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Quantitative Measurement of Molecular Diffusion Coefficients by NMR Spectroscopy

Shanmin Zhang
Sealy Center for Structural Biology, Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, Texas 77555-1157
Received February 14, 2006; E-mail: shanminz@hotmail.com

Measurement of molecular diffusion coefficients in solution by nuclear magnetic resonance (NMR) was first introduced by Stejskal and Tanner in 1965,[1] where a theoretical foundation was also laid down. Since then, this method has evolved into a number of versions for different purposes.[2−4] In particular, it was extended significantly by Johnson et al.[5,6] to multidimensional NMR, where the diffusion coefficient D serves as a new dimension of various NMR spectra to distinguish the molecules of different sizes or diffusion coefficients.

Due to the non-uniform PFG and RF fields across the sample, considerable deviation may occur when comparing diffusion data obtained from different NMR instruments. Some efforts have been made to reduce the errors[7−9] and are effective under certain conditions. In this communication, a new approach is described with an offset-independent adiabatic inversion pulse[10,11] to uniformly excite a central region of the sample (Figure 1), where the RF field is assumed to be uniform and the PFG strength can be expressed by a linear approximation (Figure 2).

Within the selected region, the decay of the peak intensity as a function of the strength of the two PFGs separated by T (Figure 3) can be described by

$$I \propto \int_{-\Delta}^{\Delta} \frac{1}{g(\delta)} \exp[-\alpha D g^2(\delta)] d\delta = \frac{2\Delta}{g_e} \exp(-\alpha D g_e^2) \quad (1)$$

where the mean value theorem for integrals is used, $g_e (g_{min} \le g_e \le g_{max})$ is termed the effective gradient strength, and α is a constant. The integration is done over the selected region from $-\Delta$ to Δ (Figure 2). The mean value of g_e in general depends on Δ, α, and D, making the diffusion process much more complicated. However, if the gradient strength $g(\delta)$ in the selected region can be described by a linear approximation (Figure 2)

$$g(\delta) = g_{max} - k\delta \quad (2)$$

where the slope $k = (g_{max} - g_{min})/\Delta$, it can be shown that $g_e \approx (g_{max} + g_{min})/2$. In addition, Δ/g_e in eq 1 becomes a constant since both g_e and Δ increase linearly as the PFG DAC unit increases, leading to

$$I \propto \exp(-\alpha D g_e^2) \quad (3)$$

The above equation implies that the molecules in the selected region behave as if they experienced the same g_e, resulting in a Gaussian decay of the peak intensity as a function of the effective gradient. Utilizing the fact that the offset is related to the z-coordinator, it can be proven that the length of the selected region

$$l = (4\pi/k\gamma)\ln(g_{max}/g_{min}) \quad (4a)$$

or

$$l \approx (4\pi\Delta/\gamma g_e), \text{ if } k\Delta \ll g_e \quad (4b)$$

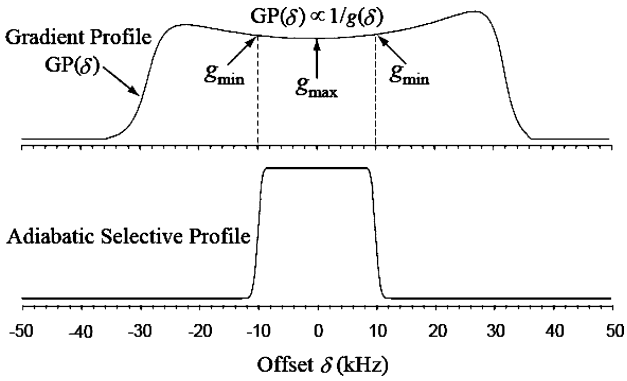

Figure 1. The gradient profile GP(δ) (top) obtained using a Varian 750 MHz NMR instrument with a 5 mm HCN triple-resonance probe, where $g_{max} = 8.900$ g/cm and $g_{min} = 8.596$ g/cm, and the adiabatic selective profile (bottom) used to excite the above region within the two dashed lines. GP(δ) $\propto 1/g(\delta)$ in the selected region, where the RF field is assumed to be uniform.

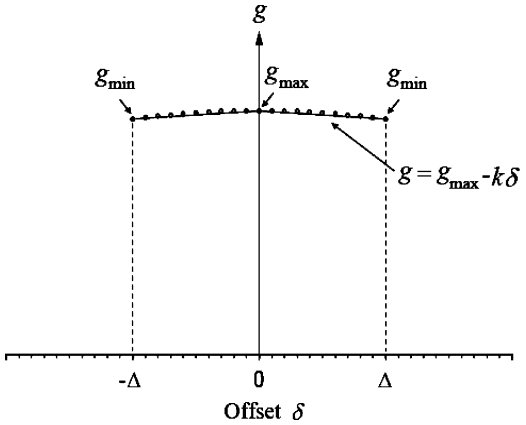

Figure 2. Gradient strength as a function of the offset, derived from the inverse of the selected region of the gradient profile in Figure 1 (circles), which is approximated by $g = g_{max} - k\delta$ (straight line).

Given the length or volume of the selected region, this formula provides a quantitative measure of the NMR spectrum. For $\Delta =$ 10 kHz, $\gamma = 26.7522128$ kHz/g,[12] and the parameters shown in Figure 1, we have $l = 0.54$ cm, about one-third of the RF coil length.

The pulse sequence is shown in Figure 3. It consists of three components: selective excitation with an adiabatic inversion pulse and a gradient pulse g_s; a standard diffusion sequence with a stimulated echo and a pair of gradient pulses separated by a time T; and a WET solvent suppression sequence composed of four selective RF pulses and gradient pulses.[13,14] If necessary, the frequency of the selective pulses can be shifted to achieve off-resonance solvent suppression, using the phase-incremented pulses.[15] The solvent suppression is not used when calibrating the effective gradient strength g_e using a sample of 90% H_2O/10% D_2O. To reduce the effects of radiation damping at high magnetic fields, a

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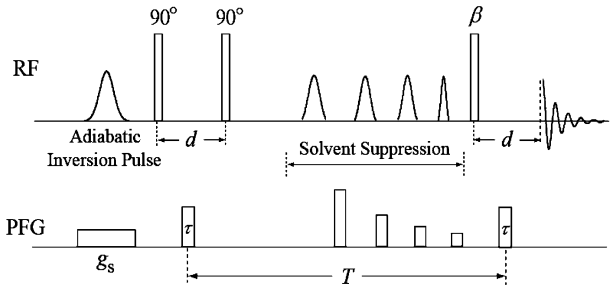

Figure 3. Pulse sequence for quantitative measurement of molecular diffusion coefficients. The phases of the three RF pulses and receiver are $x, -x, x, x; x, -x, x, -x$. CYCLOPS is used for eight scans.

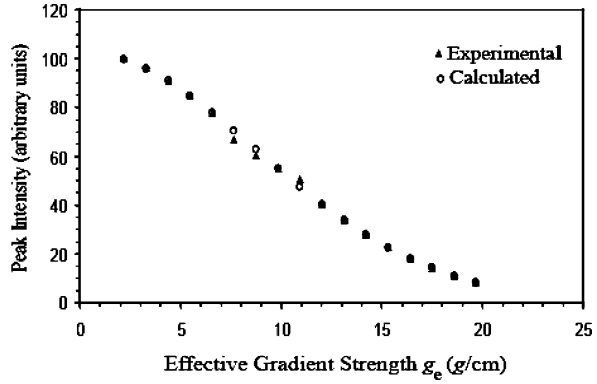

Figure 4. The diffusion decay of H_2O (in 90% H2O/10%D2O) (solid triangles) obtained with the pulse sequence shown in Figure 3 using a flip-angle $\beta = 15°$ and $T = 100$ ms at 25 °C. As shown, three data points do not match well with calculated ones, but they are of less weight in determining D.

small flip-angle β ($\leq 90°$) can be used. The width of the selective gradient pulse g_s is greater than the width of the adiabatic inversion pulse in order to dephase the transverse magnetization before and after the adiabatic inversion, leading to a pure negative z-magnetization for the selected region. The adiabatic pulse is turned on for odd scans and off for even scans, and correspondingly, the receiver alternates between positive and negative phases. After two scans, the signals from the nonselected region cancel and the selected region add. More importantly, the added magnetization is along the z-axis, and there is no $x-y$ component or phase distortion. The power of the four selective pulses for solvent suppression is reduced to zero when measuring the H_2O diffusion coefficient. To avoid glitches occurring at the center of the spectrum, the peak of H_2O is placed slightly off-resonance.

The experimental data are shown in Figure 4, along with the calculated result. As in other PFG diffusion experiments, several data points with relatively larger deviations were observed due to the nonlinearity of PFG and instability of the NMR instrument. To reduce the artifacts, especially data fluctuation in the t_1 dimension, a Java program has been developed for calculating the molecular diffusion coefficients with a graphic user interface for easy input and output of data. This program is able to determine D uniquely with a relative standard deviation (RSD). The peak intensity of the nth data as a function of gradient strength is described by

$$I_n = I_0 \exp(-D\alpha g_{en}^2) \tag{5}$$

where I_0 is the amplitude of the peak intensity, $\alpha = (T - \tau/3)(\gamma\tau)^2$, and g_{en} is the nth effective gradient strength. Since between any two data points a D can be derived, for a collection of N data points, there will be ${}_NC_2 = N(N-1)/2$ diffusion coefficients. On the basis of all these D values, an average (D_{ave}) is then calculated. To reduce the effects of data fluctuations, only 60% of the total D is used. The D that is most deviated from the D_{ave} is discarded first and a new D_{ave} is calculated using the remaining data, ${}_NC_2 - 1$. This procedure continues until 40% of the data are eliminated and the last D_{ave} is chosen as the calculated diffusion coefficient D. The relative standard deviation is calculated with the formula

$$\mathrm{RSD} = \frac{\sqrt{\sum_{i=1}^{M}(D_i - D)^2/M}}{D} \times 100\% \tag{6}$$

where D_i is the remaining 60% of the total number of diffusion data and $M = \mathrm{int}(0.6 \times {}_NC_2)$.

The calculated diffusion coefficient D is 2.28×10^{-5} cm^2/s with a small RSD = 0.60%, indicating a very good quality of data. The diffusion coefficient D measured by this method agrees well with the published value $D = 2.27 \times 10^{-5}$ cm^2/s obtained using a diffusion cell and Rayleigh interferometry.[16]

Molecular diffusion coefficient can be measured quantitatively by NMR with selective excitation of a central sample region that is sufficiently long to ignore the ending effects, yet is short enough to have a homogeneous RF field and to represent the PFG with a linear approximation. Under these conditions, the NMR signal contributed by the molecules in the excited region decays as if all molecules were experiencing the same effective field g_e. With a reference sample of 90% H_2O/10% D_2O ($D = 2.28 \times 10^{-5}$ cm^2/s) and a computer program, g_e can be determined conveniently. In a similar way, the nonlinearity of PFG as a function of the DAC unit can be compensated. As a result, the molecular diffusion coefficients measured by different instruments become comparable, resembling the PPM values of the NMR peaks obtained with different NMR spectrometers.

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Supporting Information Available: The graphic user interface of the Java program and the derivations of eqs 4a and 4b. This material is available free of charge via the Internet at http://pubs.acs.org.

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