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Multi-exponential signal decay from diffusion in a single compartment

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ABSTRACT

Multi-exponential decays in diffusion experiments are typically fitted to sums of exponentially decaying components; often this is taken as evidence for spins in multiple distinct compartments. Here we examine the signal decay due to diffusion in a single cylinder, for short diffusion times (lightly restricted). The signals are well-modeled by a sum of two exponentials, despite the single compartment housing the spins. The results agree with a previous theoretical examination of the problem. The implication for biological systems is that multiple decay signal components may not correspond to multiple physical compartments.

1. Introduction

Measurement of restricted diffusion with MRI/NMR is a valuable and well-known tool for characterizing features too small to be imaged directly [1–4]. In particular, it has been used to make surface-to-volume measurements of small features [5–9]. There are many applications of restricted diffusion MRI/NMR, particularly in the areas of biomedical systems and porous media. Diffusion data are generally obtained from the decay of a spin or gradient echo signal after the application of a pair of field gradient pulses with diffusion sensitizing strength, b (see below). For freely diffusing spins the signal decays mono-exponentially as $\exp(-b\,D_0)$, where D_0 is the free diffusion coefficient [10]. If the motion is restricted by physical barriers (such as airway or cell walls or the walls of the sample container) the measured diffusion will be reduced from the free value and the decay will generally no longer have strictly mono-exponential behavior.

In biological systems as well as porous media, one often finds that the signal decay as a function of b can be expressed as a sum of multiple exponentials. A common and seemingly natural interpretation is that the observed signal is a superposition from spins in distinct compartments of multiple types or sizes [11] or orientations [12,13], in the case of anisotropic systems. However, a recent report ([14], hereafter referred to as SAY) showed that even for certain simple, single compartment geometries, the signal S is not mono-exponential but can be well modeled as bi-exponential:

$$S = \zeta e^{-bD_S} + (1-\zeta)e^{-bD_F}. \tag{1}$$

Here ζ is the relative amplitude of the component with slow apparent diffusion D_S; the component with fast apparent diffusion is described by D_F. In our experiment, as in SAY, we examine the case of a single long cylinder of radius r, oriented perpendicular to the diffusion gradient (we emphasize that a distribution of sample orientations is not involved in this study). Now D_F describes spins far from the cylinder walls with virtually unrestricted motion, so D_F is approximately equal to the free diffusion coefficient. D_S describes spins which are near the cylinder wall and have restricted diffusion, so D_S is a fraction of the free diffusion coefficient. The relative amplitude ζ is approximately the fraction of spins that can diffuse to the wall during the diffusion time, Δ. In SAY, the key condition for a good fit of the data with the bi-exponential model is that only a small fraction of the spins are close enough to sample the barriers (here, the cylinder walls) during the diffusion time. This condition is represented by $\alpha << 1$, where the parameter α is

$$\alpha \equiv \frac{\sqrt{D_0\Delta}}{r}. \tag{2}$$

The calculations from SAY show that the relative amplitude ζ and parameter α are nearly linearly related for small α, in accord with the above explanation.

The purpose of this study is to explore multi-exponential signal decay in a simple geometry. Our work serves to verify the important message of SAY, which is to warn that multi-exponential diffusion decay may be misinterpreted in terms of spins in multiple compartments. We note that the present work is a different approach to the seminal treatment of lightly restricted diffusion and surface-to-volume ratio by Mitra and Sen [15]. In detail, Mitra and Sen examine the mean diffusion as would be extracted from the initial slope of signal as a function of b; here the entire decay is examined. The present study is also closely related to studies

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of the edge enhancement effect [16–19]; the spins near the boundaries with a lower diffusion coefficient (here D_S) suffer less attenuation, thus enhancing the relative signal near the boundaries.

2. Methods

2.1. Samples

The sample vessels used in the experiment were single smooth silica tubes from Polymicro Technologies, intended for chromatography. Two different sizes were used, with inner radii 160 ± 1 μm and 50.5 ± 1 μm. The glass tubes were filled with water with a small amount of copper sulfate ($CuSO_4$), added in order to shorten T_1 to about 300 ms. This decreased T_1 allowed more rapid signal averaging to increase the signal to noise ratio (SNR) of the data. Every point had a SNR of at least 20 even after very substantial attenuation from the diffusion gradients. Each sample was long enough (at least 8 cm) that susceptibility effects were negligible [19]. Each coil was long enough (at least 1 cm) that the effect of spins diffusing out of the rf field was also negligible.

2.2. NMR

The small volume samples dictated the use of rf coils with high filling factors. For each sample, an rf coil of small diameter wire was constructed, loosely fitting the sample tube. The active lengths of the rf coils were 1 and 2 cm for the large and small diameter samples, respectively. Hydrogen NMR measurements were performed in a 4.7 T Oxford magnet with a Varian imaging spectrometer and VnmrJ software.

2.3. Sequence

Measurements were taken with a stimulated echo sequence (Fig. 1) to minimize signal losses from diffusion of spins through background field gradients [20]. The background gradients only act upon the spins during the two relatively short periods of duration $t_e/2$, for a total here of 30 ms. For signal to noise reasons, each measurement used at least 128 averages. The duration (δ) of the diffusion gradient pulses was 3.0 ms (effective rectangular width), with the separation between gradients (Δ) being either 54 or 138 ms (for the smaller or larger cylinder, respectively). Thus the parameter α from Eq. (2) is 0.2 for the cylinder with 50.5 μm radius and 0.1 for the cylinder with $r = 160$ μm. Diffusion was measured in a direction perpendicular to the long axis of the sample; diffusion was also measured parallel to the long axis once in each sample to determine the free diffusivity D_0. Since δ is much smaller than both Δ and the diffusion time across the tube, the narrow pulse regime applies here [21]. The b-value is then the well-known Stejskal–Tanner result [22]

$$b = (\gamma G\delta)^2\left(\Delta - \frac{\delta}{3}\right) \approx (\gamma G\delta)^2\Delta. \tag{3}$$

We used a wide range of b-values, from 0.15 to 6000 mm^2/s, in order to capture signal from both the rapid and slow signal decay components in Eq. (1). Since the weighting parameter for the slow component is expected from SAY to be quite small (ζ is of order 5% in all cases), the slow component's contribution to the signal is dominant only once the signal has decayed to a few percent of the initial value.

2.4. Analysis

The fast Fourier transform of the spin echo time domain data for each set of b-values was phased and plotted in MatLab, and the real spectral peak area was determined. The peak areas were plotted as a function of b and fitted to the bi-exponential model of Eq. (1) using Origin software. Occasionally, mono-exponential fits were performed for comparison; they were always found to be vastly inferior. In order to ensure a good fit for both large and highly attenuated signals, the fitting routine was weighted appropriately.

3. Results and discussion

3.1. Diffusion perpendicular to long axis of cylinder

At the larger b-values, the signal is reduced to less than 1% of the original signal. Fig. 2 demonstrates that the decay is not mono-exponential; the dashed line represents a mono-exponential fit to the small-b data. Note that for b-values less than 2500 s/mm^2, the signal follows a mono-exponential decay quite closely. It is only after more than 97% of the original signal is attenuated that the multi-exponential nature of the decay becomes apparent. The bi-exponential fit to the same data set is shown with the solid curve.

Each data set was fitted with the bi-exponential model, as shown in Fig. 2. The parameters obtained from the bi-exponential fits are given in Table 1. Also given in the table are parameter values calculated by the authors of SAY for a range of b-values similar to those used in this study. We note that the parameter values are sensitive to the b-range of the fitting, so the theory values in Table 1 differ from those in the publication [14]. This sensitivity demonstrates that the double exponential is not an exact fit to the analytically-derived signal decay. Since the calculated diffusion parameters are dimensionless (i.e., they assume a free diffusivity of unity), we multiplied them by the free diffusion measured for our samples (see below), 0.001735 mm^2/s. The experimental results generally agree with the theory, although a fair amount of variation between trials is seen in Table 1. However, as is evident from Fig. 3, the several data sets agree closely. Thus, the variation in parameter values is due to the well-known sensitivity of bi-exponential fits to small changes in the data [23].

3.2. Diffusion parallel to long axis of cylinder

Diffusion was also measured in one instance for each sample in the direction parallel to the long axis of the cylinder, to determine the free diffusivity D_0. The results were fitted to a mono-exponential decay (Fig. 4). Clearly, the decay is mono-exponential for as long as there is sufficient signal. Since the diffusion of the spins is unrestricted down the long axis of the cylinder, the signal attenuates completely into the noise at a much lower b-value than it

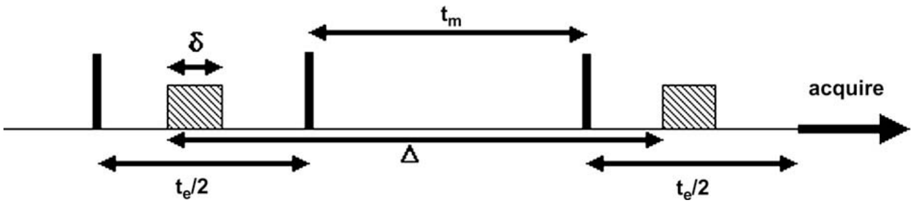

Fig. 1. Stimulated echo sequence for the experiment. Solid rectangles represent π/2 rf-pulses and shaded rectangles represent diffusion gradient pulses.

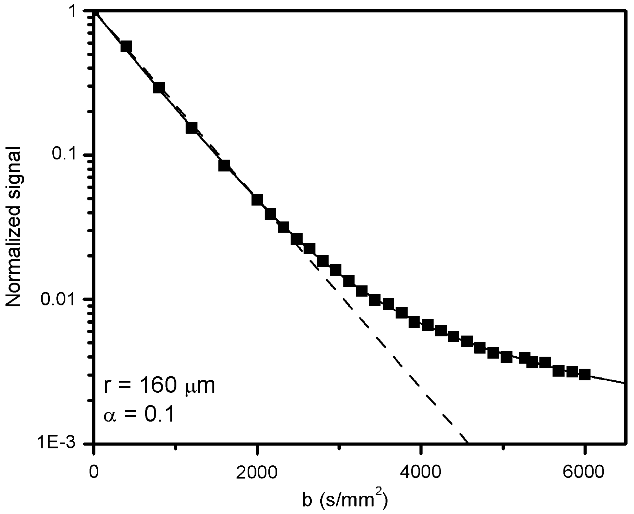

Fig. 2. Data for the large cylinder ($r = 160\ \mu m$, $\alpha = 0.1$) with diffusion gradient perpendicular to the long axis, fitted to a mono-exponential decay (dotted line) and to the bi-exponential model (solid curve). Note the logarithmic vertical axis.

does with the diffusion gradient perpendicular to the long axis. The free diffusion coefficient measured for the larger sample was 0.00173 mm^2/s and for the smaller sample was 0.00174 mm^2/s, giving an average value of 0.001735 mm^2/s. The work of Krynicki et al. suggest that the expected free diffusion coefficient for water at 17 °C (the temperature inside the bore of the magnet) is 0.00185 mm^2/s [24], making these measured free diffusion coefficients seem reasonable.

3.3. Applications

We have shown that the signal decay for lightly restricted diffusion in a single compartment can mimic the signal for spins in multiple distinct compartments. However, the dependence of the signal on the diffusion time Δ can serve as a signature, potentially providing a way to distinguish between the single and multiple compartment systems. For lightly restricted diffusion, the amplitude ζ of the slowly diffusing signal component increases with diffusion time. Very approximately, the amplitude ζ is expected to be linear in α and to vary as $\Delta^{1/2}$. At large enough Δ, corresponding to $\alpha \geqslant 1$, the decay will be nearly mono-exponential, because all spins sample all the environments (close to walls and far from

Table 1
Data for bi-exponential fits to experiment and theory.

	Trial	D_S (mm^2/s)	D_F (mm^2/s)	D_S/D_F	ζ
Larger sample $\alpha = 0.1$ ($r = 160\ \mu m$)	1	0.00034	0.00171	0.20	0.01958
	2	0.00029	0.00161	0.18	0.01700
	3	0.00036	0.00168	0.21	0.02533
	4	0.00041	0.00181	0.23	0.02796
	5	0.00037	0.00166	0.22	0.01474
	Experiment average	0.00035	0.00169	0.21	0.02092
	Theory[a]	0.00044	0.00163	0.27	0.02910
Smaller sample $\alpha = 0.2$ ($r = 50.5\ \mu m$)	1	0.00028	0.00143	0.20	0.03180
	2	0.00036	0.00159	0.23	0.04234
	3	0.00027	0.00145	0.19	0.03025
	4	0.00034	0.00159	0.21	0.03887
	5	0.00034	0.00156	0.22	0.03646
	Experiment average	0.00032	0.00152	0.21	0.0359
	Theory[a]	0.0004	0.00151	0.27	0.05037

[a] Theory values obtained from authors of Ref. [14]; dimensionless values of D_S and D_F have been scaled here by the measured free diffusion coefficient $D_0 = 0.001735$ mm^2/s.

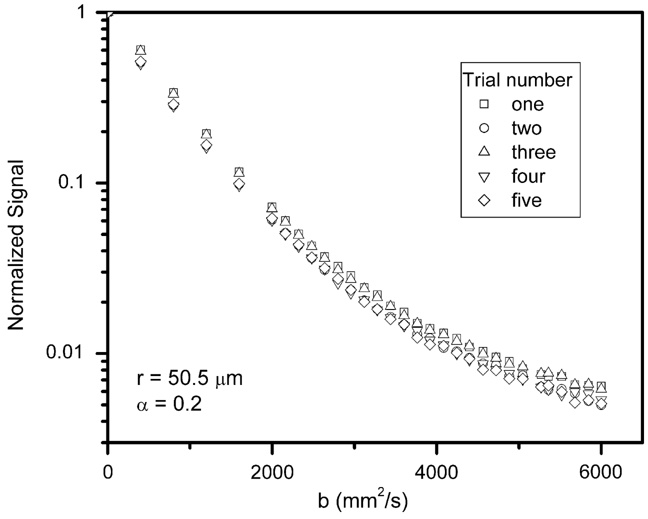

Fig. 3. The data for multiple measurements of the smaller sample. The data from all trials are quite similar.

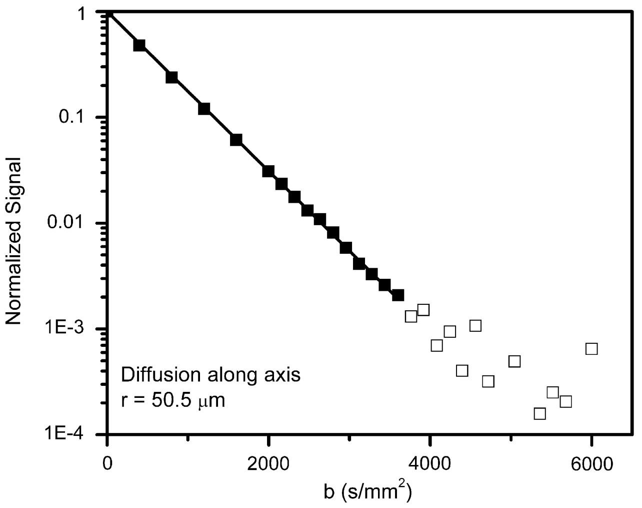

Fig. 4. The results for diffusion along the long axis of the smaller cylinder. The free diffusion coefficient was obtained by fitting the closed squares to a mono-exponential decay. The open squares were disregarded during fitting for signal to noise reasons.

walls). By comparison, in a true multiple compartment system, the amplitudes of the components are fixed at the relative numbers of spins in each (often, this is the relative volumes of the compartments). Exchange between compartments may affect this clean dichotomy, as exchange eventually yields nearly mono-exponential decays at sufficiently long diffusion times.

4. Conclusions

It is clear that care must be taken when interpreting multiple exponential signal decay in a diffusion experiment. Here we have demonstrated that even a simple container can give rise to non-mono-exponential signal decay if the conditions are right, namely, that only a small portion of the spins are allowed to sample the barrier of the space during the time that diffusion is being measured. In this case, the signal is well modeled by a bi-exponential decay in agreement with calculations published earlier. One way to distinguish a true multi-compartment system from the present case of lightly restricted diffusion in a single compartment is by the diffusion time dependence of the signal decay, particularly the relative amplitudes of the components.

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