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Water Suppression That Works. Excitation Sculpting Using Arbitrary Waveforms and Pulsed Field Gradients

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We propose a new method of selective excitation that delivers pure phase spectra with flat baselines, and that allows the spectroscopist to tailor the excitation into almost arbitrary profiles. In fact, we show how to convert *any sequence of pulses whatsoever* into such a "pure phase" sequence. This result may seem surprising, especially in view of the large number of papers on intricate excitation sequences without phase distortion, but we have verified that the idea is sound. While our technique is illustrated here with simple examples of solvent suppression, its merits in fact run far deeper: the general idea can be applied to virtually *every popular NMR experiment* for reasons quite different than mere solvent suppression, and can be used in addition to many of the existing methods. Our focus here is only to show the convenient elimination of the deleterious effects of the huge water resonance on the quality of the spectrum, using very simple pulse sequences. Based on the results obtained here, there will be little future interest in water-suppression methods that are tricky to set up, that involve trim pulses which have to be tediously optimized whenever a sample is changed or the probe is retuned, that introduce heavy phase rolls or baseline distortions across the spectrum, that present the low-field and high-field resonances with opposite polarity, that are sensitive to B_1 inhomogeneity, or that fail when radiation damping is present.

The pros and cons of the most popular water-suppression techniques have been recently reviewed in the literature (*1–3*). The methods fall into six related categories: (i) effective *saturation* of the water resonance by preirradiation (*4*), or by selective (*5*) or nonselective (*6*) inversion followed by an appropriate delay; (ii) design of frequency-selective pulse sequences, using either selective pulses (*7*) or hard pulses and appropriate delays (*8–11*), so that excitation of the water is avoided; (iii) *destruction* of the water magnetization by a B_0 or B_1 (*12*) field gradient, usually in conjunction with coherence-transfer pathway selection from a heteronucleus (*13, 14*), or by exploiting differences in spatial diffusion rates between water and a large molecule (*15*); (iv) destruction of the water magnetization by enhancing relaxation, by adding molecules that promote exchange (*16*), by adding transition metal complexes (*17*), or by isotopic substitution using oxygen-17 (*18*); (v) data-processing methods that attempt to filter or subtract out the water profile (*19, 20*); and (vi) selective detection methods using specially designed audio-frequency analog (*21*) or digital (*22*) filters. Many times several methods can be applied together.

The WATERGATE technique (*23*), one of the most promising techniques to emerge recently, relies on a refocusing pulse flanked by two symmetrical pulsed field gradients (PFGs) to attenuate the water resonance. This method evolved from the realization that echo techniques provided superior phase properties (*24*) compared with conventional selective excitation. The WATERGATE technique is restricted to refocusing elements that are *antisymmetric in time* or that have a net rotation axis that is stable as a function of offset. An antisymmetric pulse waveform with its center at $t = 0$ obeys $A(t) = A(-t)$ and $-(\phi_0 + \phi(-t)) = (\phi_0 + \phi(t))$ for the amplitude and phase, respectively, where ϕ_0 is an overall phase. Thus, both 1 1 ($\phi_0 = 0$) and 1 $\bar{1}$ ($\phi_0 = -\pi/2$) are in fact antisymmetric. An adiabatic frequency sweep, on the other hand, is not antisymmetric. Use of such a refocusing element will result in scrambled echo phases, even when a pair of strong symmetric gradients is used to select the refocused magnetization.

Our point of departure is a simple echo sequence employing a pulsed field gradient before and after the refocusing element. Normally one would use some kind of nominal 180° pulse to do the refocusing. For reasons that will become quite clear shortly, however, we solve the most general problem for a single spin neglecting relaxation. That is, let S represent the unitary transformation caused by any sequence of pulses of any phase, frequency, or amplitude modulation with or without delays, and consider the PFG echo sequence $G—S—G$. Assuming that the gradients are matched in amplitude and duration, and are sufficiently strong, the normalized signal intensity is

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$$M_\alpha = \frac{1}{2\pi}\int_0^{2\pi} \mathrm{Tr}\{I_\alpha \exp(-i\phi I_z) S \exp(-i\phi I_z)\sigma(0) \times \exp(i\phi I_z) S^\dagger \exp(i\phi I_z)\}\, d\phi, \quad [1]$$

where $\alpha = x, y, z$ and where $\sigma(0) = \mathbf{m}\cdot\mathbf{I}$ represents the arbitrary orientation of the magnetization immediately before the PFG echo. The transformation caused by S, whatever it is, can be represented by

$$S = \exp(-i\beta I_z)\exp(-i\theta I_y) \times \exp(-i\alpha I_x)\exp(i\theta I_y)\exp(i\beta I_z), \quad [2]$$

where each of the angles is understood to be some unspecified function of resonance offset, $\beta \equiv \beta(\Delta\omega)$, etc. We are interested in the net transformation of some input vector $\mathbf{m} = (m_x, m_y, m_z)$ to the output vector $\mathbf{M} = (M_x, M_y, M_z)$, as averaged by the gradient. We write this as $\mathbf{M} = \mathbf{T}\mathbf{m}$, where $\mathbf{T}$ is a 3×3 matrix. Carrying out the integration over ϕ, we find

$$\mathbf{T} = \begin{bmatrix} \cos^2\theta \sin^2(\alpha/2)\cos 2\beta & \cos^2\theta \sin^2(\alpha/2)\sin 2\beta & 0 \\ \cos^2\theta \sin^2(\alpha/2)\sin 2\beta & -\cos^2\theta \sin^2(\alpha/2)\cos 2\beta & 0 \\ 0 & 0 & \cos\alpha\cos^2\theta + \sin^2\theta \end{bmatrix}, \quad [3]$$

which immediately shows that z magnetization and transverse magnetization are not mixed by the echo sequence. We may thus consider them separately. $\mathbf{T}(3,3) = M_z/m_z$ shows the inversion performance of S as a function of offset. We can define P, the probability that a spin is flipped by S, according to the equation (*25*)

$$P = \frac{1}{2}\left(1 - \frac{M_z}{m_z}\right) = \frac{1}{2}(1 - \cos\alpha\cos^2\theta - \sin^2\theta) \equiv \cos^2\theta \sin^2(\alpha/2), \quad [4]$$

which we immediately recognize as the common term multiplying each of the other terms. That being the case, $\mathbf{T}$ simplifies to

$$\mathbf{T} = \begin{bmatrix} P\cos 2\beta & P\sin 2\beta & 0 \\ P\sin 2\beta & -P\cos 2\beta & 0 \\ 0 & 0 & 1-2P \end{bmatrix}. \quad [5]$$

Transverse magnetization is thus uniformly attenuated by a factor P, the probability that the spin is flipped by S; longitudinal magnetization is attenuated by $(1 - 2P)$. The phase shift 2β arises from an exact 180° rotation about the transverse components of the net rotation axis of S. If S is antisymmetric, or if S is designed such that the transverse components are nearly constant, then $\beta = 0$ and the magnetization has no unwanted phase shifts. This result should be fairly obvious if one imagines that the role of the gradients is to dephase all magnetization that does not receive a perfect 180° pulse.

Note, however, that $\mathbf{T}$ describes a transformation of any input magnetization, including magnetization to which $\mathbf{T}$ might have already been applied. This line of reasoning, and the convenient property that longitudinal and transverse magnetization do not get mixed by $\mathbf{T}$, allows us to remove the phase shift altogether for *any* sequence! We simply apply $\mathbf{T}$ again, noting that

$$\mathbf{T}^2 = \begin{bmatrix} P^2 & 0 & 0 \\ 0 & P^2 & 0 \\ 0 & 0 & (1-2P)^2 \end{bmatrix}. \quad [6]$$

Equation [6] is our central result. We may devise all kinds of pulse sequences simply by investigating the inversion profile: the phase is constant and zero at all offsets. The only requirement for the validity of Eq. [6] is that the gradient variable for the second application of $\mathbf{T}$ be uncorrelated with the first. In other words, we must not allow unwanted refocusing of magnetization "destroyed" by the first PFG sequence, which is easily accomplished if the gradient levels are under computer control. We find, then, for the sequence

$$G_1\text{—}S\text{—}G_1\text{—}G_2\text{—}S\text{—}G_2, \quad [7]$$

that $M_x = P^2 m_x$ and $M_y = P^2 m_y$. We conclude that a double PFG echo of any pulse sequence S whatsoever will return any transverse magnetization to its original position, attenuated by the square of the probability that a spin is flipped by S. It is not hard to see that we can continue the process as many times as we like. That, and the fact that S is arbitrary in the first place, leads to a virtually infinite number of interesting possibilities, all of which can be investigated by simply computing the spin-inversion profiles of S_1, S_2, etc., and then multiplying the probabilities P_1, P_2, etc., together. It is natural that only the probability of spin flips enters into the final result, as the gradients remove any memory of the phase of transverse magnetization, and two perfect 180° pulses constitute an effective 360° pulse, which again has no phase.

Hints that such pure phase selective excitation sequences might be possible have appeared in the literature, but to our knowledge *the generality of the approach has remained undiscovered.* For example, in devising pulse sequences for localized *in vivo* spectroscopy using a surface coil, the probability of a spin flipping was used to explain the attenuation

of magnetization at flip angles other than 180° (*26, 27*) when the refocusing pulse was phase cycled according to the Exorcycle (*28*) prescription. Exorcycle is, of course, entirely equivalent to using a strong gradient, the important distinction being that Exorcycle alone is useless for strong peak suppression because it relies on subtraction. Likewise, Wu *et al.* (*29*) used a 90°—180° sequence in a method that amounts to Exorcycle and commented on the lack of any phase shifts in the resulting excitation. Their method, once again, relies on difference spectroscopy and presumes that there is no longitudinal magnetization present at the time of the refocusing pulse, so that two of the Exorcycle steps can be eliminated. In addition, it has been known for some time that phase shifts induced by the B_1 dependence of the net rotation axis of an otherwise perfect composite 180° rotation can be canceled on the even-numbered echoes of a multiple-echo train (*30*). Finally, the expression $P = \frac{1}{2}(1 - M_z/m_z)$ can be implemented by starting from equilibrium magnetization and applying the sequence (S—G) on every other scan and following with a read pulse. The same profile is obtained as with our method, with no phase distortion, but subtraction is again required. If S is a DANTE sequence then the equivalent of the DANTE-Z method (*31*) is the result.

With an infinite number of possibilities for S, we can create very complex profiles at will. For the purposes of this Communication, however, we will restrict ourselves to two families of sequences. The first uses only hard pulses and delays, and is based on the idea that the popular binomial solvent-suppression sequences can instantly be adapted to our scheme by doubling the flip angles of each of the constituent pulses, and preceding the sequence with a conventional hard 90° pulse. Thus, 1 $\bar{1}$ or 45°_x—τ—45°_x becomes simply 90°_x—G—90°_x—τ—90°_x—G. The spectral peaks are all in positive absorption with no phase roll (except that from the first hard pulse), and the water is greatly attenuated. This sequence is similar to the "1 1 echo" sequence of Sklenar and Bax (*24*), with the substitution of a nonselective 90° pulse for the first element and the use of gradients rather than Exorcycle to select the refocused magnetization. The second set of sequences is a completely new scheme based on the simple sequence 90°_x—G—soft 180°_x—180°_x—G. We use this sequence to illustrate the phase properties of a double PFG echo applied to a non-antisymmetric sequence.

Spectra were acquired at 500 MHz using a Varian UnityPlus spectrometer equipped with a Varian pulsed-field-gradient triple-resonance probe and Varian gradient driver amplifier. The sample was a 20 mM solution of Nowick's 1,2-diaminoethane diurea molecular scaffold **1** (*32*), shown in Fig. 1, in 50% dioxane-d_8 and 50% H_2O with 10 mM aqueous phosphate buffer at pH 5.0 and 23°C. All spectra are the result of only four scans, using CYCLOPS phase cycling to remove possible receiver artifacts, superimposed on Exorcycle phase cycling of the first PFG echo in the case

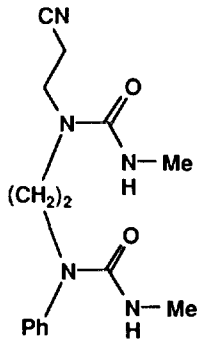

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FIG. 1. The molecule **1** under study, a "molecular scaffold" that can be used to form parallel β-sheet structures.

of the gradient spectra. Extensive time averaging and phase cycling can be used to improve the appearance of spectra by masking instrumental instabilities, and so we avoid it. For clarity, only the region ±700 Hz (~3 ppm) around the water resonance is shown. The transmitter frequency was set to the water line, and a large spectral width of 10 kHz used to minimize any phase shifts attributable to the audiofrequency filters. The 1 $\bar{3}$ 3 $\bar{1}$ sequence requires a linear phase correction; the constant and linear phase correction controls were essentially constant for the gradient spectra once the first spectrum was phased. No baseline correction or any other cosmetic routines, aside from a 1 Hz line broadening to enhance sensitivity, were used. All spectra except those in Figs. 2a and 2b were taken with identical receiver gain.

The first two spectra (Figs. 2a and 2b) serve to establish the scale of the H_2O resonance. The water signal is strong enough to cause radiation damping, as evidenced by a 12 Hz linewidth and self-regenerative FIDs after a single 180° pulse. Figure 2c shows the spectrum obtained after presaturation for 5 s ($\gamma B_2/2\pi$ = 100 Hz). The water line is attenuated by about 700 times. Figure 2d shows that 1 $\bar{3}$ 3 $\bar{1}$ with an interpulse delay of 500 μs results in far better suppression, at the cost of some baseline roll due to the linear phase correction that needs to be applied, and some loss of intensity in the desired nearby resonances.

The two hard pulse sequences 90°_x—G_1—90°_x—τ—$90^\circ_{\bar{x}}$—G_1 and 90°_x—G_1—90°_x—τ—$90^\circ_{\bar{x}}$—G_1—G_2—90°_x—τ—$90^\circ_{\bar{x}}$—G_2 (Figs. 3a and 3b) with τ = 500 μs deliver excellent suppression without the phase roll and with no optimization at all. Only modest gradients G_1 = 10.7 G/cm and G_2 = 3.1 G/cm were used, for 1 ms durations in each case. The PFG double-echo sequence results in substantial loss of intensity for lines close to the water due to the gradual spin-flip profile of the 90°_x—τ—$90^\circ_{\bar{x}}$ sequence used as an inversion sequence.

The excitation profile can be sharpened up either by introducing further pulses and delays, or by introducing a soft

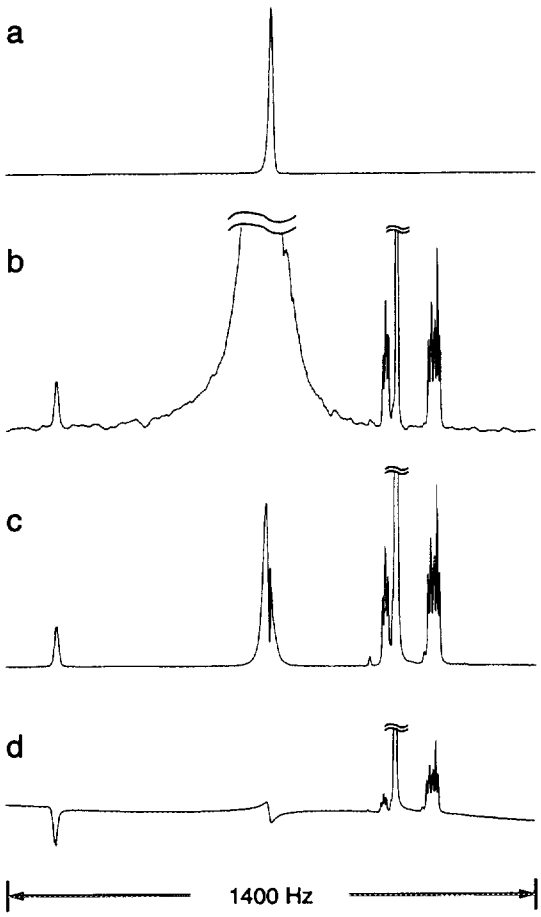

FIG. 2. Spectra obtained with a 20 mM solution of **1** in 50% dioxane-d_8 and 50% H_2O at pH 5.0 and 23°C. (a) Conventional 9.5 μs 90° read pulse, with receiver attenuation to avoid overloading the ADC. (b) Same spectrum as (a) with the vertical scale expanded 1000 times. (c) Spectrum obtained with presaturation for 5 s ($\gamma B_2/2\pi$ = 100 Hz) giving >700 suppression. (d) Spectrum obtained with the 1 $\bar{3}$ 3 $\bar{1}$ sequence, τ = 500 μs giving >7000 suppression. A linear phase correction had to be applied, resulting in some baseline roll. The upfield triplet closest to the water has been noticeably attenuated. The absolute intensity scale is the same as in (c).

pulse. To illustrate the phase properties of the PFG double echo we employ a refocusing element S = [soft 180°_x hard $180^\circ_{\bar{x}}$], where the soft square pulse of 2.2 ms is applied at the water resonance frequency. This pulse train roughly inverts everything except the water. There is a slight delay needed for the power switching. The spectrum obtained with this sequence in Fig. 3c shows significant water suppression, but is unusable on account of the wild phase behavior. However, the double application of S, including the switching delay, leads to Fig. 3d, where the spectrum has, as predicted, phased *itself*.

Our calculations assume essentially infinite gradients and assume that recovery after the gradient is instantaneous. However, because the sequence is made up of echoes anyway, there is no problem introducing minor delays in the millisecond range to let the B_0 field settle down. The actual gradient strengths needed to get good results are not excessively large, and the second pair of gradients can be significantly weaker than the first, making the technique less demanding with regard to the gradient recovery time. The sequences we have shown here are illustrative only. We will turn our attention to optimized practical schemes elsewhere.

The property that magnetization is almost magically returned to its starting point makes the double PFG echo method attractive for use in almost every conceivable experiment. It also renders many of the pulse-shaping methods obsolete, particularly long excitation sequences which try, approximately, to produce pure in-phase magnetization. We achieve pure in-phase magnetization in a single scan, and with an amplitude as a function of frequency that is limited only by the sequences S_i used to form the echoes. The mul-

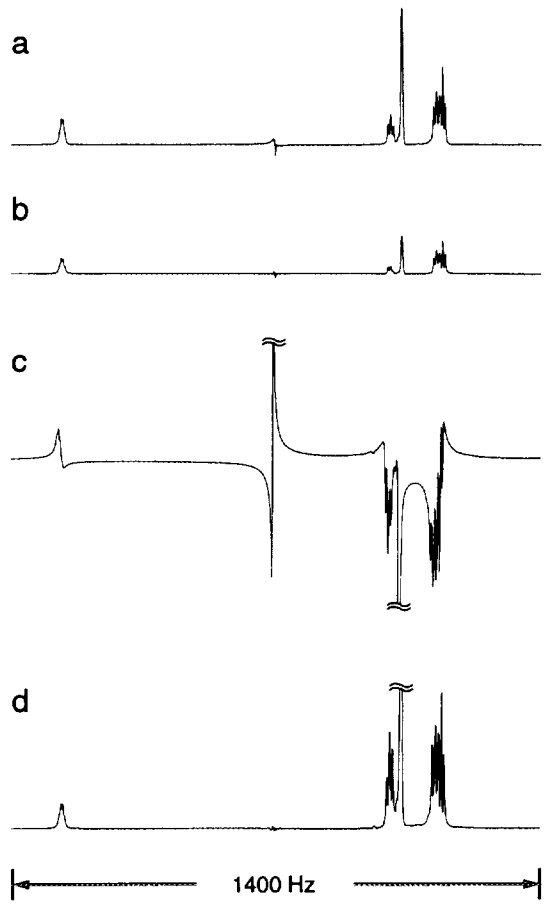

FIG. 3. Spectra obtained with PFG echo and double-echo sequences, using the indicated sequences S between the gradient pairs. All spectra are on the same absolute intensity scale as Figs. 2c and 2d. (a) Single PFG echo, $S = 90^\circ_x$—τ—$90^\circ_{\bar{x}}$, τ = 500 μs. The suppression is >9000. (b) Same as (a) but with double PFG echo. The suppression is >25,000, but there is appreciable signal loss of the nearby resonances. (c) Same as (a) with S = soft—180°_x—hard $180^\circ_{\bar{x}}$. The suppression is >400, but the phase is terrible. (d) Same as (c) using a double echo. The suppression is >30,000 and the phase is well behaved. Note the good preservation of the solute peak intensities.

tiplicative nature of the technique is far less demanding of the transmitter linearity, and complex profiles can be built up in a step-by-step fashion, much like a sculptor discards unwanted pieces of marble to reveal a sculpture. We propose the general name *excitation sculpting* for this kind of technique. Excitation sculpting can be applied to other selective-excitation problems, as we show elsewhere, including some situations where evolution under J coupling cannot be neglected. Excitation sculpting makes it possible to consider hyperbolic secant pulses (*33*), frequency sweeps (*34*), and other untried methods in constant-phase band-select or band-reject applications. It is not necessary to set the transmitter frequency to the water resonance line for these methods to work, and pulse imperfections of almost every conceivable variety are tolerated. We will report on the results of such experiments, including the effects of finite relaxation times, in a more complete description of this work.

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REFERENCES

1. P. J. Hore, *Methods Enzymol.* **176,** 64 (1989).
2. M. Gueron, P. Plateau, and M. Decorps, *Prog. NMR Spectrosc.* **23,** 133 (1991).
3. V. Sklenar, *Basic Life Sci.* **56,** 63 (1990).
4. D. I. Hoult, *J. Magn. Reson.* **21,** 337 (1976).
5. R. K. Gupta, *J. Magn. Reson.* **24,** 461 (1976).
6. S. L. Patt and B. D. Sykes, *J. Chem. Phys.* **56,** 3182 (1972).
7. A. G. Redfield, S. D. Kunz, and E. K. Ralph, *J. Magn. Reson.* **19,** 114 (1975).
8. P. Plateau and M. Gueron, *J. Am. Chem. Soc.* **104,** 7310 (1982).
9. V. Sklenar and Z. Starcuk, *J. Magn. Reson.* **50,** 495 (1982).
10. D. L. Turner, *J. Magn. Reson.* **54,** 146 (1983).
11. P. J. Hore, *J. Magn. Reson.* **55,** 283 (1983).
12. D. Canet, J. Brondeau, E. Mischler, and F. Humbert, *J. Magn. Reson. A* **105,** 239 (1993).
13. A. Bax and S. S. Pochapsky, *J. Magn. Reson.* **99,** 638 (1992).
14. R. E. Hurd and B. K. John, *J. Magn. Reson.* **91,** 648 (1991).
15. P. C. M. Van Zijl and C. T. W. Moonen, *J. Magn. Reson.* **87,** 18 (1990).
16. D. L. Rabenstein, S. Fan, and T. T. Nakashima, *J. Magn. Reson.* **64,** 541 (1985).
17. R. G. Bryant and T. M. Eads, *J. Magn. Reson.* **64,** 312 (1985).
18. M. Magnuson and B. M. Fung, *J. Magn. Reson.* **99,** 301 (1992).
19. Y. Kuroda, A. Wada, T. Yamazaki, and K. Nagayama, *J. Magn. Reson.* **88,** 141 (1990).
20. O. Gonen and G. Johnson, *J. Magn. Reson. B* **102,** 98 (1993).
21. T. E. Skinner, J. Pruski, and P. M. L. Robitaille, *J. Magn. Reson.* **98,** 604 (1992).
22. K. J. Cross, *J. Magn. Reson. A* **101,** 220 (1993).
23. M. Piotto, V. Saudek, and V. Sklenar, *J. Biomol. NMR* **2,** 661 (1992).
24. V. Sklenar and A. Bax, *J. Magn. Reson.* **74,** 469 (1987).
25. A. J. Shaka and R. Freeman, *J. Magn. Reson.* **63,** 596 (1985).
26. M. R. Bendall and R. E. Gordon, *J. Magn. Reson.* **53,** 365 (1983).
27. A. J. Shaka and R. Freeman, *J. Magn. Reson.* **59,** 169 (1984).
28. G. Bodenhausen, R. Freeman, and D. L. Turner, *J. Magn. Reson.* **27,** 511 (1977).
29. X. L. Wu, P. Xu, and R. Freeman, *J. Magn. Reson.* **83,** 404 (1989).
30. M. H. Levitt and R. Freeman, *J. Magn. Reson.* **43,** 65 (1981).
31. D. Boudot, D. Canet, J. Brondeau, and J. C. Boubel, *J. Magn. Reson.* **83,** 428 (1989).
32. J. S. Nowick, M. Abdi, K. A. Bellamo, J. A. Love, E. J. Martinez, G. Noronha, E. M. Smith, and J. W. Ziller, *J. Am. Chem. Soc* **117,** 89 (1995).
33. M. S. Silver, R. I. Joseph, and D. I. Hoult, *Phys. Rev. A* **31,** 2753 (1985).
34. J. Baum, R. Tycko, and A. Pines, *Phys. Rev. A* **32,** 3235 (1985).