Measuring small compartmental dimensions with low-q angular double-PGSE NMR

N. Shemesh[1], E. Özarslan[2], P. J. Basser[2], and Y. Cohen[1]

[1]School of Chemistry, Tel Aviv University, Tel Aviv, Israel, [2]Section on Tissue Biophysics and Biomimetics, NICHD, National Institute of Health, Bethesda, Maryland, United States

Introduction. Diffusion NMR is an important technique that can be used to non-invasively explore opaque samples by measurement of the root mean squared displacement of the diffusing moiety[1]. 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[2]. However, to measure the size of small compartments, high q-values must be reached, requiring diffusion measurements with high gradient strength and/or long gradient duration. An alternative is the employment of the double-PGSE (d-PGSE) experiment wherein the PGSE block is repeated as shown in Figure 1A. Özarslan and Basser have recently studied the d-PGSE sequence theoretically in the high q regime when the two gradients are parallel in confined geometries[3]. 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[4]. Another realization of the d-PGSE experiment involves varying the angle between the two gradient pulses, e.g., 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. This angular double PGSE experiment has been proposed to extract dimensions of confined geometries while circumventing the need for high q values[5, 6]. 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 experiments and simulations to extract sizes from well-characterized NMR phantoms consisting of water filled microcapillaries using low-q angular d-PGSE NMR[7].

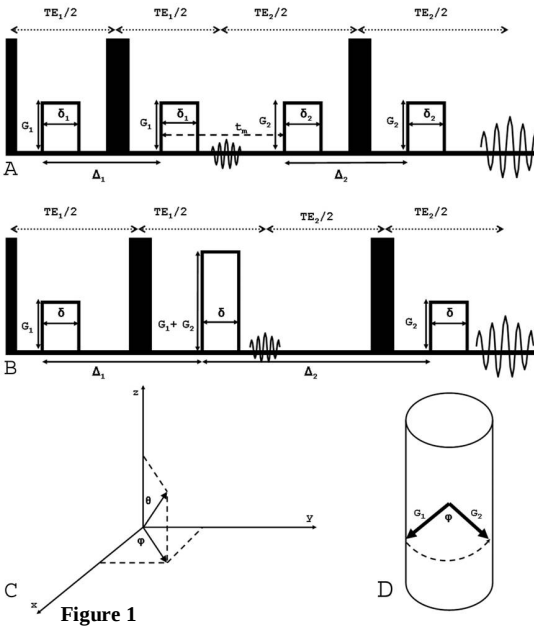

Figure 1

Materials and Methods. All measurements were performed on a Bruker 8.4 T NMR spectrometer capable of producing pulsed magnetic field gradients of magnitude up to 190 G/cm in each direction. Hollow microcapillaries with inner diameters (IDs) of 5, 9, 10 or 19 μm (Polymicro Technologies, USA) were immersed in water for several days, prior to each experiment. The microcapillaries were packed into a 4 mm glass sleeve which was inserted into a 5 mm NMR tube, aligned with the main axis parallel to the z-direction of the magnet**.** Figure 1 shows the d-PGSE sequences we used in this study. Figure 1A shows a d-PGSE sequence which employs two pairs of diffusion sensitizing gradients, with amplitudes and directions of $\mathbf{G_1}$ and $\mathbf{G_2}$ and gradient durations of δ_1 and δ_2, respectively. The diffusion times Δ_1 and Δ_2 are defined in Figure 1. In a sequence such as in Figure 1A, the mixing time is defined as the time between the end of Δ_1 and the beginning of Δ_2 periods. Figure 1B shows a variant in which the second and third diffusion sensitizing gradients are superimposed. For this sequence, $t_m = 0$ ms.

Figure 1C defines the azimuthal angle φ and the polar angle θ. In each experiment, the first gradient pair, i.e., $\mathbf{G}_1$, was aligned either along the x-axis or along the z-axis, as shown in Figure 1C. The orientation of the second gradient pair, i.e., $\mathbf{G}_2$, was varied. For example, Figure 1D shows an angular d-PGSE experiment in which $\mathbf{G}_1$ was aligned along the x-axis, and the orientation of $\mathbf{G}_2$ was varied in the X-Y plane. For all of the experiments in this study, the amplitudes of the first and second gradients were identical, i.e., $|\mathbf{G}_1|=|\mathbf{G}_2|$. The attenuation factor u^2 which was used to reduce the effects of higher order terms in the restricted diffusion experiments was calculated as follows: $u^2 = -\frac{d \log E}{dq^2}\Big|_{q=0}$

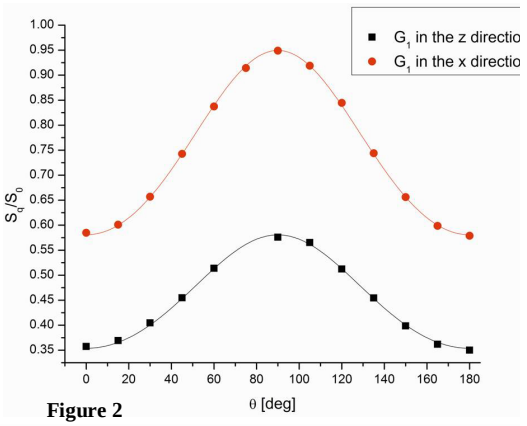

Figure 2

Results. Varying the polar angle with $\mathbf{G_1}$ set along the x or the z axis. In this type of experiment, $\mathbf{G_1}$ is fixed along one axis, and the polar angle is varied for $\mathbf{G_2}$**.** The theoretical study[6] predicted that the signal decay from such angular d-PGSE experiments should exhibit a bell-shaped function with a maximum at 90° when the signal is plotted against the polar angle θ. Figure 2 shows the normalized signal obtained from such experiments, performed on microcapillaries with a nominal ID of 9±1 μm, with q=129 cm^{-1}. Two separate experiments are shown: One with $\mathbf{G}_1$ set along the x-axis (red circles), and one with $\mathbf{G}_1$ set along the z-axis (black squares). The anticipated bell-shaped functions can be easily seen. The expected maximum signal indeed occurs at 90° for both experiments, and the simulations (solid lines) fit the experimental points (symbols) nicely.

Varying the azimuthal angle. In this type of experiment, $\mathbf{G_1}$ is fixed along the x-axis, and the azimuthal angle of $\mathbf{G_2}$ is varied. Figure 3 shows the attenuation factor u^2 as a function of the azimuthal angle φ, for three different samples of microcapillaries having nominal IDs of 5±1, 9±1 and 19±1 μm. The expected inverted bell-shaped functions can be seen for all experiments. The sizes, extracted from the fit of the angular dependence, closely correspond to the nominal ID. In this study we have also shown that the violation of the SGP approximation is accounted for by the simulations, as well as variations of the mixing time and the diffusion times. In all of these cases, provided that the diffusion period is sufficiently long to probe the boundaries of the restricting compartment, we have been able to accurately extract the sizes of the compartments.

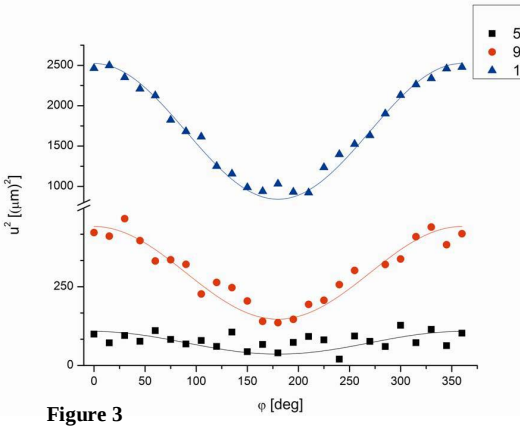

Figure 3

Conclusions. We have shown a method for extracting the compartment size in microcapillaries using low q angular d-PGSE NMR. This experimental study validates the theory that was previously published and confirms that the orientations and the dimensions of fibers can be simultaneously inferred using d-PGSE experiments at low q values. We have shown that we are able to accurately extract sizes of small compartments (5μm) using low q angular d-PGSE NMR, even when the short gradient pulse (SGP) approximation is violated and over a range of mixing and diffusion times. We conclude that the low q angular d-PGSE experiment may fill an important niche in characterizing compartment sizes in which restricted diffusion occurs**.**

References: [1] Y. Cohen et. al., (2005) Angew. Chem. Int. Ed. 44: 520-554 [2] P.T. Callaghan (1996) Magn. Reson. Imaging 14: 701-709 [3] E. Özarslan and P.J. Basser, (2007) J. Magn. Reson. 188: 285-294 [4] N. Shemesh and Y. Cohen (2008) J. Magn. Reson. 195: 153-161 [5] P.P. Mitra, (1995) Phys. Rev. B 51: 15074-15078 [6] E. Özarslan and P.J. Basser, (2008) J. Chem. Phys. 128:154511 [7] N. Shemesh et al., Submitted