One-Dimensional DOSY

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A new NMR experiment for correlating diffusion coefficients and chemical shifts is presented. This experiment provides the same information as the conventional DOSY experiment, but only requires a single dimension because a nonuniform magnetic field gradient is used to encode the diffusion information into the lineshapes of the peaks in the chemical shift dimension. By fitting the resulting lineshapes, the diffusion coefficient for each peak in the spectrum can be extracted. Using this experiment, a qualitative DOSY spectrum can be generated using the results from a single one-dimensional experiment. Quantitative results can be determined with the use of reference experiments. © 2001 Academic Press

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INTRODUCTION

In this paper, we describe a new NMR experiment that, from a single one-dimensional experiment, provides the same information as diffusion-ordered spectroscopy (DOSY) (*1, 2*). In conventional DOSY experiments, the diffusion coefficient is measured by acquiring a series of one-dimensional spectra with different amounts of diffusion weighting. The diffusion coefficients are found by fitting the variations in the intensities of the peaks between the spectra. This information is then used to generate a two-dimensional representation of the data with the estimated diffusion coefficient along one axis and the chemical shift along the other. In our technique, both the diffusion coefficients *and* the chemical shifts are measured simultaneously in a single dimension; we therefore call our experiment one-dimensional DOSY. It is also possible to generate a two-dimensional DOSY spectrum from the data acquired in our experiment.

In the one-dimensional DOSY experiment, the effects of diffusion are encoded into the lineshape of each peak in the directly acquired dimension. This is done in two parts. First, the diffusion weighting of the signal is made spatially dependent. This is accomplished by using a nonuniform gradient (i.e., a gradient that causes a nonlinear variation of the magnetic field). Second, the peaks are broadened during acquisition so that their line-

shapes reflect the spatial dependence of the diffusion weighting; this is accomplished by acquiring the signal in the presence of a weak read gradient. It is then possible to extract the diffusion coefficients by fitting the shape of each peak.

The advantage of this technique is that it does not require recording a series of diffusion-weighted spectra; the results are available after a single experiment. Although other methods for measuring the diffusion coefficient in a single experiment have been proposed (*3–7*), these methods do not allow the discrimination of signals with different chemical shifts. Therefore, they are of no use if there is more than a single peak in the sample. Although another experiment has been suggested for measuring diffusion coefficients using a nonuniform gradient (*8*), the experiment was in the context of magnetic resonance imaging and does not retain the chemical shift. Our method preserves the chemical shift; this allows estimates of the diffusion coefficient to be made for *all* the peaks in the spectrum in a single experiment. The use of a very weak gradient to broaden the lines during acquisition does, however, mean that the one-dimensional DOSY experiment sacrifices some chemical shift resolution in exchange for information about the diffusion coefficient.

In the following, we will show that a qualitative DOSY spectrum can be constructed based on a single one-dimensional NMR experiment. To make quantitative estimates of the diffusion coefficients, some instrument- and sample-dependent calibrations are required in which case more than one experiment may be necessary.

THEORY

In NMR, diffusion measurements are usually made using magnetic-field gradient pulses. A gradient pulse labels coherences with a phase that corresponds to the position and coherence order of the individual spins in the sample. Subsequently, this phase label can be removed by another gradient pulse, which is usually referred to as a refocusing gradient. If the spins remain stationary during the time between the gradient pulses, the intensity and phase of the NMR signal after the second gradient will be independent of the strength and length of the gradient pulses; the phase label is removed completely. However, if the spins move between the phase labeling and the refocusing

gradients, the intensity and/or phase of the signal will be affected. For example, molecular motion due to isotropic diffusion results in an attenuation of the signal, and it is this effect that is used to measure diffusion coefficients. A semi-classical treatment based on the Bloch equations can be used to predict the effects of diffusion (9–11).

The Bloch equation for the evolution of transverse magnetization in a frame of reference rotating at the Larmor frequency is

$$\frac{dM_+(t)}{dt} = -\frac{M_+(t)}{T_2}, \qquad [1]$$

where T_2 is the transverse relaxation time and

$$M_+(t) \equiv M_x(t) + i M_y(t).$$

$M_x(t)$ and $M_y(t)$ are the x and y components of the magnetization, respectively. This equation applies for the case of a homogenous magnetic field oriented along the z axis and in the absence of radiofrequency pulses.

In the presence of an inhomogeneous magnetic field (i.e., a gradient pulse), [1] needs to be modified to (9)

$$\frac{\partial M_+(z,t)}{\partial t} = -\frac{M_+(z,t)}{T_2} - i\gamma B(z,t)M_+(z,t) + D\frac{\partial^2}{\partial z^2}M_+(z,t),$$
$$[2]$$

where γ is the magnetogyric ratio, $B(z,t)$ is the contribution to the magnetic field at position z due to the gradient, and D is the diffusion coefficient. Here we deal with the one-dimensional case in which the only variation of the magnetic field is along the z axis; the solution for the three-dimensional case is treated in the Appendix. The first of the additional terms in [2] accounts for the change in the Larmor frequency due to $B(z,t)$. This deviation results in a spatially dependent phase. The second additional term accounts for the movement of the magnetization due to isotropic diffusion; this term is analogous to Fick's second law. Note that it is assumed that the gradient pulse only causes variations in the *magnitude* of the main magnetic field; the *direction* of the magnetic field is unaffected.

The dependence of the signal on changes in coherence order during the experiment (i.e., the effect of radiofrequency pulses) can be incorporated into the modified Bloch equation by replacing $B(z,t)$, with an *effective* magnetic field gradient, $B^*(z,t)$ (11),

$$B^*(z,t) \equiv p(t)B(z,t),$$

where $p(t)$ is the coherence order at time t. In the discussion that follows, the coherence order is either $+1$ or -1 so the relaxation rate remains the same.

In the absence of diffusion ($D = 0$), the solution to [2] is

$$M_+(z,t) = \exp\left[-i\gamma \int_0^t B^*(z,t')\,dt'\right]\exp\left(-\frac{t}{T_2}\right), \qquad [3]$$

where, for simplicity, we have assumed that $M_+(z,0) = 1$. For an appreciable amount of signal to be observable at the end of a pulse sequence, the gradient phase label needs to be refocused. This means that the phase should be independent of position; it therefore follows that the refocusing condition is

$$\int_0^t B^*(z,t')\,dt' = b,$$

where b is a constant whose value is independent of position. It is usually the case that $b = 0$, a situation that we will assume from now on.

In the presence of diffusion, a trial solution for [2] can be constructed from [3] by adding an additional term, $A(z,t)$, which reflects the attenuation of the signal due to diffusion:

$$M_+(z,t) = A(z,t)\exp\left[-i\gamma \int_0^t B^*(z,t')\,dt'\right]\exp\left(-\frac{t}{T_2}\right).$$

After substituting this trial solution into [2] (with $B(z,t) = B^*(z,t)$) and cancelling common terms, it is seen that

$$\frac{\partial A(z,t)}{\partial t} = -D\gamma^2 A(z,t)\left[\int_0^t \frac{\partial}{\partial z}B^*(z,t')\,dt'\right]^2$$
$$- 2Di\gamma\frac{\partial}{\partial z}A(z,t)\left[\int_0^t \frac{\partial}{\partial z}B^*(z,t')\,dt'\right]$$
$$- Di\gamma A(z,t)\left[\int_0^t \frac{\partial^2}{\partial z^2}B^*(z,t')\,dt'\right] + D\frac{\partial^2}{\partial z^2}A(z,t).$$
$$[4]$$

A conventional magnetic field gradient generates a magnetic field that varies linearly with distance. That is, the gradient of the effective magnetic field is spatially uniform:

$$\frac{\partial}{\partial z}B^*(z,t) = G^*(t).$$

It follows that the amount by which the signal is attenuated will also be uniform across the sample. This means that $G^*(t)$ and $A(z,t)$ are *independent* of z and therefore $\frac{\partial}{\partial z}A(z,t)$ and $\frac{\partial^2}{\partial z^2}B^*(z,t)$ are zero. Consequently, the last three terms on the right-hand side of [4] vanish.

For the one-dimensional DOSY experiment, we want to solve the more general case in which the gradient is dependent on

position:

$$\frac{\partial}{\partial z} B^*(z, t) = G^*(z, t).$$

In this case, the last three terms of [4] are nonzero. However, we will still discard these terms because, for realistic values of γ, $B^*(z, t)$, and t, these terms will be negligible in comparison to the first term. Physically what this means is that, over the distance that an individual spin will move during the experiment, the magnetic field gradient can be approximated to be uniform. Therefore [4] becomes

$$\frac{\partial A(z, t)}{\partial t} = -D\gamma^2 A(z, t) \left[\int_0^t \frac{\partial}{\partial z} B^*(z, t') \, dt' \right]^2$$

$$= -D\gamma^2 A(z, t) \left[\int_0^t G^*(z, t') \, dt' \right]^2,$$

which has the solution

$$A(z, t) = \exp\left(-D\gamma^2 \int_0^t \left[\int_0^{t'} G^*(z, t'') \, dt'' \right]^2 dt' \right). \quad [5]$$

As all the terms in the exponent of [5] are real, this result predicts that diffusion will only attenuate the signal; the signal does not change in sign or phase.

For a specific pulse sequence it is possible to use [5] to calculate the diffusion-dependent signal attenuation. For the pulsed gradient spin-echo (PGSE) experiment shown in Fig. 1, the integral in [5] can be split into three sections corresponding to the first gradient pulse ($t = 0 \to \delta$), the time between the gradient pulses ($t = \delta \to \Delta$), and the final gradient pulse ($t = \Delta \to \Delta + \delta$). Before the first gradient and after the second gradient the magnetization does not have a spatially dependent phase label, and therefore these periods do not contribute to $A(z, t)$.
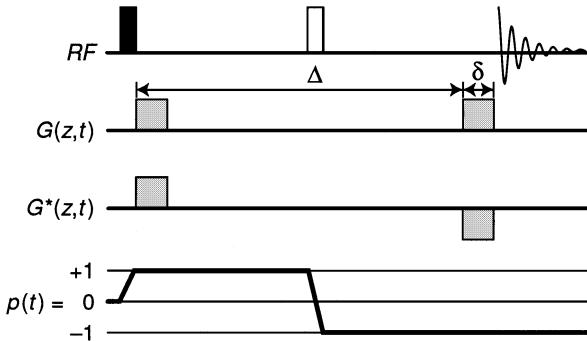

FIG. 1. The PGSE experiment. Solid rectangles represent $\frac{\pi}{2}$ pulses, open rectangles represent π pulses, δ is the length of the gradient pulses, and Δ is the time from the beginning of the first gradient to the beginning of the second gradient. $G(z, t)$, $G^*(z, t)$, and $p(t)$ are as defined in the text.

For the pulse sequence shown in Fig. 1, the exponent in [5] evaluates to

$$\int_0^t \left[\int_0^{t'} G^*(z, t'') \, dt'' \right]^2 dt'$$

$$= \int_0^\delta [g(z)t']^2 \, dt' + \int_\delta^\Delta [g(z)\delta]^2 \, dt'$$

$$+ \int_\Delta^{\Delta+\delta} [g(z)\delta - g(z)(t' - \Delta)]^2 \, dt'$$

$$= \frac{1}{3} g(z)^2 \delta^3 + g(z)^2 \delta^2 (\Delta - \delta) + \frac{1}{3} g(z)^2 \delta^3$$

$$= g(z)^2 \delta^2 \left(\Delta - \frac{1}{3} \delta \right),$$

where $g(z)$ indicates the strength of the gradient at position z. This result leads to the attenuation function

$$A(z, \Delta + \delta) = \exp\left[-D\gamma^2 g(z)^2 \delta^2 \left(\Delta - \frac{1}{3}\delta \right) \right]. \quad [6]$$

As $G^*(z, t)$ is the same for PGSE and stimulated-echo (STE) experiments, the preceding result applies in both cases as long as the timing of the gradient pulses is the same.

To proceed any further, it is necessary to assume a form for $B(z)$. If we assume that $B(z)$ can be represented by a sum of polynomials, then

$$B(z) = \sum_{n=1}^N g_n z^n,$$

where N is the maximum order of term needed to describe the magnetic field and g_n is the coefficient corresponding to the nth-order term. The gradient of the magnetic field is

$$G(z) = \frac{d}{dz} B(z) = \sum_{n=1}^N n g_n z^{n-1}.$$

For conventional gradients (i.e., those that generate a linear variation in the magnetic field and therefore a uniform gradient), this sum contains a single term corresponding to $n = 1$, and therefore [6] leads to the familiar result:

$$A(\Delta + \delta) = \exp\left[-D\gamma^2 g_1^2 \delta^2 \left(\Delta - \frac{1}{3}\delta \right) \right].$$

As described above, for the case where the gradient of the magnetic field is constant, the attenuation is independent of position in the sample. Why this is so is illustrated by the solid line in Fig. 2; moving a certain distance causes a spin to experience the same change in magnetic field regardless of its starting position. Thus, the amount by which the signal is attenuated due to diffusion is the same in all parts of the sample.
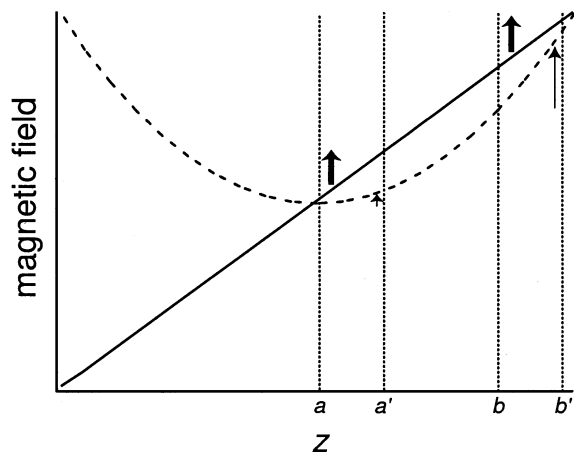
FIG. 2. The magnetic field due to z and z^2 gradients; a z gradient (solid line) causes the magnetic field to vary linearly, whereas a z^2 gradient (dashed line) causes it to vary quadratically. Movement of a spin from a to a' or from b to b' in the presence of a z gradient results in the same change in field regardless of the starting position as indicated by the thick arrows. For a z^2 gradient the change in field caused by moving from a to a' is much less than for moving from b to b' as indicated by the thin arrows; the change in field depends on the starting position.

A gradient that causes a nonlinear variation in the magnetic field will cause the diffusion-dependent signal loss to be different in different parts of the sample. The dashed line in Fig. 2 shows the field profile for a gradient that generates a quadratic variation of the magnetic field. This means that, for a spin moving a certain distance, the change in the magnetic field that it experiences will depend on its initial position. The change in the field will be greater if the molecule is at the extremes of the sample than if it is in the middle and, as a result, the amount by which the signal is attenuated due to diffusion will vary across the sample. We shall call a gradient that causes a quadratic ($n = 2$) variation in the magnetic field a z^2 gradient.

Although any nonuniform gradient can be used for the one-dimensional DOSY experiment, in practice, higher-order ($n > 2$) gradients are more useful for situations where the diffusion coefficients span a wide range, whereas lower-order gradients are better able to resolve small differences in diffusion coefficients. For practical reasons, we choose to use z^2 gradients in our experiments; in this case [6] becomes

$$A(z, \Delta + \delta) = \exp\left[-4D\gamma^2 g_2^2 \delta^2 z^2 \left(\Delta - \frac{1}{3}\delta\right)\right]. \quad [7]$$

The spatial dependence of the signal attenuation due to the z^2 gradient will not usually be visible, as the net signal from the entire sample is observed in an NMR experiment.

However, the spatial dependence of the signal attenuation predicted in [7] can be determined by using imaging techniques. After diffusion weighting by a nonuniform gradient, a one-dimensional image (profile) of the sample will reflect not only the spin density but also the spatially dependent diffusion weighting. If the spin density of an NMR sample is constant along the z axis, the resulting profile will simply reflect the attenuation due to diffusion. In the case of a z^2 gradient, the profile will consist of a Gaussian, centered at the chemical shift, with a width proportional to the diffusion coefficient.

In most imaging experiments it is desirable for the broadening of the signal due to the imaging gradient to dominate the chemical shift. If this is not the case, what will be observed is a superposition of images that are offset from one another due to the chemical shift. Such a superposition leads to a loss of resolution and to difficulties in interpreting the image. In contrast, in the one-dimensional DOSY experiment an extremely weak imaging gradient is used, allowing the chemical shift to dominate the gradient. This means that each peak in the spectrum will result in an independent profile of the sample that is resolved from the other peaks by the chemical shift. In this way, it is possible to record the sample profile, which reflects the diffusion coefficient, while retaining chemical shift dispersion.

Due to the use of a weak imaging gradient, contributions to the lineshape of the peak other than the diffusion attenuation cannot be ignored; the influence of other parameters on the lineshape, and consequently on the accuracy of the estimated diffusion coefficients, will be explored later in this paper.

EXPERIMENTAL

All spectra were acquired on a Varian Unity Inova 400 MHz spectrometer. The sample consisted of cyclohexane, acetone, and 1,1,2,2-tetrachloroethane in deuterated acetone; the sample temperature was maintained at 295 K during the experiments. For reference, the diffusion coefficients were measured using a conventional stimulated-echo experiment; these results are shown in Table 1.

The z^2 Gradient

The z^2 gradient was generated using the z^2 shim: the shim control board was modified so that an additional current could be added to that set by the shim controls, and switched on and off using a spare control line from the pulse programmer. To calibrate the strength of the z^2 gradient, the magnetic field mapping experiment shown in Fig. 3 was used; this experiment

TABLE 1
The Apparent Diffusion Coefficients Measured Using a Stimulated-Echo Experiment for the Solutes in Deuterated Acetone

Molecule	MW (g mol^{-1})	D^a (10^{-5} cm^2 s^{-1})
Acetone	58.1	3.69 (± 0.02)
Cyclohexane	84.2	2.95 (± 0.02)
1,1,2,2-Tetrachloroethane	167.9	2.36 (± 0.01)

[a] The errors given are the standard errors estimated by the fitting procedure.
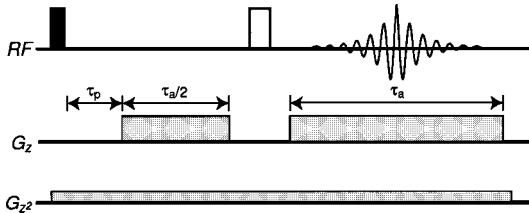
FIG. 3. The pulse sequence used for mapping the magnetic field. τ_a is the acquisition time, τ_p is varied to map the field, G_z denotes conventional gradient pulses, and G_{z^2} denotes z^2-gradient pulses.

TABLE 2
Results of Fitting the z^2-Gradient Field Map to [8]

Coefficient (units)	$N = 2^a$	$N = 4^a$
g_0 (mG)	2 (±1)	−3 (±1)
g_1 (mG cm^{-1})	−80 (±1)	−60 (±1)
g_2 (mG cm^{-2})	−436 (±3)	−360 (±1)
g_3 (mG cm^{-3})		−61 (±1)
g_4 (mG cm^{-4})		−154 (±2)

^{a} The errors given are the standard errors estimated by the fitting procedure.

is simply a one-dimensional Fourier imaging sequence with the addition of a small delay, τ_p, immediately after the first radiofrequency pulse of the sequence (12). If two experiments are performed with different values of τ_p, then the difference in phase at each point in the resulting profiles reflects the value of the magnetic field at the corresponding position in the sample.

The image that results from a field mapping experiment with the z^2 gradient switched on is shown in Fig. 4, and the corresponding field map is shown in Fig. 5a. The inhomogeneity in the main magnetic field was determined by performing the field mapping experiment without the z^2 gradient. The resulting field map, shown in Fig. 5b, demonstrates that the variation of the main magnetic field along the z axis is at least three orders of magnitude smaller than the variation due to the z^2 gradient.

The shim coil used for the z^2 gradient did not produce a purely quadratic field profile. Therefore, the field map shown in Fig. 5a was fitted with a polynomial:

$$B(z) = \sum_{n=0}^{N} g_n z^n. \qquad [8]$$

The results of fitting the field map to a second-order polynomial ($N = 2$) and a fourth-order polynomial ($N = 4$) are shown in Table 2. An attempt to interpret the one-dimensional DOSY spectra assuming that the gradient was described by only a second-order term, g_2 (which corresponds to fitting the peaks in

the spectrum using [7]), resulted in inaccurate estimates of the diffusion coefficients. Therefore, the fourth-order polynomial given in Table 2 was used to fit the one-dimensional DOSY spectra; this produced more accurate results.

The use of a shim coil to generate the z^2 gradient resulted in several difficulties. First, the maximum attainable gradient strength is much less than that offered by a conventional gradient coil. Therefore, in the one-dimensional DOSY experiment a spin echo rather than a stimulated echo was used so that the maximum amount of diffusion weighting could be achieved. Second, the lack of active shielding for the z^2 shim resulted in z^2-gradient pulses causing large and long-lived disturbances of the homogeneity of the main magnetic field during experiments. In an attempt to minimize these effects several methods were tried, including pulsing the z^0 shim simultaneously with the z^2 gradient and switching on a z^2 gradient of opposite polarity immediately before the sequence. Unfortunately, the effects that stem from a z^2-gradient pulse are complex and take place on several time scales. As we were unable to compensate for all these effects, we resorted to using a longitudinal eddy delay (LED) between the spin echo and signal acquisition to allow the system to settle.

Pulse Sequence

The pulse sequence for the one-dimensional DOSY experiment incorporating a LED is shown in Fig. 6a. The sequence consists of a spin echo with diffusion weighting by a z^2 gradient followed by a LED. A z gradient is used both as a homospoil during the LED and to image the sample. As the z^2 gradient is not powerful enough to ensure that the required coherence transfer pathway is selected by the spin echo, z-gradient pulses were placed on either side of the π pulse to restrict the coherence transfer pathway. The sequence shown in Fig. 6b is a reference experiment which, as will be explained below, is needed for quantifying the results of the one-dimensional DOSY experiment.

In both pulse sequences, the length of the z^2-gradient pulse (τ_{on}) is identical; the same is true of the time between switching the z^2 gradient off and acquiring the data (τ_{off}). This ensures that any effects due to disturbances of the main magnetic field are identical in the two experiments. As will be seen later, it is also important that the spin-echo time (τ_d) should remain the same in the two sequences.
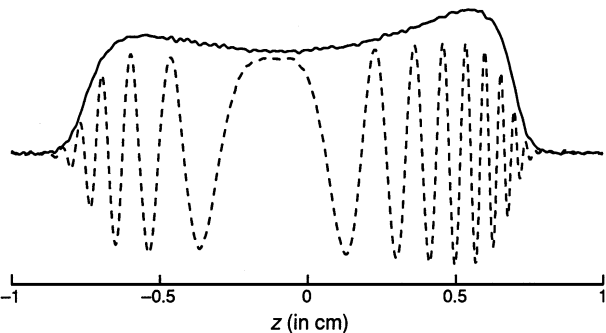

FIG. 4. Phase-sensitive profiles measured using the field mapping pulse sequence shown in Fig. 3. The profiles shown using a solid line and a dashed line are from experiments with $\tau_p = 0$ and $\tau_p = 5$ ms, respectively. The sample used to map the field was a 1% solution of H_2O in D_2O.
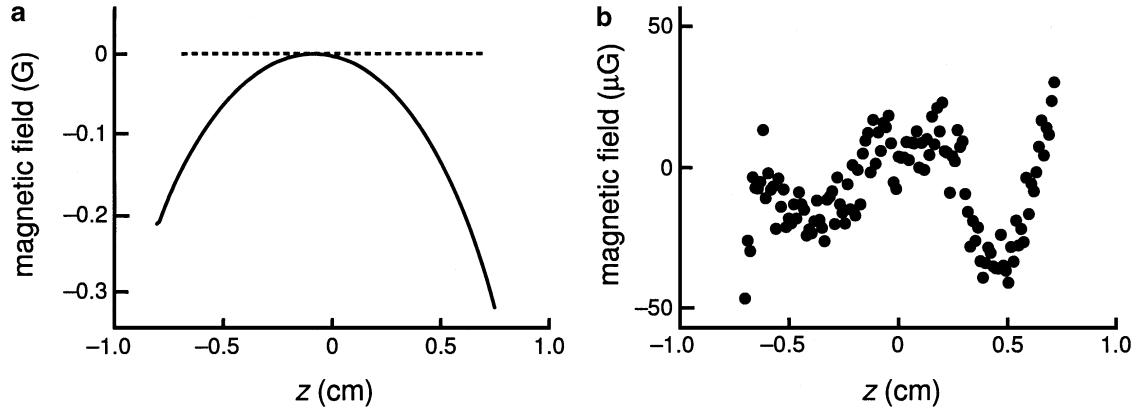
FIG. 5. (a) Maps of the magnetic field variation for the z^2 gradient (solid line) and the main field (dashed line). (b) Expansion of the main magnetic field.

In both sequences, the phases of all the pulses were the same except for the second $\frac{\pi}{2}$ pulse. The use of an unshielded z^2 gradient resulted in a fluctuation of the main magnetic field during the experiment and, as a result, the spin echo did not refocus the magnetization along the expected axis. This effect was compensated for by empirical adjustments of the phase of the $\frac{\pi}{2}$ pulse at the end of the spin echo so as to maximize the signal intensity.

For both the diffusion-weighted and the reference experiments, the spin-echo time (τ_d) was 0.174 s, τ_{on} was 0.5 s, and τ_{off} was 2 s. τ_{store} was equal to τ_{off} in the diffusion-weighted experiment and set to 0.5 s in the reference experiment. The very long value needed for τ_{off} was a result of the effects caused by unshielded z^2 gradients. All gradients had a rectangular shape. The coherence selection gradients flanking the π-pulse were 2 ms long and had a strength of 10 G cm^{-1}. The homospoil
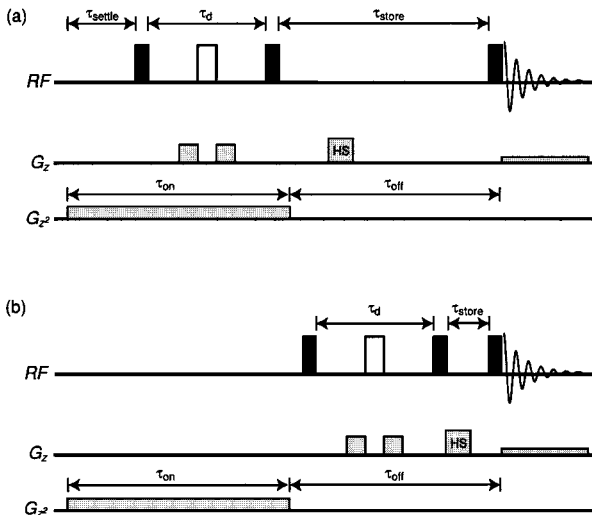

FIG. 6. The pulse sequences for (a) the one-dimensional DOSY experiment and (b) the reference experiment. The various delay times are explained in the text.
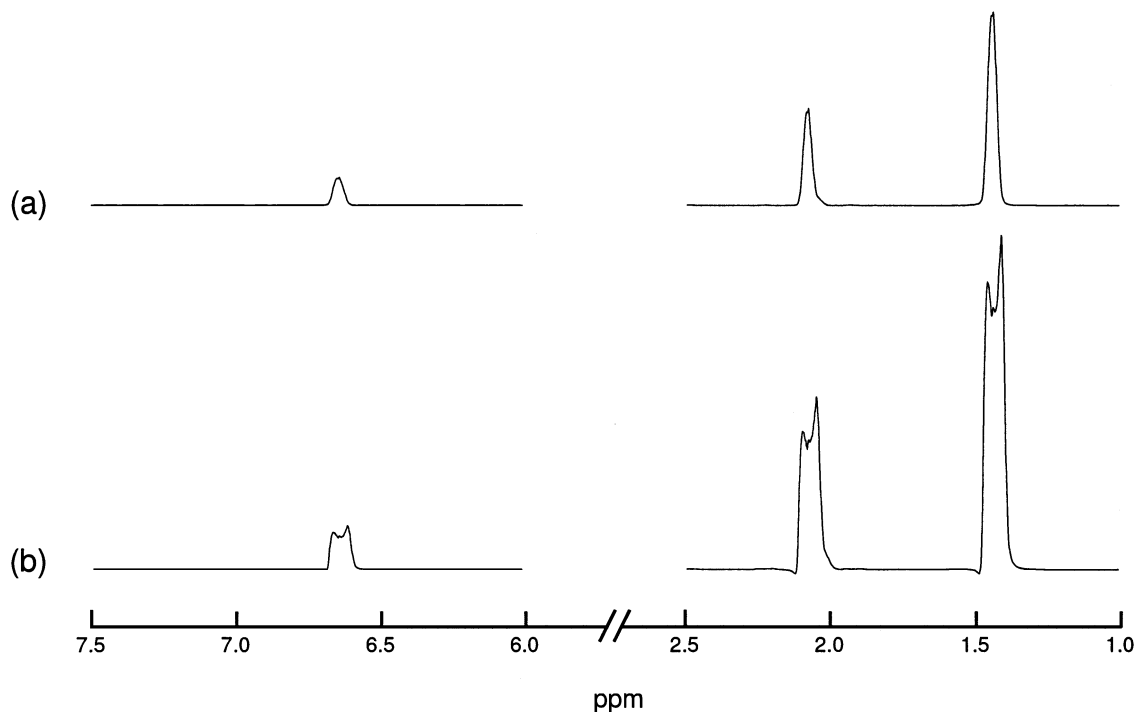
gradient pulse was 10 ms long and had a strength of 30 G cm^{-1}. The strength of the read gradient was 4.9 mG cm^{-1}.

RESULTS

Spectra acquired using the one-dimensional DOSY sequence and the reference experiment are shown in Fig. 7. To interpret these spectra, the spatially dependent diffusion weighting of the signal must be derived from [6]. As the z^2 gradient remains on throughout the experiment, $\Delta = \delta = \frac{1}{2}\tau_d$ and therefore from [6] we have

$$A(z) = \exp\left[-\frac{1}{12}D\gamma^2\mathcal{G}^2\tau_d^3\right], \qquad [9]$$

where

$$\mathcal{G} = g_1 + 2g_2 z + 3g_3 z^2 + 4g_4 z^3.$$

The values of the coefficients $g_1 - g_4$ are given in Table 2. In the presence of a conventional read gradient (G_z), the position along the z axis is related to the frequency, ν, according to

$$z = \frac{2\pi(\nu - \nu_0)}{\gamma G_z},$$

where ν_0 is the center frequency of the peak. Therefore, the signal intensity as a function of frequency for a single peak is

$$S(\nu) = S_0 A(\nu)$$
$$= S_0 \exp\left[-\frac{1}{12}D\gamma^2\left(g_1 + g_2\frac{4\pi(\nu - \nu_0)}{\gamma G_z}\right.\right.$$
$$\left.\left. + g_3\frac{12\pi^2(\nu - \nu_0)^2}{\gamma^2 G_z^2} + g_4\frac{32\pi^3(\nu - \nu_0)^3}{\gamma^3 G_z^3}\right)^2\tau_d^3\right], \quad [10]$$

where S_0 is the overall peak height. It should be noted that many of the cross terms that will result from squaring $\mathcal{G}$ are significant

FIG. 7. (a) One-dimensional DOSY and (b) reference spectra for a mixture of 1,1,2,2-tetrachloroethane, cyclohexane, and acetone in deuterated acetone. The "horns" visible in the reference spectrum are due to nonuniformity of the read gradient.

and cannot be neglected in the data analysis. To estimate the diffusion coefficients using the one-dimensional DOSY spectra, a nonlinear least-squares method was used to fit each peak. Although there are several terms in [10], there are only three adjustable parameters: the peak amplitude (S_0), the peak position (ν_0), and the diffusion coefficient (D).

After fitting each peak to determine the corresponding diffusion coefficient, it is possible to construct a conventional DOSY spectrum, such as is shown in Fig. 8a. Although the diffusion coefficients estimated from the one-dimensional DOSY experiment clearly do not agree with those determined using a conventional stimulated-echo experiment, the separation of the signals
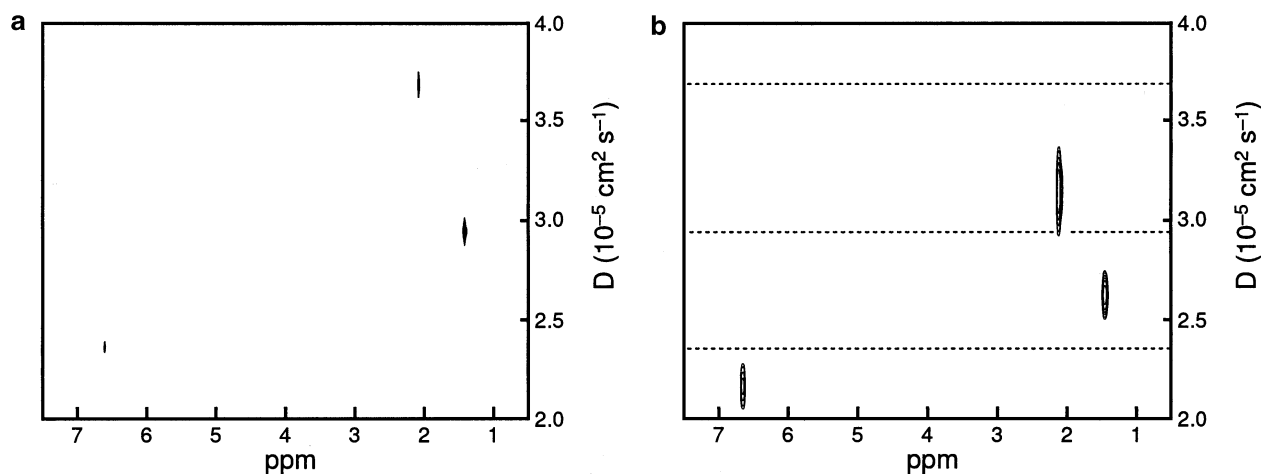

FIG. 8. DOSY spectra constructed using data from (a) a conventional (stimulated-echo) two-dimensional DOSY experiment and (b) a one-dimensional DOSY experiment. The diffusion coefficients given in Table 1 are shown as dotted lines across (b); these values were used to construct (a). For the conventional DOSY spectrum, the intensities of the peaks correspond to the first increment of the DOSY experiment. For the one-dimensional DOSY spectrum, the intensities correspond to the one-dimensional spectrum shown in Fig. 7a. In the diffusion dimension, both spectra consist of Gaussians centered at the diffusion coefficient estimated by the fitting procedure and with widths corresponding to the standard deviations of the fits. For both spectra, the contour lines correspond to 1, 2, 5, 10, 20, and 50% of the maximum intensity of the spectrum.

based on their diffusion coefficients (which is the goal of DOSY) is successful; the values are in the correct order. The difference in the linewidths in the chemical shift dimension between the DOSY spectra is due to the additional broadening necessary in the one-dimensional DOSY experiment to extract the diffusion information. This illustrates the point that to gain information about the diffusion coefficient in a one-dimensional experiment, some resolution must be sacrificed.

As described so far, the analysis of the one-dimensional DOSY experiment assumes that the only factor that influences the image profile (and therefore, the estimate of the diffusion coefficient) is the diffusion weighting. However, this is only an approximation; other factors that influence the sample profile include the z-axis dependence of the read gradient and of the radiofrequency field.

The first and most important factor that needs to be taken into account is the variation of the radiofrequency field, which determines the signal strength measured along the z axis. If the signal strength is not uniform over the entire sample, then this will be reflected in the shape of the profile. This effect can be removed by dividing each peak in the diffusion-weighted spectrum by a reference profile. In theory, this reference profile only needs to be acquired once for any given spectrometer; if a polynomial is used to fit the profile then the resulting equation can be scaled according to the size of the read gradient. In practice, we use the reference experiment described in the previous section to generate the required reference profile. To avoid difficulties such as division by zero, a minimum threshold based on the reference spectrum was used. If the intensity for a point in the reference spectrum was below the threshold value, then the corresponding point in the diffusion-weighted spectrum was ignored.

Another important instrumental factor is spatial variations of the gradient used to image the sample. This will stretch or compress the image, resulting in variations in the apparent signal intensity. Fortunately, the sensitivity profile generated from the reference image suffers from the same systematic error; dividing the one-dimensional DOSY spectrum by a reference image cancels the systematic error.

To preserve the chemical shift resolution we are forced to use a very weak read gradient, corresponding to a broadening of the lines by about 30 Hz. Therefore, the underlying linewidths of the peaks in the conventional spectrum can be sufficient to affect the profile. Attention also needs to be paid, therefore, to the effects of the underlying linewidths.

The profile of a peak in the one-dimensional DOSY spectrum is a convolution of the natural linewidth and the diffusion-weighted profile. This convolution broadens the peak profile and consequently lowers the estimate of the diffusion coefficient. To remove this effect, the spectrum needs to be deconvoluted with the natural lineshape. As convolution in the frequency domain is the same as multiplication in the time domain, the easiest way to deconvolute the spectrum is to divide the FID by a decaying exponential function that has a decay constant equal to T_2.

The effect of the instrumental lineshape is more complicated. Although in many experiments the instrumental lineshape can be removed using reference deconvolution (*13*), in the case of the one-dimensional DOSY experiment reference deconvolution is not appropriate as the spectrum is not a simple convolution of the instrumental lineshape and the sample image. The components of the instrumental lineshape due to inhomogeneities along the *transverse* axes will result in a convolution that may be different for different points along the z axis. Inhomogeneities *along* the z axis will contribute to the magnetic field generated by the read gradient. However, as was seen in Fig. 5, the magnetic field was very homogeneous, at least along the z axis, and we therefore neglect the effect of the instrumental lineshape.

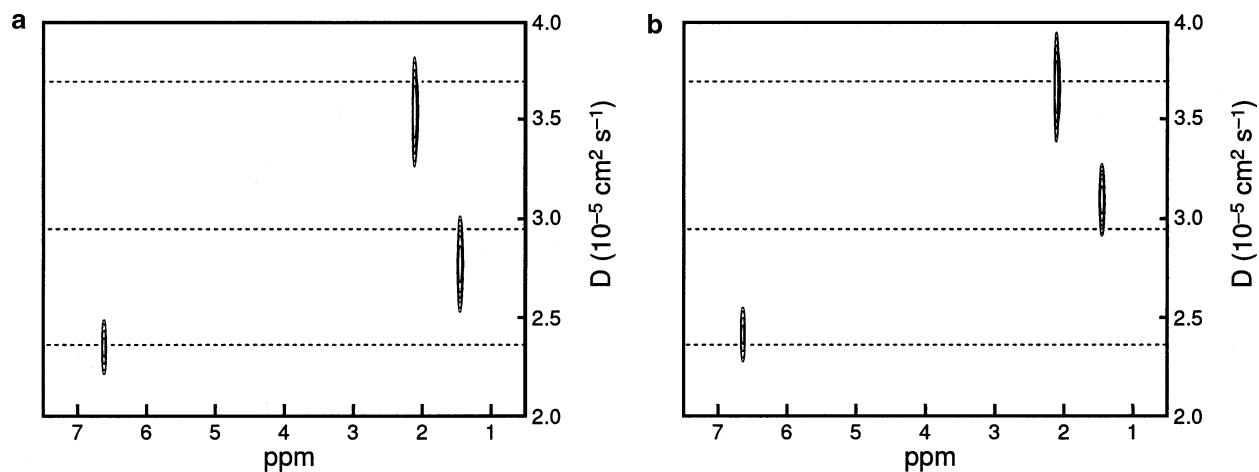As shown in Fig. 9a, the accuracy of DOSY spectra generated from the one-dimensional DOSY results is substantially

FIG. 9. DOSY spectra constructed from the spectrum shown in Fig. 7a (a) dividing by the reference spectrum shown in Fig. 7b and (b) after dividing by the reference spectrum *and* deconvoluting the peaks by their natural lineshapes. The diffusion coefficients given in Table 1 are shown as dotted lines across the spectra. Both spectra were constructed in the same manner as those of Fig. 8b.

improved by dividing the experimental data by the reference spectrum shown in Fig. 7b. The accuracy is further improved by deconvoluting the peaks according to their natural linewidths; these results are shown in Fig. 9b.

Another effect that influences the profiles of the peaks is scalar coupling. If the couplings for a peak are much larger than the broadening due to the read gradient, then the individual lines of the multiplet can be independently analyzed. If the opposite is true, then the effect of coupling can be ignored. In the intermediate case, where the broadening of the peak due to the read gradient and the coupling are of similar size, the peak in the spectrum will consist of several overlapping images. This situation can be dealt with either by fitting the peak to a sum of equations with the form of [10] or by using reference deconvolution to remove the effect of the scalar couplings on the spectrum (*13*) before fitting the peak profiles. In practice, the necessity of using a relatively lengthy spin-echo period to achieve a sufficient degree of diffusion weighting limits our experiment to the analysis of singlets; couplings result in the evolution of antiphase coherences during the spin-echo period that make the spectrum difficult to interpret. A more powerful z^2 gradient, which would allow the use of a stimulated echo for the diffusion weighting instead of a spin echo, would alleviate this problem.

CONCLUSION

One-dimensional DOSY provides the same information as a conventional DOSY experiment, but in a fraction of the experiment time. With a single experiment, it is possible to determine a rough estimate of the diffusion coefficient; if quantitative results are not needed, this is all that is required to construct a DOSY spectrum. Improved estimates of the diffusion coefficients can be obtained by further data processing using a reference experiment and by deconvoluting the spectrum.

APPENDIX

For the general case of a three-dimensional gradient, [1] needs to be modified to

$$\frac{\partial M_+(\mathbf{r}, t)}{\partial t} = -\frac{M_+(\mathbf{r}, t)}{T_2} - i\gamma B(\mathbf{r}, t) M_+(\mathbf{r}, t)$$
$$+ D\nabla^2 M_+(\mathbf{r}, t), \qquad \text{[A.1]}$$

where $B(\mathbf{r}, t)$ is the contribution to the magnetic field at position $\mathbf{r}$ due to the gradient. Defining the effective gradient as

$$B^*(\mathbf{r}, t) \equiv p(t) B(\mathbf{r}, t),$$

the solution for [A.1] is then

$$M_+(\mathbf{r}, t) = A(\mathbf{r}, t) \exp\left[-i\gamma \int_0^t B^*(\mathbf{r}, t') \, dt'\right] \exp\left(-\frac{t}{T_2}\right),$$
$$\text{[A.2]}$$

where $A(\mathbf{r}, t)$ reflects the attenuation of the signal due to diffusion.

After substituting [A.2] into [A.1] (with $B(\mathbf{r}, t) = B^*(\mathbf{r}, t)$) and cancelling common terms, it is seen that

$$\frac{\partial A(\mathbf{r}, t)}{\partial t} = -D\gamma^2 A(\mathbf{r}, t) \left\| \int_0^t \nabla B^*(\mathbf{r}, t') \, dt' \right\|^2$$
$$- 2Di\gamma \nabla A(\mathbf{r}, t) \cdot \left[\int_0^t \nabla B^*(\mathbf{r}, t') \, dt'\right]$$
$$- Di\gamma A(\mathbf{r}, t) \left[\int_0^t \nabla^2 B^*(\mathbf{r}, t') \, dt'\right]$$
$$+ D\nabla^2 A(\mathbf{r}, t). \qquad \text{[A.3]}$$

As in the one-dimensional case, the last three terms of [A.3] vanish for a conventional (uniform) gradient and are negligible for nonuniform gradients. Consequently, using the definition

$$\nabla B^*(\mathbf{r}, t) = \mathbf{G}^*(\mathbf{r}, t),$$

[A.3] becomes

$$\frac{\partial A(\mathbf{r}, t)}{\partial t} = -D\gamma^2 A(\mathbf{r}, t) \left\| \int_0^t \mathbf{G}^*(\mathbf{r}, t') \, dt' \right\|^2,$$

which has the solution

$$A(\mathbf{r}, t) = \exp\left(-D\gamma^2 \int_0^t \left\| \int_0^{t'} \mathbf{G}^*(\mathbf{r}, t'') \, dt'' \right\|^2 dt'\right). \quad \text{[A.4]}$$

As all the terms in the exponent of [A.4] are real, this result again predicts that diffusion will only attenuate the signal; the signal does not change in sign or phase. Additionally, [A.4] shows that, as would be expected, gradients along orthogonal axes contribute independently to the signal attenuation.

For the pulse sequence shown in Fig. 1, [A.4] leads to the attenuation function

$$A(\mathbf{r}, \Delta + \delta) = \exp\left[-D\gamma^2 \|\mathbf{g}(\mathbf{r})\|^2 \delta^2 \left(\Delta - \frac{1}{3}\delta\right)\right], \quad \text{[A.5]}$$

where $\mathbf{g}(\mathbf{r})$ indicates the strength of the gradient at position $\mathbf{r}$. As $\mathbf{G}^*(\mathbf{r}, t)$ is the same for PGSE and STE experiments, the

preceding result applies in both cases as long as the timing of the gradient pulses is the same.

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