is taken as positive for the time prior to the $I$-spin $\pi$ pulse and is taken as negative after the application of the $I$-spin $\pi$ pulse. The corresponding $S$-spin magnetization trajectory is shown in Fig. 3(b). The same trajectory occurs for the time up to the application of the two $\pi$ pulses as in the spin-echo experiment. Immediately following the application of the two $\pi$ pulses, the $S$-spin makes an angle of $-\phi$ with respect to the $x$ axis. Furthermore, the local field at the site of the $S$ spin has now changed orientation, but not its strength. Thus, the $S$-spin magnetization now precesses in a counterclockwise direction. Consequently, the $S$-spin magnetization accumulates an additional phase of $-\phi$ during the time $\tau$ after the $\pi$ pulses. Thus, the $S$-spin magnetization makes an angle of $-2\phi$ with respect to the $x$ axis at the time $2\tau$. The corresponding magnetization component projected onto the $x$ axis is smaller than that found when no $\pi$ pulse was applied to the $I$ spins.

![Figure 2](image_url) Models of the SEDOR and REDOR experiments. Examples of the local field at the $S$-spin site are sketched for each pulse sequence.
Figure 3 Sample spin trajectories for the SEDOR and the REDOR experiments. The magnetization vector sweeps out the paths shown. The arrows on the trajectory paths provide the direction of precession of the transverse magnetization. The length of the magnetization vector decays exponentially during the precession to simulate transverse relaxation. The magnetization returns to the x axis (a) and (c) to form echos, whereas net dephasing occurs for the trajectories shown in (b) and (d). The trajectories for the spinning sample were performed with an $S-I$ orientation defined by $\alpha = 60^\circ$ and $\beta = 30^\circ$.

At this point, it is useful to ask whether we can compare the two signal intensities obtained by the spin echo and SEDOR pulse sequences to deduce the $S-I$ dipolar interaction. Let the $S$-spin magnetization immediately following the $(\pi/2)_y$ pulse be $S_i$. The echo amplitude for the spin echo pulse sequence is the full signal intensity, $S_o$, and is given by

$$S_o(2\tau) = S_i e^{-2\tau/T_2}$$  \[2\]

The spin-echo intensity is weaker than the initial signal strength only because of $T_2$ relaxation. The spin-echo intensity for the SEDOR experiment is the cosine projection of the transverse magnetization. This signal will be taken as the reduced signal intensity, $S_r$, and is given by

$$S_r(2\tau) = S_i e^{-2\tau/T_2} \cos(2\phi)$$  \[3\]

For a static sample, the rate of precession of the $S$-spin magnetization due to the $S-I$ dipolar interaction is

$$\omega_d(\theta) = \frac{d}{2} (3 \cos^2 \theta - 1)$$  \[4\]

where the angle between the $z$ axis (taken as parallel with the Zeeman magnetic field) and the vector connecting the $S$ and $I$ spins is $\theta$ and the dipolar coupling, $d$, is equal to $\gamma_S \gamma_I h/2 \pi r^3$. Notice the strong orientational dependence of $\omega_d$. The net phase accumulation of the transverse component of the $S$-spin magnetization for the spin echo experiment is zero. However, during the time between the $(\pi/2)_y$ and $(\pi)_z$ pulses in the SEDOR experiment, the phase accumulation is $\phi = \int_0^{\tau} \omega_d \, dt$, which is equal to $\omega_d \tau$, since $\omega_d$ is time independent. Hence, $2\phi = 2\omega_d \tau$ for the SEDOR experiment, and the reduced signal becomes

$$S_r(2\tau) = S_i e^{-2\tau/T_2} \cos(2\omega_d \tau)$$  \[5\]

The ratio of the reduced and full signal is

$$S_r/S_o = \cos(2\tau \omega_d)$$  \[6\]
Equation [6] is for a single $S$-$I$ spin orientation defined by the angle $\theta$. Generally, it is simpler to work with a powder sample than with a single crystal. For powders, Eq. [6] must be summed over all sample orientations. This leads to the powder result

$$S_t/S_0 = \sum_0 \cos(2\pi \omega_d) \sin(\theta)$$  \hspace{1cm} [7]

The $\sin(\theta)$ is a geometrical weighting factor. For example, more $S$-$I$ vectors have orientation at $\theta = 90^\circ$ than $\theta = 0^\circ$. The reason that powders are easier to analyze than single crystals is that all orientations are present in a powder, whereas the orientation angle is needed for data analysis in single crystals. The ratio $S_t/S_0$ depends only on the dipolar interaction and the dipolar evolution time. This ratio is independent of $T_2$; furthermore, a more detailed analysis shows that the ratio is independent of the chemical shift as well. Thus, by simply measuring signal amplitudes it is possible to obtain the dipolar interaction and, consequently, the $S$-$I$ internuclear separation.

A two-rotor-cycle REDOR experiment is provided in Fig. 2 to illustrate its similarities and differences with SEDOR. Samples rotating at the magic angle differ from static samples, since the sample rotation makes second-rank interactions magic angle differ from static samples, since the differences with SEDOR. Samples rotating at the divided in Fig. 2 to illustrate its similarities and powder results over all sample orientations. This leads to the crystal. For powders, Eq. [6] must be summed equal to the sample rotation period, sketched as a simple sine function with a period local dipolar field strength in Figure 2 $c$ is rotor cycle is zero. For illustrative purposes, the average value of the dipolar interaction over each cycle is

$$H_{S_{\alpha}} = d(t)S_{\alpha}I_z$$  \hspace{1cm} [8]

where

$$d(t) = d\{\sin^2 \beta \cos(\alpha + \omega_{\alpha} t)$$

$$-\sqrt{2} \sin 2\beta \cos(\alpha + \omega_{\alpha} t)\}$$  \hspace{1cm} [9]

The sample spinning rate is $\omega_{\alpha}$, and $\alpha$ and $\beta$ are the azimuthal and polar angles of the internuclear vector with respect to the spinning axis (e.g., Fig. 1 in Ref. 15). Equation [9] shows that the average value of the dipolar interaction over each rotor cycle is zero. For illustrative purposes, the local dipolar field strength in Figure 2(c) is sketched as a simple sine function with a period equal to the sample rotation period, $T_0$.

Figure 2(c) shows a spin-echo experiment applied synchronously with the sample rotation. The $S$-spin $\pi$ pulse is applied after the first rotor cycle and data acquisition begins at the end of the second rotor cycle. The dipolar interaction has no effect on the detected signal amplitude, since the dipolar interaction averages to zero over each rotor period. The local field shown in Figure 2(c) has a positive average value for the first half of the rotor cycle and a negative average value for the second half of the rotor cycle. The spin trajectory for the first rotor cycle for this local field is shown in Fig. 3(c). There is dipolar dephasing of the $S$ spin during the first half of the rotor cycle; however, this dephasing is reversed during the second half of the rotor cycle. The net result is the formation of a rotational echo at the end of the rotor cycle. Note that an $S$-spin pulse is not required to refocus the $S$ magnetization. The same spin trajectory occurs for the second rotor cycle. The spin-echo sequence serves simply to refocus the isotropic chemical shift. The acquired signal, $S_0$, is the full signal and simply accounts for $T_2$ decay.

Interesting effects occur for such a periodic interaction if $\pi$ pulses are applied to both the $S$ and $I$ spins as shown in Figure 2(d). In addition to the $S$-spin spin-echo pulse sequence, $I$-spin $\pi$ pulses are applied at $T_1/2$ and $3T_1/2$. This is a two-rotor-cycle REDOR experiment. During the first half of the first rotor cycle, the average of the local dipolar field is positive. In the absence of the $I$-spin $\pi$ pulse, the average local field would be negative during the second half of the rotor period because of the spatial modulation of the dipolar interaction. However, since the dipolar interaction is a product of spatial and spin functions, the relative sign of the local dipolar field can be changed by application of an $I$-spin $\pi$ pulse. Thus, the average local field experienced by an $S$ spin during the second half of the rotor cycle is also positive because of the $I$-spin $\pi$ pulse. The average local dipolar field experienced by the $S$ spin is now positive for the first rotor cycle, not zero as before. This will result in net dipolar dephasing during the first rotor cycle. The corresponding spin trajectory for this pulse sequence is shown in Fig. 3(d). The dipolar dephasing for the first half of the rotor cycle is the same as in Fig 3(c). However, the sense of dipolar dephasing during the second half of the rotor cycle changes because of the $I$-spin $\pi$ pulse compared to the corresponding precession in Fig. 3(c). The $S$-spin magnetization does not return to the $x$-axis at the end of the rotor cycle for this pulse sequence. During the second rotor cycle, the local dipolar field of the $S$ spin is initially
negative because of the periodic nature of the dipolar interaction. The local field would go positive during the second half of this rotor cycle if it were not for the presence of the second I-spin $\pi$ pulse. This pulse causes the average of the local field experienced by the S spin to be negative during the second rotor cycle. The dashed lines show the new average of the local field for the first and second rotor periods. The dashed lines in Fig. 3(d) representing the average of $H_L$ for the rotating sample are qualitatively similar to $H_L$ found in Fig. 3(b) for the static sample.

Since the average dipolar field experienced by an S spin is positive for the first rotor cycle and negative during the second rotor cycle, the S-spin magnetization does not refocus at the beginning of data acquisition for the REDOR experiment. There is net dipolar dephasing of the S spin. This S-spin signal is the reduced signal, $S_r$. The net dephasing angle, $\phi$, for a single rotor period can be calculated by

$$\phi = \int_0^{T/2} \omega_d(t) \, dt - \int_{T/2}^T \omega_d(t) \, dt$$  \hspace{1cm} [10]

where $\omega_d(t) = \pm \alpha(t)/2$. The negative sign in front of the second term in Eq. [10] arises from the I-spin $\pi$ pulse. Evaluation of Eq. [10] for one rotor cycle of dipolar evolution yields

$$\phi = \frac{T_r d}{\pi} \sqrt{2} \sin 2\beta \sin \alpha$$  \hspace{1cm} [11]

The phase accumulation for the second rotor cycle is the same as for the first rotor cycle; thus, the total phase accumulation for two rotor cycles is twice that found for one rotor cycle.

The extension of the REDOR experiment to $N_c$ rotor cycles is shown in Fig. 4. As shown, there is a $\pi$ pulse at the start of every rotor cycle on the S channel and an interleaved train of $\pi$ pulses is applied on the I channel. In general, the total phase accumulation in an $N_c$ rotor cycle REDOR experiment is

$$\phi = \frac{N_c T_r d}{\pi} \sqrt{2} \sin 2\beta \sin \alpha$$  \hspace{1cm} [12]

Notice that there are two important quantities in this expression: the dipolar evolution time, $N_c T_r$, and the dipolar coupling, $d$. The units of $d$ are rad/sec, but we find it convenient to define $D = d/2\pi$, since $D$ has units of Hz. The evolution time and $D$ are grouped together to form the dimensionless parameter $\lambda$: $\lambda = N_c T_r D$. The reduced REDOR signal is

$$S_r = S_i e^{-N_c T_r / T_2} \cos(\phi)$$  \hspace{1cm} [13]

and the ratio of the reduced and full signals is

$$S_r/S_0 = \cos(\phi)$$  \hspace{1cm} [14]

Equation [14] is for a single S-I spin pair. For a powder sample, all internuclear orientations must be included and all values of $\alpha$ and $\beta$ must be summed over. The powder sample result is

$$S_r/S_0 = \frac{1}{2\pi} \int_0^\pi \int_0^\pi \cos(\phi) \sin \beta \, d\alpha \, d\beta$$  \hspace{1cm} [15]

where $\sin \beta$ is the geometrical weighting factor.

Figure 5 shows two universal curves for $S_r/S_0$ and for $\Delta S/S_0$ where $\Delta S/S_0 = (S_0 - S_r)/S_0$. An important feature of these graphs is that they apply to all heteronuclear spin pairs and need be calculated only once.

**Example of the REDOR Experiment**

An example of how the REDOR experiment can be used as a one-shot method for determining internuclear distances is now presented. The sample is [1-13C,15N]acetyl-L-carnitine, and its chemical structure is given in Fig. 6. The specific $^{13}$C and $^{15}$N labels are indicated. A measurement of the distance between these two labels provides the conformation (folded versus extended) of the molecule. Also marked in the figure is the C8...
Figure 5 Dependence of the REDOR ratios $S_r/S_0$ and $\Delta S/S_0$ on the dimensionless parameter $\lambda$. The dipolar evolution time is the product of the number of rotor cycles, $N_r$, in the dipolar evolution period and the sample rotation period, $T_r$. The dipolar coupling, $D$, is expressed in Hz for these graphs.

Carbon. This carbon is important because the $^{13}$C resonances from the C1 carbon ($^{13}$C-labeled) and the C8 carbon are not resolved.

Figure 7 shows $^{13}$C-observe REDOR NMR results for the powder sample. The bottom spectrum is the full spectrum (no $^{15}$N pulses) and the top spectrum is the difference spectrum. The difference spectrum is obtained by subtracting the reduced spectrum from the full spectrum. The reduced spectrum is obtained with the $^{15}$N pulses. Only $S$ spins coupled to $I$ spins contribute to the difference spectrum. The resonance at 171.9 ppm contains contributions from the C1 and C8 carbons. Experimental details are provided in the figure caption and in Ref. 28.

The C1–N distance can be obtained once $\Delta S^{(1)}/S_0^{(1)}$ is determined. The superscript designates the specific carbon (C1 or C8). For example, the ideal REDOR difference signal is $\Delta S^{(1)}$ and the REDOR full signal is $S_0^{(1)}$ for the C1 carbon. It is important to note that $S_0^{(1)}$ is understood to represent only those C1 carbons with a $^{15}$N neighbor. Some C1 carbons have $^{14}$N neighbors since the $^{15}$N enrichment is only at 95% and since the labeled molecule is in a matrix of molecules with all nuclei at their respective natural-abundance levels. Since the labeled sample is diluted with natural abundance material and since there is spectral overlap between the C1 and C8 carbon resonances, $\Delta S^{(1)}/S_0^{(1)}$ differs from the experimentally measured ratio $\Delta S/S$. The desired ratio, $\Delta S^{(1)}/S_0^{(1)}$, can be obtained by noting that the experimentally determined difference signal, $\Delta S$, is the sum

$$\Delta S = \Delta S^{(1)} + \Delta S^{(8)}$$  \[16\]

This result, divided by the measured full signal, $S$, gives the measured REDOR ratio of difference to full signals:

$$\Delta S/S = \Delta S^{(1)}/S + \Delta S^{(8)}/S$$  \[17\]

The $^{13}$C nuclei from the C1 and C8 positions contribute to $S$. The fraction of $^{13}$C spins at the C1 position and with a $^{15}$N neighbor is $\alpha = S_0^{(1)}/S$, and the fraction of $^{13}$C spins at the C8 position and with a $^{15}$N neighbor is $\beta = S_0^{(8)}/S$. (Do not confuse these definitions of $\alpha$ and $\beta$ with the powder angles.) These fractions are determined
by the isotopic enrichment and by the dilution factor of labeled material to natural-abundance material. Insertion of these fractions into Eq. [17] leads to

$$\frac{\Delta S^{(1)}}{\Delta S^{(8)}} = \frac{1}{\alpha} \frac{\Delta S}{\Delta S^{(0)}} + \frac{\beta}{\alpha} \frac{\Delta S^{(8)}}{\Delta S^{(0)}}$$

[18]

Since $\Delta S/S$, $\alpha$, and $\beta$ are experimentally determined or known, then $\Delta S^{(1)}/\Delta S^{(8)}$ can be obtained once $\Delta S^{(8)}/\Delta S^{(0)}$ is determined. For this sample, $\Delta S^{(8)}/\Delta S^{(0)}$ could be calculated from an estimate of the C8–N distance (see Ref. 28 for complete details).

Two REDOR experiments were performed for the acetyl-L-carnitine sample: one with $N_c = 34$ and the other with $N_c = 42$. Figure 8 shows $\Delta S/S_0$ versus $\lambda$ over a small range of $\lambda$. The experimentally determined $\Delta S^{(1)}/S_0^{(1)}$ values for the two REDOR experiments are shown and the corresponding values of $\lambda$ are marked. The data are summarized in Table 1 and the result is a C1–N distance of 4.96 Å. These results show that the molecule is fully extended.

This example shows that for real samples, the natural-abundance corrections are important, as is accounting for resonance overlap. For example, the desired ratio $\Delta S^{(1)}/S_0^{(1)}$ is needed to determine the C1–N distance, but the experimentally

![Figure 7](image)

Figure 7 $^{13}$C REDOR spectra for a powder sample of $[1^{-13}$C,$^{15}$N]acetyl-L-carnitine hydrochloride diluted 1 in 20 parts natural-abundance acetyl-L-carnitine. These spectra were obtained with $N_c = 34$ and a spinning speed of 3205 Hz. $^{13}$C and $^{15}$N rf field strengths were 38 kHz. The proton decoupling field strength was 110 kHz.

<table>
<thead>
<tr>
<th>Sample form</th>
<th>$N_c$</th>
<th>$\Delta S/S_0^{(8)}$</th>
<th>$\Delta S^{(8)}/S_0^{(8)}$</th>
<th>$\Delta S^{(1)}/S_0^{(1)}$</th>
<th>$D_{C1-N}$ (Hz)</th>
<th>$r_{C1-N}$ (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystalline</td>
<td>34</td>
<td>0.0498</td>
<td>0.290</td>
<td>0.0729</td>
<td>25.1</td>
<td>4.95</td>
</tr>
<tr>
<td>Crystalline</td>
<td>42</td>
<td>0.0729</td>
<td>0.416</td>
<td>0.1068</td>
<td>24.8</td>
<td>4.97</td>
</tr>
</tbody>
</table>

* Computed by integration of the centerband and spinning sidebands.
† Computed using $\alpha = 0.652$ and $\beta = 0.00785$.
§ Between the C1 and N sites.
measured ratio $\Delta S/S$ is very different from $\Delta S^{(1)}/S_0^{(1)}$ (see Table 1).

**EXPERIMENTAL DETAILS**

The REDOR experiment is a straightforward experiment to perform and analyze. The strength of the REDOR experiment is that it depends only on the dipolar interaction, which contains distance information through the $1/r^3$ dependence. However, it is important to pay close attention to the experimental details if accurate distances are to be measured. Some of the important experimental parameters for the REDOR experiment are the spinning speed control, the rf field intensities, proton decoupling power, proton decoupling offset, resonance offsets, and $\pi$ pulse phasing.

**Proton Decoupling**

Neither the strength nor the resonance offset of the proton rf field during proton decoupling has a direct influence on the outcome of the distance being measured by REDOR. However, this does not imply that it is not important to pay close attention to the proton decoupling strength and offset. In fact, the linewidth of a rare spin nucleus (such as $^{15}$N or $^{13}$C) is proportional to the square of the decoupler offset and inversely proportional to the square of the decoupling rf field strength (29). Consequently, the signal-to-noise ratio can be improved by carefully adjusting the decoupler offset and by using high decoupling rf field strengths.

Figure 9 shows the dependence of the $^{15}$N linewidth on decoupler offset for a proton decoupling field strength of 70 kHz (30). Clearly, the $^{15}$N linewidth increases as the magnitude of the offset increases. An important point is that although the REDOR ratio $\Delta S/S$ is not affected by the decoupler offset, the signal-to-noise ratio of the observe nucleus suffers because of the increased linewidth if the proton carrier is not set to resonance (nominally the water resonance). Results in Fig. 9 also suggest that it is important to use high decoupling powers. For example, a 110-kHz decoupling field applied at resonance provides a $^{15}$N linewidth about two-thirds that obtained with a 70-kHz decoupling field. Again, experimental time is significantly shortened using a higher decoupling field because the resulting narrower linewidth has a better signal-to-noise ratio.

**Spinning Speed Control**

The REDOR experiment uses $\pi$ pulse trains applied synchronously with the sample rotation. Hence, it is important that the spinning speed be relatively stable. It is easy to stabilize spinning speeds to within $\pm 2$ Hz of the desired spinning speed. Electronic circuits describing spinning speed controllers have been published (31, 32). Spinning speeds stabilized to within a fraction of 1 Hz of the desired spinning speed have been reported as well.
Figure 10 shows the effect of spinning speed stability on the REDOR ratio $\Delta S/S$. The results are for a pulse sequence timed for a spinning speed of 3205 Hz and the spinning speed intentionally misadjusted to generate the spinning speed misset. These data show that spinning speed missets of $\pm 2$ Hz from the ideal value do not effect the REDOR experiment. This is good since it is very easy to stabilize spinning speeds to within these fluctuations. However, larger spinning speed deviations from the ideal spinning speed must be avoided for quantitative work.

Aside from the electronic apparatus necessary to control the spinning speed, we have found it to be useful to dampen pressure surges that are typically associated with air compressors. For example, a typical reciprocating compressor will maintain the air pressure between two set points. When the high set point is reached, the compressor turns off. During this time, the air pressure drops to the low set point. When the low set point is reached, the compressor comes on. This sudden turning on of the compressor invariable sends a small pressure wave down the air lines which can cause a sample rotor to increase its speed by a small amount (about 1 Hz or so). We have found that by using several air ballast tanks with each stepped down in pressure effectively eliminates the pressure waves. We currently use a rotary valve compressor with an aftercooler. A rotary compressor stays on at all times and keeps the pressure between the two set points by adjusting the air intake valve to the compressor stage. The 100-gallon tank attached to the compressor is pressurized between 110 and 90 psi. This tank delivers air into a desiccant dryer. After the dryer, the air is delivered into a 60-gallon tank maintained at 80 psi, followed by a 60-gallon tank maintained at 60 psi, and finally followed by a 40-gallon tank regulated at 40 psi. This arrangement provides a very stable source of compressed air for our use.

**Pulse Imperfections**

Generally, a single $\pi$ pulse works reasonably well to invert the longitudinal magnetization (flip-spin orientations) provided that it is of the proper duration and that it is not applied too far from resonance. Figure 11 shows results of the dependence of the $^{13}$C $\Delta S/S$ on the flip angle of the single $^{15}$N pulse in a one rotor cycle $^{13}$C--$^{15}$N REDOR experiment. This experiment was designed to see how strongly the REDOR experiment is affected by pulse-length misset. As can be seen from the data and simulation, the one-rotor-cycle REDOR experiment is tolerant of pulse length missets of approximately $\pm 10\%$ from the ideal $\pi$ pulse length. It is experimentally easy to set $\pi$ pulse lengths within this range. Larger pulse length missets result in smaller values of $\Delta S/S$ because only a fraction of the $^{15}$N spins change their respective spin orientations for pulses deviating from a $\pi$ pulse. Those $^{13}$C spins coupled to $^{15}$N spins that do not change their spin state because of the pulse length misset will not dephase. Consequently, the $^{13}$C $\Delta S/S$ will be

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**Figure 10** Dependence of $^{13}$C $\Delta S/S$ on the misset of the spinning speed. The sample was the acetyl-$l$-carnitine sample described in Fig. 7. The data were obtained with the REDOR pulse sequence timing set for a spinning speed of 3205 Hz. The solid line is an eye guide.

**Figure 11** Dependence of $^{13}$C $\Delta S/S$ on the $^{15}$N flip angle for a one-rotor-cycle REDOR experiment. The sample is $[4-^{13}$C,amide-$^{15}$N]$l$-asparagine.
smaller when the $^{15}$N pulse deviates from a $\pi$ pulse. Thus, it is important to determine the proper $\pi$ pulse length prior to performing the REDOR experiment.

Even though a single-pulse, one-rotor-cycle REDOR experiment is tolerant of modest pulse errors, a train of same-phase $\pi$ pulses applied with a small pulse-length misset and/or applied off-resonance can result in a substantial errors in the REDOR experiment. Figure 12 shows $^{13}$C-$^{15}$N REDOR data taken with the original REDOR pulse sequence (see Ref. 14). Measurements of the $^{13}$C $\Delta S/S$ when all of the $^{15}$N pulses have the same phase show a strong dependence on the offset of the applied $^{15}$N pulses. There is a clear oscillation in the data. Clearly, unless this experiment is performed at exact resonance for the $^{15}$N nuclei, no quantitative REDOR results can be obtained. Performing the REDOR experiment with all of the $\pi$ pulses having the same phase is unacceptable. The offset dependence of $\Delta S/S$ for same-phased $\pi$ pulses indicates that there is a loss of longitudinal order of the $^{15}$N longitudinal magnetization when the $\pi$ pulses are applied with a resonance offset. That is, the $^{15}$N magnetization is not simply toggled by the $^{15}$N $\pi$ pulses in an up-down fashion synchronously with the sample rotation. Simulations shown in Fig. 13 show how the $z$ component of the magnetization does not simply toggle between plus and minus values here between $+1$ and $-1$ as the number of pulses increases for a pulse train with identically phased $\pi$ pulses. It is this loss of the simple toggling of the magnetization in an up-down fashion that causes the deviation of $\Delta S/S$ from its ideal value.

Fortunately, it is possible to eliminate pulse errors arising from resonance offset by using compensated $\pi$ pulse trains (34). Figure 12 also shows $^{13}$C-$^{15}$N REDOR data taken with $\pi$ pulses phased according to the $xy$-$4$ phasing scheme (35). In the $xy$-$4$ phasing scheme, the $\pi$ pulse phases alternate between $x$ and $y$ phase. With $xy$-$4$ phasing of the $^{15}$N $\pi$ pulses, the $^{13}$C $\Delta S/S$ is nearly independent of the $^{15}$N offset. Figure 13 shows that with $xy$-$4$ phasing of the pulses, the magnetization toggles between plus and minus values very effectively. This is why $xy$-$4$ phasing provides $\Delta S/S$ values independent of resonance offset. Typically, the $xy$-$8$ phasing of the $\pi$ pulses is used in REDOR experiments; the REDOR pulse sequence in Fig. 4 is shown with $xy$-$8$ phasing on both the S and I channels. Higher $xy$ phasing schemes may be necessary for very large numbers of $\pi$ pulses, but we find that $xy$-$8$ phasing is adequate for most of our work. Table 2 shows the $xy$-$4$ cycle and other $xy$ supercycles phase schemes.
Table 2 Phases of \( xy \)-4 Cycle and Supercycles

<table>
<thead>
<tr>
<th>Supercycle</th>
<th>rf phases</th>
</tr>
</thead>
<tbody>
<tr>
<td>( xy )-4</td>
<td>((xyxy)^n)</td>
</tr>
<tr>
<td>( xy )-8</td>
<td>((xyx yxy xyy xyx)^n)</td>
</tr>
<tr>
<td>( xy )-16</td>
<td>((xxy yxy yxy yxy yxy yxy xxy yxy xyx)^n)</td>
</tr>
<tr>
<td>( xy )-32</td>
<td>((xxy yxy yxy yxy yxy yxy yxy yxy yxy yxy)^n)</td>
</tr>
<tr>
<td>( xy )-64</td>
<td>((xxy yxy yxy yxy yxy yxy yxy yxy yxy yxy yxy yxy)^n)</td>
</tr>
</tbody>
</table>

CONCLUSIONS

The R\(E\)DOR experiment is useful for measuring distances between heteronuclear spin pairs. The experiment is straightforward to implement, requiring only high-power decoupling, \( xy \)-8 phase cycling, and stable spinning speed. Data analysis requires knowledge of the fraction of coupled spin pairs; once this fraction is known, the dipolar interaction between the coupled pair can be recovered from the universal \( \Delta S/S_0 \) curve. Dipolar dephasing of the observe spins depends only on the dipolar interaction.

If the fraction of coupled spin pairs is not known, it is possible to perform two-dimensional R\(E\)DOR experiments or several R\(E\)DOR experiments to obtain the dipolar interaction. Descriptions of these additional experiments can be found in Refs. 15 and 24. These references also contain more general details about the experiment.

REFERENCES

19. C. Fyfe, K. T. Mueller, H. Grondey, and K. C. Wongmoon, “Dipolar Dephasing between Quadrupolar and Spin-\(\frac{1}{4}\) Nuclei: R\(E\)DOR and T\(E\)DOR


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