diffusion-fundamentals

The Open-Access Journal for the Basic Principles of Diffusion Theory, Experiment and Application

Red Blood Cell Shape Evolution Probed by Fast-Diffusion Nuclear Magnetic Resonance Measurements

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1. Introduction

Diffusion NMR measurements have been extensively used in order to study water and solute mobility, ligand binding to macromolecules, and membrane transport in cellular systems, and more recently the shape transformations of red blood cells (RBCs) [1,2]. A *q*-space plot obtained from pulsed field gradient spin echo (PGSE) NMR experiments can exhibit a 'diffraction pattern' [2,3] of the sample and, from the position of minima, the mean diameter of the RBCs can be extracted. A method to process the data involves taking the second derivative of the *q*-space data, applying a Blackman-Harris digital filter and Fourier transforming the result [4,5]. The displacement probability density (or average propagator) is the outcome of the analysis. One of the major drawbacks of this experiment is the time it takes to obtain a *q*-space plot; this is usually ~1 h. For each *q*-value, a minimum of 16 transients is recorded to sample the radio-frequency (RF) phase cycle.

Only a few pulse sequences have been described to measure the diffusion coefficient more rapidly [6]. Normally, unwanted coherence pathways and hence unwanted signals are removed by phase cycling the RF pulses. In a 'bipolar pulse' sequence, 16 or 64 transients must be recorded in order to have complete RF phase cycling. So as to decrease the number of transients per value of the magnetic field gradient-strength Morris et al. [7] proposed the use of unbalanced gradient-pulse pairs. Introducing the unbalancing factor has the same theoretical consequence as phase cycling the RF pulses. Thus only one transient per gradient-strength value needs to be recorded, decreasing the time of the experiment to a few minutes.

We applied this gradient-pulse sequence and obtained *q*-space plots of high quality from suspensions of human RBCs in ~5 min. The information extracted from the datasets was in excellent agreement with that from the classical RF-phase-cycling experiments [2-5]. Adding sodium fluoride to the sample rapidly changes the shape of the RBCs (minute time scale) so we studied this shape evolution by using fast-diffusion NMR measurements.

2. Results and Discussion

The first part of this work was to implement and evaluate the fast-diffusion NMR pulse sequence of Morris et al. [7] and to test if is performed well with the intense magnetic field gradient pulses used in *q*-space analysis of RBCs (~9 T m^{-1}) [2-5]. To obtain exploitable *q*-space plots, we needed to run 2 transients per gradient strength, to remove unwanted signals that occurred at high gradient strengths. The signal decays obtained with a classical pulsed field-gradient stimulated echo (PGSTE) experiment, and

a fast-diffusion pulse sequence were seen to be comparable for discocyte-shaped RBCs, as shown in Fig. 1. We processed our *q*-space data by using the 'second derivative-Blackman-Harris procedure' [4,5]. Fig. 2 shows results obtained for the two experiments in Fig. 1. The mean diameter obtained was ~5.6 μm, in excellent accordance with the accepted value [2-5].

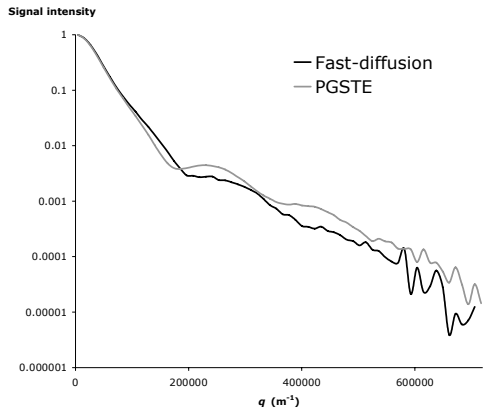

Fig. 1. Comparison of *q*-space data obtained with a PGSTE pulse sequence (grey) and the fast-diffusion experiment (α = 0.30; black) applied to discocyte-shape RBCs. Intensity decay as a function of the magnitude of the **q** vector. The diffusion time Δ was 20 ms for both experiments instead of the length of the gradient impulsion δ was 2 and 3 ms, respectively, for PGSTE and fast-diffusion experiments. Gradient values were adjusted in order to have the same maximal *q* value.

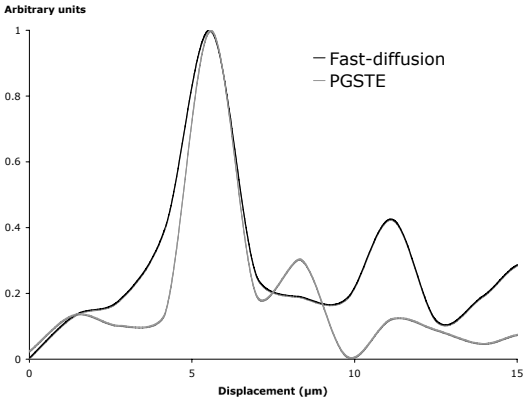

Fig. 2. 'Second derivative-Blackman-Harris procedure' [4,5] applied to the data in Fig.1. Both estimates of the mean diameter of the RBCs were the same.

The RBCs were then treated with sodium fluoride (final concentration 20 mM) in order to accelerate the change in their shape from discocytes to spherocytes. This shape

evolution was able to be measured by PFG NMR because the mean diameter decreased to a value that reflected itself well in *q*-space plots, like those of Fig. 1. Fig. 3 shows a processed dataset from fast-diffusion experiments for these two different RBC shapes. It is easy to see the difference between both plots, and that the mean diameter evolved from ~5.6 μm (discocyte) to ~2.8 μm (spherocyte).

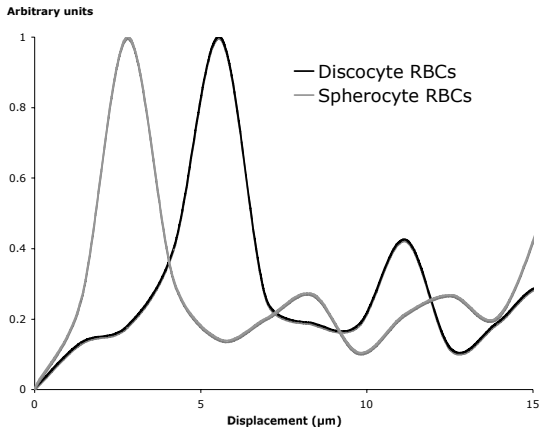

Fig. 3. Influence of RBC shape on the mean diameter measured by *q*-space analysis. RBCs with a mean diameter of 5.6 μm corresponding to discocyte shape (black), instead the mean diameter was 2.8 μm due to spherocytic RBCs (grey).

At 37°C, after only 2 h the sample contained 50% of each shape and after 4 h the RBCs were almost all spherocytes (Fig. 4).

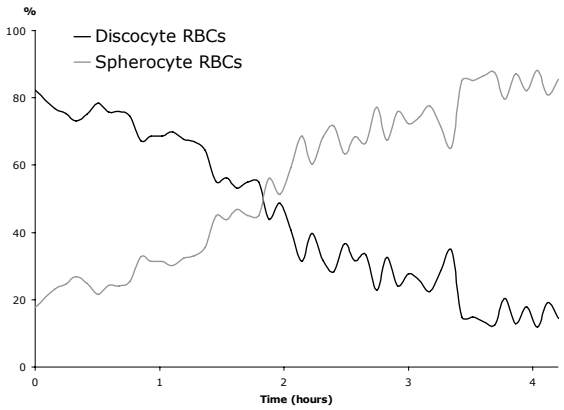

Fig. 4. Evolution of RBC shapes probed with ~50 fast-diffusion NMR *q*-space measurements over 4 h. Black line denotes discocyte-shaped RBCs and the grey line denotes spherocytic RBCs.

3. Conclusions

We present the first application, to our knowledge, of fast-diffusion NMR measurements to obtain *q*-space plots to monitor the evolution of cell-shape changes. The mean RBC diameter measured by the fast-diffusion pulse sequence was in excellent agreement with the values obtained with more classical pulse field-gradient methods such as PGSTE.

We demonstrate the capability of recording a kinetic time courses of the average-shape evolution of RBCs under conditions of relatively fast shape changes.

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