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Measuring small compartments with relatively weak gradients by angular double-pulsed-field-gradient NMR

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ABSTRACT

NMR diffusion–diffraction patterns observed in compartments in which restricted diffusion occurs are a useful tool for direct extraction of compartment sizes. Such diffusion–diffraction patterns may be observed when the signal intensity $E_{(\mathbf{q},\Delta)}$ is plotted against the wave-vector $\mathbf{q}$ (when $\mathbf{q}=(2\pi)^{-1}\gamma\delta\mathbf{G}$). However, the smaller the compartment sizes are, the higher are the q-values needed to observe such diffractions. Moreover, these q-values should be achieved using short gradient pulses requiring extremely strong gradient systems. The angular double-pulsed-field gradient (d-PFG) NMR methodology has been proposed as a tool to extract compartment sizes using relatively low q-values. In this study, we have used single-PFG (s-PFG) NMR and angular d-PFG NMR to characterize the size of microcapillaries of about $2\pm1\,\mu m$ in diameter. We found that these microcapillaries are characterized by relatively strong background gradients that completely masked the effects of the microscopic anisotropy (μA) of the sample, resulting in a completely unexpected $E(\varphi)$ profile in the angular d-PFG NMR experiments. We also show that bipolar angular d-PFG NMR experiments can largely suppress the effect of these background gradients resulting in the expected $E(\varphi)$ profile from which the compartment dimensions could be obtained with relatively weak gradient pulses. These results demonstrate that the above methodology provides a quick, reliable, non-invasive means for estimating small pore sizes with relatively weak gradients in the presence of large magnetic susceptibility.

1. Introduction

Non-invasive measurement of small size compartments is of great importance in a myriad of chemical and biological applications [1–3]. The pulsed-gradient spin-echo (PGSE) NMR experiment, which was introduced by Stejskal and Tanner in 1965 [4], is indeed the most widely used method for measuring diffusion [1–6], and for extracting micro-structural information non-invasively in systems such as porous materials [1,2,7–9], emulsions [10], rocks [11–13], cells [14,15] and neuronal tissues [3]. Diffusion NMR was also used to study organometallic complexes [16], supramolecular system [17], nanoparticles [18] and nanotubes [19].

It is well known that free diffusion is Gaussian and that there the signal decay can be analyzed using the well-known Stejskal–Tanner equation from which the diffusion coefficient can be extracted. In free diffusion the root mean square displacement (rmsd) increases with the square root of the diffusion time ($\sqrt{t}$). In restricted compartments that are mono-disperse in size, when the signal intensity ($E(\mathbf{q})$) in the single-pulsed-field-gradient (s-PFG) NMR experiment is plotted as a function of the wave-vector $\mathbf{q}$ (when $\mathbf{q}=(2\pi)^{-1}\gamma\delta\mathbf{G}$), where γ is the magnetic gyro ratio, δ and $\mathbf{G}$ are the pulse gradient duration and intensity, respectively), diffusion–diffraction minima should be and are observed experimentally [20,21]. It was shown that from such diffusion–diffractions the compartment size can be extracted directly for simple known geometries [22]. However, these diffusion–diffraction minima may easily disappear as a result of size poly-dispersity [23] or because of susceptibility effects induced by the inhomogeneity of the sample [23]. The use of bipolar (bp) gradient pulses [24,25] in such NMR experiments can recover the diffusion–diffractions minima in inhomogeneous samples and may allow extracting the relevant microstructural information, but only in systems that are mono-disperse in size [26].

Many chemical and biological systems are characterized by size poly-dispersity where diffusion–diffraction minima are generally not observed when s-PFG NMR experiments are performed. In such cases the way to extract the compartment size is by Fourier transformation of the signal decay ($E(\mathbf{q})$). The methodology was originally developed by Cory and Garroway [27]. There the compartment size can, in principle, be estimated from full-width at half-height (FWHH) or from the base of the displacement probability distribution profile [28–32]. Extraction of the correct displacement distribution profile in a system with restricted diffusion is possible under the long diffusion time scale and short gradient pulse (SGP) approximation [20,21].

Another way to extract the compartment size in systems which are characterized by size poly-dispersity is by using the double-PFG (d-

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PFG) NMR experiment. The d-PFG NMR experiment was first proposed by Cory et al. in 1990 [33]. The d-PFG NMR sequence employs two diffusion sensitizing gradient pairs, G_1 and G_2, with duration δ_1 and δ_1, respectively, that are separated by a mixing time t_m. The two gradient pairs may be applied collinearly or with an angle between them yielding the radial and angular d-PFG NMR experiments, respectively [33,34]. Two diffusion time intervals, Δ_1 and Δ_2, exist in these sequences. Both theoretical and experimental studies of the signal decay in d-PFG experiments performed on systems exhibiting restricted diffusion demonstrated that zero-crossings of the signal that are analogous to the diffusion–diffraction minima in s-PFG experiments should be and are observed in such NMR experiments [35,36]. In addition, these zero-crossings were very recently found to be more robust toward poly-dispersity in sizes [23].

To characterize compartments with an inner diameter of about 2 μm (comparable to the diameter of axons in WM) using the diffusion–diffractions or the zero-crossings observed in the single or double PFG MR experiments, respectively, would necessitate reaching q-values of more than 6100 cm^{-1}. To fulfill the SGP approximation, extremely strong gradients of more than 1400 G/cm in the s-PFG experiment should be used assuming δ is set to 1 ms. Gradient systems capable of producing only up to less than 100 G/cm are now common on small animal MRI scanners and those of clinical scanners are capable of producing only 5–8 G/cm because of safety issues and technological limitations. Thus a possible means for measuring small compartments of about 2 μm with common gradient systems seems to be the use of the angular d-PFG NMR methodology introduced by Mitra [34,37–39]. Indeed, in recent years, angular d-PFG NMR is attracting much attention [37–47].

In the present study we challenged the ability of single-PFG and double-PFG NMR experiments to probe the compartment sizes as small as 2 μm. Both single- and angular double-PFG NMR experiments and their bipolar versions were used to characterize non-invasively the inner diameter of such microcapillaries where the ground truth is known a priori. The data from all NMR experiments were fitted to the appropriate cylindrical model. We found that the bipolar (bp) angular d-PFG NMR experiments can characterize such systems which are also characterized by relatively large magnetic inhomogeneity.

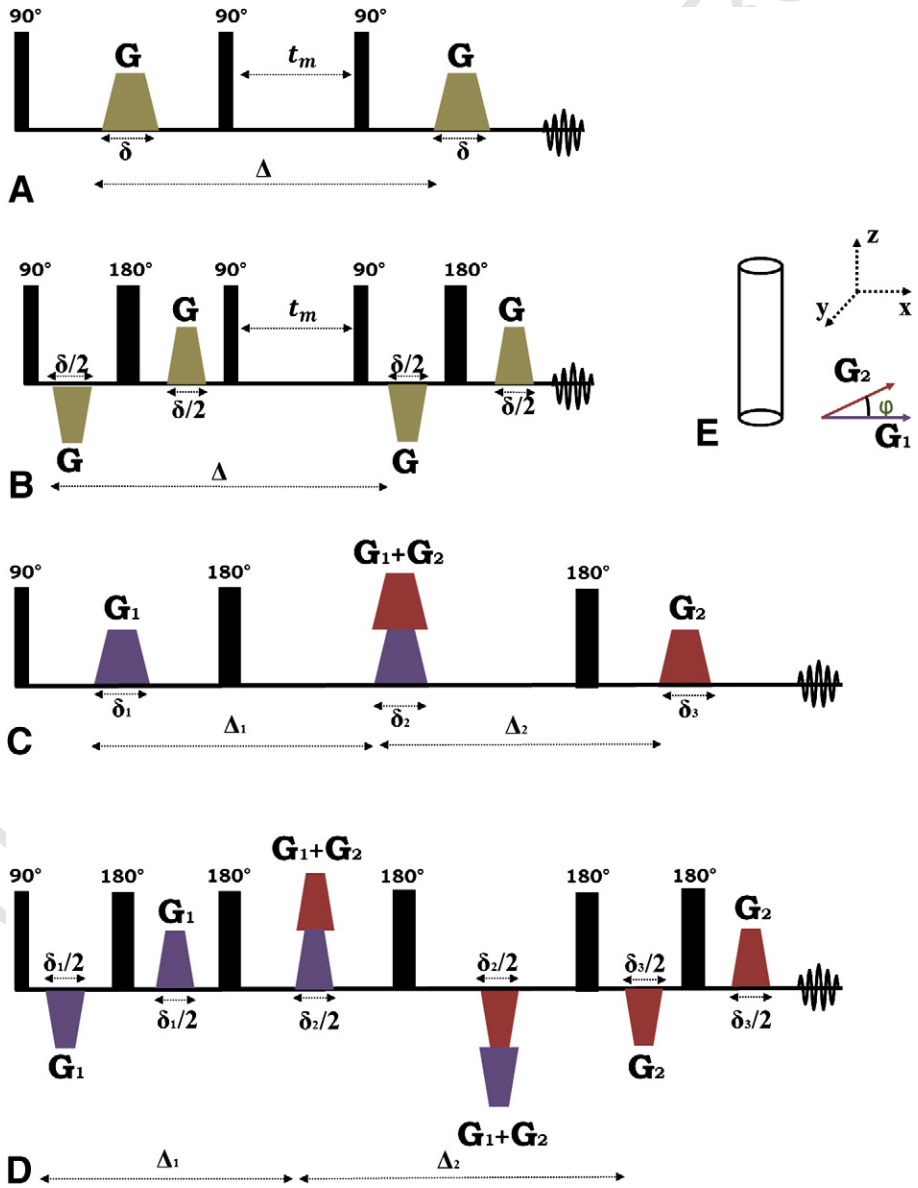

Fig. 1. Single-pulsed-gradient stimulated echo (s-PGSTE) with (A) monopolar and (B) bipolar gradients. The double-pulsed-gradient spin echo (d-PGSE) sequences with $t_m = 0$ with (C) monopolar and (D) bipolar gradients. (E) The angular d-PFG is performed by setting $|G_1| = |G_2|$ and by varying only the angle φ between the two gradient pairs.

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2. Experimental methods

2.1. General

The NMR experiments were performed on a Bruker 8.4T NMR spectrometer capable of producing up to 190G/cm along each of the three directions. Microcapillaries with a known inner diameter of 2± 1μm and outer diameter of 150±1μm (Polymicro Technologies, Phoenix, AZ, USA) were cut to micro-tubes of about 3cm in length and dipped in water for a week, prior to each experiment. The microcapillaries were then packed into a glass sleeve (8mm) and inserted into a 10mm NMR tube. The NMR tube was aligned parallel to the z direction of the magnet.

2.2. NMR experiments

The s-PFG and d-PFG NMR experiments were first performed using conventional monopolar gradients, and then the experiments were repeated using bipolar gradients. The gradients in the monopolar sequences are characterized by their duration δ and amplitude G. To reach the same q value in the bipolar sequences, the gradient amplitude G was kept the same as in the monopolar version, and the gradient pulse duration was split into two parts with duration δ/2, and a π pulse was symmetrically placed between the two gradient pulses.

The single-pulsed-gradient stimulated-echo (s-PGSTE) and the bp-s-PGSTE NMR experiments shown in Fig. 1A and B, respectively, were performed in the x-direction with the following parameters: 40 q values were collected with G_{max}=160G/cm, δ=4ms, resulting in a maximal q value of 2724cm^{-1}, Δ was set to 30ms.

The angular double-pulsed-gradient spin-echo d-PGSE and the bp-d-PGSE NMR experiments shown in Fig. 1C and D, respectively, 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 , 8 q values were collected with G_{max}=80G/cm, t_m, = 0ms, $\delta_1=\delta_2=\delta_3$=4ms, $\Delta_1=\Delta_2$, = 30ms, resulting in a q_{max}=1362cm^{-1} for both experiments. The fitting of the angular bp-d-PGSE NMR was performed according to references [38] and [39]. Note that this fitting takes into consideration the finite values of δ. The fitting of the s-PFG was performed as described below.

2.3. s-PFG data fittings

Let $E_s(q)$ denote the theoretical magnetization in an *s-PFG* sequence and $E_d(q)$ the theoretical magnetization in a *d-PFG* sequence according to Eq. (21) in reference [42]. Specifically,

$$E_d(\mathbf{q}_1,\mathbf{q}_2,r_0) = [\exp\{-\Lambda\delta_1 + i2\pi\mathbf{q}_1\cdot\mathbf{A}\}\exp\{-\Lambda(\Delta_1-\delta_1)\} \times \exp\{-\Lambda\delta_1 - i2\pi\mathbf{q}_1\cdot\mathbf{A}\}\exp\{-\Lambda(t_m-\delta_1)\} \times \exp\{-\Lambda\delta_2 - i2\pi\mathbf{q}_2\cdot\mathbf{A}\}\exp\{-\Lambda(\Delta_2-\delta_2)\} \exp\{-\Lambda\delta_2 - i2\pi\mathbf{q}_2\cdot\mathbf{A}\}]_{0,0},$$

and

$$E_s(\mathbf{q},r_0)=[\exp\{-\Lambda\delta + i2\pi\mathbf{q}\cdot\mathbf{A}\}\exp\{-\Lambda(\Delta-\delta)\}\exp\{-\Lambda\delta - i2\pi\mathbf{q}\cdot\mathbf{A}\}]_{0,0},$$

where the multiplications of the exponentials result in a matrix and we only take its (0,0) element. The matrices Λ and **A** depend on the geometry of the compartment.

In the case of microcapillaries which correspond to an infinite cylinder geometry with radius r_0 and diffusion coefficient d_0 [39],

$$\Lambda_{km,k'm'}(r_0) = \delta_{kk'}\delta_{mm'}\frac{\alpha_{km}^2 D_0}{r_0^2}$$

$$\mathbf{A} = \begin{pmatrix} X \\ Y \end{pmatrix},$$

where

$$X_{km,k'm'}(r_0) = r_0\delta_{m,m'\pm1}\sqrt{1+\delta_{m,0}+\delta_{m',0}}\beta_{km}\beta_{k'm'}\frac{\alpha_{km}^2+\alpha_{k'm'}^2-2mm'}{(\alpha_{km}^2-\alpha_{k'm'}^2)^2}$$

$$Y_{km,k'm'}(r_0) = ir_0\left(\delta_{m,m'+1}-\delta_{m,m'-1}\right) \times\sqrt{1+\delta_{m,0}+\delta_{m',0}}\beta_{km}\beta_{k'm'}\frac{\alpha_{km}^2+\alpha_{k'm'}^2-2mm'}{(\alpha_{km}^2-\alpha_{k'm'}^2)^2},$$

$\delta_{mm'}$ stands for the Kronecker delta, and α_{km} is the kth zero of the derivative of the mth Bessel function satisfying $J'_m(\alpha_{km})=0$.

Finally

$$\beta_{km} = \begin{cases} \dfrac{1}{\alpha_{km}} & k=m=0 \\ \dfrac{1}{\sqrt{\alpha_{km}^2-m^2}} & otherwise \end{cases}.$$

We denote the measured magnetization as $M_s(\mathbf{q})$ *and* $M_d(\mathbf{q})$ for the single and double sequences respectively. The estimation of the microstructure radius r_0 is performed by minimizing the mean least square error,

$$r_0 = \underset{r}{\operatorname{argmin}}\sum_i (E_s(\mathbf{q}_i,r)-M_s(\mathbf{q}_i))^2,$$

or

$$r_0 = \underset{r}{\operatorname{argmin}}\sum_i (E_d(\mathbf{q}_1 i,,\mathbf{q}_{2i},r)-M_d(\mathbf{q}_{1i},,\mathbf{q}_2 i))^2.$$

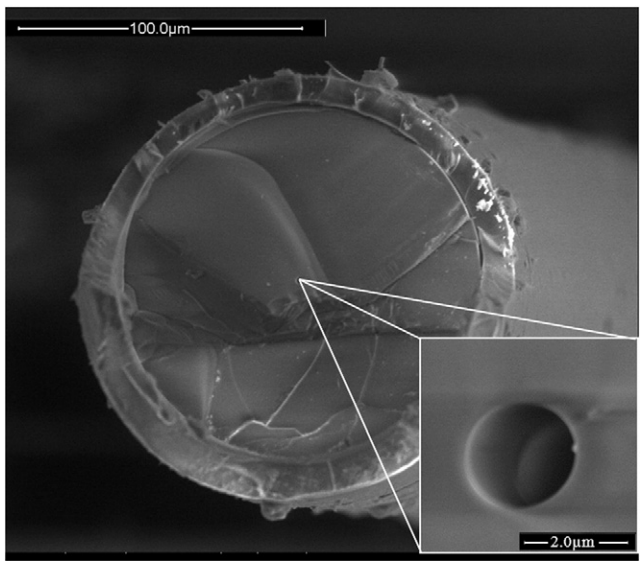

Fig. 2. The low vacuum scanning electron microscopy (SEM) image of 2±1μm microcapillaries at two different magnifications.

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The minimization was carried out using the Matlab® (R2008a, MathWorks, MA, USA) library function lsqnonlin.

The SEM images were collected on a FEI Quanta 200 field emission gun (FEG) environmental scanning electron microscope (ESEM).

3. Results and discussion

To validate the accuracy of the microstructural information extracted by different methods, microcapillaries of known diameters should be studied. In such systems the accuracy of the different methods and approaches can be challenged and evaluated. Fig. 2 shows the SEM image of such microcapillaries showing that their size is indeed about 2 μm. Clearly, in a sample of an ensemble of such microcapillaries most of the volume is that of the solid materials of the microcapillaries and not of the enclosed liquid in the pores. Therefore both the signal to noise ratio (SNR) and the background gradients can be a problem when studying such systems with NMR.

Fig. 3A shows the signal decays of s-PGSTE NMR (black circles) and bp-s-PGSTE NMR (red squares) experiments performed on an ensemble of 2±1 μm microcapillaries in the x-direction with G_{max} of 160 G/cm and δ of 4 ms. The signal decay appears larger in the s-PGSTE NMR experiment as compared with the signal decay observed in the bp-s-PGSTE NMR experiment, which corrects for much of the effects of the background magnetic field. However, no diffusion–diffraction minima were observed in these two s-PFG NMR experiments, despite the fact that the microcapillaries are mono-disperse in size and are coherently aligned along the z-direction. It should be noted that such diffusion–diffractions in s-PFG NMR experiments performed on such systems with larger diameters can be easily observed [48]. Fig. 3B shows an attempt to FT the data presented in Fig. 3A. Here, in principle, the mean diameter of the microcapillaries should be extractable from

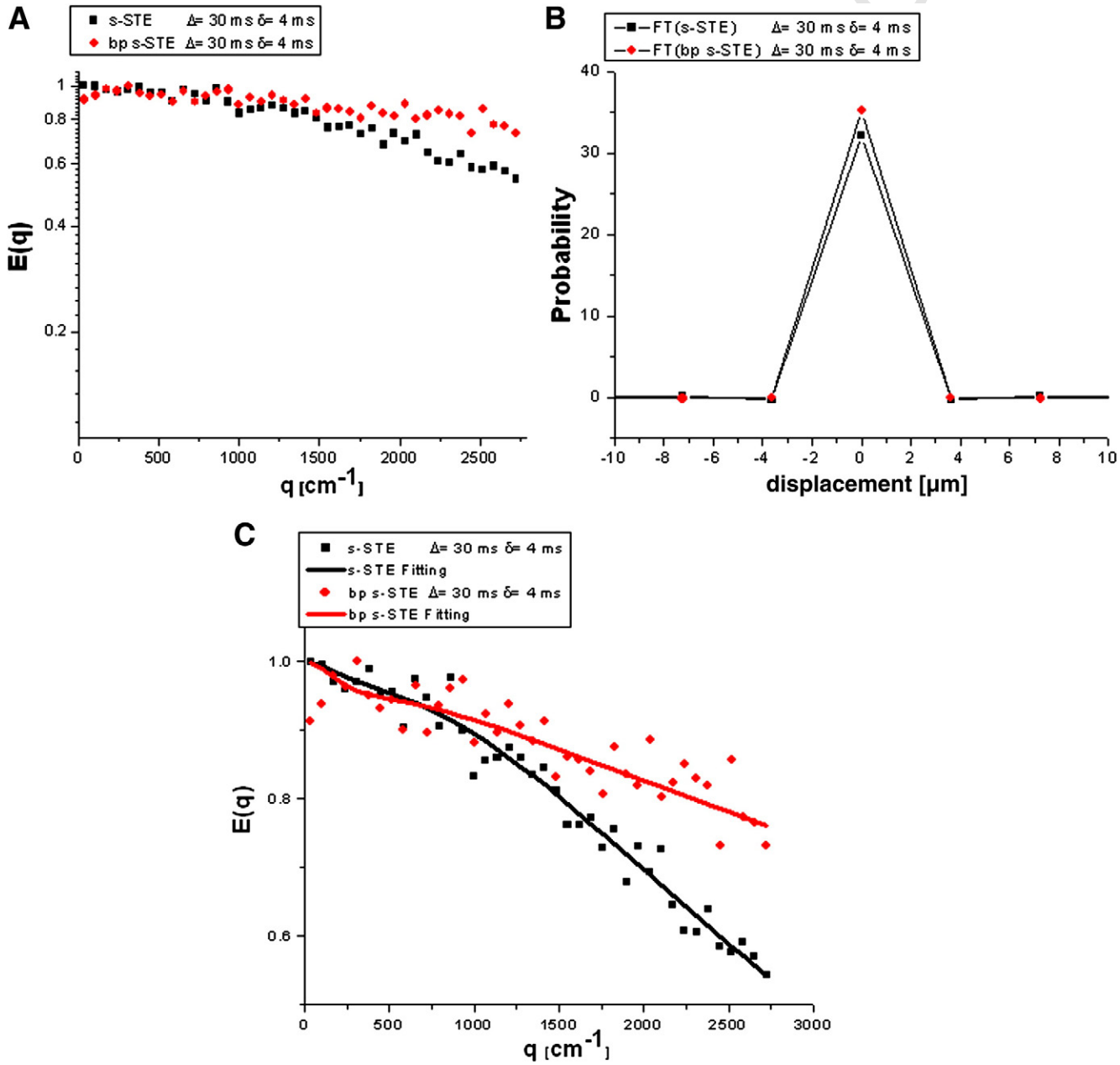

Fig. 3. s-PFG experiments performed on an ensemble of 2±1 μm microcapillaries. (A) The signal decay (Eq) as a function of q-values in a conventional (monopolar) s-PGSTE experiment (black circles) and in a bipolar s-PGSTE experiment (red squares). The two diffusion NMR experiments were performed with the same parameters. (B) Displacement distribution profiles obtained by Fourier transformation of the data shown in (A). (C) s-PFG fittings of the data shown in (A). For the fitting procedure see the experimental section.

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the base of the displacement distribution profile, since it describes the maximum available displacement in the sample. Despite the two different decay curves (Fig. 3A) the obtained profiles (Fig. 3B) are similar and the extracted mean diameter for both s-PFG NMR experiments is found to be about 3.6 μm for the 2±1 μm capillaries, clearly indicating that there is a problem with such a straightforward analysis of the data. However, when we fitted the data according to the method presented in the experimental section, the mean diameters extracted from s-PGSTE and bp-s-PGSTE experiments were found to be 4.2±0.2 and 2.6±0.3 μm, respectively (Fig. 3C). Clearly the compartment size extracted from fitting the data of the bp-s-PGSTE NMR experiment seems to be adequate.

The results from the angular d-PGSE NMR experiments with q of 1362 cm^{-1} are presented in Fig. 4. In such d-PFG NMR experiments with zero mixing time (see Fig. 1D), the angular dependence of the signal i.e. $E(\varphi)$, is predicted to follow a bell-shaped function [34,37–41]. However, completely contrary to theoretical predictions, the results of the d-PGSE experiments presented in Fig. 4A show that $E(\varphi)$ increases up to 90°, then decreases up to 180°. This behavior is nearly mirrored from 180° to 360°. These observations are inconsistent with the expected $E(\varphi)$ profile, when $E(\varphi)$ is governed by microscopic anisotropy (μA) that arises from the boundaries of the restricting compartment [37–45]. We hypothesized that here the effect of μA is completely masked by susceptibility effects, which arise from the relatively strong internal gradients within the sample. It was previously shown that the cross-term induced by background gradients can be effectively suppressed using bipolar pulse gradients [24–26]. Therefore, we repeated the experiments by introducing bipolar pulse gradients to the angular d-PFG NMR sequence with the hope of suppressing these effects.

Fig. 4B shows the results obtained from the bipolar angular d-PFG NMR experiment performed on the same sample with the same parameters as the monopolar angular d-PFG experiment (open red squares). Here the $E(\varphi)$ regains its expected form, showing a bell-shaped dependency implying that the susceptibility effects are significantly suppressed. In this case, a size of 3.07±0.04 μm was obtained from fitting the signal decay according to the derivation of Özarslan [37–39]. It should be noted that the size extracted from the angular bp d-PFG NMR seems to be somewhat larger than the nominal size and the size seen on the SEM images. The reason is most likely the fact that our bipolar gradients could not completely suppress the effect of the background gradients. As a result, the $E(\varphi)$ regains its expected form but the obtained size was still somewhat larger than the nominal size of the microcapillaries.

Noninvasive measurement of compartment size in opaque systems is of paramount importance in many fields and the ability of different methods to convey such information should be challenged, first, in systems where the "ground truth" is known. Diffusion–diffractions are indeed an excellent indicator of restricted diffusion and are a useful tool to report on compartment sizes at least for compartments of simple geometries [1,2,20–33,49]. However, for small compartments, such diffusion–diffractions are only observed at very high q-values which can be achieved only with very strong gradient systems which are not available in most laboratories. Indeed for observing even the first diffraction of a compartment of 2 μm, gradient systems capable of producing gradient pulses of more than 1400 Gauss/cm are needed (assuming δ of 1 ms). In addition, such diffusion–diffraction patterns generally disappear with size polydispersity. Here we found that FT of the s-PFG data could not convey a meaningful estimate of the compartment size. However, fitting the data of only the bp-s-PFG NMR experiment assuming a cylindrical shape provides an accurate estimate of the compartment size in this mono-disperse sample.

In the present study we used angular d-PFG NMR [37,40–46] to obtain such information with relatively much weaker gradient pulses of about 80 Gauss/cm. Indeed, in recent years we have seen an increase in the use of angular d-PFG MR to obtain information on the compartment size in different samples, but there, in many studies, the ground truth was hard to estimate [45–47,50,51]. The smallest compartment that was measured using this angular d-PFG NMR methodology in a system in which the ground truth is known was of 5±1 μm [52]. Clearly pushing the limit toward smaller compartments in the range of two micrometers or less is both important and rather difficult. Here we chose to use even smaller microcapillaries, i.e. microcapillaries having a nominal diameter of only 2±1 μm which is reminiscent to the diameters of axons in the nervous system [31]. Such microcapillaries allow one to challenge the accuracy of the different methods used. In the present system, we found that bipolar gradients are vital for obtaining the expected signal dependency because of background gradients. Indeed we found that with the correct fitting both the bipolar s-PFG and the bipolar angular d-PFG NMR experiments enable one to extract sizes of about 2 μm with good accuracy even with relatively weak gradients. Fitting the bipolar s-PFG

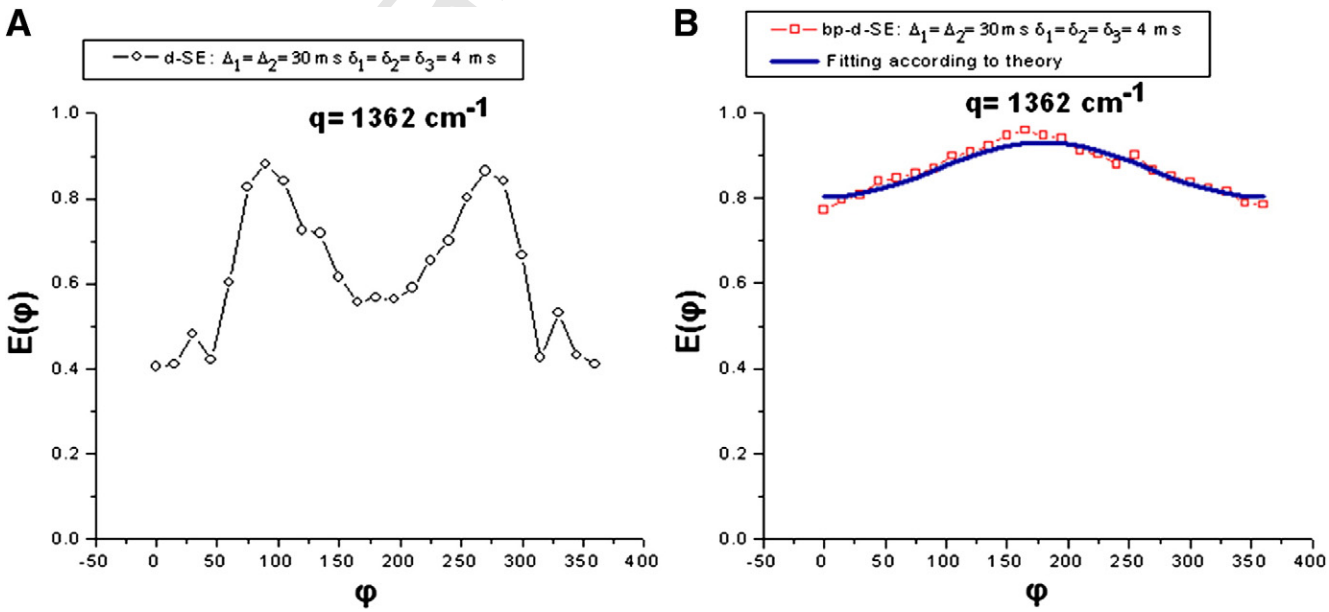

Fig. 4. The $E(\varphi)$ profiles of the angular d-PGSE experiments performed on an ensemble of 2±1 μm microcapillaries with q=1362 cm^{-1}. (A) Angular d-PGSE with monopolar gradients (open black circles). (B) The $E(\varphi)$ profile of the angular bp-d-PGSE (open red squares) with fitting according to theory in references [38–40] (blue line).

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and angular d-PFG NMR data afforded a good estimate of the size of the $2 \pm 1\,\mu m$ microcapillaries.

4. Conclusions

In the present study we challenged the ability of both s-PFG and angular d-PFG NMR methodologies to characterize microcapillaries with an inner diameter of about $2 \pm 1\,\mu m$ with gradient systems of only moderate strength. The diffusion–diffraction minima were not observed in the s-PGSTE NMR experiments performed on these microcapillaries. Trying to obtain an estimation of the compartment dimensions by FT of the signal decay was found to be unreliable. Fitting the bipolar s-PFG NMR data assuming a cylindrical shape allowed for a good estimate of the size of the microcapillaries.

The presence of strong susceptibility effects was also observed in the angular d-PFG experiment resulting in an unexpected $E(\varphi)$ profile. We found that bipolar gradients in the angular bp-d-PFG can suppress most of these effects and the information on the underlying compartment size can be recovered.

These results demonstrate the ability of angular bp-d-PFG NMR experiments to non-invasively measure compartments with size of about $2\,\mu m$ with only moderate gradient systems, even in the presence of significant magnetic inhomogeneity as one should expect to find in porous materials for example.

Acknowledgments

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