Microstructural Information by double-pulsed-field-gradient NMR: From Model Systems to Nerves

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Introduction: Double-pulsed-field gradient (d-PFG) MR techniques are attracting more attention in recent years [1]. The technique, that was proposed first by Cory et al. in 1990 [2] is an extension of the single PFG MR sequence, that employs two diffusion sensitizing gradient pairs, G_1 and G_2, with duration δ_1 and δ_2, respectively, that are separated by a mixing time t_m. Two diffusion time intervals, Δ_1 and Δ_2, exist in these sequences [2]. Moreover, these two gradient pairs may be applied collinearly or with an angle between them yielding the radial and angular d-PFG NMR experiments, respectively [2,3]. The angular d-PFG MR sequence enables one to detect at zero mixing time microscopic anisotropy (μA) even in macroscopically isotropic samples and with relatively weak gradients [3]. Mitra and then Özarslan predicted that such experiments would yield an angular dependence (E(φ)), that resembles a bell-shaped curve from which the compartment size can be obtained by fitting the data to a suitable model [3,4]. Özarslan demonstrated that at finite mixing time one can obtain information on the sample eccentricity. This methodology was recently used to qualitatively describe diffusion in neuronal tissues [5,6]. However before studying quantitatively complex biological specimens it is important to test the accuracy and performance of our modeling approach on real, complex samples were the ground truth is known a priori.

Objectives: To challenge a new model for studying diffusion in angular d-PFG NMR experiments using phantoms of increasing complexity where the ground trough is known a priori. Subsequently to use it to obtain structural information of pig optic nerves.

Methods: The experiments were performed on a Bruker 8.4 T NMR spectrometer equipped with Micro5 probe capable of producing pulsed gradients of up to 190 G/cm in x-, y- and z- directions. The angular double-pulsed-gradient-spin-echo d-PGSE and the bipolar d-PGSE (bp-d-PGSE) NMR experiments were performed in the x-y plane in which G_1 was fixed along the x-axis and the orientation of G_2 was varied. The measurements were conducted for 25 different values of φ between 0° and 360°. A mixtures of microcapillaries with known inner diameters (ID) of 5 ± 1, 9 ± 1, 15 ± 1 and 23 ± 1 were used both in the absence or presence of different amount of water. For mixtures of 23±1 and 14±1 μm (1:1 volumetric ratio) microcapillaries the angular d-PGSE experiments were performed with $\delta_1 = \delta_2 = \delta_3 = 2$ ms and G_{max} of 80 G/cm, resulting in a maximal q value of 681 cm^{-1}. Δ_1 and Δ_2 were set to 150 ms and the t_m was set to 0**.** For the mixture of 9±1 and 5±1 μm (1:1 volumetric ratio) microcapillaries the angular bp-d-PGSE experiments were collected with $\Delta_1 = \Delta_2$ =50 ms, t_m=0 ms, $\delta_1 = \delta_2 = \delta_3 = 4$ ms and G_{max} of 80 G/cm, yielding a maximal q value of 1362 cm^{-1}. The pig optic nerve was studied with diffusion times of 30 ms, the mixing time was 0 ms, the pulse gradient durations were set to 2 or 4 ms and the G_{max} was 80 G/cm, yielding a maximal q value of 681 or 1362 cm^{-1}, respectively. This model is trying to fit the signal decay by a superposition of free Gaussian diffusion and a series of restricted diffusion in cylindrical geometries. No assumption is made on the number of compartments and only a size range has to be inserted a priori.

Results and Discussion: Figures 1A-B show the results obtained from the angular monopolar and bipolar d-PFG NMR experiments performed on mixtures of 23±1:14±1 μm and 9±1:5±1 μm microcapillaries, respectively, with increasing amounts of free diffusing water. Figure 1 shows only a few q-values for each sample, before and after addition of free diffusing water to the sample. The numerical values obtained from the fitting procedure are presented in Table 1. For the mixture of 9±1:5±1 μm microcapillaries we had to perform angular bp-d-PGSE experiments to suppress the effects of background gradients. As expected $E(\varphi)$ profile, showing a bell-shaped dependency can be observed in both samples. $E(\varphi)$ is governed by microscopic anisotropy (μA) that arises from the boundaries of the restricting compartment. Moreover, when increasing amounts of free diffusing water were added to the samples the $E(\varphi)$ profile seems to be suppressed. In our modeling no assumption was made regarding the number of compartments that need to be identified, however the fitting was able to detect the sizes and fractions of the restricted compartments and to detect the increase in the fraction of free diffusing water in the sample. The extracted sizes are in very good agreement with nominal sizes. Moreover, when the amount of free diffusing H_2O was increased the fractional volume ratio extracted for the two restricted components remained nearly the same. After verifying that the fitting provide accurate information in samples where the ground truth is known, we used the same approach to study diffusion in optic nerve. Figures 1C-D show $E(\varphi)$ profiles of optic nerve obtained from angular bp-d-PGSE experiments. The fittings of data presented in Figures 1C-D were obtained while assuming that the diameters that need to be found are in range of 0.6 to 12 μm. The numerical values obtained from the fittings are tabulated in Table 1. We found that the results which were obtained under the different experimental conditions are similar. The extracted mean axonal diameter was found to be 2.0±0.2 μm for the different experiments performed on the nerve.

Conclusion: We could demonstrate that our modeling is able, without assuming the number of compartments or the modes of diffusion that prevail in the sample to detect the sizes of the restricted compartments, as identify their relative populations, and characterize free diffusion when presents in the sample. This model seems to be suitable for obtaining accurate detailed microstructural information in neuronal tissues from angular d-PFG experiments**.**

Figure 1. The $E(\varphi)$ profiles of the angular d-PGSE and bp-dPGSE experiments performed on a phantoms of increasing complexity (A-B) and on optic nerve (C-D). Symbols indicate experimental data and the solid lines represent the fitting curves.

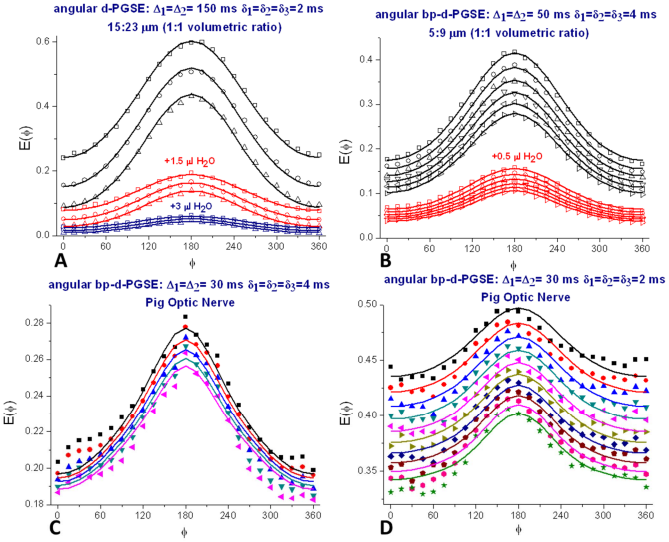

Table 1. Compartment sizes and volume fractions of the phantoms comprising of two type microcapillaries with increasing amount of free diffusing water and optic nerve as obtained from the PGSTE experiments.

sample	free water	ID [μm]	restricted diffusion fraction	free diffusion fraction	standard deviation
15:23μm	1ml D_2O	14.9 22.7	0.46 0.48	0.06	0.0068
	1 ml D_2O + 1.5 μl H_2O	14.9 22.7	0.15 0.14	0.71	0.0026
	1ml D_2O + 3 μl H_2O	15.0 22.9	0.04 0.04	0.92	0.0010
5:9 μm	1ml D_2O	4.8 8.3	0.35 0.45	0.20	0.0071
	1 ml D_2O + 0.5 μl H_2O	4.7 8.0	0.12 0.17	0.71	0.0050
optic nerve	a	2.1	0.33	0.67	0.0075
	b	2.1 6.4	0.25 0.16	0.59	0.0049

a). $\delta_1 = \delta_2 = \delta_3 = 2, \Delta_1 = \Delta_2 = 30$ ms **b).** $\delta_1 = \delta_2 = \delta_3 = 2, \Delta_1 = \Delta_2 = 30$ ms

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