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NMR Diffusion Experiments for Complex Systems

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1. Introduction

The last decade has seen a considerable increase in the number of biochemical and biomedical applications of PFG NMR diffusion methods. However, despite commercial availability of powerful, stable, and reproducible gradient units, ^{1}H diffusion measurements of low-concentration solutes in non-deuterated media can still present a challenging problem. Large solvent peaks can make integration of small solute peaks extremely difficult. Complex mixtures can also be difficult to work with, due to spectral crowding. At the same time, non-deuterated, spectrally crowded systems must routinely be dealt with in many of the practically relevant experimental situations, such as NMR of biofluids, proteins, or cellular systems. Our ongoing work in this area has been directed at the development of NMR diffusion experiments which deal with these problems by using contemporary solvent suppression and multiple-quantum filtering techniques.

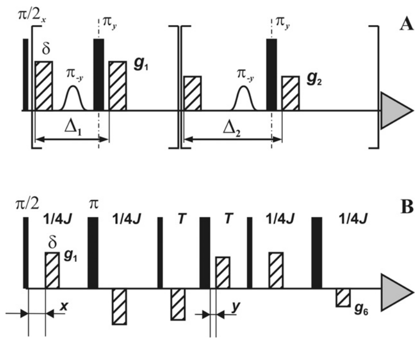

Fig. 1. Pulse sequences of: (A) CONVEX; (B) DQDiff.

2. The CONVEX experiment

CONVEX [1] is a double-echo PGSE which incorporates excitation-sculpting solvent suppression [2]. Its pulse sequence is shown in Fig. 1A. Within each bracketed excitation-sculpting block, the on-resonance solvent peak experiences a cumulative 0° rotation and is not refocused. Off-resonance solute peaks undergo 180° rotations and thus experience a double spin-echo [3]. The gradient amplitudes g_1 and g_2 relate as prime numbers in order to eliminate solvent signal leakage. Signal attenuation for off-resonance peaks is given by

$$S(g) = S(0)e^{-D\gamma^2 g_1^2 \delta^2\left[\Delta_1(1+C) - \frac{\delta(1+C^2)}{3}\right]}$$

where $C = g_2 : g_1$. Convection compensation is achieved by setting $\Delta_1 : \Delta_2 = g_2 : g_1$. The CONVEX experiment was tested on a number of samples, including simple solutions of small molecules; solutions of proteins and peptides in phosphate-buffered saline; and hydrogels. A representative comparison of CONVEX and PGSE measurements of hydrogel systems is shown in Fig. 2 [4]. CONVEX provides superb water suppression and almost no baseline distortions, compared to conventional PGSE or convection-compensated double PGSE.

3. DQDiff

DQDiff diffusion experiment (shown in Fig. 1B) is another recent development [5]. This is a double-quantum filtered diffusion experiment which selects a single coherence transfer pathway using an asymmetric set of gradient pulses. Diffusion sensitization and convection compensation are both built into the gradient CTP selection. The resulting diffusion spectra are phase-sensitive, which eliminates the need for a magnitude Fourier transform required by traditional DQF diffusion measurements. The analytic expression for the signal attenuation in DQDiff is rather cumbersome, but produces a linear Stejskal–Tanner plot with the negative slope proportional to D. The experiment was tested on solutions of small molecules; a protein; and a micellar solubilizate. It does not appear to offer an across-the-board improvement over the existing convection-compensating methods, but provides a potential advantage where DQF editing of crowded diffusion spectra is required.

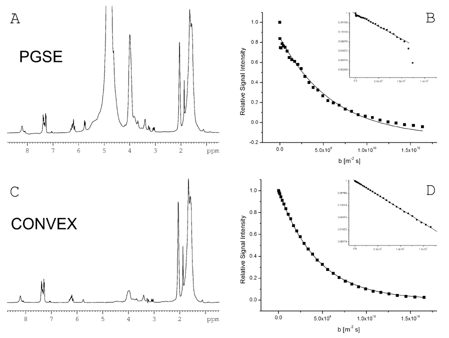

Fig. 2. Representative spectra and attenuation plots from conventional PGSE (A, B) and CONVEX (C, D).

4. Pure-phase acquisition using OGSE

Also presented are new applications of oscillating-gradient spin echo (OGSE) [6]. Traditionally, OGSE has been advantageous in measuring the apparent short-Δ diffusion coefficient in the presence of restricted diffusion [7,8]. We demonstrate that single-echo OGSE provides convection compensation which compares well with that afforded by double-echo PGSE. We also show that, in the presence of homonuclear scalar couplings, setting the OGSE echo time to $1/2J$ enables acquisition of pure-phase diffusion spectra and yields more reliable D estimates than mixed-phase PGSE or OGSE spectra. Pure-phase OGSE acquisition is compatible with measurements of the apparent diffusion coefficient at an arbitrary diffusion time. These features of OGSE can be valuable in diffusion measurements of scalar-coupled small-molecule probes in cellular and other heterogeneous systems.

References

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