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An Application of Pulse-Gradient Double-Quantum Spin Echoes to Diffusion Measurements on Molecules with Scalar-Coupled Spins

LEWIS E. KAY* AND J. H. PRESTEGARD†

*Department of *Molecular Biophysics and Biochemistry and †Chemistry,*
Yale University, New Haven, Connecticut 06511

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The theory and an application of double-quantum coherence for the measurement of diffusion of a molecule containing a scalar-coupled ^{19}F spin system, 1,1,3-trichloro-2,2,3-trifluorocyclobutane, is presented and discussed. It is shown that the technique is capable of measuring diffusion of molecules in viscous media with application of only modest field gradients. T_2 relaxation studies, showing multiple-quantum T_2's to be slightly longer than one-quantum T_2's, suggest a possible sensitivity advantage of the multiple-quantum method over the more conventional spin-echo diffusion experiment. © 1986 Academic Press, Inc.

Pulse-gradient NMR has long been recognized as a useful means of measuring diffusion of small molecules in both simple and complex fluids (*1–3*). Labeling and spatial resolution of molecules is accomplished by rf pulse perturbation of magnetic nuclei in the molecule of interest and application of linear field gradients which make Larmor precessional frequencies for the nuclei a function of position in the sample. This method offers advantages in that diffusion is measured in the absence of concentration gradients, in that the movement of specifically spin-labeled molecules can be followed, and in that diffusion within small homogeneous regions of otherwise heterogeneous media can be measured. Despite these advantages, most measurements have been confined to relatively simple, highly fluid systems (*1–4*). In part, this is because of experimental difficulties in applying the high gradients necessary for measurement of small diffusion constants while maintaining the resolution and sensitivity necessary to focus on a particular molecular species.

Recently, the application of gradients during the evolution of multiple-quantum, as opposed to single-quantum, coherence has been proposed as a means of reducing requirements for high-field gradients (*5, 6*). The effects of gradients are predicted to scale up by n^2, where n is the order of the coherence. Double-quantum coherence is thus four times as sensitive to the effects of a field gradient as single-quantum coherence. Use of double-quantum coherence requires coupled spin systems with well resolved one-quantum transitions which can be differentially excited to produce multiple-quantum coherence. In applications to date, this resolution has been provided by dipolar coupling between pairs of spin-$\frac{1}{2}$ nuclei in ordered media (*5, 6*). The expected enhancement of the effects of field gradients has been realized and the general idea of using multiple-quantum coherence would seem reasonable. However, systems of interest are not always easily oriented and do not always have pairs of strongly dipolar coupled nuclei.

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In this manuscript we present an application which makes use of scalar coupling rather than dipolar coupling to overcome some of these restrictions. The nuclei to be monitored are ^{19}F nuclei in the molecule 1,1,3-trichloro-2,2,3-trifluorocyclobutane. The gem difluoro pair in this molecule presents a particularly large scalar coupling, 200 Hz, and the inherent asymmetry of the gem difluoro site produces a near-first-order spectrum at moderate field strengths. Measurement of diffusion constants for this molecule is of more than passing interest because the molecule is closely related to a number of fluorinated molecules used as general anesthetics and their movement in model and biological membranes is of some importance (*7, 8*). We will outline the theory necessary to understand the diffusion measurements and show that diffusion can be measured in viscous media (ethylene glycol) with application of modest field gradients.

THEORY

The pulse sequence and phase cycling to be used in these studies are presented in Fig. 1 and Table 1, respectively. For simplicity we will consider only the first line of the phase-cycling scheme. The sequence can be analyzed in three parts. The first part, consisting of the 90°_x–τ–180°_x–τ–90°_x rf pulse sequence at the ^{19}F resonance frequency, produces multiple-quantum coherence. The 180°_x pulse in the middle allows for uniform excitation in the presence of different chemical shifts and ^{1}H–^{19}F couplings. The second part, consisting of a 180°_x rf pulse in the middle of the time period, $2 \times t_1$, produces a multiple-quantum echo attenuated by the application of field gradients of length δ and movement of molecules during the delay Δ. This 180° pulse also refocuses heteronuclear couplings and allows us to view our molecule as containing only an AMX system of ^{19}F spins. The third part, consisting of a 90°_x pulse and acquisition during t_2, mixes coherence remaining at the peak of the multiple-quantum echo into observable transverse magnetization which can be Fourier transformed to a frequency domain and areas of peaks interpreted in terms of echo amplitudes.

The effect of each part of the sequence on the ^{19}F AMX spin system of 1,1,3-trichloro-2,2,3-trifluorocyclobutane is conveniently described in terms of the product

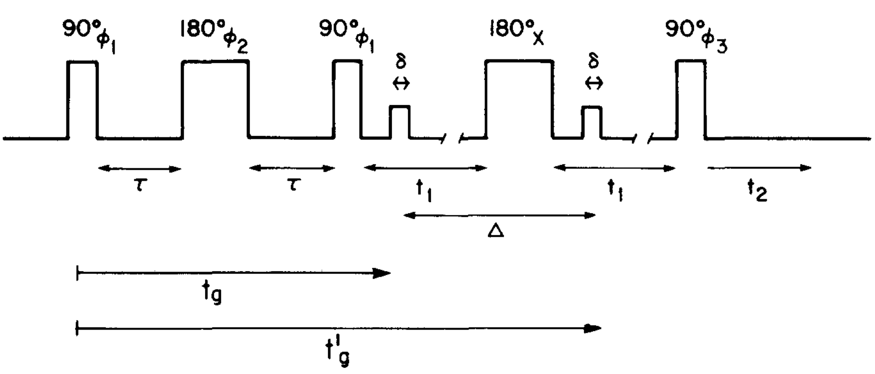

FIG. 1. Double-quantum spin-echo pulse sequence used to measure translational diffusion. An echo occurs at $2 \times t_1$ with an amplitude related to the diffusion constant, *D*.

TABLE 1

Phase Cycling Employed in the Selection of Double-Quantum Spin Echoes

ϕ_1	ϕ_2	ϕ_3	Receiver phase
x	x	x	x
y	y	x	$-x$
$-x$	$-x$	x	x
$-y$	$-y$	x	$-x$
x	x	$-x$	$-x$
y	y	$-x$	x
$-x$	$-x$	$-x$	$-x$
$-y$	$-y$	$-x$	x

operator formalism of Sørensen *et al.* (*9*) and Van De Ven *et al.* (*10*). The excitation sequence, 90°_x–τ–180°_x–τ–90°_x, has been described previously in the literature and leads at the end of 2τ to the following expression for the density matrix (ρ):

$$\frac{1}{2}\sum_{\substack{i,j,k=1\\ i\neq j\neq k}}^{3}[I_{zi}\cos(2\pi J_{ij}\tau)\cos(2\pi J_{ik}\tau) + 2I_{xi}I_{yk}\cdot 2\cos(2\pi J_{ij}\tau)\sin(2\pi J_{ik}\tau) - 4I_{zi}I_{yj}I_{yk}\sin(2\pi J_{ij}\tau)\cdot\sin(2\pi J_{ik}\tau)]. \quad [1]$$

In this expression J_{ij} refers to the coupling between spins i and j and I_{qi} ($q = \{x, y, z\}$) is the projection of the spin angular momentum of spin i along q. ($i = 1, 2$ refers to the geminal fluorines and $i = 3$ refers to the vicinal ^{19}F in this molecule.) Analogous expressions are obtained with other phase combinations as represented in Table 1.

The linear combinations of these expressions will result only in the retention of components proportional to double-quantum coherence defined as follows:

$$\{2QT_y\}_{ij} = 1/2\{2I_{xi}I_{yj} + 2I_{xj}I_{yi}\}. \quad [2]$$

In general, coherences involving all ij combinations will be retained but these are weighted by factors such as $\cos\{2\pi J_{ij}\tau\}$ and $\sin\{2\pi J_{ij}\tau\}$ with $i, j = \{1, 2, 3\}$. Since we choose $\tau = 1/(4J_{12})$ and since $J_{12} \gg J_{13}, J_{23}$, we can focus on a component of $\rho(2\tau)$ written as follows:

$$\rho_{2QT}(2\tau) \propto 2\left[\cos\left[\frac{\pi J_{13}}{2J_{12}}\right] + \cos\left[\frac{\pi J_{23}}{2J_{12}}\right]\right]\{2QT_y\}_{1,2}. \quad [3]$$

Analysis of the evolution of magnetization during the period 2 × t_1 is simplified by the fact that at $t = 2 \times t_1$ magnetization evolving as I_z, which includes chemical shift as well as both heteronuclear dipolar and heteronuclear scalar couplings, will be refocused. Thus, neglecting relaxation as well as the above effects, the rotating frame Hamiltonian (in radians) for the period 2 × t_1 reduces to:

$$\begin{aligned}\hat{\mathscr{H}} &= 2\pi \sum_{\substack{k\\ k\neq m}} J_{mk} I_{zm} I_{zk}, \quad 2\tau < t < t_g, \quad t_g + \delta < t < t'_g, \quad t'_g + \delta < t < 2t_1 + 2\tau \\ \hat{\mathscr{H}} &= \sum_{k=1}^{3} -\gamma \mathbf{r}_k \cdot \nabla \mathbf{H} I_{zk} + 2\pi \sum_{\substack{k\\ k\neq m}} J_{mk} I_{zm} I_{zk}, \quad t_g < t < t_g + \delta, \quad t'_g < t < t'_g + \delta.\end{aligned} \qquad [4]$$

In expression [4] J_{mk} refers to spin coupling between "passive" nucleus m and nucleus k actively involved in the two quantum transition, ∇ is the "del" operator, $\mathbf{r}$ denotes the position of a spin relative to some convenient origin and t_g and t'_g are the times of application of the first and second gradient pulses, respectively. The geometry of the gradient coils in our probe is such that the gradients are applied in the z direction and thus $\mathbf{r} \cdot \nabla \mathbf{H}$ can be simplified to

$$\frac{z\partial H_z}{\partial z} \equiv zg.$$

The effect of the Hamiltonian described in Eq. [4] on the density matrix given in Eq. [3] during interval t, $2\tau < t < 2\tau + t_1$, is given by

$$R[F(J)\{2QT_y\}_{1,2}]R^{-1} \qquad [5]$$

where

$$R = \exp(i2\gamma g z_1 \delta \sum_{k} I_{zk}) \exp(-\pi i \sum_{\substack{k\\ k\neq m}} J_{mk} 2 I_{zm} I_{zk} t_1)$$

and

$$F(J) = 2\left[\cos\left[\frac{\pi J_{13}}{2J_{12}}\right] + \cos\left[\frac{\pi J_{23}}{2J_{12}}\right]\right].$$

In this expression, δ denotes the duration of the gradient pulse and z_1 is the position of the diffusing spin at the time of application of the gradient pulse. We assume that δ is short compared to Δ and that no movement occurs during δ. Subsequent application of a 180°_x refocusing pulse followed by evolution during the second t_1 interval yields, after some simplification,

$$R'[-F(J)\{2QT_y\}_{1,2}]R'^{-1} \qquad [6]$$

where

$$R' = \exp(-i2\gamma g(z_2 - z_1)\delta \sum_{k} I_{zk}) \exp(-\pi i \sum_{\substack{k\\ k\neq m}} J_{mk} 2 I_{zm} I_{zk} 2t_1)$$

and z_2 is the position of the diffusing spin at the time of application of the second gradient pulse. Equation [6] can be simplified to give

$$\begin{aligned}\rho(2\tau + 2t_1) \propto{} & -F(J)\cos\{2\pi(J_{13} + J_{23})t_1\}[\{2QT_y\}_{1,2}\cos\{2\gamma g(z_2 - z_1)\delta\} \\ & - \{2QT_x\}_{1,2}\sin\{2\gamma g(z_2 - z_1)\delta\}] + F(J)\sin\{2\pi(J_{13} + J_{23})t_1\} \\ & \times [\{2QT_x\}_{1,2}\cos\{2\gamma g(z_2 - z_1)\delta\} + \{2QT_y\}_{1,2}\sin\{2\gamma g(z_2 - z_1)\delta\}] \cdot 2I_{z3}\end{aligned} \qquad [7]$$

where $\{2QT_x\} = 1/2\{2I_{x1}I_{x2} - 2I_{y1}I_{y2}\}$. Application of the final $(\pi/2)_x$ pulse gives rise to components of one-quantum coherence:

$$\rho_{1Q} \propto -1/2F(J)\cos\{2\pi(J_{13} + J_{23})t_1\}\cos\{2\gamma g(z_2 - z_1)\delta\}\{2I_{x1}I_{z2} + 2I_{x2}I_{z1}\}$$
$$+ 1/2F(J)\sin\{2\pi(J_{13} + J_{23})t_1\}\cos\{2\gamma g(z_2 - z_1)\delta\}\{4I_{y3}I_{z1}I_{z2}\}. \quad [8]$$

The second term in Eq. [8] is proportional to y magnetization of spin 3 out of phase with both spins 1 and 2. Since the coupling between spin 3 and the other spins is small and since the resolution afforded by our gradient probe is only $\approx$30 Hz we are unable to detect the antiphase multiplet corresponding to this term. We, therefore, focus only on the first term in this expression:

$$\rho_{1Q} \propto -1/2F(J)\cos\{2\pi(J_{13} + J_{23})t_1\}\cos\{2\gamma g(z_2 - z_1)\delta\}\{2I_{x1}I_{z2} + 2I_{x2}I_{z1}\}. \quad [9]$$

For an ensemble of diffusing molecules this term must be weighted by the probability of a molecule diffusing from (x_1, y_1, z_1) to (x_2, y_2, z_2) in the time between application of the two gradient pulses, Δ. The probability distribution is a solution to the diffusion equation

$$\frac{\partial P}{\partial t} = D\nabla^2 P \quad [10]$$

where $P(x_1, y_1, z_1|x_2, y_2, z_2, \Delta)$ is the conditional probability of a molecule originally at (x_1, y_1, z_1) diffusing to (x_2, y_2, z_2) in time Δ and D is the diffusion constant. The solution to Eq. [10] for the isotropic three-dimensional case at hand is

$$P(x_1, y_1, z_1|x_2, y_2, z_2, \Delta) = \frac{1}{(4\pi D\Delta)^{1.5}}\exp(-(\mathbf{r}_2 - \mathbf{r}_1)^2/4D\Delta) \quad [11]$$

where $\mathbf{r}_i = (x_i, y_i, z_i)$ and the result of averaging over the dimensions of the sample gives

$$\rho_{1Q} \propto \iiint_{-\infty}^{\infty} P(x_1, y_1, z_1|x_2, y_2, z_2, \Delta)\cos\{2\gamma g\delta(z_2 - z_1)\}$$
$$\times\, d(x_2 - x_1)d(y_2 - y_1)d(z_2 - z_1) = \exp(-\gamma^2 4g^2\delta^2 D\Delta) \qquad \Delta \gg \delta. \quad [12]$$

For the case where $\Delta \gg \delta$ does not hold, Δ is to be replaced by $(\Delta - 1/3\delta)$ in analogy with the spin-echo case (*1*).

Finally, magnetization evolves in t_2 according to

$$\rho_{1Q} \propto -F(J)\cos\{2\pi(J_{13} + J_{23})t_1\}\exp(-\gamma^2 4g^2\delta^2 D\Delta)$$
$$\times \sum_{\substack{i,j=1 \\ i\neq j}}^{2} [1/2\cos(\pi J_{i3}t_2)\cos(\pi J_{12}t_2)\cdot(2I_{xi}I_{zj}\cos(\Delta\omega_i t_2) + 2I_{yi}I_{zj}\sin(\Delta\omega_i t_2)$$
$$+1/2\cos(\pi J_{12}t_2)\sin(\pi J_{i3}t_2)\cdot(4I_{z3}I_{zj}I_{yi}\cos(\Delta\omega_i t_2) - 4I_{z3}I_{zj}I_{xi}\sin(\Delta\omega_i t_2))$$
$$+1/2\sin(\pi J_{12}t_2)\cos(\pi J_{i3}t_2)\cdot(I_{yi}\cos(\Delta\omega_i t_2) - I_{xi}\sin(\Delta\omega_i t_2))$$
$$-1/2\sin(\pi J_{12}t_2)\sin(\pi J_{i3}t_2)(2I_{xi}I_{z3}\cos(\Delta\omega_i t_2) + 2I_{yi}I_{z3}\sin(\Delta\omega_i t_2))] \quad [13]$$

where we have neglected the effects of heteronuclear couplings. Thus, the magnitudes of the resonances at ω_1 and ω_2 are proportional to $F(J)\cos\{2\pi(J_{13} + J_{23})t_1\} \times \exp(-\gamma^2 4g^2\delta^2 D\Delta)$.

The dependence of two-quantum magnetization on the gradient is identical to that predicted by Eq. [1] of Martin *et al.* (*5*). However, our Eq. [13] shows an additional amplitude modulation by $\cos\{2\pi(J_{13} + J_{23})t_1\}$. This is to be expected for two-quantum coherence in a three-spin system. Its presence in normal two-quantum spectroscopy provides the interesting possibility of determining the relative signs of coupling constants. In diffusion experiments the J modulation is an undesirable complication since it requires choosing t_1 delays that optimize echo amplitudes. The effects are, however, less complicated than J modulation for one-quantum spin echoes (*11*) and can be eliminated entirely by observing triple-quantum coherence.

EXPERIMENTAL

All spectra were acquired on a Bruker CXP 200 spectrometer operating in the Fourier transform mode. The spectrometer was equipped with a Bruker pulse-gradient unit (B-KR 300 Z 18) and a 7 mm, high-power, gradient probe. The probe was constructed to provide gradients up to ≈400 G/cm in the z direction and employs a Helmholtz coil to give a 7 μs 90° pulse with approximately 1000 V peak to peak rf input. A Bruker pulse programmer and associated software were employed to generate appropriate rf pulse sequences and a trigger for the pulse gradient unit. The temperature for the experiments was maintained at either 298 or 293 K using a Bruker VT regulator unit.

The gradient was calibrated using the known diffusion constant of water (*12*) and the spin-echo pulse sequence. A conventional spin-echo pulse gradient experiment was also run for comparison with the data obtained using multiple-quantum methods. In the spin-echo experiment typically four transients were summed and the FID was Fourier transformed starting at 2τ. Spectra were magnitude corrected ($\sqrt{(\text{real})^2 + (\text{imaginary})^2}$) to avoid some of the problems of complex J modulation in the trifluorocyclobutane case and 2τ was chosen to minimize 60 Hz noise. The area under the peaks in the transformed spectra was calculated in the presence and absence of a field gradient as a function of g. D was then calculated by a least-squares linear regression computer program using the Stejskal-Tanner Eq. [1]. In the double-quantum experiment two cycles were completed through the eight-scan sequence depicted in Fig. 1 and the FIDs were Fourier transformed as a function of t_2. Delays, Δ, of either 16.7 or 50.0 ms were chosen to maximize signal and minimize stray 60 Hz noise. Spectra were magnitude corrected and the total area under doublets from both geminal fluorines was calculated as a function of g for fixed diffusion time Δ. D was calculated using Eq. [12] and a least-squares linear fit computer program.

One-quantum T_2 values were obtained by using the spin-echo sequence, 90°–τ–180°–τ, with values chosen so that the cosine terms modulating the echo amplitudes were ± 1. The data were magnitude corrected and fitted to an equation of the form $A\exp(-t/T_2) + B$. Multiple-quantum T_2 values were obtained by using the sequence of Fig. 1 without application of gradients. The data were magnitude corrected so that only positive intensities were recorded and fitted to an equation of the form $A|\cos\{2\pi J_{\text{eff}} t_1 + \phi\}|\exp(-t_1/T_2) + B$.

Samples consisted of a 10% vol/vol solution of 1,1,2-trichloro-2,3,3-trifluorocyclobutane (PCR, Inc., Gainesville, Fla.) dissolved in either cyclohexane (η = 0.90 cp at 298 K for pure cyclohexane (*13*)) or ethylene glycol (EG) (η = 19.9 cp at 293 K for pure EG (*14*)).

RESULTS AND DISCUSSION

Figure 2 shows a high-resolution one-quantum ^{19}F spectrum of 1,1,2-trichloro-2,3,3-trifluorocyclobutane dissolved in cyclohexane. The spectrum is complicated by both homonuclear and heteronuclear J couplings. The large geminal ^{19}F–^{19}F coupling ($|J|$ = 201.7 Hz) gives rise to multiplets centered at −26.3 and −35.7 ppm relative to 0.1 M trifluoroacetic acid in water at 298 K. In addition to the geminal coupling, these resonances show couplings to the equivalent proton pair and the adjacent ^{19}F nucleus which is centered at −38.6 ppm. The magnitudes of the additional couplings are $|J_{\mathrm{HF1}}|$ = 10.4 Hz, $|J_{\mathrm{HF2}}|$ = 10.7 Hz, $|J_{\mathrm{F2F3}}|$ = 10.7 Hz, and $|J_{\mathrm{F1F3}}|$ = 2.8 Hz.

Figure 3 shows a typical two-quantum filtered spectrum which was acquired with a delay optimal for the excitation of double-quantum coherence between the geminal ^{19}F pair and a fixed t_1 value of 16.7 ms. The spectrum corresponds to the entire region presented in Fig. 2 but because of the low resolution of the gradient probe does not show any of the fine structure. As expected, for $\tau = 1/4J_{12}$ the resonance multiplet for fluorine 3 (region C in Fig. 2) is suppressed and the components from the coupling of spins 1 and 2 (regions A and B in Fig. 2) are in anti-phase. The amplitudes of these antiphase peaks are expected to decay with a multiple-quantum T_2 and to be modulated by J_{eff} as t_1 is varied.

Figure 4 shows a multiple-quantum spin-echo relaxation study of the fluorinated cyclobutane derivative in ethylene glycol at 293 K obtained by varying t_1 in the absence of gradients. The sinusoidal modulation arising from $J_{\mathrm{eff}} = J_{13} + J_{23}$ is to be contrasted with the complex modulation of peak amplitudes obtained by Fourier transformation of the normal spin echo. In the normal spin-echo case modulation arises due to coupling of spin i to all its homonuclear spin neighbors j. In the multiple-quantum case only J_{eff} persists and the value of J_{eff} obtained from the $|\cos\{2\pi J_{\mathrm{eff}} t_1\}|$ modulation provides a convenient determination of the relative signs of J_{13} and J_{23}. Comparing $|J_{\mathrm{eff}}|$ = 7.8

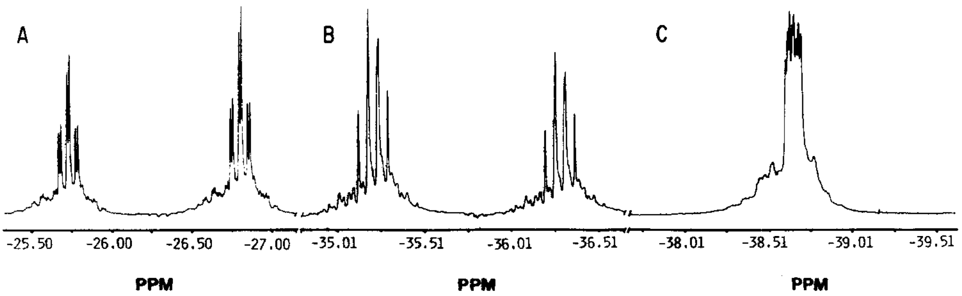

FIG. 2. High-resolution ^{19}F spectrum of 1,1,3-trichloro-2,2,3-trifluorocyclobutane (10% by volume) dissolved in cyclohexane at 298 K. 10 scans of 32K data were acquired. The standard used was 0.1 M trifluoroacetic acid dissolved in water at 298 K.

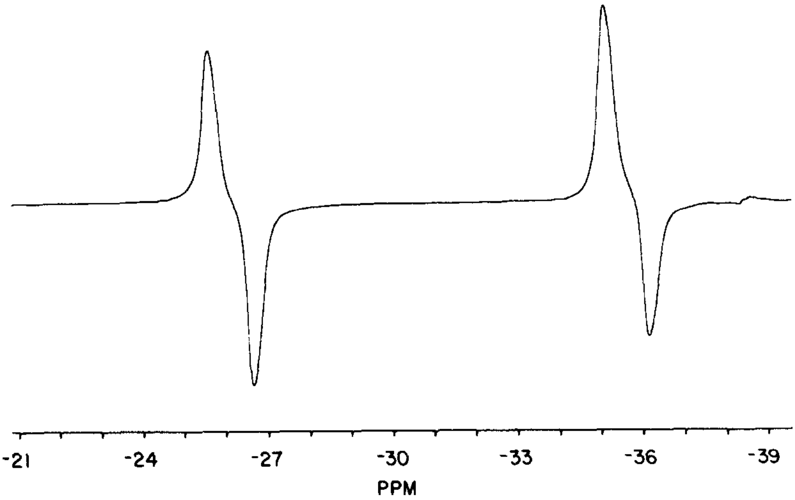

FIG. 3. Double-quantum filtered ^{19}F spectrum of 1,1,3-trichloro-2,2,3-trifluorocyclobutane (10% by volume) dissolved in cyclohexane at 298 K. The spectrum was acquired using the pulse sequence and phase cycling of Fig. 1. A Bruker high-power gradient probe was employed which has an optimal resolution of only ≈30 Hz. Therefore, the fine structure of Fig. 2 is not observed.

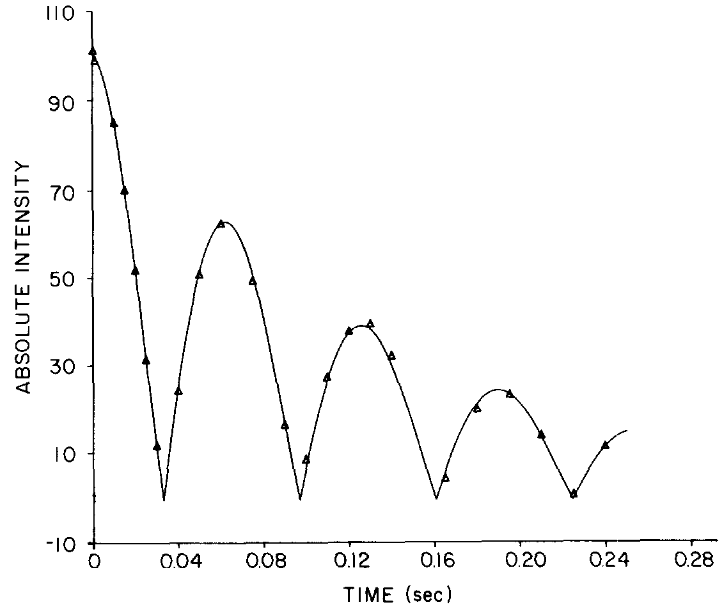

FIG. 4. ^{19}F multiple-quantum spin-echo relaxation study of the cyclobutane derivative dissolved in ethylene glycol (10% by volume) at 293 K. Data were obtained from the resonance centered at −35.3 ppm. Experimental data are indicated with the triangular points and the solid curve is the best fit of the data.

Hz with $|J_{23}| = 10.7$ Hz and $|J_{13}| = 2.8$ Hz obtained from the high-resolution spectrum of Fig. 2 implies that J_{13} and J_{23} have opposite signs.

The value of T_2 obtained from the multiple-quantum data, $T_2 = 0.137 \pm 0.004$ s (the error being given as 1 standard deviation) is larger than T_2 spin echo $= 0.10 \pm 0.01$ s. (T_2 values were measured in both cases for the ^{19}F resonance centered at −35.3 ppm.) This trend to a longer T_2 is expected in systems dominated by dipole or quadrupole relaxation, especially in the limit of slow motions (*15*). Multiple-quantum T_2's can become shorter as CSA contributions become large or effects such as chemical exchange occur. Where multiple-quantum T_2's are longer, however, they provide a distinct advantage for the multiple-quantum method. Longer T_2's allow for an increased Δ at constant S/N ratios which allows for measurement of smaller diffusion constants.

Examination of Eq. [12] suggests that pulsed gradient diffusion experiments could be performed either by varying the interval between application of gradient pulses, Δ, at fixed gradient g or by varying g at fixed Δ. In practice variation of Δ is complicated by effects such as those produced by small amounts of stray 60 Hz modulation. We have chosen to vary g and fix Δ at an integral multiple of 16.7 ms. The time interval t_1 is chosen to be greater than $1/2\Delta$ and to equal an integer multiple of $1/2J_{\text{eff}}$ in order to maximize signal amplitude.

Figures 5 and 6 show plots of echo attenuation as a function of the square of the field gradient for the spin-echo and the multiple-quantum pulse sequences, respectively, using ethylene glycol as solvent. Similar plots were obtained with cyclohexane as sol-

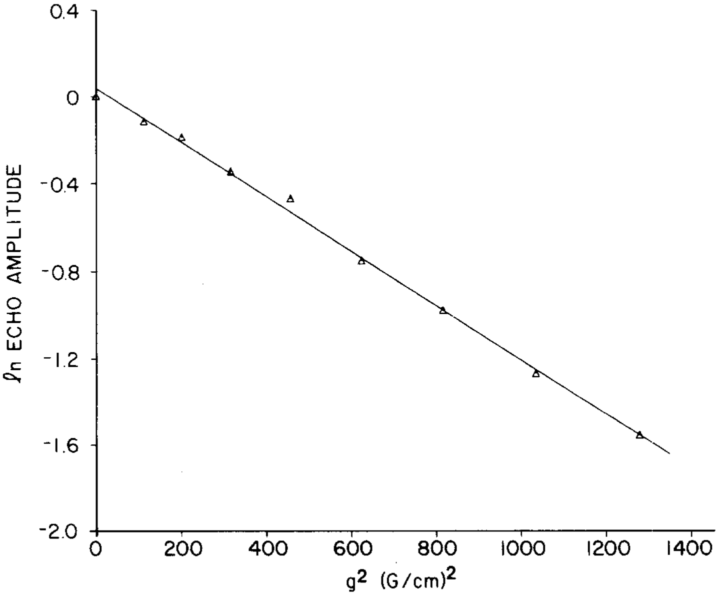

FIG. 5. Attenuation of the single-quantum spin echo as a function of the square of the field gradient, g^2. The data were normalized so that at $g = 0$ the echo amplitude is unity. The sample consisted of a 10% by volume solution of 1,1,3-trichloro-2,2,3-trifluorocyclobutane dissolved in ethylene glycol at 293 K.

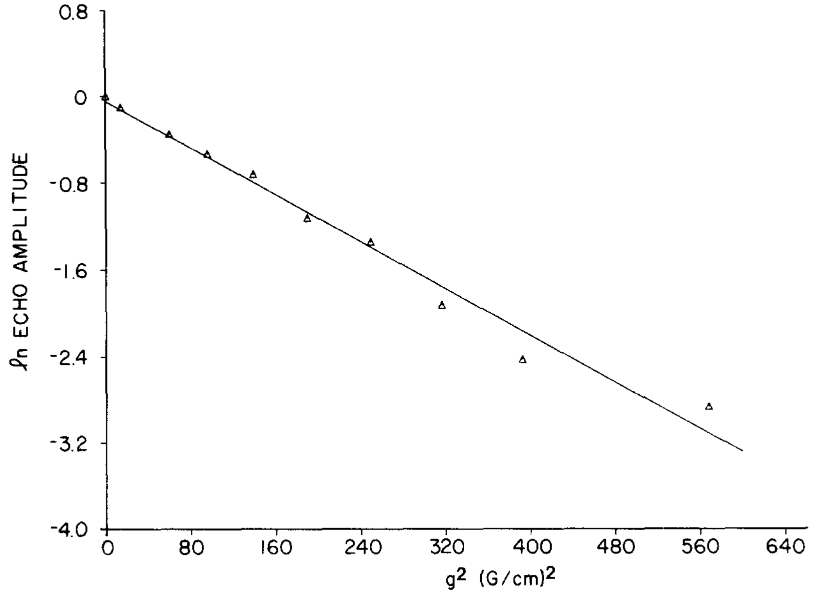

FIG. 6. Double-quantum spin-echo attenuation as a function of g^2. The echo amplitude was normalized to 1 at $g = 0.0$. Same sample as for Fig. 5.

vent. Note that the range of field gradients is approximately one-half as large in the multiple-quantum experiment for equal signal attenuation. The average values of D obtained for the spin-echo experiment, $D_{\mathrm{ave}} = 1.73 \times 10^{-5}$ cm²/s for diffusion in the solvent cyclohexane at 298 K and $D_{\mathrm{ave}} = 4.46 \times 10^{-6}$ cm²/s for ethylene glycol as solvent at 293 K, show good agreement with average diffusion constants obtained from the double-quantum experiment, $D_{\mathrm{ave}} = 1.62 \times 10^{-5}$ and $D_{\mathrm{ave}} = 4.56 \times 10^{-6}$ for diffusion in cyclohexane at 298 K and ethylene glycol at 293 K, respectively. Repeat determinations for a given pulse sequence gave diffusion constants differing by no more than 10% indicating good reproducibility for both methods.

In conclusion, we have shown that the multiple-quantum sequence developed for measurement of diffusion in ordered systems with resolved dipolar coupling can be extended to molecules with scalar coupled spin systems. Previously suggested advantages over the spin-echo experiment are confirmed (*5*, *6*), and applications to ^{19}F carrying anesthetics in biological membranes seems worthy of pursuit.

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