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High-Resolution NMR Measurement of Molecular Self-Diffusion by Fast Multi-Spin-Echo Diffusion Sequences

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There is currently much interest in probing the structure as well as the hydrodynamics of molecular species in solution. The steady growth of chemical and biomedical applications including the measurement of molecular transport, the study of reaction dynamics, the identification of metabolites, and the measurement of their transport *in vivo* demands methods that are fast, accurate, and free from artifacts. It therefore seems worthwhile to develop experiments that could address both classes of issues with the same general approach. NMR has proved to be a powerful technique on both counts; traditionally, however, NMR protocols for structural work have relied on high-resolution strategies, while transport and relaxation have been investigated by time-domain measurements. We report here a novel approach to the rapid measurement of molecular self-diffusion coefficients D, employing high-resolution NMR. Our approach is based on encoding molecular self-diffusion as a linewidth parameter, in a simple one-dimensional multiecho pulse sequence. The measurement protocols we report are basically two-scan procedures—one with pulsed diffusion encoding, and a second without—that can typically be executed in under ten seconds if the signal-to-noise ratio is adequate. The sequences sample the tops of spin echoes and are hence completely free from inhomogeneity and susceptibility artifacts. They are thus suited to the investigation of solution-state self-diffusion in heterogeneous environments. We also emphasize that our methods are suitable for single-component systems as well as for multicomponent systems, and are applicable regardless of echo modulations. They may be readily performed on the present generation of high-resolution NMR spectrometers that support gradient-accelerated spectroscopy.

Self-diffusion is measured in magnetic resonance by applying either steady or pulsed field gradients (*1–4*), so that there is a variation of the field over the sample. This renders the Larmor precession frequency position-dependent, providing the frequency or phase labeling of molecules that is required to retrieve information about molecular transport processes. The spatial phase which accumulates during molecular self-diffusion leads to signal attenuation in the ensemble average. This constitutes the basis for extracting D by traditional methods. Field gradients may in fact be applied in any arbitrary direction, permitting the measurement of individual components of the diffusion tensor in its principal-axis system.

The phenomenon of molecular self-diffusion in constant gradients was recognized early by Hahn (*1*) as a mechanism of echo attenuation. Later Stejskal and Tanner (*4*) developed pulsed-gradient methods that overcome problems associated with measurements of D in constant gradients; this was then followed by the development of other refinements (*5–10*) as well as a recent revival of interest in extremely large constant gradients (*11*).

NMR methods for measuring D, including PGSE (*4*) (pulsed-gradient spin echo) and PGSTE (*6*) (pulsed-gradient stimulated echo), sample the echo top, the second half of the echo (FT-PGSE/DOSY) (*8, 9*), or the entire echo. Each of these modes of operation has its own merits and demerits. When the echo top is sampled, the measurement is free of susceptibility and inhomogeneity artifacts. The disadvantage of this mode of operation however is the loss of chemical-shift information, restricting its validity to single-component systems. For multicomponent systems, the measurement must be performed by sampling either the second half of the echo or the entire echo. This mode is, however, susceptible to inhomogeneity and susceptibility artifacts. Further, if the echo time τ is varied, echo modulation must be taken into account. Clearly, satisfactory experiments need to be developed to handle multicomponent systems in heterogeneous liquid-like environments.

Our earlier work (*12*) in this direction involved the development of novel two-dimensional methods employing single-quantum or multiple-quantum echoes. The disadvantage of the 2D family of methods (*12–14*), however, is the measurement time (an hour or longer), since a pair of 2D experiments is required, one with evolution-dependent and a second with evolution-independent diffusion encoding.

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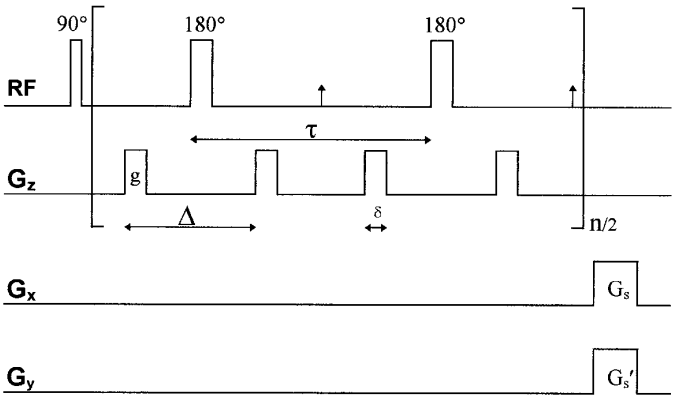

FIG. 1. Pulse sequence for 1D echo spectra with diffusion encoding. Diffusion-encode gradients (amplitude g and duration δ) are imposed symmetrically on each π pulse in the train. Spoilers G_s and G_s' are issued at the end of each scan to destroy any remaining transverse magnetization at the end of signal acquisition. The arrow indicates signal sampling at the echo top.

In the novel one-dimensional experiment that we report here, a first scan is acquired with the pulse sequence shown in Fig. 1, which involves application of the Carr–Purcell–Meiboom–Gill (CPMG) train of refocusing pulses, with suitably placed constant-amplitude diffusion-encoding gradients flanking each 180° pulse symmetrically. A second scan is acquired with an identical pulse sequence, except there are now no diffusion-encoding gradients. The echo tops are sampled midway between the 180° pulses. On Fourier transformation, Lorentzian lines result at each of the echo-modulation frequencies, the linewidths reflecting echo damping due to T_2 relaxation and the diffusional random walk that occurs on this time scale. Note that the echo spectrum exhibits significant line narrowing in comparison to the FID spectrum in most systems of interest.

Considering the first scan with diffusion-encoding gradients, one may readily derive the expression for the amplitude $I(n\tau)$ of the nth echo that occurs at time $t = n\tau$ from the cumulative effect of echo attenuation (*4*),

$$I(t = n\tau) = I(0)\prod_{i=1}^{n}\exp\left(-\frac{\tau_i}{T_2}\right) \times \exp\left[-\gamma^2 g_i^2 \delta_i^2 D\left(\Delta_i - \frac{\delta_i}{3}\right)\right]$$

$$= I(0)\exp\left(-\frac{n\tau}{T_2}\right) \times \exp\left[-n\gamma^2 g^2 \delta^2 D\left(\Delta - \frac{\delta}{3}\right)\right]. \quad [1]$$

The final form above results because the echo time τ, gradient pulse width δ, and amplitude g, as well as gradient interval Δ, are all constant for the entire sequence. Expressing δ and Δ by the parameters k_δ and k_Δ in terms of τ, we have from Eq. [1]

$$k_\delta = \frac{\delta}{3\tau}\,;\; k_\Delta = \frac{\Delta}{\tau} \Rightarrow I(t) = I(0)\exp\left(-\frac{t}{T_2}\right)\exp[-\gamma^2 g^2 \delta^2 D t(k_\Delta - k_\delta)]. \quad [2]$$

The effects of constant gradients $\mathbf{g}_0$ have been ignored here, this being a realistic approximation. On the other hand, the amplitude of the nth echo that occurs at time $t = n\tau$ in the case of the scan without diffusion-encoding gradients is given by the first two factors of Eq. [2]. (It may be noted that an expression of the form of Eq. [2], with the appropriate D values, is in reality associated with each modulation frequency.)

Fourier transformation of the damped echo modulation in the diffusion-encoded scan may be easily shown to result in the following expression for the full width at half-height (*15, 16*) (FWHH), $\Delta\nu_{1/2}$, of the frequency-domain absorption signal:

$$\Delta\nu_{1/2} = \frac{1}{\pi}\left[\frac{1}{T_2} + \gamma^2 g^2 \delta^2 D(k_\Delta - k_\delta)\right]. \quad [3]$$

The FWHH for the second scan run without diffusion encoding is given by the first term in parentheses in Eq. [3]. From these two runs we may readily determine the diffusional contribution to the linewidth, which is given by the second term in parentheses in Eq. [3]. The value of k_Δ may be selected by suitably changing the position of diffusion-encoding gradients with respect to the 180° pulses. In our current implementation, experiments are performed with $\Delta = \tau/2 \Rightarrow k_\Delta = 1/2$. We therefore have for the diffusion-controlled linewidth

$$\Delta\nu_{\mathrm{D}} = \frac{\gamma^2 g^2 \delta^2 D}{2\pi}(1 - 2k_\delta). \quad [4]$$

In the case of non-Lorentzian lineshapes and/or overlapping echo-modulation frequencies, suitable deconvolution procedures may be adopted.

An alternative, straightforward solution in the case of overlaps in the echo spectrum is to employ shift-selective excitation. Figure 2 shows the corresponding shift-selective pulse sequence with diffusion encoding.

It may be noted that our sequences differ vitally from some other single-shot experiments that have been published recently by Li *et al.* (*17*), van Gelderen *et al.* (*18*), and Doran *et al.* (*19*). In particular, the latter protocols rely as

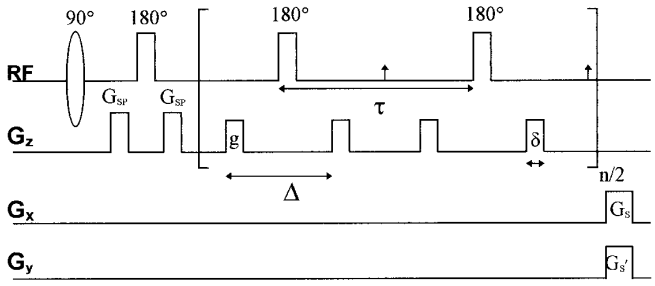

FIG. 2. Pulse sequence for 1D chemical-shift-selective echo spectra with diffusion encoding. The shaped 90° pulse has optimum bandwidth to select the desired chemical shift in single-/multicomponent systems. A refocusing π pulse with symmetric spoiler gradients G_{sp} is applied thereafter; this counteracts dephasing during the shaped pulse. The π pulse train then commences after the appropriate rephasing delay. All other information is to be read as in the legend to Fig. 1.

usual on measuring the attenuation of spin echoes (*17, 19*) or gradient echoes (*19*). Further, in single-shot mode, echo attenuation due to diffusion must be carefully unraveled from *J*-modulation effects in these strategies. Note also that our sequences involve neither amplitude variation nor alternation of gradients.

The conversion of intensity information into frequency information by our method results in high accuracy of measurement of D. Our experiments were all performed at 23°C on a Bruker MSL 300 P system with an actively shielded microimaging probe employing a 5 mm RF insert; 128–300 echo tops were collected in the multiecho train and zero-filled to 2K points, while the spectral width is between 10 and 25 Hz, corresponding to the maximum proton-multiplet splitting. This results in a digital resolution of at least 0.01 Hz per point and enables extremely accurate measurement of linewidths and hence of D. Accordingly, with the modest gradient pulse areas $g\delta$ that we have employed, about 10^{-2} G cm^{-1} s, the error in linewidth measurement amounts to $\pm 3\%$, translating directly to $\pm 3\%$ error in the measured diffusion coefficient. It is to be emphasized, however, that with gradient pulse areas only a modest factor of 3.2 higher, the error in the measured D drops to $\pm 0.3\%$. These absorption-mode experiments may also be optimized in single-detection mode for higher digital resolution. Further, a two-scan procedure employing two different $g\delta$ values may be employed as an alternative to the procedure with one zero and one nonzero value of $g\delta$.

Application of the pulse sequences corresponding to Fig. 1 on ethanol resulted in a 1D *J* spectrum (*20*) that displays frequencies corresponding to *J* modulation ($\pm J/2$, $\pm J$, $\pm 3J/2$, and 0) arising from the methyl and methylene groups of the molecule. The diffusional broadening measured on the intense peaks at $\pm J$ hertz and 0 hertz amounts to 0.18 Hz, resulting from the application of the diffusion gradient (11.66 G cm^{-1}); this corresponds to $D = 1.16 \times 10^{-5}$ cm^2 s^{-1}, which is in excellent accord with the value obtained by standard NMR methods (*21, 22*).

The shift-selective pulse sequences corresponding to Fig. 2 were applied on an equimolar binary system, viz., *n*-butanol and benzene. In this case, the methyl triplet of *n*-butanol was selected. Here, the frequencies corresponding to *J* modulation are $\pm J$ and 0. The diffusional line broadening now amounted to 0.28 Hz, resulting from the application of the diffusion gradient (16.33 G/cm). This corresponds to $D = 0.92 \times 10^{-5}$ cm^2 s^{-1}. Application of the shift-selective methods to the signal from benzene in the same sample, on the other hand, generates an additional broadening of the zero-frequency peak by 0.3 Hz, on application of the diffusion gradient (11.66 G cm^{-1}); this corresponds to $D = 1.93 \times 10^{-5}$ cm^2 s^{-1}. For comparison, our experiment performed on pure samples of *n*-butanol and benzene yielded D values of 0.46×10^{-5} and 2.3×10^{-5} cm^2 s^{-1}, respectively, in agreement with standard values. The mixture thus exhibits a characteristic viscosity-controlled effect on the diffusion coefficient of the individual components which is in excellent accord with results from standard NMR methods (*23*).

Figure 3 shows the application of the shift-selective pulse sequence to the methyl doublet of 100 m*M* *N*-acetylalanine in D_2O. In this case, the additional broadening of 0.14 Hz that results from the application of the diffusion gradient of 16.33 G cm^{-1} corresponds to a diffusion coefficient of 0.46×10^{-5} cm^2 s^{-1}.

We have employed our sequences to measure D on multitube phantoms as well, each tube containing a different molecular species. As expected, the glass wall boundaries have no effect on the D values measured from the echo spectra.

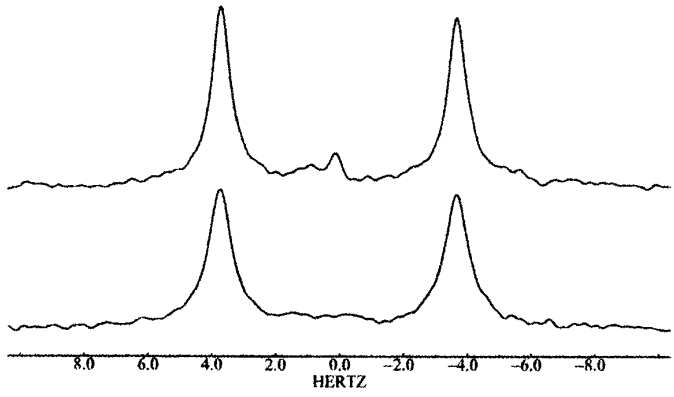

FIG. 3. Application of shift-selective FAMOUS to the methyl doublet of 100 m*M* *N*-acetylalanine in D_2O. The lower trace displays the diffusion-encoded *J* spectrum with a linewidth of 0.81 Hz for the $\pm J/2$ peaks. The upper trace displays the spectrum obtained without diffusion encoding with a linewidth of 0.67 Hz for the $\pm J/2$ peaks. The additional broadening of 0.14 Hz resulting from the application of the diffusion gradient of 16.33 G cm^{-1} corresponds to a diffusion coefficient of 0.46×10^{-5} cm^2 s^{-1}. Shift selection was achieved with a Gaussian pulse of duration 20 ms, issued at the methyl chemical shift.

We propose for these experiments, which may also be readily modified to run in slice-selective mode, the generic name of *fa*st *m*ulti-spin-echo diff*u*sion *s*equences (FAMOUS).

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