Pore sizes and directionality in microcapillaries from angular double-pulsed-field-gradient NMR

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A B S T R A C T

Angular double-pulsed-field gradient (d-PFG) MR methodology is increasingly used to non-invasively obtain pore sizes in opaque chemical and biological systems. In such MR experiments, the angular dependency of the signal at zero mixing time, through modeling, can be used to extract the pore size. In many systems not only the pore sizes but also their directions are of importance. Before applying d-PFG NMR to complex systems, it is of value to challenge the ability of the methodology to extract these microstructural parameters in samples where the ground truth is known. In the present study we explored whether modeling of the signal in angular d-PFG NMR experiments at zero mixing time, can simultaneously provide the size and the direction of tilted compartments with little prior knowledge. We showed that the angular d-PFG MR methodology enables simultaneous extraction of the pore size and the direction of mono-dispersed phantoms and of phantoms where the restricted compartments have different pore sizes. However, we found that in phantoms with two or more pore sizes, only averaged pore sizes were extracted for large azimuthal and polar angles.

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1. Introduction

Porous materials are widespread both in nature and in man-made materials [1]. In many porous materials and biological systems, pore size may affect physical and chemical characteristics of materials and the functions of biological systems. For example, pore size is an important parameter in the characterization of sediments in the logging and petroleum industries [1], and the sizes of axons in neuronal tissues affect conduction velocity [2]. Therefore, non-invasive determination of pore size in opaque materials is of prime importance in numerous fields [3–5]. In many porous materials and biological systems, pores may have different shapes, are polydisperse in size, and may point in different directions resulting in complex microstructural networks.

NMR relaxation techniques were first used to study porous materials [1], and, subsequently, diffusion NMR and MRI have been used to study both artificial and natural porous materials [1,3,4,6]. Since diffusion MR provides a means to study pore sizes, totally non-invasively, diffusion MRI is of paramount importance not only in material sciences [1,3,4] but it is also extremely important in the study of biological tissues in general and neuronal tissues in particular [5,7]. Most diffusion MRI and MRI applications in material and biological sciences are in fact based on the Stejskal and Tanner design [8]. This design is known as the single-pulsed-field-gradient (s-PFG) MR experiment and can be classified as a single diffusion encoding (SDE) experiment. Recently, MR methods that use oscillating gradients [9–11] or asymmetrical gradient pulses have attracted interest [12].

Double-PFG (d-PFG) MR experiments, that can be classified as double diffusion encoding (DDE) methods, were suggested as an additional tool for studying microstructural features of compartments where restricted diffusion occurs [13]. This methodology, first proposed by Cory et al., is an extension of the well-known s-PFG MR method. The d-PFG MR sequences contain two diffusion encoding periods (Δ_1 and Δ_2) that may be separated by a mixing
time \( (t_m) \), each weighted for diffusion by the wave vectors \( \mathbf{q}_1 \) and \( \mathbf{q}_2 \), respectively. These wave vectors may be applied collinearly [13] or with an angle between them [14] resulting in the radial or angular d-PFG NMR experiments, respectively [13–17]. The signal decay in the angular d-PFG MR experiment is sensitive to the microscopic anisotropy (\( \mu \AA \)) of the sample, which may be present even in macroscopically isotropic samples [18]. When an angular d-PFG experiment is conducted at zero mixing time, the angular dependence of \( E(\phi) \) (where \( \phi \) is the angle between the two pairs of gradient pulses) may provide, after proper modeling, information on the compartment size [14–18]. Moreover, when the mixing time is sufficiently long, one may glean information about the eccentricity of the sample from such angular d-PFG MR experiments [19].

DDE MR methodologies have been used to study and characterize biological [20] and chemical systems [21] and to obtain structural information in different neuronal tissues [22–28]. In many of these cases, however, the ground truth of the studied samples is not known; therefore, it is difficult to evaluate the accuracy and, more importantly, the limitations of the new methodological approaches used. Therefore, other investigators and our group have, in recent years, used microcapillaries phantoms of different complexities to evaluate the potential of d-PFG MR to provide structural information that can be obtained by modeling signals in different NMR experiments [29–31]. Such phantoms have been used to validate angular d-PFG NMR experiments in samples where the ground truth is known [32–37] and to test approaches to pore imaging [12,38–40]. Very recently, however, the similarities in the data that can be obtained using s-PFG MR and d-PFG MR were outlined by Jespersen [41].

In the present paper, we modeled the signal in angular d-PFG NMR experiments performed on coherently organized microcapillaries in which the main axis was tilted by a known amount. The purpose of this study was to evaluate the ability of angular d-PFG diffusion MR to simultaneously provide, after proper modeling, both the size and the tilt angle or angles (i.e., the direction of the sampled compartment) with very little prior knowledge. Simulations, experiments, and modeling were performed on monodispersed, tilted compartments and on phantoms where the restricted compartments have two discrete sizes. The d-PFG MR experiments were also performed on samples in which pore size and the polar and azimuthal angles were unknown.

2. Materials and methods

2.1. MR instrument and phantoms

Diffusion measurements were performed on an 8.4 T NMR spectrometer (Bruker, Karlsruhe, Germany) equipped with a Micro5 probe and a gradient system (Bruker) capable of producing pulse gradients of 1900 mT/m in the x-, y-, and z-directions. Fused silica microcapillaries (Polymicro Technologies, Phoenix, AZ, USA) having inner diameters (ID) of 23 ± 1, 15 ± 1, 9 ± 1, 4.6 ± 0.3, and 5 ± 1 \( \mu \)m were cut into 4-cm long microcapillaries and dipped in water for two weeks before the MR experiments. Before the measurements, the outside of microcapillaries were carefully dried, packed into a 4-mm glass sleeve, and inserted into a 5-mm NMR tube, which was aligned parallel to the z direction of the magnet essentially as described previously [34,35]. Experiments were performed on microcapillaries with IDs of 23 ± 1 and 4.6 ± 0.3 \( \mu \)m and on 1:1 mixtures of microcapillaries with IDs of 23 ± 1 and 15 ± 1 \( \mu \)m and with IDs of 9 ± 1 and 5 ± 1 \( \mu \)m. All diffusion experiments were performed at 298 K.

2.2. NMR experimental details

Angular double pulsed-gradient spin-echo (d-PGSE) MR experiments (see Fig. 1A) were performed when the orientation of the first gradient pair \( (G_1) \) was in the x-direction while the second gradient pair \( (G_2) \) was varied in the xy-plane using 25\( \phi \) values between 0° and 360°. Since we could not physically rotate the microcapillaries in our 10-mm imaging probe, we rotated the coordinates of \( G_1 \) and \( G_2 \) so as to achieve an effective known rotation. \( G_1 \) was rotated from the xz-plane from \( \alpha \) of 0° (\( G_1 \) in the x-direction) to \( \alpha \) of 90° (\( G_1 \) in the z-direction) while \( G_2 \) was rotated accordingly from the xy-plane when \( \alpha \) was 0° to the xz-plane when \( \alpha \) was 90° (see Fig. 1C for coordinate definitions). In different d-PGSE MR experiments \( G_1 \) was rotated in the xy-plane from \( \beta \) of 0° (\( G_1 \) in the x-direction) to \( \beta \) of 90° (\( G_1 \) in the y-direction), while \( G_2 \) was rotated from the xy-plane when \( \beta \) was 0° to the xy-plane when \( \beta \) was 90° (see Fig. 1C for coordinate definitions). Note that in all the d-PGSE MR experiments performed herein \( \mathbf{q}_1 \) was equal to \( \mathbf{q}_2 \), so that \( \mathbf{q}_1 = \mathbf{q}_2 = \mathbf{q}_0 \).

The angular d-PGSE NMR experiments performed on the 23 ± 1-\( \mu \)m microcapillaries and on the 23 ± 1 and 15 ± 1 \( \mu \)m mixture were collected for \( \alpha \) values that were changed in 5° steps with the following parameters: 20\( \phi \) values were collected with \( G_0 = 80 \ G/cm \) and \( \delta_1 = \delta_2 = \delta_3 = 2 \) ms, resulting in a maximal \( \mathbf{q} \) value of 681 cm\(^{-1}\); the diffusion times, \( \Delta_1 \) and \( \Delta_2 \), were set to 150 ms; and the \( t_m \) was set to zero. In these MR experiments, the TR and TE were 4000 and 311 ms, respectively, and the number of averages collected (NA) was set to 16. For each \( \phi \) value, 25\( \phi \) angles, 20\( \phi \) angles were collected between 0° and 360° (i.e., 0°, 15°, 30°, 45°, 60°, 75°, 90°, ..., 360°). The signal to noise ratio (SNR) of the angular d-PGSE MR experiment performed on the 23 ± 1 \( \mu \)m and the 23 ± 1 and 15 ± 1 \( \mu \)m samples at \( q_{\min} \), when \( \phi \) was set to zero were about 6000 and 4000, respectively. For the 23 ± 1 \( \mu \)m phantom, additional d-PGSE MR experiments were performed when both \( \alpha \) and \( \beta \) were non-zero. For the 23 ± 1 \( \mu \)m phantom, d-PGSE MR experiments were also performed when \( \delta_1 = \delta_2 = \delta_3 \) were set to 2, 4, and 8 ms keeping all other parameters constants. In these experiments \( q_{\max} \) values of 681, 1362, and 2274 cm\(^{-1}\) were obtained.

Angular bipolar (bp) d-PGSE MR experiments (Fig. 1B) performed on the 4.6 ± 0.3-\( \mu \)m microcapillaries and on the mixture of 9 ± 1- and 5 ± 1-\( \mu \)m microcapillaries were collected using the above parameters, with the only differences being that \( \delta_1 = \delta_2 = \delta_3 \) were set to 4 ms (\( q_{\max} \) of 1362 cm\(^{-1}\)) and \( \Delta_1 \) and \( \Delta_2 \), were set to 50 ms. In these d-PGSE MR experiments the TR and TE were set to 4000 and 158 ms, respectively, and the NA was set to 32. The SNRs in these d-PGSE MR experiments for \( q_{\min} \) when \( \phi \) was zero were 690 for the 9 ± 1.5 ± 1 \( \mu \)m mixture and 537 the 4.6 ± 0.3 \( \mu \)m phantom.

2.3. Simulations and fitting of the experimental data

In the simulations, the angle \( \alpha \) or \( \beta \) was changed by steps of 10°, and microcapillaries of 23.4, 15, 5, and 2 \( \mu \)m were investigated. For \( \alpha \) or \( \beta \) in the range of 0°–10° and in the range of 80°–90°, simulations with 2.5° steps were also performed. Simulations of the d-PGSE MR experiment where both \( \alpha \) and \( \beta \) angles were non-zero were also performed.

For the modeling we used capped cylinders with radius \( r_0 \) and length \( L \), oriented along an arbitrary direction \( \mathbf{u} \). In these simulations, \( q_z \) is the component of \( \mathbf{q} \) that is parallel to the cylinder axis, and \( q_x, q_y \) are the components in the perpendicular plane such that \( q_x = q \mathbf{u} \), \( q_y = q \hat{\mathbf{v}} \), \( q_z = q \hat{\mathbf{w}} \). Since the unit vector \( \mathbf{u} \) is associated with the polar angle \( \theta \) and azimuthal angle \( \phi \) we obtain,
\[ u = \begin{pmatrix} \cos \phi \sin \theta \\ \sin \phi \sin \theta \\ \cos \theta \end{pmatrix}, \quad v = \begin{pmatrix} \sin^2 \phi + \cos \theta \cos^2 \phi \\ (\cos \theta - 1) \sin \phi \cos \phi \\ -\sin \theta \cos \phi \end{pmatrix}. \]

\[ w = \begin{pmatrix} (\cos \theta - 1) \sin \phi \cos \phi \\ \cos^2 \phi + \cos \theta \sin^2 \phi \\ -\sin \theta \sin \phi \end{pmatrix}. \]

The magnetization \( E(\mathbf{q}) \) in the d-PFG MR sequence, according to Refs. [18] and [42], is given by \( E(\mathbf{q}) = E_A E_1 \), where \( E_A \) and \( E_1 \) stand for the parallel and perpendicular magnetization components, respectively. \( E_A \) and \( E_1 \) are given by Eq. (2).

\[
\begin{align*}
E_A & = \left[ e^{-A_1 \delta + i2\pi \phi_1 T_e} e^{-A_1 \delta - i2\pi \phi_1 T_e} e^{-A_1 \delta + i2\pi \phi_1 T_e} e^{-A_1 \delta - i2\pi \phi_1 T_e} e^{-A_1 \delta + i2\pi \phi_1 T_e} e^{-A_1 \delta - i2\pi \phi_1 T_e} \right]_{0,0} \\
E_1 & = \left[ e^{-A_1 \delta + i2\pi \phi_1 T_e} e^{-A_1 \delta - i2\pi \phi_1 T_e} e^{-A_1 \delta + i2\pi \phi_1 T_e} e^{-A_1 \delta - i2\pi \phi_1 T_e} e^{-A_1 \delta + i2\pi \phi_1 T_e} e^{-A_1 \delta - i2\pi \phi_1 T_e} \right]_{0,0} \\
\end{align*}
\]

The multiplications of the exponentials result in a matrix, and we take only its (0,0) element. Here,

\[
\begin{align*}
(A_A)_{km,k'm'} & = \delta_{kk'} \delta_{mm'} \frac{\alpha_{km}^2 D_0}{r_0^2}, \\
(A_1)_{k,k'} & = \delta_{kk'} \frac{D_0 \pi^2 k^2}{r_0^2},
\end{align*}
\]

where \( \delta_{kk'} \) stands for the Kronecker delta and \( \alpha_{km} \) is the kth zero of the derivative of the nth Bessel function satisfying \( J'_n(\alpha_{km}) = 0 \). Finally,

\[
P_{kk'} = \begin{cases} 
L/2, & k = k' \\
L \epsilon_k \frac{(-1)^{k+k'} - 1}{\pi^2 (k^2 - k'^2)^2} & \text{otherwise}
\end{cases}
\]
\[(T_a)_{km,m'\pm1}(r_0) = r_0^2 \delta_{m,m'\pm1} \sqrt{1 + \delta_{m,0} + \delta_{m',0} \beta_{km,km'} \left( \frac{a_{km}^2 + a_{k'm'}^2 - 2mm'}{a_{km}^2 - a_{k'm'}^2} \right)^2} \]

\[(T_b)_{km,m'\pm1}(r_0) = r_0^2 \delta_{m,m'\pm1} - \delta_{m,m'\pm1} \sqrt{1 + \delta_{m,0} + \delta_{m',0} \beta_{km,km'} \left( \frac{a_{km}^2 + a_{k'm'}^2 - 2mm'}{a_{km}^2 - a_{k'm'}^2} \right)^2} \]

\[\beta_{km} = \begin{cases} 1 & \text{if } k = m = 0 \\ \frac{a_{km}}{\sqrt{a_{km}^2 - m^2}} & \text{otherwise} \end{cases} \]

In our modeling, \(M(q)\) denotes a measured signal at a given \(q\). In this work we modeled the signal as a linear combination of signals at different geometries (cylinder radii at different orientations). This means that for a set of \(m\) radius candidates \(r_1,r_2,\ldots,r_m\), \(n\) polar angles \(\theta_1,\theta_2,\ldots,\theta_n\), and \(z\) azimuthal angles \(\phi_1,\phi_2,\ldots,\phi_z\), the \(M(q)\) is given by:

\[M(q) = \sum_{jk} W_{jk} E(q, r_j, \theta_j, \phi_k)\]

The vector \(W = [W_{11}, W_{12}, \ldots, W_{mnm}]\) stands for the non-negative weights of the different signals that sum to 1. These weights were then calculated according to the measured signal. The analysis and optimization was carried out using the CVX tool (Matlab software for disciplined convex programming, http://cvxr.com) as described previously [34–39]. This procedure yielded the weight of every cylinder radius and angle to the measured signal. A specific cylinder was considered active if its weight exceeded some threshold (generally 0.5%). This formulation shows that there is no limitation to the number of active cylinders. We found that the contribution of inactive cylinders was negligible.

In our simulations we consider the cylinder length \(L\) to be infinite (1000 \(\mu m\)). The \(\theta_i\) candidates were quantized, and \(\phi_i\) was set to 0 since in some of the experiments the angle \(\alpha\) rotates in the xy-plane and therefore the azimuthal angle is 0. The optimization was carried out in the fixed coordinates system where the cylinder is rotated at angle \(\theta\) relative to the z axis. This angle \(\theta\) is actually the angle \(\alpha\) in the rotated system: The cylinder is oriented along the z axis and the plane is tilted with angle \(\theta\) as shown in Fig. 1. In other simulations and d-PFG MR experiments, angles \(\theta\) (angles \(\alpha\)) were kept constant (at zero or other values) and angles \(\phi_i\) (angles \(\beta\)) were rotated and the optimization was carried out in the same manner.

The fittings of the experimental data shown in Figs. 4 and 3 (see Supplementary data) were performed by assuming the angle \(\alpha\) is known. For the fittings of the data presented in Fig. 4, it was assumed that the restricted compartments had diameters in the range of 16–40 \(\mu m\), and the numbers of steps used were 20, 40, or 60. All the above experimental data were also fitted assuming a diameter range of 4–40 \(\mu m\) using 40 steps. For the fittings of the experimental data shown in Fig. 3, it was assumed that the restricted compartments had diameters in the range of 10–40 \(\mu m\), and the numbers of steps used for the simulations were 20, 40, or 60. These data were also fitted assuming a diameter range of 4–40 \(\mu m\) using 40 steps. The extracted microstructural parameters were found to be practically the same in all the above fittings. Therefore, the microstructural parameters presented in Table 1A and B are those extracted from the fittings performed assuming a diameter range of 4–40 \(\mu m\) using 40 steps.

The fittings of the experimental data shown in Figs. 5 and 5S were performed as for the data presented in Figs. 3 and 3, respectively, but in these cases both the IDs and the \(\alpha\) were unknown. Here again the number of steps used in the fittings and the diameter ranges used had no significant effect on the extracted parameters. Therefore the microstructural parameters presented in Table 2 are those obtained by fitting the experimental data assuming a diameter range of 4–40 \(\mu m\) using 40 steps. The fittings presented in Fig. 9 were performed with the above fitting parameters assuming that \(\beta\) and the pore size are unknown (see

<table>
<thead>
<tr>
<th>A</th>
<th>ID [(\mu m)]</th>
<th>Standard deviation of fit</th>
<th>Normalized RMSD</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>23.6 ± 0.4</td>
<td>3.17 × 10⁻²</td>
<td>3.93 × 10⁻²</td>
</tr>
<tr>
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<td>23.5 ± 0.3</td>
<td>3.58 × 10⁻²</td>
<td>4.86 × 10⁻²</td>
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<td>23.5 ± 0.3</td>
<td>3.34 × 10⁻²</td>
<td>4.48 × 10⁻²</td>
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<td>23.6 ± 0.4</td>
<td>4.84 × 10⁻²</td>
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<tr>
<td>30</td>
<td>23.9 ± 0.5</td>
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<table>
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<tr>
<th>B</th>
<th>ID [(\mu m)]</th>
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<tbody>
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<td>2.46 × 10⁻²</td>
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<td>7.74 × 10⁻²</td>
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<td>22.8 ± 0.4</td>
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<td>0.128</td>
<td>0.149</td>
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<th>C</th>
<th>(\alpha)</th>
<th>(\beta)</th>
<th>ID [(\mu m)]</th>
<th>Measured (\alpha) [(\mu m)]</th>
<th>Measured (\beta) [(\mu m)]</th>
<th>Standard deviation of fit</th>
<th>Normalized RMSD</th>
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<td>0</td>
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<td>0.0 ± 0.1</td>
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<td>3.37 × 10⁻²</td>
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<tr>
<td>10</td>
<td>23.0 ± 0.5</td>
<td>21.6 ± 0.1</td>
<td>9.5 ± 3.6</td>
<td>4.51 × 10⁻²</td>
<td>6.67 × 10⁻²</td>
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<tr>
<td>20</td>
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<td>21.6 ± 0.3</td>
<td>21.7 ± 9.2</td>
<td>2.39 × 10⁻²</td>
<td>3.31 × 10⁻²</td>
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Table 2
Compartment sizes, αs, and relative fractions obtained from the fittings of the angular d-PGSE MR data presented in (A) Fig. S5 and (B) Fig. 5. In (A) pore size and as were unknown, and in (B) pore sizes, fractions and as were taken as unknown.

<table>
<thead>
<tr>
<th>ID [µm]</th>
<th>Measured α [°]</th>
<th>Standard deviation of fit</th>
<th>Normalized RMSD</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>23.5 ± 0.3</td>
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<td>3.02 × 10⁻²</td>
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<td>3.51 × 10⁻²</td>
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<td>5</td>
<td>23.4 ± 0.2</td>
<td>5.1 ± 1.2</td>
<td>3.29 × 10⁻²</td>
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<td>23.5 ± 0.3</td>
<td>10.0 ± 0.8</td>
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<td>5.96 × 10⁻²</td>
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<td>22.6 ± 0.5</td>
<td>31.4 ± 0.4</td>
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<tr>
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<td>1.93 × 10⁻²</td>
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<td>10</td>
<td>0.54</td>
<td>5.2 ± 1.5</td>
<td>5.4 ± 1.4</td>
<td>1.80 × 10⁻²</td>
<td>2.02 × 10⁻²</td>
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<tr>
<td>15</td>
<td>0.46</td>
<td>5.6 ± 1.4</td>
<td>10.4 ± 1.2</td>
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<td>30</td>
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<td>9.5 ± 0.7</td>
<td>15.9 ± 0.8</td>
<td>8.60 × 10⁻²</td>
<td>9.74 × 10⁻²</td>
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Table 3
Compartment sizes, αs, and relative fractions obtained from the fittings of the angular d-PGSE MR data performed on phantoms consisting of (A) microcapillaries having an ID of 46 ± 0.3 µm and (B) a 1:1 mixture of 3 ± 1-µm and 9 ± 1-µm microcapillaries. Pore sizes, fractions, and as were taken as unknown.

(A) | measured α [°] | standard deviation of fit | normalized RMSD |
<table>
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<tbody>
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<td>4.6 ± 0.2</td>
<td>0 ± 0</td>
<td>0.108</td>
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<td>10</td>
<td>4.5 ± 0.1</td>
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<td>6.99 × 10⁻²</td>
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(B) | measured α [°] | standard deviation of fit | normalized RMSD |
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<tbody>
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<td>4.9 ± 0.2</td>
<td>0.52</td>
<td>4.79 × 10⁻²</td>
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<tr>
<td>8.0</td>
<td>0.2 ± 0.4</td>
<td>0 ± 0</td>
<td>5.04 × 10⁻²</td>
</tr>
<tr>
<td>5</td>
<td>4.9 ± 0.2</td>
<td>0.54</td>
<td>5.2 ± 1.1</td>
</tr>
<tr>
<td>8.2</td>
<td>0.4 ± 0.4</td>
<td>5.4 ± 2.1</td>
<td>7.36 × 10⁻²</td>
</tr>
<tr>
<td>10</td>
<td>4.9 ± 0.9</td>
<td>10.7 ± 0.5</td>
<td>4.89 × 10⁻²</td>
</tr>
<tr>
<td>8.3</td>
<td>1.1 ± 0.4</td>
<td>10.2 ± 1.4</td>
<td>4.89 × 10⁻²</td>
</tr>
</tbody>
</table>

Table 1C. The fittings of the experimental data collected on the 4.6 ± 0.3-µm microcapillaries and on the 9 ± 1- and 5 ± 1-µm mixture (data shown in Fig. 8 and Table 3) were performed by assuming that both the IDs were in the range of 2–12 µm; the number of steps used was 40. In these cases both the pore size and the α angles were unknown. The diffusion coefficient of the microcapillaries along the z-axis, where diffusion is free and Gaussian, was found to be 2 × 10⁻⁹ m²/s, therefore in all fittings D₀ was set to this value. To evaluate the quality of the fits we computed the standard deviation (SD) of the fit and the normalized root mean square deviation (RMSD) of the fitted and the experimental points. The SD and the normalized RMSD values are given in Tables 1–3.

3. Results

To evaluate experimentally whether d-PFG NMR can be used to evaluate parameters of complex systems, we explored whether modeling of the signal in angular d-PFG NMR experiments at zero mixing time was able to accurately determine the sizes of pores and the direction of tilted compartments in microcapillaries with IDs of 23 ± 1 and 46 ± 0.3 µm and on a 1:1 mixture of microcapillaries with IDs of 23 ± 1 and 15 ± 1 µm and with IDs of 9 ± 1 and 5 ± 1 µm.

Fig. 2 shows the simulated E(φ) signals of angular d-PGSE MR experiments with zero mixing time for microcapillaries having IDs of 23.4 µm (Fig. 2A–C) or 5.0 µm (Fig. 2D–F) aligned in different directions (i.e., for α values from 0° to 90°). In Fig. 2A and D ten different α angles from 0° to 90° were used. Clearly, when α is equal to zero (G₁ is along the x-direction and G₂ is rotated in the y-plane), the signal intensity is the highest, as expected, for φ = 180°. As α becomes larger, the E(φ) signal changes. For α values larger than 20°, two maxima were observed for each E(φ) profile at φs of 90° and 270° with minima observed for φ = 0°, 180° and 360° as expected (Fig. 2A and D). Fig. 2D also shows that for the 5.0-µm microcapillaries the two maxima in the E(φ) profile were observed at smaller α than for the 23.4-µm microcapillaries. This is even more apparent in Fig. 2B and E, which show the E(φ) signals for as of 0° and 10°. Fig. 2B and E shows that the differences in the simulated E(φ) signals for αs of 0° and 10° were greater for 5.0-µm microcapillaries than for 23.4-µm microcapillaries. However, the E(φ) signals for as of 80° and 90° appear to be very similar, indicating that the perpendicular restricted direction is in fact more sensitive to the actual direction of the microcapillaries than is the free parallel direction, at least for the 23.4- and 5.0-µm ID microcapillaries. To visualize these changes and differences even better, Fig. S1 (see Supplementary data) shows the results of the same simulations when the E(φ) signals for all α values were normalized to φ = 0°.

Fig. 3 shows the simulated E(φ) signals of angular d-PGSE MR experiments with zero mixing time for microcapillaries having IDs of 23.4, 5.0, and 2.0 µm for different β angles when β angles were varied between 0° and 90°. When both αs and βs are non-zero, more complex behavior of the E(φ) signals was observed. Fig. S2 (see Supplementary data) shows the same simulations when the E(φ) signals were normalized to φ = 0°.

Fig. 4 presents the E(φ) signals and fits of angular d-PGSE MR experiments performed on microcapillaries having an ID of 23 ± 1 µm at five αs. Fig. S3 (see Supplementary data) shows the same data for a 1:1 mixture of microcapillaries with IDs of 23 ± 1 and 15 ± 1 µm. The fittings of the experimental data shown in Figs. 4 and S3 were made by assuming that the angle α is known a priori. Clearly, fits are in very good agreement with the experimental results. The E(φ) signals for α angles of 0° and 5° are very
similar, showing a maximal signal for $\phi$ of 180° for all $q$ values (Fig. 4A,B). However when $\alpha$ increased a plateau appeared first (Fig. 4C,D). When $\alpha$ was equal to 30° or higher (Fig. 4E) the plateau disappeared, and two maxima for $E(4)$ were observed.

Fig. S4 (see Supplementary data) shows the $E(4)$ signals for five different $\alpha$s at two $q$ values of 86.9 cm$^{-1}$ and 191.6 cm$^{-1}$ for microcapillaries having an ID of 23 ± 1 μm. It is easy to see that the fitted curves are all in very good agreement with the experimental results. In this figure one can also see that the differences in $E(\phi)$ for the different $\alpha$s become more apparent as the $q$ value increases. For example, for a $q$ value of 191.6 cm$^{-1}$ (Fig. S4B in Supplementary data) the difference in the $E(\phi)$ signals between $\alpha$ of 0° and 5° is clearly observed (Fig. S4B, black rectangles and red circles, respectively), whereas for a $q$ value of 86.9 cm$^{-1}$ very similar curves were obtained (Fig. S4A). It is important to note that no assumption was made regarding the number of compartments that need to be identified and that the model looks for an unknown number of compartments having a cylindrical geometry when $\alpha$ (i.e., the direction of the microcapillaries with respect to gradient pulses) is known a priori. The values extracted from the fittings of the experimental data presented in Figs. 4 and S3 are summarized in Table 1.

To further challenge the fitting procedures, we attempted the fittings when both $\alpha$ (i.e., the direction) and the size of the microcapillaries were assumed to be unknown. Figs. S5 (see Supplementary data) and 5 show $E(\phi)$ signals of the angular d-PGSE MR experiments and their fittings for a phantom consisting of microcapillaries with an ID of 23 ± 1 μm and of a 1:1 mixture of microcapillaries having IDs of 23 ± 1 and 15 ± 1 μm, respectively. The fitting parameters for Figs. 3, S3, 5 and S5 were all similar. Here again there was an excellent agreement between the experimental data and the fitting curves. The values extracted from the fittings of the data presented in Figs. 5 and S5 are summarized in Table 2. Despite the good fit of the experimental data
collected when $\alpha$ was 30° or higher, it is evident that the modeling, with the current input, was unable to characterize the different sizes of the restricted compartments in the phantom and only a weighted average of the pore size could be extracted for these cases.

Fig. 6 shows the $E(\varphi)$ signals of the angular d-PGSE experiments performed on 23 ± 1-μm microcapillaries and the fit to the data without prior knowledge of the $\alpha$ or compartment size for $\varphi$ values of 86.9 cm$^{-1}$ and 191.6 cm$^{-1}$. Fig. 7 shows the same data for a ~1:1 mixture of microcapillaries having IDs of 23 ± 1 and 15 ± 1 μm. Here again, there was good agreement between the fits and the experimental data. To compare the two fitting procedures, we evaluated the experimental $E(\varphi)$ signals of the angular d-PGSE MR experiments and their fittings for the 23 ± 1-μm microcapillaries phantom and for the phantom consisting of a ~1:1 mixture of microcapillaries with the current input. With and without prior knowledge of the $\alpha$ values during the fitting procedure (Figs. S6 and S7). The results of the two fittings were very similar and in good agreement with the experimental data. The above results show that the modeling procedure is indeed able to provide both the main direction and the size of the microcapillaries with accuracy and with very little prior knowledge even when the phantoms are not monodisperse in size. The modeling is accurate only for $\alpha$ of less than 30°, however, for $\alpha$ angles of 30° or greater, one obtains only the weighted average of the sizes of the microcapillaries.

To challenge the modeling even further additional bp d-PGSE MR experiments were performed on two phantoms which have significantly smaller IDs using bipolar d-PGSE MR experiments [35]. Phantoms consisting of microcapillaries having IDs of 4.6 ± 0.3 μm and of a ~1:1 mixture of 5 ± 1-μm and 9 ± 1-μm microcapillaries were studied. Fig. 8 shows the fittings of the experimental data obtained for the 4.6 ± 0.3-μm phantom for several values of $\alpha$. The microstructural parameters extracted from the fitting of the d-PGSE MR experiments are presented in Table 3. Fig. 8 shows that there is a good agreement between the experimental data and the fittings for the phantom with microcapillaries of one diameter. For the 4.6 ± 0.3-μm microcapillaries, the extracted pore sizes and $\alpha$s are in good agreement with the microstructural features of the phantoms. However, only the average pore size could be extracted from the data collected on the mixture of the 5 ± 1 and 9 ± 1 μm phantom when $\alpha$s were about 20°.

Based on the simulations presented in Figs. 3 and S3 (see Supplementary data) we next performed d-PGSE MR experiments in the three-dimensional space where both the polar and azimuthal angles were non-zero for a phantom consisting of 23 ± 1-μm microcapillaries. The results obtained from these experiments and their fittings are presented in Fig. 9 and the extracted

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**Fig. 3.** Simulations of $E(\varphi)$ signals for angular d-PGSE MR experiments performed on microcapillaries of (A, D, G) 23.4 μm, (B, E, H) 5.0 μm, and (C, F, I) 2.0 μm for different $\alpha$s for $\beta$s from 0° to 90°.
microstructural parameters are summarized in Table 1C. From these data, it is clear that one can obtain pore size as well as the orientation when the pore size and both the polar ($\alpha$) and azimuthal angles ($\beta$) are unknown.

Fig. 4. $E(\varphi)$ signals of the angular d-PGSE MR experiments (symbols) and fits (solid lines) performed on 23 ± 1-μm microcapillaries with $\alpha$s of (A) 0°, (B) 5°, (C) 10°, (D) 15°, and (E) 30°. The angular d-PGSE MR measurements were performed with $\Delta_1 = \Delta_2 = 150$ ms, $\delta_1 = \delta_2 = \delta_3 = 2$ ms, and $t_m$ of zero. The fits of the experimental data were performed by assuming that $\alpha$ was known a priori.

Fig. 5. $E(\varphi)$ signals of the angular d-PGSE MR experiments (symbols) and fits (solid lines) performed on a 1:1 mixture of 23 ± 1-μm and 15 ± 1-μm microcapillaries with $\alpha$s of (A) 0°, (B) 5°, (C) 10°, (D) 15°, and (E) 30°. The angular d-PGSE measurements were performed with $\Delta_1 = \Delta_2 = 150$ ms, $\delta_1 = \delta_2 = \delta_3 = 2$ ms, and $t_m$ of zero. The fits were performed assuming that both the pore size and $\alpha$ were unknown.
In the present study, microcapillaries (23 ± 1 or 4.6 ± 0.3 μm) and mixtures of microcapillaries (23 ± 1 and 15 ± 1 μm or 9 ± 1 and 5 ± 1 μm) with well-defined IDs were used to evaluate the ability of angular d-PFG MR experiments to provide, through modeling, both the size and the main direction of tilted compartments simultaneously.

Simulations of the angular d-PFG MR experiments conducted with zero mixing time and with increasing α angles showed that the expected $E(\phi)$ profiles obtained changed as α was increased. At small angles, $E(\phi)$ profiles had single maxima, whereas two maxima

4. Discussion

Fig. 6. $E(\phi)$ signals of the angular d-PGSE MR experiments (symbols) and fits (solid lines) performed on 23 ± 1-μm microcapillaries assuming that neither α nor ID were known with $q$ values of (A) 86.9 cm$^{-1}$ and (B) 191.6 cm$^{-1}$.

Fig. 7. $E(\phi)$ signals of the angular d-PGSE MR experiments (symbols) and fits (solid lines) performed on a 1:1 mixture of 23 ± 1- and 15 ± 1-μm microcapillaries assuming that neither α nor ID were known with $q$ values of (A) 156.7 cm$^{-1}$ and (B) 331.2 cm$^{-1}$.

Fig. 8. $E(\phi)$ signals of the angular bp-d-PGSE MR experiments (symbols) and fits (solid lines) performed on 4.6 ± 0.3-μm microcapillaries with αs of (A) 0°, (B) 5°, and (C) 10° assuming that neither pore size nor α were known. The angular bp-d-PGSE MR measurements were performed with $D_1 = D_2 = D_3 = 50$ ms, $\delta_1 = \delta_2 = \delta_3 = 4$ ms, and $t_m$ of zero.
were observed at larger angles as expected. Indeed, when $a$ was increased to 90° (i.e., when $G_1$ points along the $z$-direction in which diffusion is free in the microcapillaries), restriction was expected and observed when $\varphi$ was 90° or 270°. We also observed significant changes in the $E(\varphi)$ signals when $\alpha$ was varied in the range of 0°–10°. These changes were even more pronounced for smaller diameter microcapillaries. The $E(\varphi)$ signals when $\alpha$ was in the range of 80°–90°, however, appear similar to each other. This occurs since in angular d-PGSE experiments, in which $G_1$ is aligned or nearly aligned with the $z$-axis (i.e., the direction of free diffusion), the $E(\varphi)$ signals are dominated by free diffusion.

We found that the fitting curves presented in Figs. 4 and S4 are in good agreement with the experimental results. From the experimental data we also observed that differences in $E(\varphi)$ as a function of $\alpha$ became more pronounced when the $q$ values were increased. Table 1 summarizes the compartment sizes obtained from the fittings of angular d-PGSE data presented in Figs. 4 and S4. The fits were made by assuming that the angle $\alpha$ is known a priori. Only a range of diameters that need to be explored was inserted with no prior knowledge about the number of compartments that needs to be found or their sizes. For the single size microcapillaries phantoms, sizes that are in excellent agreement with the nominal compartmental sizes were found for $\alpha$ up to 50°. For larger $\alpha$, however, the extracted pore sizes were about 29 µm for the 23 ± 1-µm microcapillaries. For phantoms consisting of mixture of microcapillaries, very accurate microstructural information was obtained for $\alpha$ of less than 30°. However, despite the very good agreement between the experimental data and the fittings for $\alpha$ of 30° or more the extracted sizes were a weighted average of the two sizes of the compartments present in the sample.

When both the sizes and the directions of the microcapillaries were taken as unknown, the curve fits were also in very good agreement with experimental data (Figs. 5 and S5). Table 2 summarizes the compartment sizes and $\alpha$ extracted from the fittings of angular d-PGSE data shown in Figs. 5 and S5. Table 2 shows that the modeling procedure was indeed able to characterize both the size and the main direction of microcapillaries accurately. Good agreement was found between the extracted values from the fitting of the experimental data and the known microstructural features of the phantoms. Here again, when $a$ was equal or larger than 30°, only a weighted average of the sizes of the compartments in the sample could be obtained.

The results presented in Fig. 8 and Table 3 show that similar microstructural information can be obtained also when the IDs of the microcapillaries are significantly smaller and when the SNRs are lower. In addition, we found that microstructural information was accurately extracted by modeling the d-PGSE MR signal when polar and azimuthal angles and pore size were unknown (Fig. 9 and Table 1C). However, as expected, as the number of unrelated parameters to be determined (pore sizes, fractions, angles) increases it is increasingly likely that only averaged microstructural information will be obtained. In addition, when measurements are carried out along the direction where the diffusion is free, it is, as expected, much more difficult to obtain detailed microstructural information.

We found a somewhat better agreement between the fittings and the experimental data when both the sizes of the compartments as well as the direction of the microcapillaries ($\alpha$ angles) were fitted. When more parameters are fitted there is a better chance for a better fitting but this by no means implies that these are indeed better solutions. Nevertheless, since phantoms where the ground truth is known were used, it is clear that both size and direction can be obtained by modeling and fitting the signal of angular d-PFG NMR experiments. Our analysis demonstrates that the two major features of the microstructure of the phantom, size and direction of microcapillaries, can be determined with good accuracy. However, we note that when there was more than one size microcapillaries in the phantom and $\alpha$ were higher than 30°, despite the good agreement between fittings and the experimental data only a weighted average of sizes was extracted.

The microstructural information obtained by modeling the d-PGSE MR signal was obtained with little prior knowledge. The only inputs were the range of sizes that need to be explored which has only to encompass the sizes to be found and $D_0$. $D_0$ affected the extracted sizes; however, in the current systems its value could be determined quite accurately. So on the one hand the known $D_0$ simplify the modeling by excluding one parameter that need to be found, but on the other hand one has to generate the correct pore sizes and directions in all systems with a single, discrete well defined $D_0$ value.

5. Conclusions

In the present study we explored the ability to extract, through modeling, the size as well as the direction of compartments in defined microcapillaries systems using zero mixing time angular d-PFG NMR. To evaluate the accuracy of our fitting procedure, we first performed a series of simulations on cylindrical phantoms having known IDs, which directions were also known a priori. We then applied the same methodology to study single size and
double size microcapillaries phantoms. We found that modeling and fitting the experimental data obtained from angular d-PFG NMR experiments enabled one to extract the compartment size of tilted microcapillaries with high precision. Since the fitted curves were in good agreement with the experimental data, we used the same approach to determine simultaneously the size and the tilting, i.e. the direction of the microcapillaries in these phantoms. Here the fittings were also in good agreement with the experimental data, and we could determine with high accuracy both the size and the direction of the microcapillaries in the phantom, even when samples in which the microcapillaries were of two different diameters. However, for samples consisting of compartments with different sizes, when \( \alpha = 30^\circ \) or higher, only an averaged size was extracted from the fitting of the d-PFG MR data. The microstructural information was obtained with little prior knowledge. Only the pore size range, which can be large, that encompass the pore sizes to be extracted and the \( D_0 \) are needed as inputs. In future work we plan to extend our model to obtain structural information in more complex systems where both size and angle distributions occur and where both free and restricted diffusions coexist. For more complex systems, clearly it will be of value to obtain such information in a rotational invariant acquisition as proposed very recently [43,44].

### Acknowledgment

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### Appendix A. Supplementary data

Supplementary data related to this article can be found at [http://dx.doi.org/10.1016/j.micromeso.2015.11.029](http://dx.doi.org/10.1016/j.micromeso.2015.11.029).

### References