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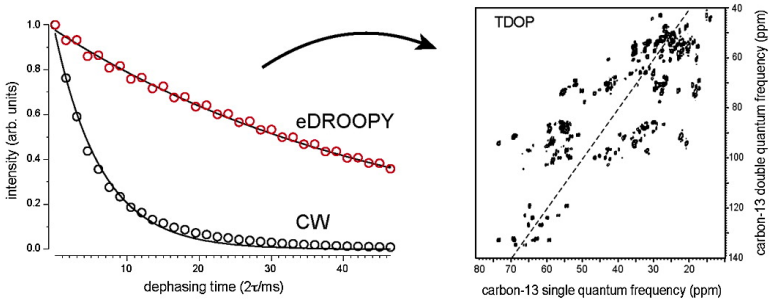

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Transverse Dephasing Optimized Solid-State NMR Spectroscopy

G. De Paëpe,[§] N. Giraud,[§] A. Lesage,[§] P. Hodgkinson,[‡] A. Böckmann,[†] and L. Emsley*,[§]

Laboratoire de Chimie, UMR 5182 CNRS/ENS, Laboratoire de Recherche Conventionné du CEA (no. 23V), Ecole Normale Supérieure de Lyon, 69364 Lyon, France, Department of Chemistry, University of Durham, South Road, Durham DH1 3LE, United Kingdom, and Institut de Biologie et de Chimie des Proteines, UMR 5086 CNRS, 69367 Lyon, France

Received July 11, 2003; E-mail: Lyndon.Emsley@ens-lyon.fr

Solid-state NMR spectroscopy is becoming a powerful tool to characterize a range of molecular systems as diverse as inorganic oxide glasses through plaque-forming protein systems.[1] The primary limitation to the application of solid-state NMR to more complex, or less concentrated, systems is sensitivity. This limitation is particularly acute for the multidimensional experiments that are the cornerstone of all the spectral assignment and structure determination methods. Sensitivity is routinely improved by cross-polarization and by spin decoupling, which concentrates the intensity into a narrow resonance. In many solids, however, decoupling only improves resolution and sensitivity up to a point where the remaining line width is dominated by susceptibility effects or chemical shift distributions.[2,3]

Here we show that even once the limiting line width has been reached, decoupling sequences continue to act strongly on the transverse dephasing times that determine the sensitivity of many multidimensional or multipulse experiments. We show that we can actively develop decoupling sequences that increase the coherence lifetimes in solids by up to a factor of 2. We then implement transverse dephasing optimized NMR for the disorded solid cellulose and for a microcrystalline protein, where we obtain sensitivity improvements of up to a factor of 5 in INADEQUATE spectra.

Figure 1a shows the resolution obtained using different heteronuclear decoupling sequences for powdered [^{13}C-2]glycine. We note that there is a remarkable improvement from continuous wave (CW) decoupling compared to the three more sophisticated schemes, SPINAL64,[4] TPPM,[5] or eDROOPY,[6] but that there is only a very small (almost negligible) difference in line width (and the corresponding decay time T_2^*) observed between the three latter sequences. Thus, one may think that the limit for decoupling has been reached in these organic systems.

In many solid-state NMR experiments, however, the preparation and mixing periods employ effective Hamiltonians that refocus linear interactions, and the relevant time constant is the coherence lifetime (T'_2). This is true in most dipolar recoupling experiments, notably REDOR,[7] as well as for experiments utilizing J couplings for coherence transfer.[8,9] We have recently observed large differences in these coherence lifetimes measured in MAS experiments using different decoupling sequences.[10] To understand this difference between T_2^* and T'_2, we consider the three contributions to residual broadening: (i) broadening due to transverse relaxation induced by *incoherent motions*, analogous to liquid-state NMR,[2] and by possible fluctuations in the proton dipolar network; (ii) broadening due to *coherent residuals* arising from the incomplete averaging of the dipolar interactions by magic-angle spinning and decoupling; and (iii) broadening due to *distributions of chemical shifts* arising from disorder, B_0 inhomogeneity, or magnetic susceptibility effects.[3] These three terms may contribute very differently to NMR experiments. If we consider the effect of a π pulse on the terms described above, the first term is unaffected, the second term may behave in a less predictable manner, and the third term is completely refocused.[9,11] As the first term (incoherent residuals) is expected to be extremely small in most solids, we expect the second term (coherent residuals) to dominate coherence lifetimes in solid-state MAS experiments. This is confirmed in Figure 1c, where we show the results of a spin−echo measurement for the sample of glycine, with a significant increase in coherence lifetimes on going from CW decoupling to either TPPM or SPINAL.

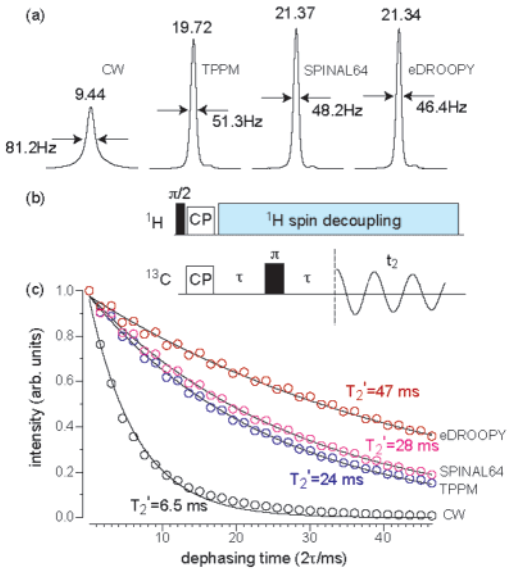

Figure 1. α-^{13}C resonance in [^{13}C-2]glycine recorded on a Bruker Avance 500-MHz spectrometer using four different decoupling schemes is shown in (a) with $\omega_1^H = 80$ kHz, $\omega_r = 10$ kHz. TPPM was implemented with $\beta = 158°$ and $\phi = 15°$. SPINAL is described in ref 4. The eDROOPY sequence is described in the text. (c) The transverse dephasing curves for the same sequences obtained using the simple spin−echo acquisition procedure shown in (b). The pulse sequences and phase cycles used in this work are available on our Web site[12] or upon request.

As a consequence of these observations, *it should be possible to develop decoupling pulse sequences specifically to increase coherence lifetimes*, either through analytical or semianalytical approaches[13] or by using the direct spectral optimization approach that we recently introduced.[6] In the latter approach, an iterative cycle is implemented on the spectrometer in which the response of the spin system is refined with respect to an ideal target. Previously, this target was simply the intensity of the peak in the normal 1D spectrum. But this approach can be adapted to obtain longer coherence lifetimes if the optimization is carried out on the peak intensity after a spin−echo. We have implemented this procedure (with $\tau = 15$ ms) using a pure phase modulation with the form $\phi = a\cos(\omega_c t)$, and the optimized parameters under the experimental conditions used to record the data shown in Figure 1 were found

[§] Ecole Normale Supérieure de Lyon.
[‡] University of Durham.
[†] Institut de Biologie et de Chimie des Proteines.

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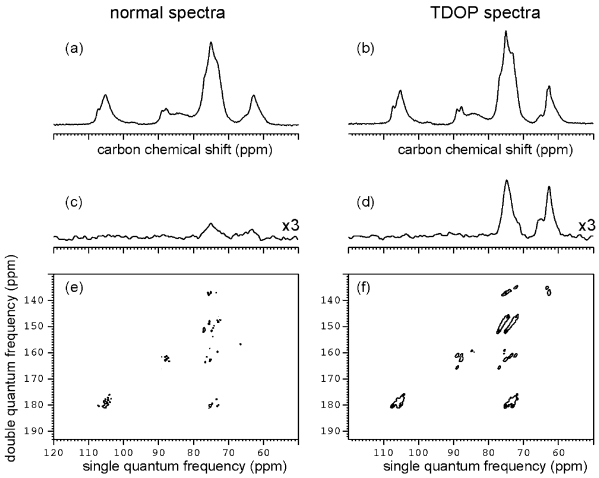

Figure 2. 1D ^{13}C CPMAS spectra of 10% [^{13}C]cellulose from wood obtained with (a) CW and (b) transverse-dephasing-optimized (TDOP) eDROOPY decoupling. Two-dimensional refocused INADEQUATE spectra are shown in (e) and (f) for the two decoupling sequences, and (c) and (d) show traces through the spectrum taken parallel to F2 at 137 ppm. The spectra were acquired with a 4-mm double-tuned CPMAS probe with a full rotor and with 160 scans for each of 256 increments in t_1, with $\omega_1^H = 100$ kHz, $\omega_r = 12.5$ kHz. The eDROOPY sequence was optimized at values of $a = 33.5°$ and $\omega_c = 110.3$ kHz.

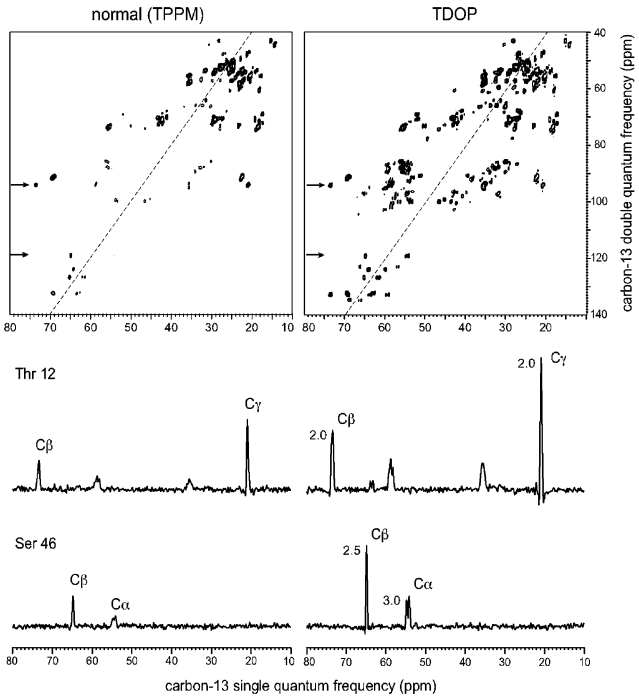

Figure 3. Aliphatic region of the 2D refocused INADEQUATE spectra of microcrystalline Crh, acquired with TPPM (left) and transverse-dephasing-optimized eDROOPY decoupling (right), with a 4-mm double-tuned CPMAS probe with a full rotor and with 64 scans for each of 700 increments in t_1, with $\omega_1^H = 75$ kHz, $\omega_r = 10$ kHz, and $T = -10$ °C. The eDROOPY sequence was optimized to have values of $a = 18.3°$ and $\omega_c = 86.4$ kHz. Below the 2D spectra are traces taken for two (typical) different residues at the frequencies indicated by arrows. The enhancement factors obtained with eDROOPY are indicated.

to be $a = 16.6°$ and $\omega_c = 97.2$ kHz. (A range of eDROOPY parameters for different conditions is given in the Supporting Information.) *The optimized sequence provides coherence lifetimes nearly a factor of 2 longer than the existing sequences (Figure 1c), although there is virtually no difference in the 1D spectra*. Notably, a CH_2 refocused line width as small as 7 Hz was measured using eDROOPY.

The effect of using decoupling sequences with optimized coherence lifetimes is shown in Figure 2 for a sample of 10% fully carbon-13-enriched cellulose.[14] The figure shows only a very small difference between 1D spectra recorded using CW and those recorded using the eDROOPY sequence derived for these conditions using glycine, since the spectrum is dominated by broadening due to structural disorder. However, there is a spectacular difference in sensitivity in 2D refocused INADEQUATE spectra[9] recorded using the two schemes. Under these conditions, transverse dephasing optimized eDROOPY provides nearly a factor of 5 improvement in the sensitivity of the spectrum.

Similarly, Figure 3 shows the effect on microcrystalline Crh, an 85-residue protein involved in carbon catabolite repression in *Bacillus subtilis* for which solid-state chemical shifts were assigned recently.[15] In this case, under moderate acquisition conditions, we see an average increase in sensitivity of over a factor of 2, with maximum enhancements of up to a factor of 5. Notably the Cα,Cβ cross-peaks and cross-peaks between side-chain carbons are very significantly enhanced. Even the correlations with the non-protonated carbonyl resonances are increased typically by a factor around 1.5. In this case, *the refocused line widths for the solid are actually narrower than those obtained in solution for the same protein*.

In conclusion, we have shown how the residuals that contribute to transverse dephasing times in solids can be coherently controlled, and that new decoupling schemes can be obtained which optimize coherence lifetimes. These schemes provide substantial sensitivity improvements. The methods are applicable to almost all types of samples and have been demonstrated on a disordered polymer system as well as a microcrystalline protein. The approach will yield improvements to most multipulse or multidimensional NMR experiments, including popular experiments such as REDOR[7] (where the REDOR curves will be extended in time and thus permit a more reliable measurement of longer distances), RFDR,[16] *J*-spectroscopy,[17] or any experiment where longer coherence lifetimes lead to better data.

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Supporting Information Available: Details of the optimization procedure, and parameters for a range of spinning speeds and decoupling frequencies for 4- and 2.5-mm probes (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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