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Communication

PGSTE-WATERGATE: An STE-based PGSE NMR sequence with excellent solvent suppression

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Abstract

A new stimulated-echo based pulsed gradient spin-echo NMR diffusion sequence incorporating WATERGATE solvent suppression, PGSTE-WATERGATE, is presented. The sequence provides superb solvent suppression without any phase distortions. The sequence is simple to set up and particularly suited to measuring diffusion coefficients in aqueous solution such as is commonly required in pharmaceutical and combinatorial applications. The utility of the sequence is demonstrated on samples containing lysozyme and sucrose. Importantly, the high degree of phase-distortion suppression allows more complicated selective π pulses to be used to enhance the selectivity of solvent suppression.

Keywords: Diffusion; PGSE; PGSTE; Solvent suppression; WATERGATE

1. Introduction

Due to solubility problems, limited sample availability and/or aggregation, solvent signals in NMR are typically 4–5 orders of magnitude higher than the solute signals. In pulsed field gradient spin-echo (PGSE) NMR measurements [1–3], strong solvent signals hamper the determination of diffusion. Many solvent suppression methods have been proposed [4]. However, only a subset of these methods may be combined with PGSE diffusion sequences due to the deleterious effects of radiation damping and long range dipole–dipole interactions [4–6]. WATERGATE [7–9] is one of the most efficient and easily implemented suppression techniques which can be combined with PGSE diffusion experiments. Stimulated-echo (STE) PGSE sequences outperform Hahn spin-echo PGSE sequences in determining the diffusion of molecules with short T_2 relaxation times (e.g., protein) or in magnetically inhomogeneous samples (e.g., human tissue) and are also widely used for small molecule samples (e.g., ligands, carbohydrates). In 1996, the diffusion and relaxation editing (DIRE) sequence was developed by using a bipolar gradient pair with a soft π pulse (i.e., a single WATERGATE unit) in a bipolar STE-PGSE sequence [10]. However, the use of only one WATERGATE unit in an STE-PGSE sequence may result in phase distortions caused by the application of the requisite selective pulse (the same phenomenon was found in Hahn spin-echo PGSE experiments by Hwang and Shaka [11]).

In this paper, we present a new STE-PGSE NMR diffusion sequence, PGSTE-WATERGATE (Fig. 1), which provides superb solvent suppression, pure phase spectra, and also high coherence-pathway selectivity with only a 4-step phase cycle (Fig. 1). This sequence is complementary to the PGSE-WATERGATE sequence reported by Price et al. [12].

2. Theory

For the non-solvent resonances, the effects of the PGSTE-WATERGATE sequence can be described by the

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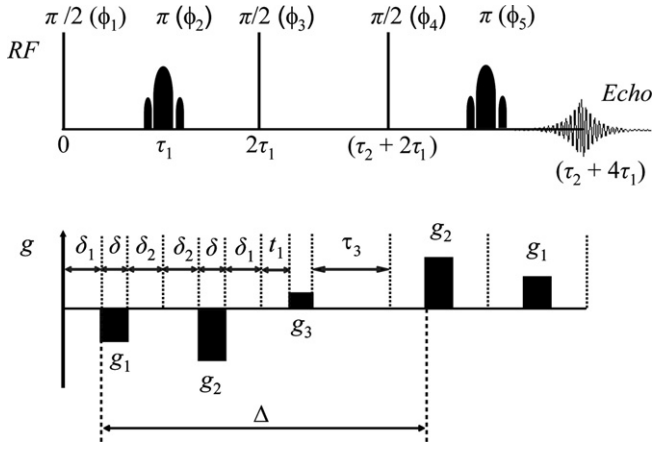

Fig. 1. The PGSTE-WATERGATE sequence. The narrow bars represent $\pi/2$ RF pulses, g_1, g_2 and g_3 are rectangular gradient pulses with different amplitudes, and the shaped rectangles represent "W5" binomial π pulses [18]. The phase cycle for the pulse sequences is $\phi_1 = x, -x$; $\phi_2 = y, y, -x, -x$; $\phi_3 = x, y, -x, -y$; $\phi_4 = x, y, -x, -y$; $\phi_5 = y$; ϕ_r (receiver phase) $= x, -x, -x, x$. For $\phi_2 = y$ (or $\phi_5 = y$), the phasing of the "W5" binomial pulse train is $(x)_5 - (-x)_5$; for $\phi_2 = -x$, the phasing is $(y)_5 - (-y)_5$.

use of a transformation matrix [11]. The input and output magnetization vectors can be written as $\mathbf{m} = [m_x, m_y, m_z]'$ and $\mathbf{M} = [M_x, M_y, M_z]'$, respectively, and the transformation caused by the pulse sequence can be written as $\mathbf{M} = \mathbf{Tm}$, where $\mathbf{T}$ is a 3-by-3 matrix which represents the transformation caused by the pulse sequence. By the use of density matrices and rotation operators [13,14], the transformation matrix for the PGSTE-WATERGATE sequence with the 4-step phase cycle shown in Fig. 1 is given by

$$\mathbf{T} = \begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & \frac{1}{2} \\ 0 & 0 & 0 \end{bmatrix}. \tag{1}$$

This matrix shows the two important properties of the PGSTE-WATERGATE sequence: first, no phase factors are introduced by the application of the selective pulses; and second, the sequence gives a pure coherence-pathway (i.e., the stimulated-echo) which only transforms half of the z magnetization to y magnetization.

Though it has been proved by Pelta et al. that only one transient (i.e., no phase cycle) is sufficient to obtain the desired stimulated-echo signal by the use of asymmetric bipolar gradient pairs (NB their phase cycle is different than that used in PGSTE-WATERGATE), a 16-step phase cycle was used to suppress residue unwanted signals and obtain accurate diffusion data in their study [15]. The same asymmetric bipolar gradient pairs as used in the PGSTE-WATERGATE sequence (Fig. 1) but with g_1/g_2 at a "magic ratio" were developed to suppress the effects of background gradients in NMR diffusion experiments [16,17], and an 8-step phase cycle was used in ref. [16]. In the present study, because of the use of the two soft π pulses, a 4-step phase cycle is necessary to suppress unwanted signals and obtain proper excitation profiles (i.e., no spectral distortions).

The diffusion-based attenuation of the non-solvent resonances in the PGSTE-WATERGATE sequence can be written as

$$\ln(E) = -\gamma^2 D\delta^2 \left[\left(\Delta - \frac{4}{3}\delta - 2\delta_2 \right)(g_2 - g_1)^2 - \frac{2}{3}\delta(g_2 - g_1)g_1 + \left(4\delta_2 + \frac{4}{3}\delta \right) g_1^2 \right], \tag{2}$$

where E is the diffusion-based spin-echo attenuation, D ($m^2\ s^{-1}$) is the diffusion coefficient and γ (rad s^{-1} T^{-1}) is the gyromagnetic ratio of the nucleus being used, and Δ, δ, δ_1, δ_2, g_1 and g_2 are defined in Fig. 1. According to Eq. (2), in the limit of $\Delta >> \delta$ and δ_2, the effective diffusion time for the PGSTE-WATERGATE sequence can be defined as $\Delta - 2\delta_2 - 4\delta/3$.

The solvent resonances are unaffected by the two selective π pulses so a normal STE-PGSE sequence is experienced with effective gradient sequences in the first $2\tau_1$ interval being inverted from that in the second $2\tau_1$ interval. This sequence chooses the STE#2 coherence-pathway as referred to by Kingsley [19], which has the same coherence level in the first $2\tau_1$ interval and the second $2\tau_1$ interval. The STE#2 coherence of the solvent is attenuated by molecular diffusion, and the residual coherence can be further attenuated by the phase cycle. The diffusion-based attenuation of the solvent resonance can be written as

$$\ln(E) = -\gamma^2 D\delta^2 \left[\left(\Delta - \frac{4}{3}\delta - 2\delta_2 \right)(g_1 + g_2)^2 + \frac{2}{3}\delta(g_1 + g_2)g_1 + \left(4\delta_2 + \frac{4}{3}\delta \right) g_1^2 \right]. \tag{3}$$

Comparison of Eqs. (2) and (3) reveals that the solvent resonance experiences a much higher applied gradient (i.e., $g_1 + g_2$ terms) than the resonances of interest, which experience $g_2 - g_1$ terms. Further, most solvent molecules (e.g., H_2O) have far higher diffusion coefficients than the molecules of interest (e.g., protein, peptide, carbohydrate). For a typical solvent like water, a suppression factor of 10^4 can be easily achieved by using gradient strengths of $g_1 = 0.110$ T m^{-1} (20% of the full gradient strength in our study) and $g_2 = 0.138$ T m^{-1} (25% of the full gradient strength in our study) when typical parameters are $\Delta = 90.4$ ms, $\delta = 3$ ms, $\delta_1 = 0.2$ ms and $\delta_2 = 2$ ms. Thus, the NMR signals from the solvent molecules can be totally suppressed while the resonance of the molecules of interest is left intact.

3. Results and discussion

In a typical diffusion experiment, g_1 and g_2 ($g_2 - g_1 = 0.005$ T m^{-1}) should be large enough to suppress the solvent resonance, in a measurement g_1 is kept constant whilst g_2 can be varied up to the available gradient strength to attenuate the resonances of the molecules of interest. For a slowly diffusing molecule (e.g., $D = 3 \times 10^{-11}$ m^2 s^{-1}) an attenuation factor of 10 can be easily achieved by varying

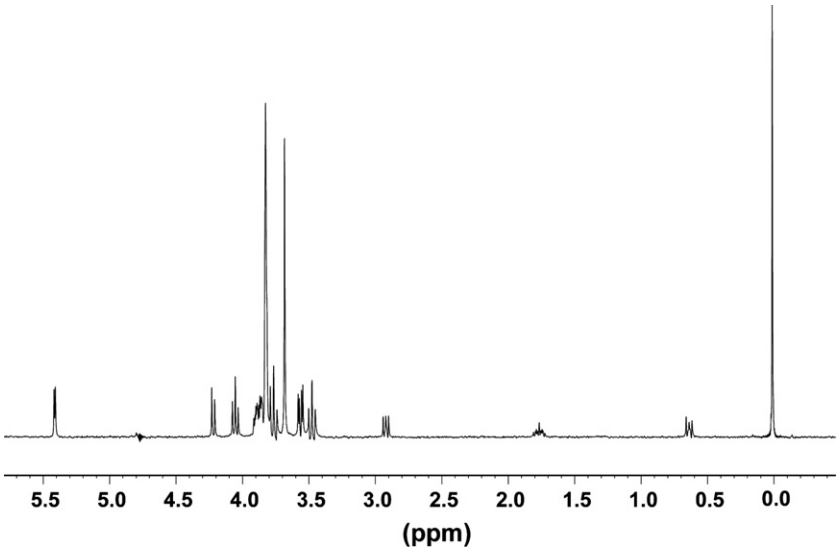

Fig. 2. A 400 MHz ^{1}H spectrum of a sample containing 2 mM sucrose, 0.5 mM DSS and 2 mM NaN_3 in water (10:90 D_2O/H_2O) at 298 K using the PGSTE-WATERGATE sequence. Acquisition parameters were number-of-scans = 64, $\Delta = 69.4$ ms and $\delta = 2.5$ ms with the strengths of g_1 and g_2 at 0.193 T m^{-1} and 0.221 T m^{-1}. The inter-pulse delay in the binomial pulses was set to 400 μs.

g_2 from 0.138 T m^{-1} to 0.65 T m^{-1} with $g_1 = 0.132$ T m^{-1}, $\delta = 4$ ms and $\Delta = 132.4$ ms.

As mentioned above, for the PGSTE-WATERGATE sequence, the resonances of interest only see a gradient strength of $g_2 - g_1$, which is very small (e.g., 0.005 T m^{-1}) at the beginning of a diffusion experiment, so a good signal-to-noise ratio can be obtained even with low concentration samples. In comparison, both DIRE, which uses symmetric bipolar gradient pairs and a 16-step phase cycle, and PGSE-WATERGATE require the solvent resonances, and consequently the resonances of interest, to see a gradient strength of at least 0.1–0.2 T m^{-1} for good solvent suppression. As this determines the smallest values of the diffusion gradients that can be used in these sequences, it results in lower signal-to-noise ratios when measuring the diffusion of low molecular weight species.

The utility of the sequence is demonstrated on samples containing sucrose (Fig. 2) and lysozyme (Fig. 3). As shown in Figs. 2 and 3, high quality water suppression was achieved without phase distortions. The lysozyme peaks close to the water resonance are also observable, which shows the selectivity of the suppression.

To illustrate the advantage of PGSTE-WATERGATE in removing phase distortions caused by the use of soft pulses, the PGSTE-WATERGATE sequence was compared with the PGSE-WATERGATE sequence and a modified PGSTE-WATERGATE sequence in which the second soft π pulse was replaced with a hard π pulse. To make the comparison more rigorous, "$\overline{1}12$" binomial π pulses [11], well-known for causing significant spectral phase roll [11], were used for the soft pulses. As shown in Fig. 4B and C, for the modified PGSTE-WATERGATE and PGSE-WATERGATE sequence, the use of the "$\overline{1}12$" pulse introduced serious phase distortions since both sequences contain only one WATERGATE unit. However, as shown in Fig. 4A, no phase distortion was observed for the PGSTE-WATERGATE sequence containing "$\overline{1}12$"

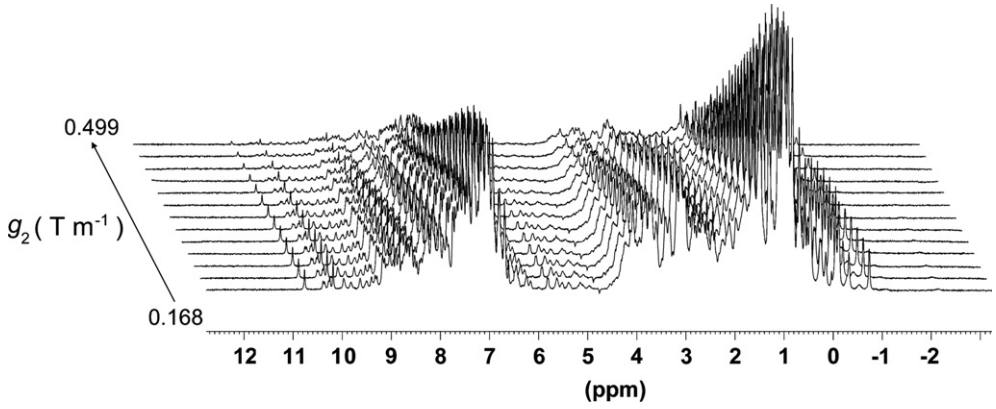

Fig. 3. A series of 500 MHz ^{1}H PGSTE-WATERGATE spectra of a sample containing 2 mM lysozyme in water (10:90 D_2O/H_2O) at 298 K. Acquisition parameters were number-of-scans = 32, $\Delta = 93.7$ ms and $\delta = 4$ ms with the strengths of g_1 at 0.1 T m^{-1} and g_2 varying from 0.168 T m^{-1} to 0.499 T m^{-1} in equal increments. The inter-pulse delay in the binomial pulses was set to 250 μs.

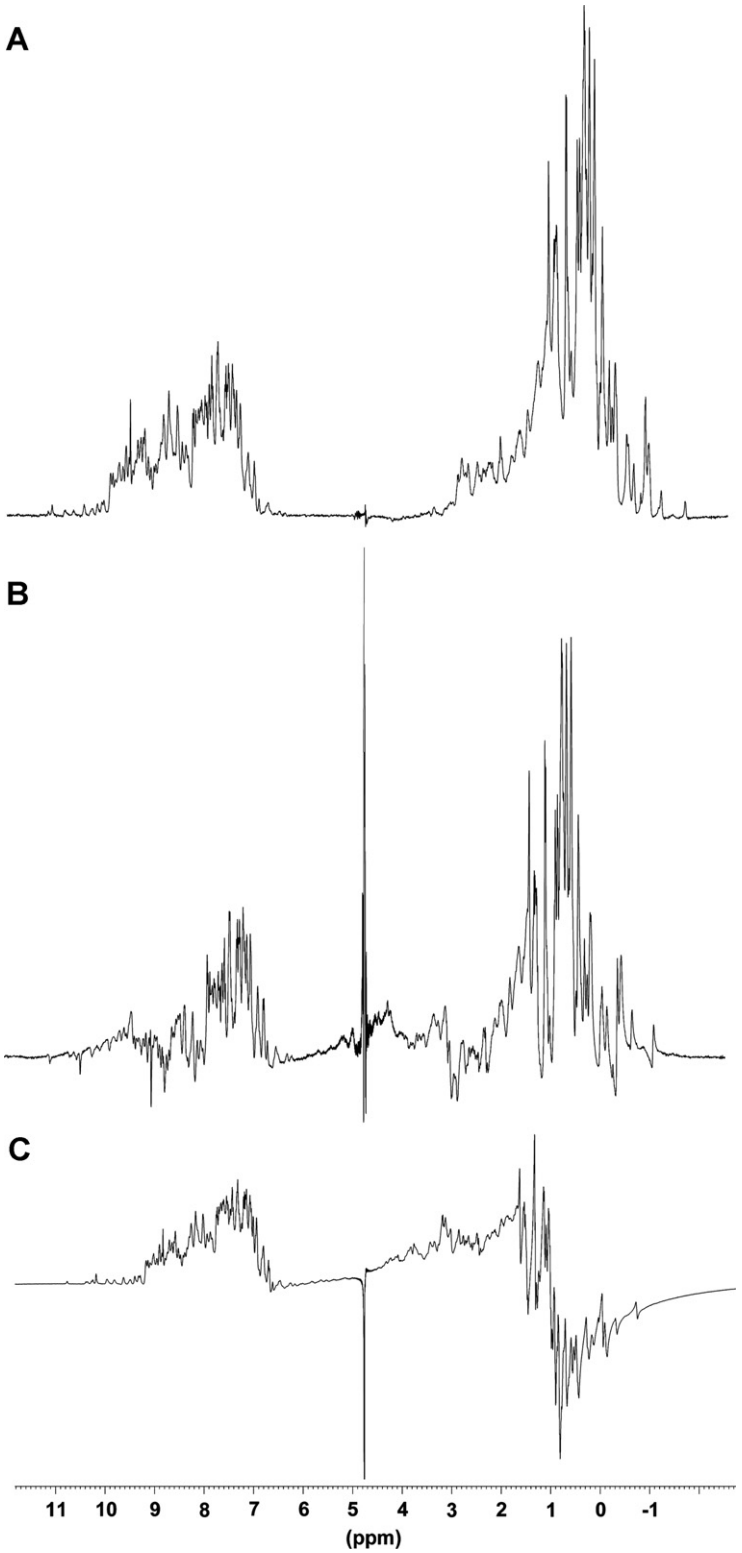

Fig. 4. 500 MHz ^{1}H spectra of 2 mM lysozyme in water (10:90 D_2O/H_2O) at 298 K acquired with (A) PGSTE-WATERGATE (number-of-scans = 32, $\Delta = 91.9$ ms, and $\delta = 4$ ms with the strengths of g_1 and g_2 at 0.1 T m^{-1} and 0.105 T m^{-1}), (B) the modified PGSTE-WATERGATE (number-of-scans = 32, $\Delta = 91.9$ ms and $\delta = 4$ ms with the strengths of g_1 and g_2 at 0.152 T m^{-1} and 0.157 T m^{-1}), (C) PGSE-WATERGATE [12] (number-of-scans = 32, $\Delta = 14.1$ ms and $\delta = 3$ ms with a gradient strength of 0.315 T m^{-1}). In each case the "$\overline{1}12$" pulse sequence was used for the soft π pulses. The inter-pulse delay in the binomial pulses was set to 250 μs.

pulses due to the symmetrical nature of the PGSTE-WATERGATE sequence.

To illustrate the ability of the PGSTE-WATERGATE sequence to determine diffusion, two diffusion experiments were performed. The first diffusion experiment was performed on the residual HDO in D_2O at 298 K by setting the transmitter frequency 1250 Hz away from the HDO resonance frequency so that the HDO resonance was fully inverted by the binomial π pulse. The diffusion coefficient of HDO was determined to be $1.90 \pm 0.01 \times 10^{-9}\,m^2\,s^{-1}$, which is in agreement with the diffusion coefficient ($1.900 \pm 0.004 \times 10^{-9}\,m^2\,s^{-1}$) obtained with the standard Hahn spin-echo PGSE sequence on the same sample and literature values [20]. The second diffusion experiment was performed on a sample containing 2 mM lysozyme in water (10:90 D_2O/H_2O) at 298 K (Fig. 3), and a diffusion coefficient of $1.08 \pm 0.01 \times 10^{-10}\,m^2\,s^{-1}$ was obtained for the lysozyme. The result is in line with the diffusion coefficient ($1.04 \pm 0.02 \times 10^{-10}\,m^2\,s^{-1}$) obtained with the standard STE-PGSE sequence on the same sample and also literature values [21].

4. Conclusions

The PGSTE-WATERGATE sequence provides superb solvent suppression and pure phase spectra. The sequence is simple to set up and particularly suited to measuring diffusion in aqueous solution such as is commonly required in pharmaceutical and combinatorial applications. Importantly, the high degree of phase-distortion suppression allows more sophisticated selective π pulses to be used to enhance the selectivity of solvent suppression.

Acknowledgments

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References

[1] P. Stilbs, Fourier transform pulsed-gradient spin-echo studies of molecular diffusion, Prog. Nucl. Magn. Reson. Spectrosc. 19 (1987) 1–45.
[2] C.S. Johnson Jr., Diffusion ordered nuclear magnetic resonance spectroscopy: principles and applications, Prog. Nucl. Magn. Reson. Spectrosc. 34 (1999) 203–256.
[3] W.S. Price, Gradient NMR, in: Annu. Rep. NMR Spectrosc., Academic Press, New York, 1996, pp. 51–142.
[4] W.S. Price, Water signal suppression in NMR spectroscopy, in: G.A. Webb (Ed.), Annu. Rep. NMR Spectrosc., vol. 38, Academic Press, London, 1999, pp. 289–354.
[5] W.S. Warren, S.L. Hammes, J.L. Bates, Dynamics of radiation damping in nuclear magnetic resonance, J. Chem. Phys. 91 (1989) 5895–5904.
[6] Y.Y. Lin, N. Lisitza, S. Ahn, W.S. Warren, Resurrection of crushed magnetization and chaotic dynamics in solution NMR spectroscopy, Science 290 (2000) 118–122.
[7] M. Piotto, V. Saudek, V. Sklenář, Gradient-tailored excitation for single-quantum NMR spectroscopy of aqueous solutions, J. Biomol. NMR 2 (1992) 661–665.
[8] V. Saudek, M. Piotto, V. Sklenár, Bruker Report, 1994, p. 6.
[9] V. Sklenář, M. Piotto, R. Leppik, V. Saudek, Gradient-tailored water suppression for ^1H–^{15}N HSQC experiments optimized to retain full sensitivity, J. Magn. Reson. Ser. A 102 (1993) 241–245.
[10] M.L. Liu, J.K. Nicholson, J.C. Lindon, High-resolution diffusion and relaxation edited one- and two-dimensional ^1H NMR spectroscopy of biological fluids, Anal. Chem. 68 (1996) 3370–3376.
[11] T.L. Hwang, A.J. Shaka, Water suppression that works. Excitation sculpting using arbitrary wave-forms and pulsed-field gradients, J. Magn. Reson. Ser. A 112 (1995) 275–279.
[12] W.S. Price, F. Elwinger, C. Vigouroux, P. Stilbs, PGSE-WATERGATE, a new tool for NMR diffusion-based studies of ligand-macromolecule binding, Magn. Reson. Chem. 40 (2002) 391.
[13] T.C. Farrar, Density matrices in NMR spectroscopy: part I, Concepts Magn. Reson. A 2 (1990) 1–12.
[14] M.H. Levitt, Spin Dynamics: Basics of Nuclear Magnetic Resonance, John Wiley & Sons, Chichester, New York, 2001.
[15] M.D. Pelta, G.A. Morris, M.J. Stchedroff, S.J. Hammond, A one-shot sequence for high-resolution diffusion-ordered spectroscopy, Magn. Reson. Chem. 40 (2002) 147–152.
[16] P.Z. Sun, J.G. Seland, D. Cory, Background gradient suppression in pulsed gradient stimulated echo measurements, J. Magn. Reson. 161 (2003) 168–173.
[17] P. Galvosas, F. Stallmach, J. Kärger, Background gradient suppression in stimulated echo NMR diffusion studies using magic pulsed field gradient ratios, J. Magn. Reson. 166 (2004) 164–173.
[18] M.L. Liu, X.A. Mao, C.H. Ye, H. Huang, J.K. Nicholson, J.C. Lindon, Improved WATERGATE pulse sequences for solvent suppression in NMR spectroscopy, J. Magn. Reson. 132 (1998) 125–129.
[19] P.B. Kingsley, Product operators, coherence pathways, and phase cycling. Part III: phase cycling, Concepts Magn. Reson. 7 (1995) 167–192.
[20] R. Mills, Self-diffusion in normal and heavy water in the range 1–45 deg, J. Phys. Chem. 77 (1973) 685–688.
[21] W.S. Price, F. Tsuchiya, Y. Arata, Lysozyme aggregation and solution properties studied using PGSE NMR diffusion measurements, J. Am. Chem. Soc. 121 (1999) 11503–11512.