JOURNAL OF MAGNETIC RESONANCE **132,** 125–129 (1998)
ARTICLE NO. MN981405

Improved WATERGATE Pulse Sequences for Solvent Suppression in NMR Spectroscopy

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Received October 17, 1997; revised January 26, 1998

Modifications to the WATERGATE method for removing the solvent resonance from ^{1}H NMR spectra are presented. In the conventional WATERGATE, a pulse train with three pairs of symmetric pulses in the form $3\alpha-\tau-9\alpha-\tau-19\alpha-\tau-19\alpha-\tau-9\alpha-\tau-3\alpha$ (designated here W3), where $26\alpha = 180°$, is used, while the improved versions use four and five pairs of symmetric pulses (designated here W4 and W5), respectively. The modified methods provide narrower noninversion regions and hence enhance the sensitivities of the peaks close to the water resonance. The inversion and suppression profiles of the pulse trains W3, W4, and W5 are compared theoretically and experimentally and good agreement is obtained. © 1998 Academic Press

Key Words: **NMR; solvent suppression; pulsed field gradients; WATERGATE; proton exchange.**

INTRODUCTION

The suppression of solvent resonances in ^{1}H FT NMR spectra has been a necessity because of the high dynamic range of solution concentrations found in proton-containing solvents. Numerous techniques have been proposed and reviewed (*1*), of which one of the most promising is the WATERGATE method (water suppression by gradient-tailored excitation) (*2, 3*), which resembles a spin-echo sequence with the refocusing pulse flanked by two symmetrical gradient pulses as shown in Fig. 1a. Transverse coherences are dephased by the first gradient and they can be rephased by the second gradient, provided they experience a 180° rotation by the selective pulses or pulse train, denoted W. This can be a hard 180° pulse sandwiched by two soft 90° pulses (*2*), or a frequency-selective pulse train of form $3\alpha-\tau-9\alpha-\tau-19\alpha-\tau-19\alpha-\tau-9\alpha-\tau-3\alpha$ (denoted W3) with $62\alpha = 180°$, where τ is a short delay, usually a few hundreds of microseconds, which is used to control the null-inversion points. In both cases, the spectral resonances experience a 180° rotation and will be rephased by the second gradient, while the net flip angle at the water resonance frequency approaches zero and thus the water signal will be dephased by both gradient pulses. These two methods give comparable results, but for equivalent total pulse lengths, the selective pulse method has a wider noninversion region by about 30%. One advantage of using the W3 pulse train is that the null points can be easily modified for off-resonance water suppression (*3*). However, there is a disadvantage of using the hard pulse train, since the peak elimination region is wider than when presaturation is used and thus any resonances close to the solvent peak will be suppressed. The double gradient-echo method proposed by Hwang and Shaka provides much better suppression and phase properties (*4*), but the intensities of any peaks near the solvent together with resonances from exchangeable protons in aqueous solution will be reduced because its suppression profile is just the square of that of W3 and, in addition, the total pulse sequence duration is doubled.

In order to reduce the suppression bandwidths, we demonstrate here a modified version of the WATERGATE method. The relative efficiency and inversion profile of the new solvent resonance suppression schemes have been investigated and compared to the WATERGATE W3 method. The modifications comprise the incorporation of one and two more pulse pairs into the W3 pulse train. The new methods may be regarded as W4 and W5 pulse trains respectively, which provide narrower noninversion regions at the null points than the original scheme, W3. The bandwidths and nonsuppression profiles of the various schemes have been compared experimentally and using theoretical simulations. The results show that when more pulse elements are used in the pulse train, the suppression region becomes narrower, particularly in double gradient echo experiments.

EXPERIMENTAL

The experiments were carried out at 500.13 MHz using a Bruker ARX-500 instrument with a BGU-10 field gradient accessory capable of delivering z-field gradients up to 480 mT/m. Sine-shaped gradients of 1 ms duration were used and each was followed by a 200 μs delay for gradient recovery. For each one-dimensional spectrum, typically 64 tran-

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1090-7807/98 $25.00

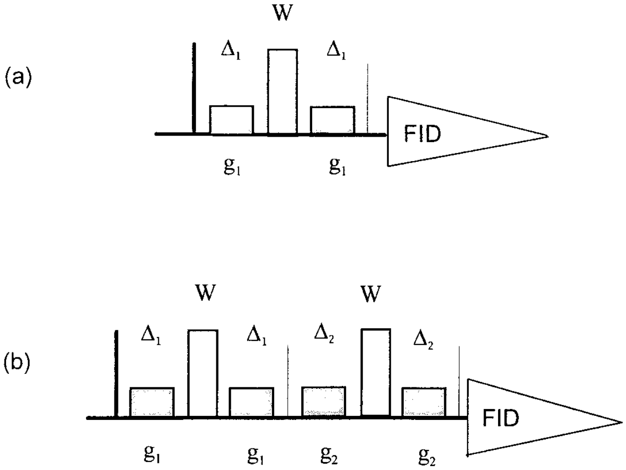

FIG. 1. The NMR pulse sequences used for solvent resonance elimination: (a) WATERGATE and (b) WATERGATE with double gradients. The bar symbol represents a 90° pulse and the open symbols represent the frequency selective pulse train, W3, W4, W5, as described in the text. The component pulses, flip angles, and phase program for the different schemes are listed in Table 1. The time intervals Δ_1, Δ_2 should be just enough to allow gradient switching and recovery.

sients were acquired using a spectral width of 7500 Hz into 16K data points. The free induction decays (FIDs) were weighted by a 90° phase-shifted sine-bell function and zero-filled to 32K before Fourier transformation. The preacquisition delay was adjusted to give a recycle time of 2.5 s. The WATERGATE pulse sequence is given in Fig. 1a. The use of a double gradient-echo method was explored using the pulse sequence of Fig. 1b. Gradient strengths of 48.0 and 33.6 mT/m were used for g1 and g2, respectively. A delay (τ) of 333.3 μs was used for the WATERGATE pulse trains, corresponding to a bandwidth of 3000 Hz between two adjacent null points, unless otherwise described in the text.

RESULTS

WATERGATE W4 and W5 pulse trains and their corresponding inversion profiles. The various WATERGATE pulse sequences tested here are shown in Fig. 1. The pulse train for W4 has flip angles of 10.4°, 29.4°, 60.5°, and 132.8°, and those for W5 are 7.8°, 18.5°, 37.2°, 70.4°, and 134.2°. When the phase of the component pulses in the first part of the pulse train is ϕ and that in the second half is $\phi + \pi$, the resonances at offsets of $\pm k/\tau$ ($k = 0, 1, 2, 3, \ldots$) experience a 0° rotation and will be destroyed by the pulsed field gradients, while the resonances between these null points receive a 180° inversion and will be refocused. If a different sequence of phasing of ϕ and $\phi + \pi$, as shown in Table 1, is used for the component pulses, the null points are shifted to $(2k + 1)/2\tau$. This phasing property had been noted for the W3 pulse train (*3*) and is also applicable to all pulse trains with more than two element pulses. The pulse flip angles, the normal and alternative phasing programs, and the null-point positions are all listed in Table 1.

Figure 1b shows the WATERGATE pulse sequence with double gradient echoes. Here the time intervals Δ_1 and Δ_2 can have equal or different values and should be long enough for the gradient to switch on and off and to allow recovery. Different gradient strengths of g_1 and g_2 are recommended in order to avoid breakthrough of undesired coherences.

Simulated excitation profiles for the various WATERGATE sequences with the composite pulse trains of (a) W5, (b) W4, and (c) W3 are shown in Fig. 2. The simulation calculation was carried out using a PC as follows. The flip angles of the individual pulses were optimized to give the narrowest suppression region at the null points and the flattest inversion profile between them. The simulation was based on the theoretical calculation of the spin operator I_ρ ($\rho = x, y, z$) using the effect of the RF pulses and chemical shift precession. Effects of J coupling and relaxation were not included in the simulation because of the short durations involved. To obtain the W4 pulse sequence, an extra pulse and delay period was inserted into both parts of the W3 sequence and the pulse angles were optimized by varying each of the four pulse element lengths (ensuring that the sum corresponded to 180°) using a grid search to give the widest and flattest inversion profile. The W5 pulse sequence was obtained in a similar fashion by starting from the W4 sequence. A delay time (τ) of 333.3 μs was used for the simulation and this corresponds to a bandwidth of 3000 Hz between the null points. The resulting frequency widths which provide inversion of more than 90% are 1720, 2000, and 2200 Hz for W3, W4, and W5, respectively, corresponding to an increase in the inversion bandwidth of 280 Hz for W4 and 480 Hz for W5, compared with the original pulse train of W3. The frequency width of the suppression region which produces less than 10% of the intensity are 290, 230, and 180 Hz for the pulse trains of W3, W4, and W5, respectively, which are equivalent to narrowing the suppression region by 21% for W4 and 38% for W5. The simulation shows that use of more component pulses results in a simultaneous widening of the inversion profile and a narrowing of the suppression region.

Experimental NMR results at 500 MHz using the pulse trains of (a) W5, (b) W4, and (c) W3 on a sample of HOD in D_2O are shown in Fig. 3. The experiments were carried out in the one-dimensional mode with frequency shifting of 50 Hz after each scan. The parameters were the same as those used for the simulation in Fig. 2. The experimental bandwidths which correspond to inversion of over 90% are 1800, 2000, and 2200 Hz for W3, W4, and W5, respectively, corresponding to an increase in the inversion bandwidth of 200 Hz for W4 and 400 Hz for W5, compared with the original pulse train of W3. The widths of the suppression region which produce less than 10% of the intensity are 280, 220, and 170 Hz for the pulse trains of W3, W4, and W5, respectively, which are equivalent to narrowing the suppres-

TABLE 1
WATERGATE Pulse Sequence Parameters

Pulse type	Pulse train composition (°)[b]	Phasing for null points[c] at k/τ	Phasing for null points[c] at $(2k+1)/\tau$
W1	90, 90	$\phi-\theta^{d}$	$\phi-\phi$
W2	45, 135, 135, 45	$(\phi)_2-(\theta)_2$	$\phi-(\theta)_2-\phi$
W3[a]	20.8, 62.2, 131.6, 131.6, 62.2, 20.8	$(\phi)_3-(\theta)_3$	$\phi-\theta-(\phi)_2-\theta-\phi$
W4	10.4, 29.4, 60.5, 132.8, 132.8, 60.5, 29.4, 10.4	$(\phi)_4-(\theta)_4$	$\phi-\theta-\phi-(\theta)_2-\phi-\theta-\phi$
W5	7.8, 18.5, 37.2, 70, 134.2, 134.2, 70, 37.2, 18.5, 7.8	$(\phi)_5-(\theta)_5$	$\phi-\theta-\phi-\theta-(\phi)_2-\theta-\phi-\theta-\phi$

[a] Original WATERGATE pulse sequence (*10*).
[b] Each pulse element is separated by a period τ.
[c] $k = 0, 1, 2, 3, \ldots$.
[d] $\theta = \phi + \pi$.

sion region by 21% for W4 and 39% for W5. There is therefore good agreement between the experimental results and the theoretical simulation.

The dependence of the frequency width of the suppression region on τ is shown in Fig. 4. The spectra were acquired using the pulse trains of W5 (Figs. 4a–c) and W3 (Figs. 4d–f) with τ values of 333.3 μs (Figs. 4a,d), 250 μs (Figs. 4b,e), and 200 μs (Figs. 4c, f), corresponding to a bandwidth of 3000, 4000, and 5000 Hz between two adjacent null points. The sample used was HOD in D_2O. The results show that the suppression region gets larger when a wider bandwidth is required. The experimental results (and corresponding simulation, not shown) both indicate that the width of the suppression region at half-height is inversely linearly proportional to the τ value, with a coefficient of 0.0768 for W5, 0.0956 for W4, and 0.1225 for W3. It can be seen from Fig. 4 that the peak intensity at 100 Hz offset is increased

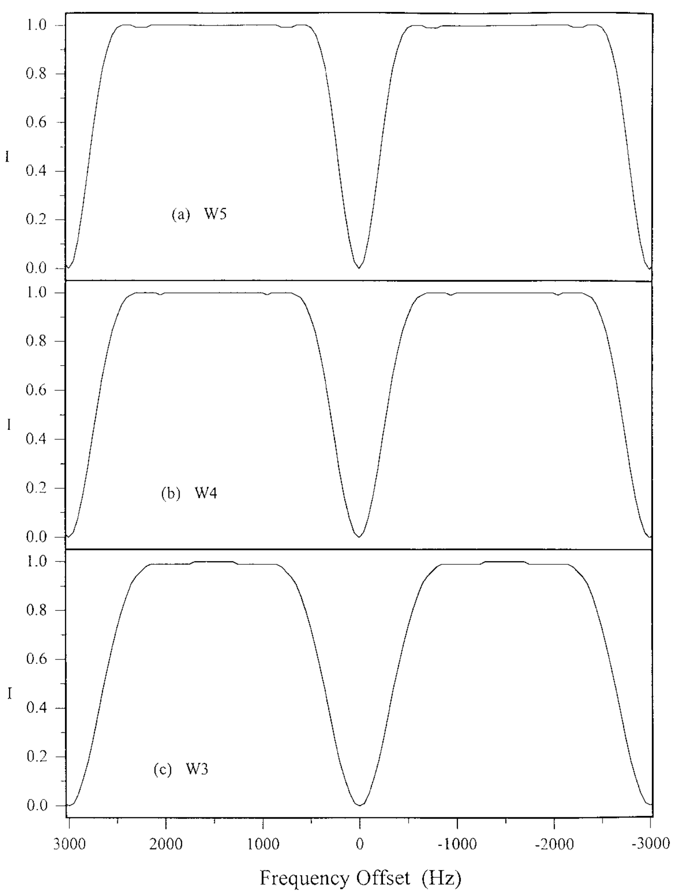

FIG. 2. Simulation of the excitation profiles of the NMR pulse sequences for (a) W5, (b) W4, and (c) W3. The bandwidth was set nominally to 3000 Hz.

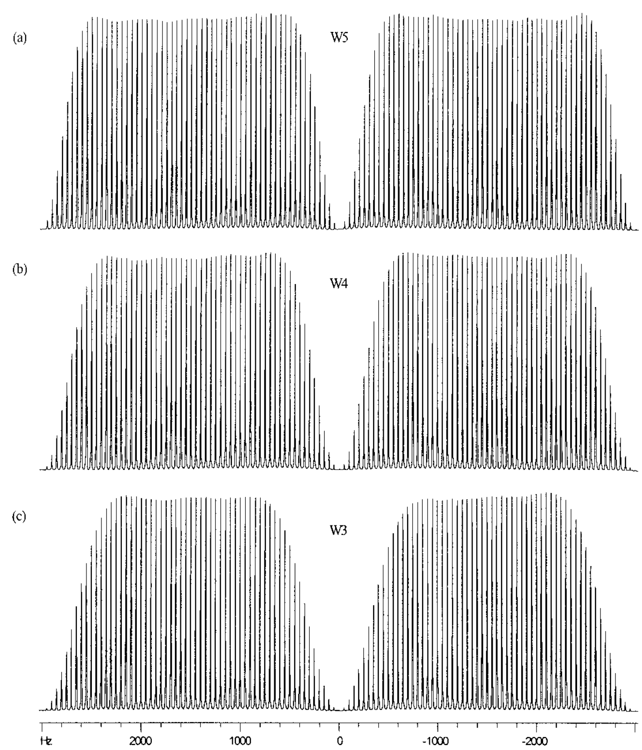

FIG. 3. Experimental excitation profiles of the NMR pulse sequences using W3, W4, and W5 methods. The experiments were carried out at 500 MHz using same parameters as those used for simulation in Fig. 2.

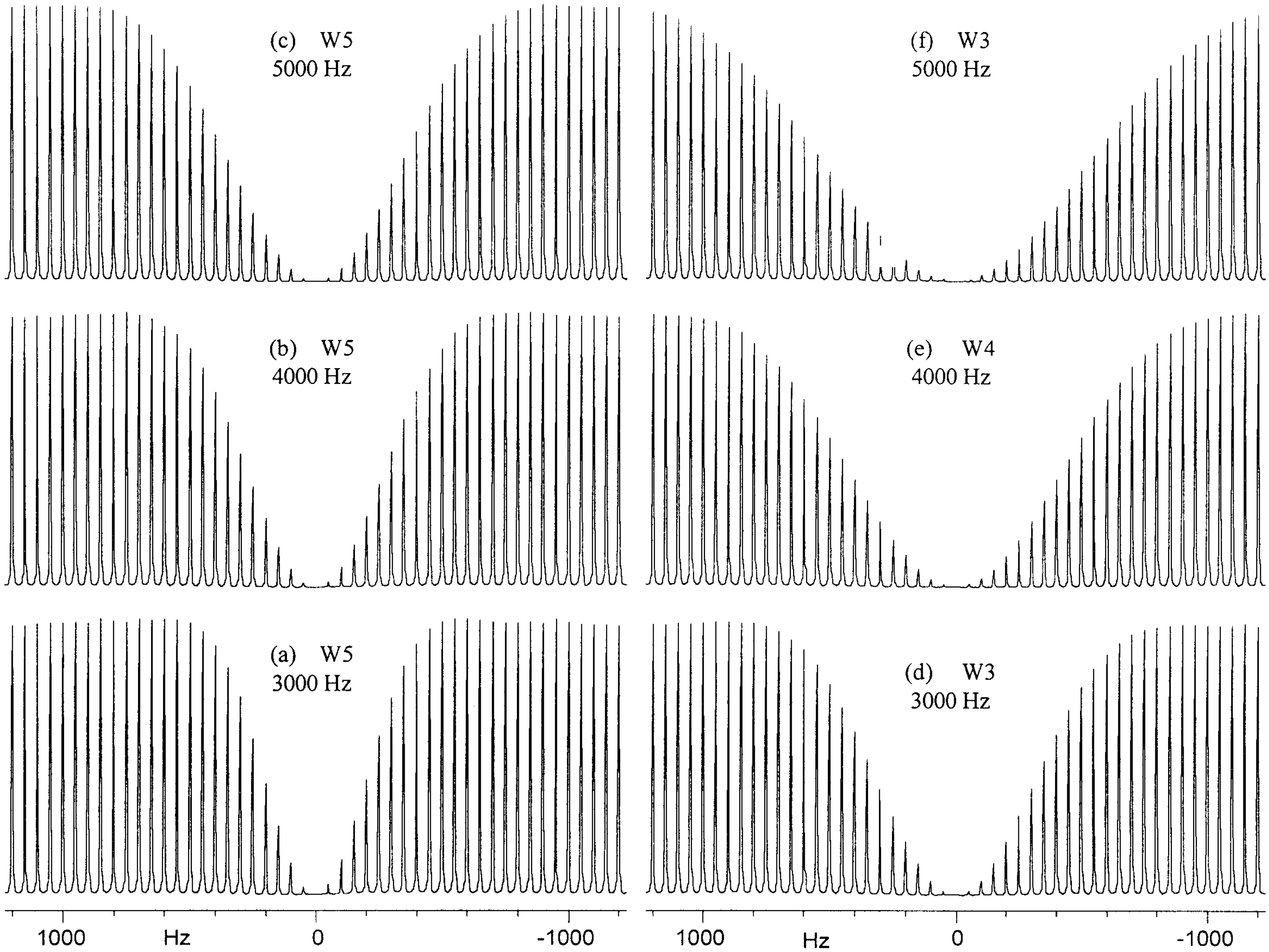

FIG. 4. Dependence of suppression profiles on bandwidths of 3000 Hz (a, d), 4000 Hz (b, e) and 5000 Hz (c, f) for W5 (a, b, c) and W3 (d, e, f).

more than twofold in all bandwidths when the W5 pulse train is used, thereby producing a narrower excitation null region.

Figure 5 shows the experimental results on a sample of H_2O(90%)/D_2O(10%) with pulse trains of W5 (Figs. 5a,b) and W3 (Figs. 5c,b) and with a bandwidth of 3000 Hz. Figures 5b and 5d were obtained using the double gradient echo method (*4*) with gradient strengths of 52.8/52.8 and 33.6/33.6 mT/cm. It has been shown that the suppression profile of the double gradient echo method is a squared form of that of the single-echo approach with the same inversion element (*4*). This has a significant effect on the suppression regions. It can be seen that for W3 with double gradient echoes (Fig. 5d), the intensity at an offset of 230 Hz has been suppressed by over 90% and a 10% effect is still observed when the peak is 650 Hz away from the water resonance. When the W5 pulse train with double gradient echoes is used (Fig. 5b), the offsets for the suppression of over 90% and 10% are 150 and 450 Hz, which corresponds to a narrowing of the suppression region by 35% and 31% at these intensity levels. Thus, the signals of the resonances close to water can be enhanced when the pulse trains of W4 and W5 are used in the WATERGATE sequence.

It is interesting to note that the half-width of the suppression profile is narrower when a high proton concentration water sample (H_2O) is used (Figs. 5a,d) than when using a dilute sample (HOD) (Figs. 4a, c). This is the known phenomenon of radiation damping (*5*), which provides an important decay mechanism for the time domain signal other than transverse relaxation (*5, 6*). The strength of the radiation damping effect is strongly dependent on the magnitude of the transverse magnetization. When the RF carrier frequency is set to the center of the water peak, the highest suppression efficiency is achieved and thus there is no radiation damping. When the carrier frequency is shifted away from the center of the water resonance, the suppression efficiency is reduced. The transverse magnetization increases and reaches a maximum value in the noninversion region. Hence, the radiation damping attenuation takes the same profile as the transverse magnetization. There is no attenua-

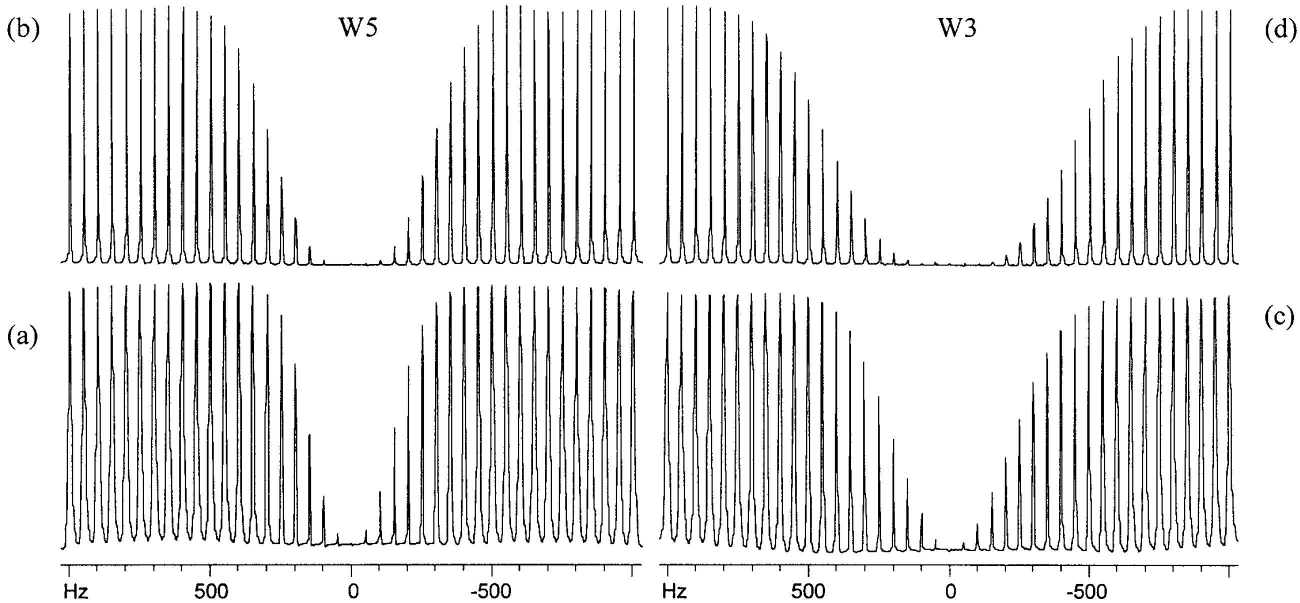

FIG. 5. Comparison of the suppression profiles of W5 (a, b) and W3 (c, d) on a sample of 90%H_2O/10%D_2O using conventional WATERGATE (a, c) and double gradient-echo WATERGATE (b, d).

tion at the null point, but when the offset increases, attenuation gets larger. The largest attenuation takes place in the nonsuppression region because the transverse magnetization is at its full intensity. Thus, the normalized suppression region is narrower than that in the dilute solution.

Application of the modified WATERGATE methods to peaks close to water. The 500-MHz NMR spectrum of human milk has been acquired using WATERGATE with the W5 pulse train, the W4 pulse train, and the W3 pulse train at 289.6 K. Examination of the spectra in the sugar (lactose) region highlights the effect of WATERGATE on NMR peaks close to the water resonance at δ4.7. The relative intensities of α-glucose (H1), β-glucose (H1), and β-galactose (H1) resonances are listed in Table 2 together with the simulated results at the same offset. It can be seen that the pulse trains have a significant effect on the peak intensity close to water. With the W5 pulse train, the intensity of β-glucose (H1) at approximately 100 Hz offset from the null point is enhanced more than twofold compared with approximately W3 pulse train of the original WATERGATE.

TABLE 2
Relative Intensities of Simulated and Observed NMR Peaks of Lactose in Human Milk at Different Offsets from the Null Point

	H1 (β-glucose)	H1 (α-glucose)	H1 (β-galactose)
Offset (Hz)	98.6	181.3	309.6
W3 (experimental)[a]	1.0	1.0	1.0
W3 (simulation)[a]	1.0	1.0	1.0
W4 (experimental)	1.6	1.4	1.4
W4 (simulation)	1.6	1.5	1.5
W5 (experimental)	2.2	1.8	1.8
W5 (simulation)	2.4	2.2	2.1

[a] Used as reference.

SUMMARY

Improved WATERGATE pulse sequences, designed principally for ^{1}H NMR observation, for solvent resonance elimination have been presented. The inversion profile is improved, and there is a narrower suppression region. Consequently, those peaks close to water are less affected by suppression. The improved methods are suitable for the study of exchange processes and assignment of labile proton NMR peaks in aqueous solution.

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