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Measuring small compartmental dimensions with low-*q* angular double-PGSE NMR: The effect of experimental parameters on signal decay

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ABSTRACT

In confined geometries, the MR signal attenuation obtained from single pulsed gradient spin echo (s-PGSE) experiments reflects the dimension of the compartment, and in some cases, its geometry. However, to measure compartment size, high *q*-values must be applied, requiring high gradient strengths and/or long pulse durations and diffusion times. The angular double PGSE (d-PGSE) experiment has been proposed as a means to extract dimensions of confined geometries using low *q*-values. In one realization of the d-PGSE experiment, the first gradient pair is fixed along the *x*-axis, and the orientation of the second gradient pair is varied in the *X*–*Y* plane. Such a measurement is sensitive to microscopic anisotropy induced by the boundaries of the restricting compartment, and allows extraction of the compartment dimension. In this study, we have juxtaposed angular d-PGSE experiments and simulations to extract sizes from well-characterized NMR phantoms consisting of water filled microcapillaries. We are able to accurately extract sizes of small compartments (5 μm) using the angular d-PGSE experiment even when the short gradient pulse (SGP) approximation is violated and over a range of mixing and diffusion times. We conclude that the angular d-PGSE experiment may fill an important niche in characterizing compartment sizes in which restricted diffusion occurs.

1. Introduction

Diffusion NMR [1] has become important in MR measurements due to its ability to non-invasively measure the mean displacement of molecules [2]. The single pulsed gradient spin echo (s-PGSE) method employs one pair of pulsed magnetic field gradients (PFG) with amplitude and direction given by the vector **G**, which are applied for a duration δ, separated by a diffusion time, Δ, when spins may diffuse due to Brownian motion. The resulting NMR signal is governed by the phase distribution of molecules produced during the diffusion time, Δ. The signal attenuation is directly related to the root mean-squared displacement (rmsd) of the diffusing species, which in turn, is directly related to the diffusion coefficient, D, in the case of free diffusion. Since the D is also an indirect measure of the size of molecules, diffusion MR is invaluable for studying supramolecular systems and mixtures of substances [3], degrees of aggregation [4], chemical complexes [5] and even ligand-protein interactions [6].

In isotropic samples, where molecules are free to diffuse in all directions, the logarithm of the NMR signal attenuation is directly related to $b = (\gamma\delta|\mathbf{G}|)^2 t_d$, where γ is the gyromagnetic ratio of the spins, and t_d is the diffusion time. Especially interesting is when molecules diffuse in a confined geometry, in which case the logarithm of the signal attenuation is no longer linear with respect to b at high *b*-values [7]. In systems which do not exhibit free diffusion, only an apparent diffusion coefficient (ADC) can be extracted from the plot of the signal decay versus the b values. In recent years it has been shown that the geometry and size of the compartment can be inferred from such s-PGSE experiments, a property which has been utilized in a variety of applications ranging from porous media [8], to biological tissue [9,10]. By measuring the signal decay in several directions, the diffusion tensor can also be estimated [11], providing intrinsic MR parameters that are invariant to rotation. While diffusion tensor imaging (DTI) studies are intended to be performed with low *b* values ($b < 1500$ s/mm^2), others have conducted diffusion measurements with high b values [9,10,12–16]. In isotropic freely diffusing systems characterized by one compartment, the information that is extracted is the same for both high and low *b* values, because the natural logarithm of the signal decays linearly when plotted as a function of *b*. However, in systems characterized by multiple compartments and/or in which different modes of diffusion exist, the attenuation curve deviates from linearity. A diffusion measurement in such systems with high b values can suppress the fast decaying component and accentuate the slow decaying components. Suppressing the fast component often leads

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to a better estimate of the compartment size [17,18], since the slow component usually arises from spins that remain confined in the restricting compartment whose dephasing is limited.

One of the most important applications of s-PGSE experiments in confined geometries is to extract the size of the compartment. Callaghan et al. showed that in diffusion NMR experiments in regular, ordered confined geometries, a phenomenon called diffusive-diffraction occurs, which is manifested by the non-monotonicity of the signal decay when plotted as a function of $|\boldsymbol{q}|$, where $\boldsymbol{q} = (2\pi)^{-1}\gamma\delta\mathbf{G}$ [19]. The size of the compartment can be extracted from the minima of the signal attenuation curves when plotted as a function of q or from the full-width-at-half-maximum (FWHM) of the probability distribution function (PDF), i.e., from the Fourier Transform (FT) of the signal decay, $E(q)$, as a function of q, provided that the diffusion time is sufficiently long to probe the boundaries of the compartment [20–22]. Exact solutions have been derived for idealized geometries [23–26] and some of these solutions were verified experimentally [22]. Several studies have shown how variation of experimental parameters affects the compartment size that was extracted, including variation of δ, Δ and the rotational angle in both phantoms and neuronal tissue [21,22,27,28], however, the need to apply very strong gradients limits the ability to probe the finest spatial dimensions using high **q** experiments.

The double-PGSE (d-PGSE) experiment (Fig. 1), which has two pairs of diffusion sensitizing gradients separated by a mixing time (t_m), was first proposed by Cory et al. [29] and was applied to studying porous media and flow phenomena [30], 2D diffusion measurements [31,32], suppression of convection artifacts [33], and dispersion and velocity correlation [34,35]. Using the d-PGSE sequence, Callaghan and Komlosh showed microscopic anisotropy in macroscopically isotropic polymers [36]. Recently, Komlosh et al. extended these findings to grey matter and grey matter NMR and MRI phantoms [37,38].

Özarslan and Basser have recently studied the d-PGSE sequence theoretically in the high q regime in confined geometries when the two gradients are parallel [39]. Their findings suggested some unexpected phenomena such as zero-crossings in the NMR signal profile (which result in negative diffractions) that they predicted to be sensitive to prolongation of the mixing time. Shemesh and Cohen have corroborated these findings experimentally, and have shown that, as predicted by simulations in [39], the d-PGSE experiment is sensitive and robust (compared to the s-PGSE) for inferring structural information that can be obtained from complex samples such as mixtures of microcapillaries having different diameters [40].

A limitation of both single and double-PGSE diffraction experiments is that in order to extract the dimension of the restricting compartment, relatively high q values must be reached. Since the q value at which diffractions occur is proportional to the reciprocal of the compartment size, the smaller the compartment, the higher the q that one needs to measure the compartment dimensions. This means that to probe small compartments, which are prevalent, for example, in the central nervous system, there is a need to apply very high q values [23,28], requiring either very strong gradients or very long δs. In such experiments, the finite length of the pulsed gradients makes matters worse, since "motional narrowing" pushes the diffraction minima towards even higher q values, due to a violation of the short gradient pulse (SGP) approximation. To accurately measure the compartment size, δ is assumed to be negligible compared to Δ. In other words, the SGP approximation assumes that the mean-squared displacement of molecules during the pulsed gradient is insignificant with respect to the mean-squared displacement the molecules will experience during the diffusion period. However, in small compartments, the Δ needed for molecules to completely probe the boundaries of the restricting compartment is very short; therefore, δ becomes comparable to Δ, violating the SGP approximation and resulting in inaccurate size

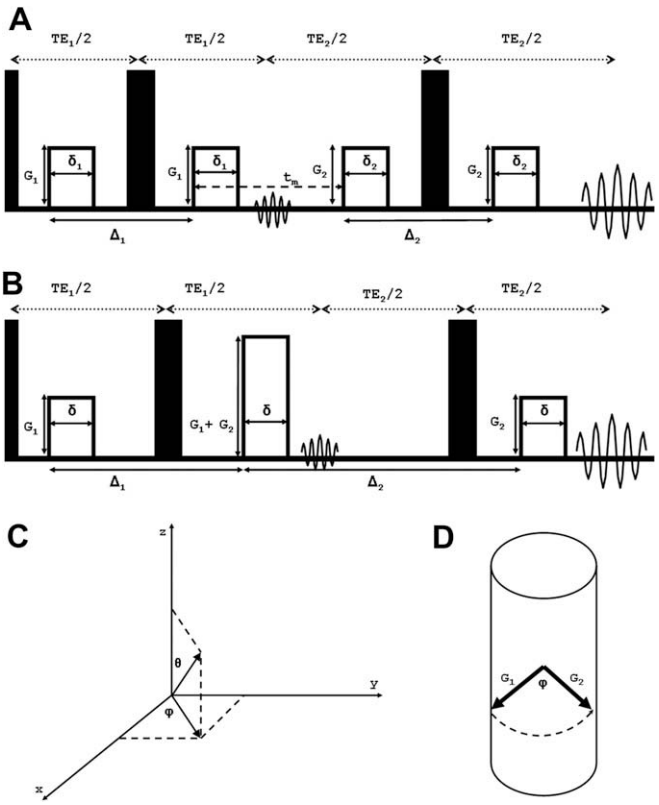

Fig. 1. Sequences and orientation schemes. (A) The d-PGSE experiment in which the mixing time is defined between the beginning of the second diffusion sensitizing gradient and the beginning of the third diffusion sensitizing gradient. (B) The d-PGSE experiment in which the mixing time is inherently 0. The second and the third diffusion sensitizing gradients are superimposed. (C) The azimuthal and polar angles φ and θ, respectively. (D) The orientation of the gradients in one of the angular d-PGSE experiments used in this study, in which the azimuthal angle is varied. In this experiment, $\mathbf{G}_1$ is set along the x-axis, i.e. with $\varphi = 0°$ and $\theta = 90°$, and $\mathbf{G}_2$ is varied along φ in the X–Y plane. Note that the cylinders are aligned with their long axis parallel to the z-axis, which is also the direction of B_0.

measurements. An alternative is to employ extremely strong gradient amplitudes for very short periods; however, such strong gradients also pose a challenge to hardware, and are unlikely to be deemed safe for clinical use because of the large electric fields they can induce. Therefore it remains a challenge to accurately extract the size of small compartments with diffusion MR methods, particularly in a biological or clinical setting.

Mitra studied the angular d-PGSE experiment as early as 1995 [41] in which he predicted the angular dependence of the signal decay from d-PGSE experiments in confined geometries. The theory developed by Mitra did not take into account finite durations of the diffusion sensitizing gradients or the mixing time. However, very recently, Özarslan and Basser addressed the angular dependence of the signal decay in confined geometries theoretically [42] with "arbitrary timing parameters". This study shows, *inter alia*, that when the first gradient is fixed along the axis perpendicular to the fiber, and the direction of the second gradient is varied in the X–Y plane from 0° to 360°, the signal decay exhibits an angular dependence, which can be interpreted as arising from microscopic anisotropy due to the borders of the confining geometry. The theory presented in [42] took into account the duration of the gradient pulses and the mixing time. Moreover, Özarslan and Basser showed that one could circumvent the need for high gradients, and extract the compartment size, even in very small compartments at low q values provided that, $2\pi qa < 1$ for a compartment of size 'a'. Since this method obviates applying very strong gradients for extracting sizes, we have sought to test and