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A New Water Suppression Technique for Generating Pure-Phase Spectra with Equal Excitation over a Wide Bandwidth

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A large variety of new selective excitation methods recently has been proposed for suppressing the H_2O resonance from the NMR spectra of samples dissolved in H_2O. These methods include time-shared hard pulse sequences (*1–4*), combinations of hard and soft pulses (*5, 6*), and "two-stage" time-shared hard pulse sequences (*7, 8*). In the two-stage suppression, the suppression per individual scan is relatively low but sufficient to avoid dynamic range problems in the spectrometer receiver; after the second stage (consisting of EXORCYCLE (*9*) phase cycling of a 1–1 refocusing pulse) very high suppression can be obtained. All approaches mentioned above, as well as a large number of other schemes proposed previously, are well suited for generating conventional 1D NMR spectra. Phase distortions in the 1D NMR spectra obtained with most of these methods can be removed to a large extent by linear or higher order phase corrections, and the remaining baseline distortions generally do not prohibit accurate measurement of peak positions or intensities. However, when generating phase-sensitive 2D NMR spectra, baseline distortions remaining after the first Fourier transformation give rise to serious distortions in the final 2D spectrum, especially in the vicinity of intense resonances. Even relatively small phase distortions (*4, 5*) can cause severe baseline problems in the 2D spectrum. Only a few methods are available that yield spectra without any phase distortion. These include (a) the 90°_x–$90^\circ_{\bar{x}}$ (1–1 or jump-and-return) sequence (*1*), (b) a soft (Gaussian)-shaped 90°_x pulse followed by a non-selective $90^\circ_{\bar{x}}$ pulse (*5*), and (c) the "1–1 echo" scheme (*7*). Schemes (a) and (b) provide relatively poor suppression of the H_2O resonance due to the sharp null in the excitation profile; scheme (c) provides high suppression but has an undesirable $\sin^3$ offset dependence of the excitation profile. Here we present a new method, of the two-stage type, that offers very good water suppression and a nearly ideal excitation profile.

The pulse scheme is depicted in Fig. 1a. The sequence starts with a soft $90^\circ_{\bar{y}}$ pulse that rotates the H_2O magnetization to the x axis of the rotating frame. A subsequent nonselective 90_ϕ pulse followed by a short (about 2 ms) spin lock is used to measure the remaining z magnetization. The water suppression obtainable from a single ex-

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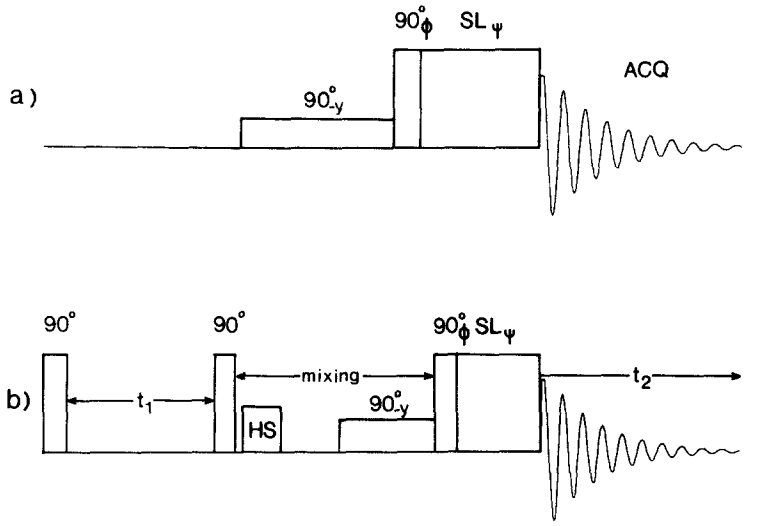

FIG. 1. (a) Excitation scheme for water suppression. The carrier is positioned at the H_2O frequency. The pulse width of the soft $90°_y$ pulse is typically 3–5 ms. The duration of the spin-lock pulse is adjusted to about 2 ms; fine adjustment by incrementing this width by amounts of up to the 180° pulse width may be necessary for optimum suppression from a single scan. The four-step phase cycle is as follows: $\phi = x, -x, x, -x$; $\psi = y, y, -y, -y$; Receiver +, −, +, −. In addition, the phase of the soft $90°_y$ pulse may be inverted after four scans without changing the receiver phase, yielding slightly better suppression. (b) Incorporation of this water suppression scheme in the NOESY experiment.

periment is limited by RF inhomogeneity and the width of the hump of the water resonance. On our 500 MHz spectrometer the H_2O intensity in a single transient is suppressed by a factor of 30–50, using a 4 ms soft pulse. As shown below, further suppression can be obtained by appropriate phase cycling of the phases ϕ and ψ.

The soft 90° pulse can be described by a Cartesian rotation matrix **R**, with elements R_{11}, R_{12}, etc. Neglecting off-resonance effects, the subsequent nonselective α_x ($\alpha \approx 90°$) pulse is described by the matrix

$$\mathbf{A} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & \cos\alpha & \sin\alpha \\ 0 & -\sin\alpha & \cos\alpha \end{bmatrix}. \qquad [1]$$

The spin lock along the y axis corresponds to a flip angle β (which will vary strongly across the sample) and is described by the rotation matrix

$$\mathbf{B} = \begin{bmatrix} \cos\beta & 0 & -\sin\beta \\ 0 & 1 & 0 \\ \sin\beta & 0 & \cos\beta \end{bmatrix}. \qquad [2]$$

Starting with magnetization along the z axis, represented by a vector (0, 0, 1), one obtains after the first scan a vector **M** given by

$$\mathbf{M} = \mathbf{B} \cdot \mathbf{A} \cdot \mathbf{R} \cdot \begin{bmatrix} 0 \\ 0 \\ 1 \end{bmatrix} = \begin{bmatrix} \cos\beta R_{13} - \sin\beta(R_{33}\cos\alpha - R_{23}\sin\alpha) \\ R_{33}\sin\alpha + R_{23}\cos\alpha \\ R_{13}\sin\beta + \cos\beta(R_{33}\cos\alpha - R_{23}\sin\alpha) \end{bmatrix}. \quad [3]$$

The x component of this vector depends on $\sin\beta$ and $\cos\beta$ which vary strongly across the sample because of RF inhomogeneity, and therefore the x component will be very small. For a value of α near 90° the y component depends mainly on the magnitude of R_{33}. Near the center of the H_2O resonance, the components R_{23} and R_{33} of the soft $90°_y$ pulse are very small and consequently very little transverse H_2O magnetization is generated. Further suppression of transverse H_2O magnetization is obtained by phase cycling the nonselective 90° pulse and the spin-lock pulse. After four scans with the phase cycling indicated in the legend to Fig. 1 the vector $\mathbf{M}^+$ is represented by

$$\mathbf{M}^+ = (0, R_{33}\sin\alpha, -\cos\beta\sin\alpha R_{23}). \quad [4]$$

Note that the amount of transverse magnetization generated now is independent of the length of the spin-lock pulse; i.e., the greatest possible water suppression does not require poor RF homogeneity or very long spin-lock pulses. The length of the selective pulse is set to near 90° on resonance and then is varied systematically to

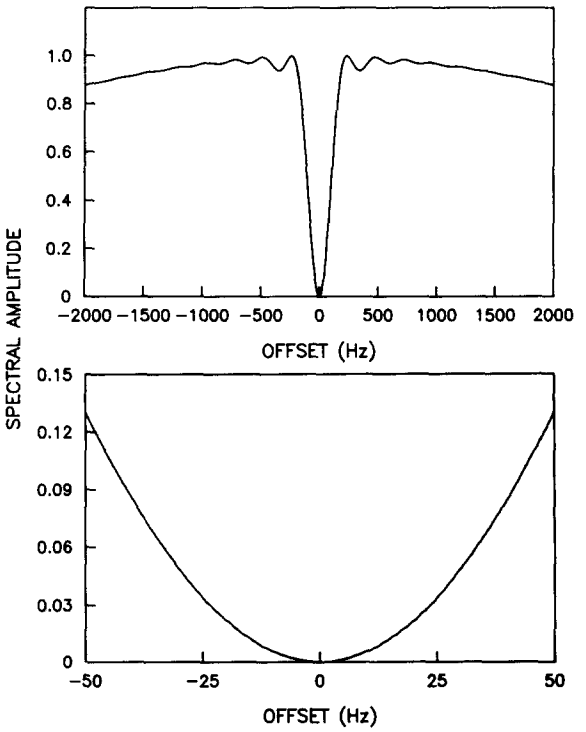

FIG. 2. The simulated intensity profile of the excitation scheme of Fig. 1a upon completion of four scans with the phase cycling of Fig. 1a, using RF field strengths of 61 and 6750 Hz for the low-power and high-power pulses, respectively.

obtain the best suppression. It follows from Eq. [4] that the phase of the resonance is independent of resonance offset and the amplitude is proportional to R_{33}. Figure 2 shows this offset dependence, also accounting for the finite RF pulse power of the nonselective 90° pulse and of the spin lock which causes the intensity to droop slightly toward the edges of the spectrum. This profile agrees well with the experimental offset dependence shown in Fig. 3. Since the suppression depends strongly on the size of the matrix element R_{33}, i.e., on the flip angle of the soft 90° pulse, one might expect the suppression to be affected by RF inhomogeneity. However, it is the R_{33} value averaged over the entire sample that determines the level of suppression. Therefore, RF inhomogeneity does not create any problems; on the contrary, it is used to maximize H_2O suppression in a single scan.

The maximum degree of H_2O suppression obtainable with the scheme of Fig. 1a depends on the lineshape of the H_2O and on the RF field strength of the soft pulse. The higher the RF power of the soft pulse, the wider the bandwidth where signals are suppressed. Typically, with a soft pulse of 60 Hz RF field strength the water signal is suppressed by a factor of about 300 on our 500 MHz spectrometer, more than sufficient to avoid any interference of the H_2O resonance with the rest of the spectrum. More importantly, only a very narrow band of resonances (±0.25 ppm) around the H_2O frequency is attenuated by more than 30%.

The water suppression scheme of Fig. 1a can be incorporated into a number of 2D NMR experiments. Here we demonstrate its application in the NOESY experiment (Fig. 1b). In this scheme, the final 90° pulse of the regular NOESY three-pulse sequence (*10*) is replaced by the composite unit of Fig. 1a. Figure 4 shows the 2D NOE spectrum of hen egg white lysozyme recorded with this method. The t_1 noise near the H_2O frequency is largely caused by phase instability of the transmitter relative to the receiver. The t_2 streak at the H_2O F_1 frequency is caused by a very small baseline distortion after the first Fourier transformation. This distortion is present in regular NOE spectra recorded in D_2O and therefore is not an artifact induced by the composite read pulse.

The water suppression scheme presented here has an ideal phase profile and a nearly

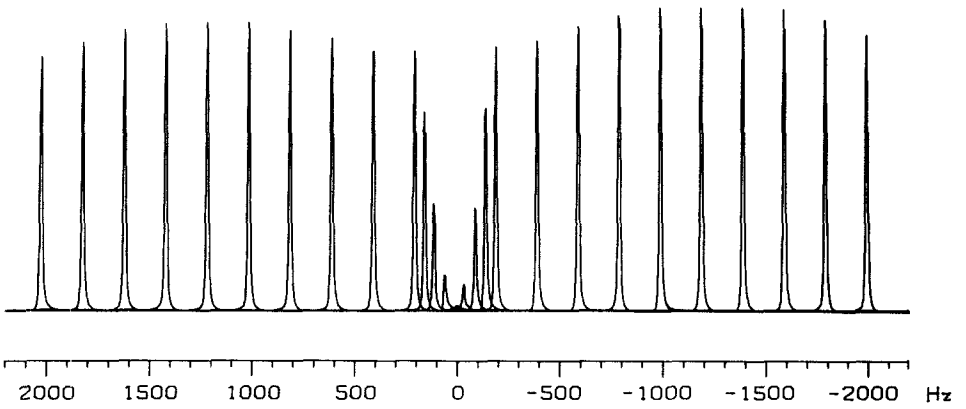

FIG. 3. Experimental intensity profile obtained with the scheme of Fig. 1a upon completion of four scans using RF field strengths of 62 and 8000 Hz for the low-power and high-power pulses, respectively.

ideal amplitude profile. Although the suppression of the H_2O resonance is not as high as can be obtained with some other schemes, the phase of the residual H_2O signal is absorptive and the residual signal therefore does not interfere with the rest of the spectrum. This new composite water suppression pulse is easily incorporated into standard 2D NMR experiments and may make the commonly used presaturation of the H_2O resonance unnecessary. Of course, the scheme proposed here is also a satu-

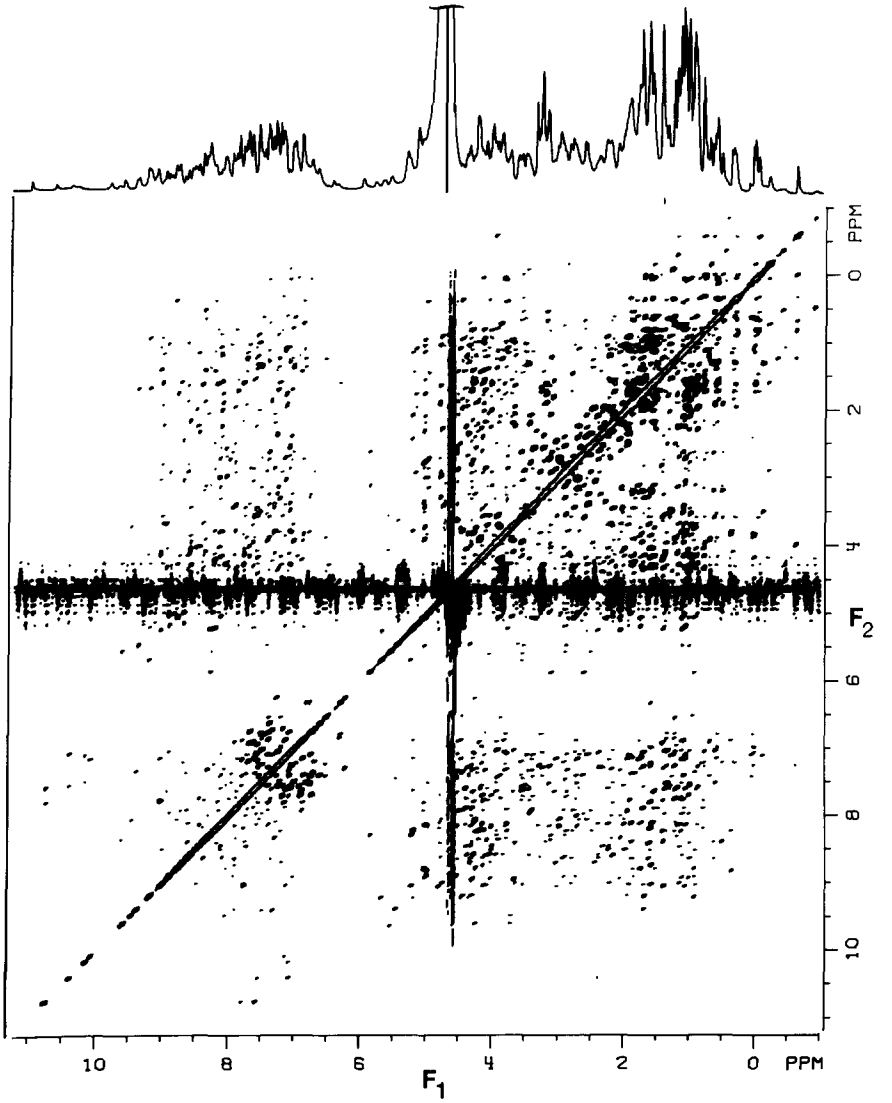

FIG. 4. A 500 MHz phase-sensitive NOESY spectrum of hen egg white lysozyme. Experimental conditions: mixing time 150 ms; concentration 5.5 m*M*; 100 m*M* NaCl; T = 42°C, pH 5.8; acquisition times of 70 and 47 ms in t_2 and t_1 dimensions, respectively; 61 and 6.75 kHz RF field strength for low-power and high-power RF pulses; spin-lock duration of 2.42 ms.

ration-type method but since the saturation occurs in a few milliseconds, the usual disadvantages of presaturation do not apply to this scheme.

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