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# A New Method for Water Suppression in the Proton NMR Spectra of Aqueous Solutions

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We suggest a new method of obtaining proton NMR spectra of dilute solutes in $H_2O$, which suppresses the intense water resonance by more than three orders of magnitude. This is achieved with a sequence of short, strong radiofrequency pulses designed to be insensitive to many of the imperfections of Fourier transform NMR spectrometers. High quality spectra can therefore be obtained without the need for fine adjustment of experimental parameters (pulse lengths, phase shifts, delays, and transmitter frequency). The performance of this sequence surpasses all existing methods of solvent suppression.

The ability to measure routinely proton NMR spectra of $H_2O$ solutions is essential for the observation of exchangeable protons that cannot be seen in $D_2O$. Much attention, for example, has focused on the NH protons of nucleic acids and proteins. The problems associated with the detection of weak solute resonances in the presence of an intense signal from the 110 *M* water protons have been comprehensively reviewed (*1–3*). Of the various techniques devised to overcome these difficulties, those employing selective excitation (*1–10*) are probably the most successful, the most widely used being Redfield's 214 composite soft pulse (*1, 6*). Other approaches (*11*) involve presaturation, partial inversion recovery, and rapid scan correlation spectroscopy. Solvent suppressions of a few hundred can only be obtained by careful variation of several parameters to minimize the amplitude of the free induction decay or the spectrum baseline curvature.

In looking for a more satisfactory selective pulse sequence, we have used the criterion that it must give a broad flat region of near zero excitation around the solvent frequency and yet appreciably excite relatively distant resonances. Thus small static field inhomogeneities and errors in the transmitter position could be tolerated. We were guided in this by the approximate Fourier transform relationship (*5–7*) between the pulse sequence in the time domain and its frequency domain "excitation spectrum" (excited transverse magnetization as a function of offset frequency $\nu$).

A function in the frequency domain possessing the desired properties is $S_n(\nu) = \sin^n(\pi\nu\tau)$ with $n$ a positive integer. All derivatives of $S_n(\nu)$ are zero at $\nu = 0$ up to and including the $(n-1)^{\text{th}}$, thus satisfying the first part of the above criterion. The Fourier transform of $S_n(\nu)$ is proportional to

$$\sum_{k=0}^{k=n} (-1)^k \binom{n}{k} \delta(t + [k - n/2]\tau), \qquad [1]$$



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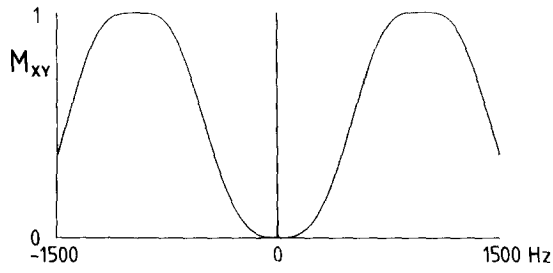


FIG. 1. Transverse magnetization ($M_{XY}$) resulting from the pulse sequence $1\bar{3}3\bar{1}$ as a function of frequency offset from the transmitter. Full scale corresponds to complete conversion of $Z$ into $XY$ magnetization.

i.e., $n + 1$ equally spaced delta functions with alternating signs, and amplitudes given by the binomial coefficients $\binom{n}{k}$. This expression suggests the family of pulse sequences which we designate $1\bar{1}$, $1\bar{2}1$, $1\bar{3}3\bar{1}$, $1\bar{4}6\bar{4}1$.... The numbers give the relative pulse lengths, the overbars denote a 180° phase shifted pulse, and equal delays ($\tau$) between the pulses are understood. The corresponding cosine functions have similar properties and give rise to the sequences 11, 121, 1331, 14641, ... (all pulses with same phase) which should give zero net excitation at $\nu\tau = \pm 1/2$. Some of the early members of these series ($1\bar{1}$, $1\bar{2}1$, and 121), derived using arguments based on the Bloch equations, have recently been proposed for solvent suppression (*9, 10*).

In practice $1\bar{3}3\bar{1}$ outperforms the other sequences up to $n = 4$: in consequence the remainder of this communication will be devoted to it. Higher sequences gave only modest improvements on $1\bar{3}3\bar{1}$.

Figure 1 shows a theoretical excitation spectrum for $1\bar{3}3\bar{1}$, calculated by exact solution of the Bloch equations for a single spin, with $\alpha = 11.25°$ (the flip angle of the "1" pulses), $\tau = 500$ μsec and a radiofrequency field $B_1$ of 5 kHz. As anticipated, there is a flat excitation null around the transmitter frequency ($M_{XY} < 10^{-3}$ in the range ±5 Hz). The basic experiment is

$$\alpha(\pm X)\text{–}\tau\text{–}3\alpha(\mp X)\text{–}\tau\text{–}3\alpha(\pm X)\text{–}\tau\text{–}\alpha(\mp X)\text{–acquisition}(\pm). \qquad [2]$$

The pulse and receiver phases are inverted on alternate scans to cancel any solvent excitation arising from slightly imperfect 180° phase shifts. The phases of all four pulses and the receiver are then incremented together in 90° steps as in the CYCLOPS (*12*) scheme. The transmitter is set at the exact resonance frequency of the solvent and $\tau$ chosen to excite the desired spectral region. No further adjustment or refinement is necessary.

The performance of $1\bar{3}3\bar{1}$ is demonstrated by the 200 MHz spectrum of 50 m*M* viomycin, a cyclic peptide antibiotic of molecular weight 685, in 90% $H_2O$ + 10% $D_2O$ (Fig. 2). It was recorded on a Varian XL-200 spectrometer with $\tau = 500$ μsec, $B_1 = \sim 5$ kHz, $\alpha = \sim 5°$, and a relaxation delay between scans of 10 sec. The residual $H_2O$ resonance at 4.77 ppm has been reduced in intensity by a factor of more than 1000. As expected from Fig. 1, the viomycin resonances close to the water are also suppressed but the exchangeable NH protons between 7.2 and 9.4 ppm are clearly visible. By increasing the spacing of the pulses, one can observe lines closer to the

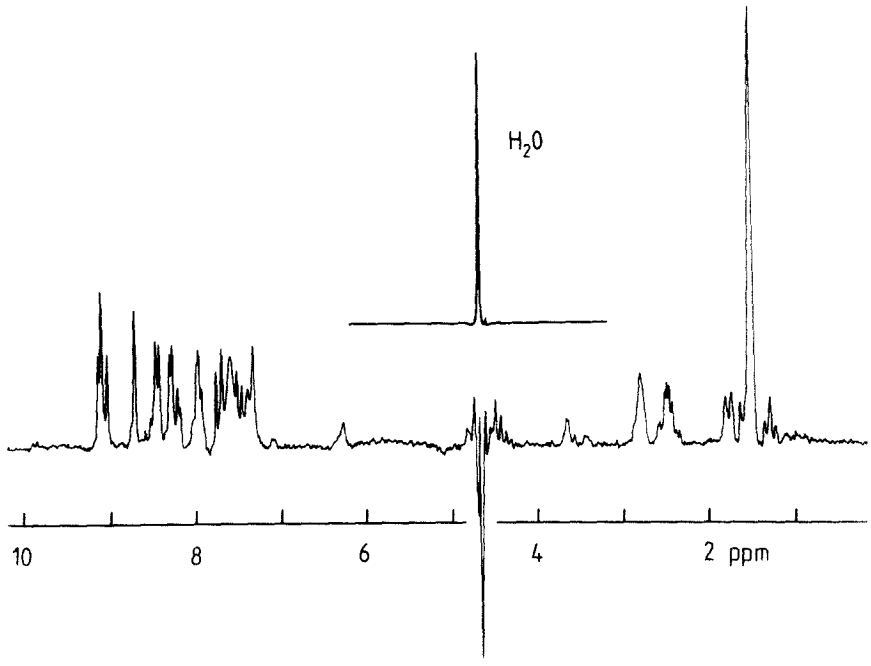


FIG. 2. The 200 MHz proton NMR spectrum of 50 m$M$ viomycin in 90% $H_2O$ + 10% $D_2O$ obtained with the $1\bar{3}3\bar{1}$ selective excitation sequence. For comparison, the $H_2O$ resonance resulting from nonselective excitation under the same conditions is shown, scaled down by a factor of 1000. Chemical shifts are relative to $H_2O$ at 4.77 ppm.

water without difficulty. Because of the relatively broad null (Fig. 1) spinning sidebands on the water resonance are also suppressed. The phase error in the spectrum resulting from $1\bar{3}3\bar{1}$ excitation is essentially linear in the offset frequency and therefore easily removed with standard first order phase correction routines.

The reason why both $1\bar{2}1$ and $1\bar{4}6\bar{4}1$ are much less successful than $1\bar{3}3\bar{1}$, although they are predicted to be superior, probably derives from their sensitivity to the effects of nonideally shaped pulses. Thus, if the relative pulse flip angles in $1\bar{3}3\bar{1}$ are not exactly 3:1, the symmetry of the sequence ensures that there is still a null, albeit less flat, at $\nu = 0$. No such self-compensation occurs for the central $\bar{2}$ and 6 pulses of $1\bar{2}1$ and $1\bar{4}6\bar{4}1$. For example, a ±5% error in the length of either of these pulses would reduce the solvent suppression at $\nu = 0$ to ~25-fold.

The sequences 11, 121, 1331, ... with zero excitation $1/2\tau$ Hz from the carrier are inferior due to their greater sensitivity to off resonance effects of the finite $B_1$ field and to the exact transmitter frequency.

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