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Difftrain: A Novel Approach to a True Spectroscopic Single-Scan Diffusion Measurement

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***D*iffusion-*o*rdered *s*pectroscop*y* (DOSY) has gained considerable attention over the past decade as a useful tool for calculating diffusion-related parameters or in the analysis of complex (reaction) mixtures. A major drawback of the established methods are the relatively long recording times needed to acquire the spectra, excluding the monitoring of rapidly progressing reactions and (hence) measurements of less stable components. In order to overcome these shortcomings a new pulse sequence, *Difftrain*, has been developed. The sequence involves stimulated echo attenuation, multilow flip angle excitation, and multiple sampling of the FID during the longitudinal storage. The calculated diffusion parameters obtained by Difftrain were compared with those obtained by the established sequence BPPSTE (*b*ipolar *p*ulse *p*air *s*timulated *e*cho) and were in good agreement. For systems with moderate to good signal-to-noise ratios the Difftrain building block yields significant saving in recording time (single-shot acquisition instead of acquiring *n*-different gradients strengths), thus opening up new applications in nonequilibrium systems and screening of compositions and/or interactions of (larger) compound arrays.** © 2001 Academic Press

INTRODUCTION

Although field gradient experiments were already described at the onset of NMR spectroscopy (*1*, *2*), these techniques have only recently emerged as a powerful tool for analysis of complex (reaction) mixtures. In this respect, the improved quality of the pulsed field gradient equipment showed to be the major enabler. Experiments that possess the capability of hyphening both diffusional and chemical shift information, commonly denoted as DOSY methodology, have been designed (*3*). Using differences in diffusional behavior, separate components within mixtures can be analyzed without the need of physical separation. In addition, the diffusional parameters of the observed species can be used to assess molecular size (viz. by hydrodynamic radius) and aggregation behavior (*4–6*), hence providing physico-chemical information. As NMR is a noninvasive technique, equilibration and the progress of certain reactions can be monitored using NMR without interfering with the reaction and/or reaction conditions, providing an excellent analytical platform for the study of (bio)-organic reaction pathways. Most DOSY experiments (*7*) are based on the BPPSTE (*b*ipolar *p*ulse *p*air *s*timulated *e*cho) pulse sequence (*8*) (Fig. 1a). In this experiment spins are spatially encoded with a bipolar gradient pulse pair, then decoded after a certain delay by a second bipolar gradient pulse pair, thus producing an echo of the signal. The echo attenuation is described by

$$\ln(I/I_0) = -(\gamma g \delta)^2(\Delta - \delta/3 - \tau/2)D, \qquad [1]$$

where γ is the gyromagnetic ratio and g and δ are the strength and length of the gradient pulses, respectively (τ is the 90°–180° pulse distance in the BPPSTE sequence). D is the diffusion constant of the spin system studied, and Δ is the diffusion time between the encoding and decoding bipolar gradient pairs. Commonly, to obtain values for D, spectra are recorded and collected by varying the gradient strength. The duration of such experiments is typically on the order of 10^2–10^3 s. Although DOSY is an elegant tool for noninvasive assessment of complex (reaction) mixtures, the relatively long acquisition delays have hampered its applicability to study dynamic (nonequilibrium) systems and its deployment as a tool for rapid screening of compositions and interactions of (larger) compound arrays (*9*).

These drawbacks urged us to develop and explore the "single-shot" *Difftrain* pulse sequence. Previously, single-shot methods have been suggested (*10–13*) allowing diffusion experiments to be recorded in lesser time, but these suffered from a loss in chemical shift resolution or even the complete lack of chemical shift information, thus only allowing bulk diffusion parameters to be recorded. By using Difftrain, a major part of the chemical shift information can be regained by combining features of both single-shot diffusion and relaxation experiments. In order to retain the chemical shift information, it is essential that no read gradient is applied during acquisition, as is the case in most single-shot diffusion experiments suggested so far. As is shown in Fig. 1b, we can acquire all the information in one single run by encoding the spins *once* and decoding them *multiple* times. By merging this feature with a single-scan FT

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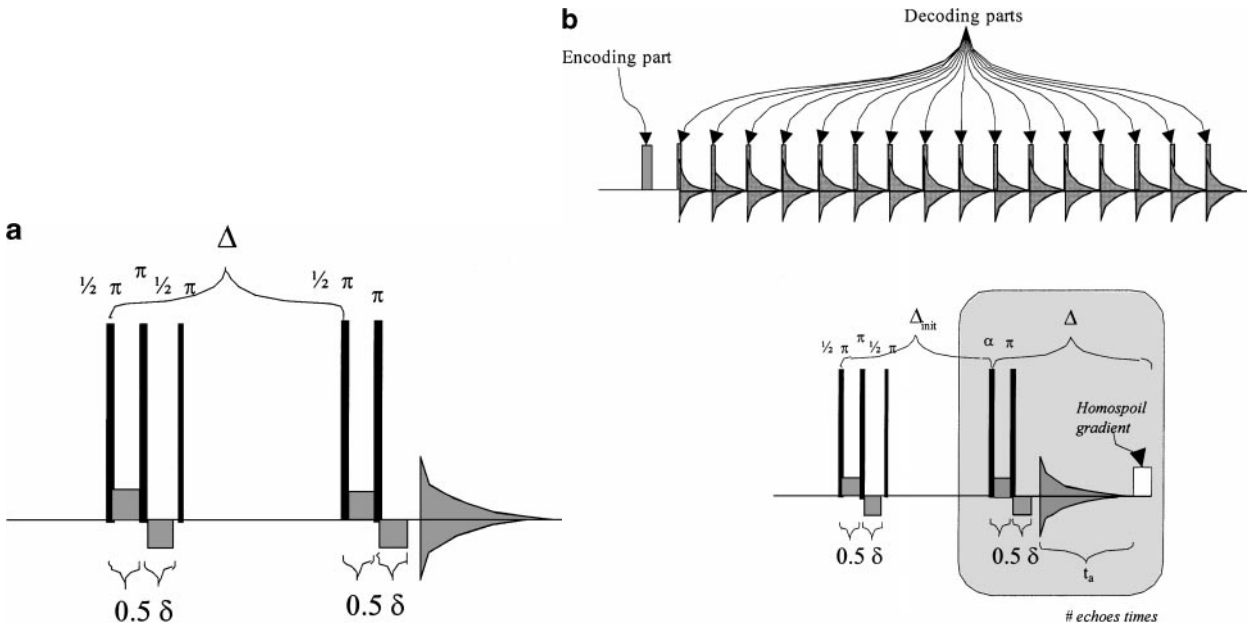

FIG. 1. (a) The basic BPPSTE pulse sequence, where δ is the gradient length, g the gradient strength, and Δ the diffusion delay. (b) The proposed Difftrain pulse sequence, schematic (upper) and detailed (lower). t_a is the acquisition time and Δ is the diffusion delay (see text). In the implementation of the Difftrain experiment, the same phase cycle as in previous BPPSTE implementations was used (see text).

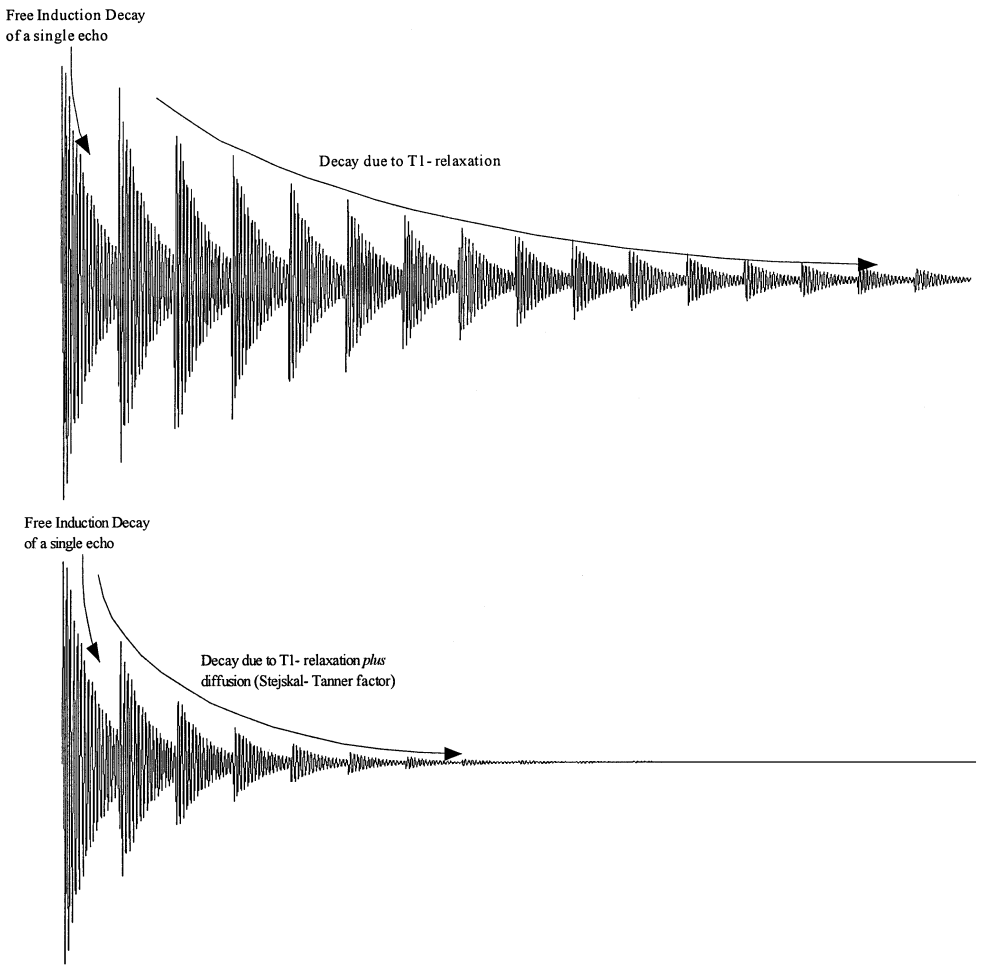

FIG. 2. FIDs of the reference (upper) and the diffusion experiment (lower). The spectra were recorded with a Varian Unity 400 WB NMR spectrometer operating at 400 MHz, equipped with a Performa II Model L700 gradient driver (max. gradient strength approx. 75 G/cm). A Varian 400 MHz PFG Indirect Detection probe was used.

transform pulse sequence, previously used for longitudinal relaxation measurements (*16*), we can assess chemical shift information. During this experiment, the spins are encoded once in the transversal plane and brought back into the longitudinal state, and subsequently, a small amount out of the spatially encoded longitudinal "reservoir" is brought into the transversal plane. Subsequently, decoding of the transversal magnetization "aliquots" takes place and the FID is recorded. After the acquisition, this transversal magnetization aliquot is scattered by means of a gradient homospoil pulse, allowing the next echo to be recorded. This sequence is repeated 16 times, typically. It must be emphasized that each echo acquisition delay adds up to the diffusion time for every next echo. Hence, multiple spectra can be acquired with different diffusion times in a single run, allowing the diffusion constant to be computed for every separate signal in the chemical shift domain. After each echo, only $\cos(\alpha)$ of the signal remains longitudinal; therefore after n echoes, the remaining signal will be dropped to $\cos^n(\alpha)$ of the original signal, if T_1 relaxation and diffusion are neglected. When the spectra are recorded over time, signal attenuation will not only occur due to the diffusion according to Eq. [1], but will also be induced by T_1 relaxation and the inevitable loss of "tapping" magnetization aliquots out of the reservoir. To compensate for this, the experiment must be recorded once *with* and once *without* diffusion gradients, the last serving as a reference, as the spectral values of the diffusion experiment need to be divided by the reference values.

RESULTS

The pulse sequence of Difftrain was implemented in a straightforward manner using a phase-cycle canceling nonphase-encoded magnetization. The phase cycle stores the longitudinal reservoir alternatingly along the positive and negative z-axis so that magnetization arising from longitudinal relaxation is canceled (*7, 14*). In addition, the phase cycle also eliminates secondary echoes (*15*). The experimental parameters of Difftrain must be selected with care in relation to each other. As the acquisition time determines the diffusion times between the spectra, by increasing the resolution, both the diffusion gradient strength and the number of echoes need to be decreased in order to achieve a recording time of less than approx. 2^*T_1. These considerations were amply discussed in the implementation of single-scan relaxation (*16*) and imaging (e.g., FLASH (*17*)) experiments. Examples of series of FIDs of both reference and diffusion experiments are shown in Fig. 2, which clearly illustrates the faster decay as a result of dephasing due to diffusion under the influence of z-oriented gradients. Assuming that all the attenuating effects in the experiment behave exponentially (diffusion, T_1, and tapping the aliquots), the decay constant of the diffusion experiment and that of the reference can be computed separately, after which the net diffusion constant is obtained from the difference. These calculations were performed with an in-house written software package, applying a gradient-expansion algorithm to compute a nonlinear least-squares fit to a user-defined function. The software can handle raw FID files, and all spectral preprocessing algorithms, e.g., time domain weighting and reference deconvolution by the *Fiddle* algorithm (*18*), have been implemented. The Fiddle procedure removes experimental

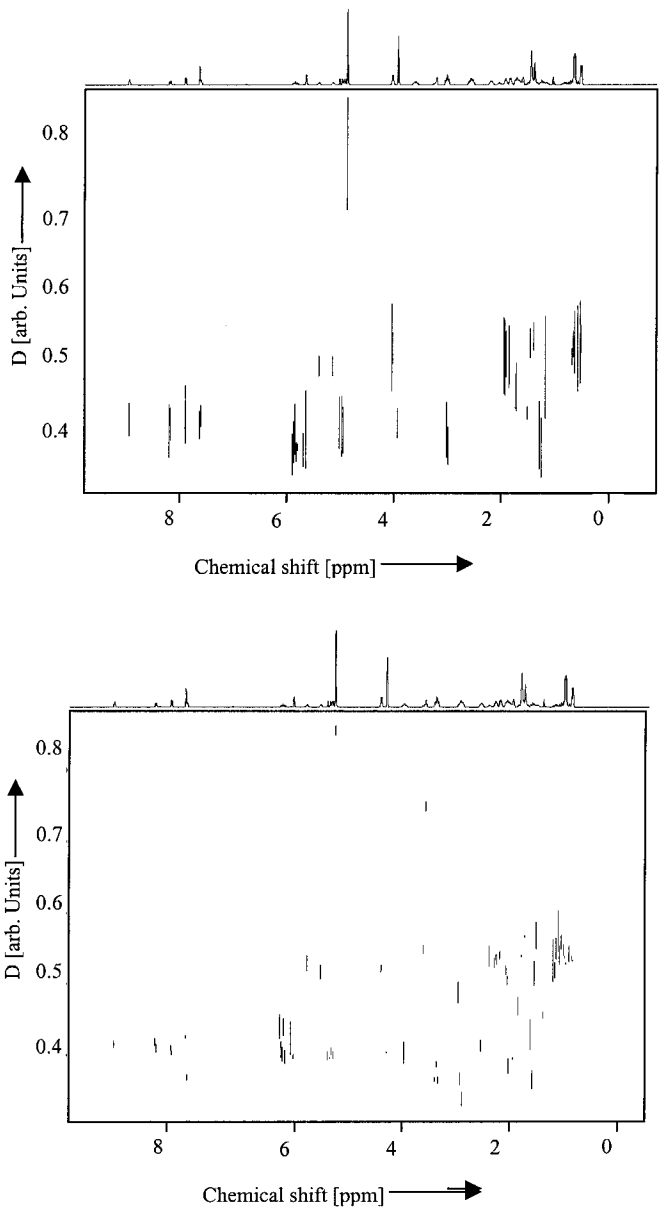

FIG. 3. DOSY representations of BPPSTE (upper) and Difftrain (lower) experiments performed on a solution of 0.10 M L-menthol, 0.10 M quinine, and 0.10 M geraniol dissolved in CD_3OD (99.8% deuteration). The BPPSTE experiment was recorded at 298 K, with 49,984 data points in the FID, $\Delta = 0.1$ s, and $\delta =$ ms, and 25 gradient levels ranging between 0.7 and 55 G/cm were used. The Difftrain experiment shown was recorded at 291 K, with 16 echoes of 2048 datapoints, $\alpha = 15^\circ$, $\delta = 0.5$ ms, and a gradient level of approx. 8 G/cm. The processing of the raw Difftrain data was done by the P-Nmr Experiment software (IDL 5.2, for Linux, with a built-in development environment, IDLDE). Further experimental details are explained in the legend of Fig. 2.

lineshape distortions by Fourier deconvolution with the disturbing function. Due to the relatively short echo sampling times we sacrifice spectral resolution; however, by reference deconvolution we were able to regain much of the spectral quality.

An example of the results obtained using the Difftrain as well as the BPPSTE sequence is shown (in DOSY format) in Fig. 3. When the diffusion spectra of Difftrain are compared with those of BPPSTE, one can note that there is a good correspondence between the diffusion constant values. However, in the diffusion dimension of the Difftrain experiment one can observe in general larger linewidths, which corresponds to a smaller precision of the diffusion constant values in this experiment. This is explained by the number of data points used, BPPSTE 49 K versus Difftrain generated with 2048 data points, typically, which is a factor of approx. 25 less.

DISCUSSION AND CONCLUSIONS

The experiments indicate that Difftrain is a promising single-shot NMR diffusion technique, with a performance comparable to the established BPPSTE pulse sequence. With respect to signal-to-noise yield there is only a moderate advantage of Difftrain over the conventional BPPSTE sequence. Although in Difftrain the complete diffusion decay can be obtained in a single scan, the signal per scan is less because only a minor part of the longitudinal magnetization is excited per echo. For a given number of echoes, the optimal excitation pulse angle (α) (*16*) and the signal yield of the diffusion decay is readily calculated. Compared to a typical BPPSTE experiment ($\Delta = 200$ ms, n transients times 16 gradient strengths), the corresponding single-scan Difftrain experiment (optimal pulse angle $\alpha = 22^\circ$) yields 40% of the original signal. Hence we need 7^*n Difftrain acquisitions to obtain a comparable signal yield. Taking into account that also a reference experiment must be recorded, in total 14^*n acquisitions are needed, leading to a minor time saving compared to the n^*16 acquisitions in BPPSTE. We note that for BPPSTE experiments the number of gradients can be reduced (<16), thus leading to a further reduction of the signal yield advantage. Although the efficiency in terms of signal yield per time unit may be moderate, a major advantage of the Difftrain sequence is in the rapid acquisition of DOSY spectra of samples where sensitivity is not an issue.

Since the T_1 correction procedure only works for discrete values, Difftrain is not suitable for polydisperse samples. We do not consider this a serious drawback with respect to the conventional DOSY sequences, since these also have problems with the assessment of polydisperse samples. Such samples tend to produce data sets with distributions of diffusion constants that require nontrivial DOSY data-processing techniques (*5, 19*). With respect to the range of accessible diffusion constants there is a limit with respect to the duty cycle of the gradients during the gradient train, and the eddy currents they induce. With the commercial equipment used in this study, we could apply gradient strengths of approx. 10 G/cm without compromising spectral quality. Under these conditions, diffusion constants as low as 10^{-11} m^2 s^{-1} are within the range of the Difftrain experiment.

We foresee applications for monitoring rapidly proceeding reactions or aggregation phenomena. The Difftrain sequence may also become a building block in three-dimensional DOSY experiments (*20, 21*). The main problem of such experiments is the considerably long recording time, which can be decreased to a clearly more acceptable time when 2D sequences are diffusion weighted with Difftrain instead of established diffusion experiments like BPPSTE. In the same run, Difftrain also provides the T_1 times of the spin system, which may for example be used to aid in the assignment of the species in the complex mixture and provide physico-chemical parameters. For systems which moderate to good signal-to-noise ratio the Difftrain building block yields significant savings in the overall recording time, opening up new applications in nonequilibrium systems and in screening of molecular compositions and interactions.

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