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Binomial frequency response to non-binomial pulse sequences for efficient water suppression

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SUMMARY

This article reports on the use of short-hard pulse and spin-lock pulse combinations giving a binomial-like frequency response for the measurement of NMR spectra in aqueous solutions of quite dilute samples. The pulse sequence proposed provides excellent water suppression and does not introduce any linear or higher order phase errors. Application to the measurement of 2D NOESY data of a 0.25 mM solution of a double-stranded DNA fragment is presented.

Over the last two decades a great number of methods have been proposed for the suppression of the H_2O resonance in NMR spectra of water-soluble compounds. These methods include presaturation (Hoult, 1976; Zuiderweg et al., 1986), selective excitation with soft-pulse sequences (Redfield et al., 1975; Plateau and Guéron, 1982) or binomial hard-pulse sequences (Plateau and Guéron, 1982; Sklenàr and Starcuk, 1982; Hore, 1983; Plateau et al., 1983), combination of hard and soft pulses (Sklenàr et al., 1982), spin-lock pulses (Piveteau et al., 1987; Messerle et al., 1989; Tate et al., 1991) and shaped pulses (Smallcombe, 1993). Among these, presaturation is the oldest and most widely used method because of its simplicity. The H_2O resonance is presaturated using low-power irradiation or soft pulses prior to the observation pulse. However, this technique leaves residual artifacts, and signals near the water frequency are also saturated. The major drawback of the method is that it also saturates protons that are in chemical exchange with the bulk water. This is disadvantageous for nucleic acid studies, which need information coming from the exchangeable imino and amino protons. Many of the alternative water suppression techniques are aimed at exciting as much of the spectrum as possible while the solvent signal remains on the z axis; of these techniques binomial hard-pulse sequences have become most widely used. The 'jump and return' sequence (Plateau et al., 1983) is the simplest composite pulse for water

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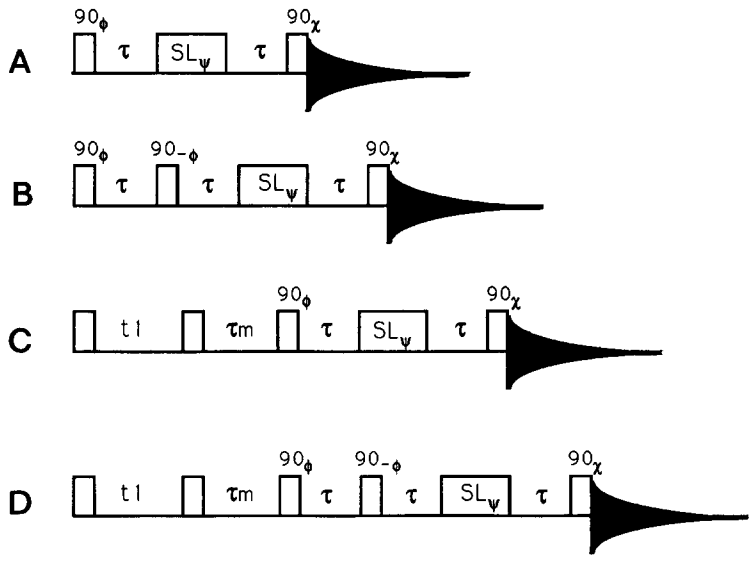

Fig. 1. Schemes for water suppression using a combination of short-hard pulses and spin-lock pulses. (A) the $\sin^2(\omega\tau)$ spin-lock scheme and (B) the $\sin^3(\omega\tau)$ spin-lock scheme for generating a 1D spectrum. (C) and (D) modified NOESY schemes with the two proposed pulse sequences. The phase cycles are φ (x,x,−x,−x), ψ (x,x,x,x), χ (y,−y,y,−y) and acq (y,y,−y,−y) for schemes (A) and (C) and φ (x,x,x,x), −φ (−x,−x,x,x) ψ (y,y,y,y), χ (x,−x,x,−x) and acq (x,x,x,x) for schemes (B) and (D), respectively.

suppression, involving a straightforward two-pulse sequence where the frequency response is sinusoidal with a sharp null at the offset frequency. Better solvent suppression is achieved by more sophisticated composite pulse sequences which represent higher order terms of the Pascal triangle (Hore, 1983). Although these methods provide much better suppression, the phase distortions resulting from this type of excitation are severe, which leads to problems when this type of composite pulse is used in phase-sensitive 2D NMR experiments. Indeed, the resulting frequency-dependent phase-shift produces baseline rolling. Some optimized binomial-based sequences have been proposed using additional pulses (Guéron et al., 1992) or a combination of soft and hard pulses (Sklenàr et al., 1982). However, the growing interest in these types of pulse sequences has been spurred by the recent determination of water molecules bound to proteins and DNA (Otting and Wüthrich, 1989; Otting et al., 1991, 1992; Holak et al., 1992; Kubinec and Wemmer, 1992). For this purpose, new techniques based on spin-lock, which is known to enhance the suppression of undesired proton magnetization, have been developed by Otting et al. (Otting and Wüthrich, 1989; Otting et al., 1992). Although, the sinusoidal response of these sequences provides a very sharp null at the offset frequency, the strong solvent signal that remains is quite a problem when very dilute samples in non-deuterated water have to be studied. This article reports on the use of short-hard pulse and spin-lock pulse combinations that can provide very good water suppression giving a binomial-like frequency response.

Conceptually, the method is simple. Following the product operator notation (Sørensen, et al., 1983) and considering the scheme shown in Fig. 1A, the magnetization is aligned along Iz at equilibrium. The first pulse, which produces an effective field along the Oy direction of the rotating frame, rotates all spins from Oz to Ox. The spins then precess in the transverse plane

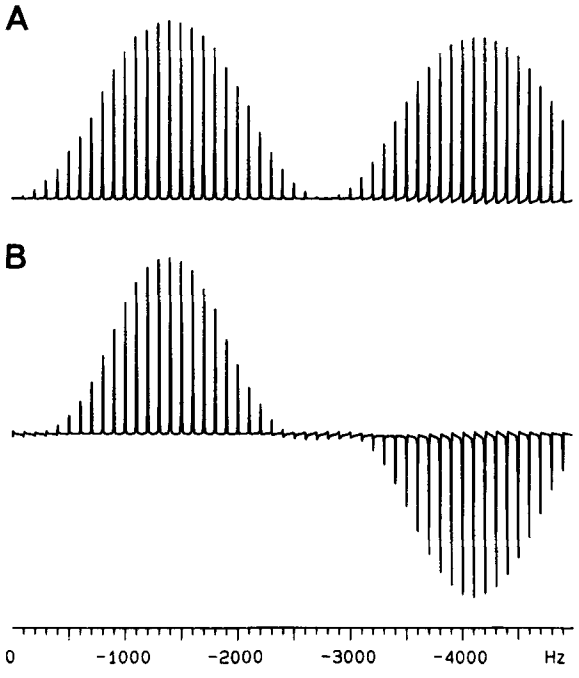

Fig. 2. Experimental offset dependence of the $\sin^2(\omega\tau)$ spin-lock excitation scheme 1A and $\sin^3(\omega\tau)$ spin-lock excitation scheme 1B. The offset was increased by steps of 100 Hz, starting from the water resonance; no linear phase correction has been applied and four scans were acquired.

during the following τ period under chemical shift Hamiltonian. At the end of this period the in-plane magnetization is: $Ix\cos(\omega\tau) + Iy\sin(\omega\tau)$. If the carrier is placed on the H_2O resonance, the protons of the bulk solvent remains on the x axis and are represented by the in-phase magnetization Ix. The spin lock is then applied along the y axis retaining the Iy magnetization unperturbed and purging the water magnetization by its radiofrequency field inhomogeneity in the xz plane. Efficient water suppression is achieved with spin-lock pulses of 2 ms with the same RF amplitude as the other pulses. Then, during the second period τ, the remaining spins which are aligned along the y axis, $Iy\sin(\omega\tau)$, will precess again under chemical shift Hamiltonian giving rise to the in-plane magnetization $Iy\sin(\omega\tau)\cos(\omega\tau) - Ix\sin^2(\omega\tau)$. The last pulse brings the Iy component on the z axis and selects the in-phase magnetization that has precessed to the x axis during the delay τ. The response to this sequence is therefore $\sin^2(\omega\tau)$, where ω denotes the angular frequency offset of the spins with respect to the RF frequency. This sequence provides excellent water suppression and does not introduce any linear or higher order phase errors.

The sequence presented in Fig. 1A gives a frequency profile that depends on $\sin^2(\omega\tau)$ while the one in Fig. 1B gives a frequency profile depending on $\sin^3(\omega\tau)$. The relevant evolution of the different spins under scheme 1B is described as follows where I denotes a proton spin:

$$Iz \xrightarrow{(\pi/2)_y} Ix \xrightarrow{\tau} Ix\cos(\omega\tau) + Iy\sin(\omega\tau) \xrightarrow{(\pi/2)-y}$$

$$Iz\cos(\omega\tau) + Iy\sin(\omega\tau)\cos(\omega\tau) - Ix\sin^2(\omega\tau) \xleftarrow{\tau} Iz\cos(\omega\tau) + Iy\sin(\omega\tau)$$

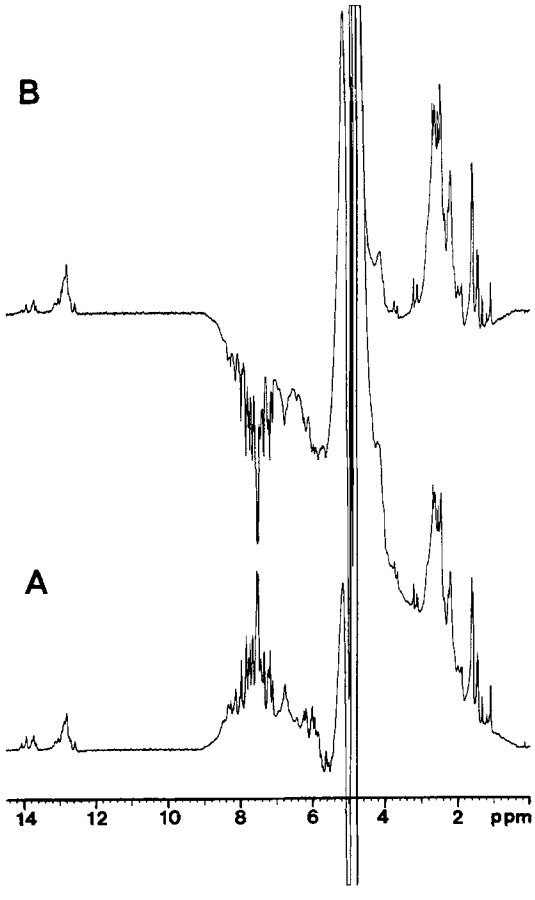

Fig. 3. One dimensional spectra of a 0.25 mM sample of a double-stranded DNA fragment solution composed of two complementary strands d(GGGGACTTTCCAGGGAGGCGTGGC), and d(GCCACGCCTCCCTGGAAAGTCCCC) measured in 90% H_2O at 25°C, pH 7, 110 mM KCl, 16 mM NaCl and 15 mM $MgCl_2$. The spectra were obtained on a Varian Unity 500 operating at 500 MHz with 256 scans. (A) $\sin^2(\omega\tau)$ spin-lock scheme; (B) $\sin^3(\omega\tau)$ spin-lock scheme.

Then a purging spin-lock is applied on the x axis. This high-power spin-lock of 2 ms duration will defocus any magnetization not aligned along the spin-lock axis and so will randomize all the magnetizations that are in the yz plane. Since the water resonance is placed at the offset frequency, its magnetization, which is in the yz plane, will be destroyed, keeping Ix $\sin^2(\omega\tau)$ untouched. This in-phase magnetization will then evolve under the chemical shift Hamiltonian. Before the last pulse is applied, the in-plane magnetization will therefore be: Ix $\sin^2(\omega\tau)\cos(\omega\tau)$ + Iy $\sin^3(\omega\tau)$. The subsequent pulse brings the Ix component on the z axis and selects the observable in-phase magnetization Iy $\sin^3(\omega\tau)$. The response of such a sequence will therefore be modulated by the function $\sin^3(\omega\tau)$.

Figure 2 displays the experimental offset dependence of the two excitations, showing the absorption character of the resonance independent of the offset and the respective $\sin^2(\omega\tau)$ and $\sin^3(\omega\tau)$ intensity profiles. The overwhelming advantage of these experiments is that they are

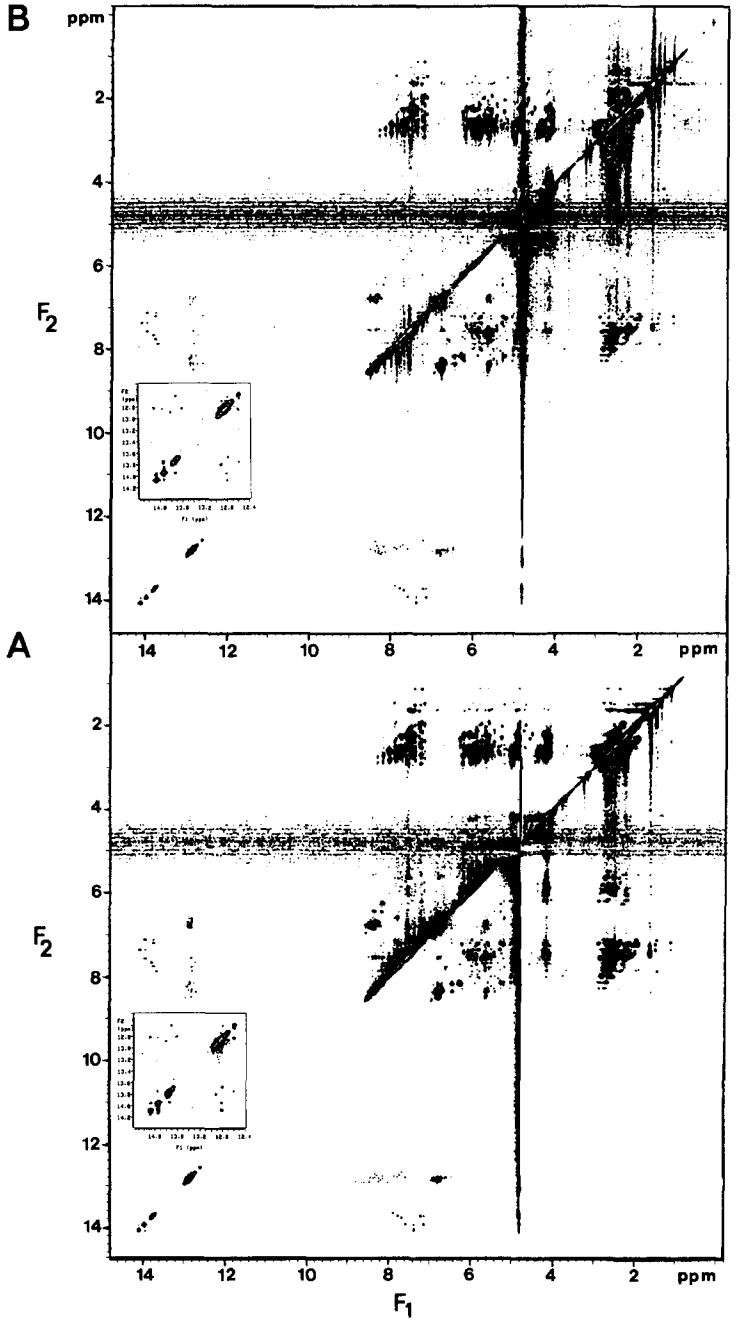

Fig. 4. NOESY spectra of the double-stranded DNA fragment recorded in the phase-sensitive mode with quadrature detection in both dimensions using a hypercomplex method with 3008 points in t_2 and 600 in t_1 for a spectral width of 10 000 Hz. For each t_1 value 96 scans were collected with a relaxation delay of 1.3 s between transients. The mixing time was 120 ms. The τ delay was 165 μs and a 2-ms spin-lock pulse was applied. Both dimensions were processed with a sine-squared 60° phase-shifted function. Finally, the shifted time-domain convolution difference procedure (Sodano and Delepierre, 1993) was applied to both spectra with M = 21, (A) $\sin^2(\omega\tau)$ spin-lock scheme 1C; (B) $\sin^3(\omega\tau)$ spin-lock scheme 1D.

extremely simple to conduct. Furthermore these excitation profiles show that frequency-dependent phase correction is not needed.

To demonstrate the solvent signal cancellation efficiency, we performed the 1D spectra shown in Fig. 3, obtained with a very dilute DNA sample (about 0.25 mM). The delay τ was set to 160 μs, which corresponds to having the minima at the water frequency and the first null at 10.8 ppm, i.e., between the amino and the imino proton region, while maxima are at 13.83 ppm in the imino proton region and at 7.8 ppm in the amino and adenine H_2 proton region. These spectra show the water suppression efficiency of the two pulse sequences and that the Fourier transformed spectra are devoid of linear phase shift.

These schemes can be easily appended to multidimensional experiments such as NOESY. The great importance of such water suppression schemes for the characterization of water molecules bound to proteins and DNA has been demonstrated recently. Figures 1C and 1D show NOESY pulse sequences that include the two water suppression schemes. These seem very suitable for DNA studies by 2D NMR. Indeed, the labile protons of DNA duplexes exhibit cross peaks in two quite narrow and well-separated spectral regions. Therefore a non-uniform excitation profile can be selected. This leads to minimal loss of information since peaks that are close to the water and which are missing in the final 2D spectrum do not contain valuable information. The first pulse brings the equilibrium magnetization in the transverse plane and their frequency is then labelled during the t_1 evolution period. The second pulse will convert in-phase magnetization on the z axis and protons will exchange information during the following NOESY mixing time. Then the reading pulse scheme will destroy water and peaks that are lying close to it. But as the water is destroyed only before acquisition, exchange cross correlation can be detected on the F_1 water resonance of the 2D spectrum. A possible pitfall of the present water suppression schemes lies in the possible transfer of magnetization that could result from spin-lock via TOCSY or ROESY pathways. Otting et al. (1991) have demonstrated that during spin-lock, the extent of such coherent or incoherent transfers is negligible.

As an example, the 2D NOE spectra of a 0.25 mM solution of a double-stranded DNA fragment composed of two non-palindromic complementary strands d(GGGGACTTTC-CAGGGAGGCGTGGC) and d(GCCACGCCTCCCTGGAAAGTCCCC) measured in 90% H_2O at 25 °C, pH 7, are shown in Fig. 4. The insets shown contain cross peaks between different imino protons and are drawn at lower contour levels.

Although the pulse schemes presented here suffer from non-uniform excitation, there is no baseline distortion and a reasonably good water suppression is achieved. These schemes are a good alternative to short-pulsed magnetic field gradients (Piotto et al., 1992) as the null can always be set in a region free of signals for most biological materials studied, i.e., proteins or DNA.

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REFERENCES

Guéron, M., Plateau, P., Kettani, A. and Decorps, M. (1992) *J. Magn. Reson.*, **96,** 541–550.
Holak, T.A., Wiltscheck, R. and Ross, A. (1992) *J. Magn. Reson.*, **97,** 632–636.
Hore, P.J. (1983) *J. Magn. Reson.*, **45,** 283–300.
Hoult, D.I. (1976) *J. Magn. Reson.*, **21,** 337–347.
Kubinec, M.G. and Wemmer, D.E. (1992) *J. Am. Chem. Soc.*, **114,** 8739–8740.
Messerle, B.A., Wider, G., Otting, G., Weber, C. and Wüthrich, K. (1989) *J. Magn. Reson.*, **85,** 608–613.
Otting, G. and Wüthrich, K. (1989) *J. Am. Chem. Soc.*, **111,** 1871–1875.
Otting, G., Liepinsh, E. and Wüthrich, K. (1991) *Science,* **254,** 974–980.
Otting, G., Liepinsh, E. and Wüthrich, K. (1992) *J. Am. Chem. Soc.*, **114,** 7093–7095.
Otting, G., Liepinsh, E., Farmer II, B.T. and Wüthrich, K. (1991) *J. Biomol. NMR,* **1,** 209–215.
Piotto, M., Saudek, V. and Sklenàr, V. (1992) *J. Biomol. NMR,* **2,** 661–665.
Piveteau, D., Delsuc, M-A., Guittet, E. and Lallemand, J.-Y. (1987) *J. Magn. Reson.*, **71,** 347–348.
Plateau, P. and Guéron, M. (1982) *J. Am. Chem. Soc.*, **104,** 7310–7311.
Plateau, P., Dumas, C. and Guéron, M. (1983) *J. Magn. Reson.*, **54,** 46–53.
Redfield, A.G., Kunz, S.D. and Ralph, E.K. (1975) *J. Magn. Reson.*, **(19),** 114–117.
Sklenàr, V., Tschudin, R. and Bax, A. (1982) *J. Magn. Reson.*, **75,** 352–357.
Sklenàr, V. and Starcuk, Z. (1982) *J. Magn. Reson.*, **50,** 495–501.
Smallcombe, S.H. (1993) *J. Am. Chem. Soc.*, in press.
Sodano, P. and Delepierre, M. (1993) *J. Magn. Reson.*,**103**, in press.
Sørensen, O.W., Eich, G.W., Levitt, M.H., Bodenhausen, G. and Ernst, R.R. (1983) *Prog. NMR Spectrosc.*, **16**, 163–192.
Tate, S., Masui, Y. and Inagaki, F. (1991) *J. Magn. Reson.*, **94,** 394–400.
Zuiderweg, E.R.P., Hallenga, K. and Olejniczak, E.T. (1986) *J. Magn. Reson.*, **70,** 336–343.