Improved Solvent Suppression in One- and Two-Dimensional NMR Spectra by Convolution of Time-Domain Data

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Numerous methods are available for suppression of the H$_2$O resonance in the $^1$H NMR spectra of water-soluble compounds (1). On average, these methods present a trade-off between the excitation profile and the level of suppression of the water resonance: with few exceptions, methods that yield the best solvent suppression per single scan tend to have a worse excitation profile than the simple “jump-and-return” or “1-1” sequence (2) which has a relatively good, sine-bell-shaped excitation profile. For H$_2$O suppression in 2D experiments, a second and even more important criterion is the reproducibility of H$_2$O suppression from one scan to the next. Poor reproducibility leads to $t_1$ noise in the vicinity of the H$_2$O $F_2$ frequency and the tails of this unsuppressed H$_2$O resonance can extend into the spectral region of interest. Here we demonstrate that treatment of the time-domain data is an effective method for removing the residual H$_2$O signal. This process does not require any operator interaction and therefore it can be easily included in automated data processing.

Our approach utilizes a convolution difference of the time-domain data to remove the undesired solvent signal component from the free induction decay. The process is most easily applied for the case where the solvent signal is on-resonance, although, as discussed later, it can be applied independent of the position of the solvent resonance provided that complex data are acquired. Figure 1A shows the FID obtained with a 1-1 sequence for a 1.5 mM solution of the protein calmodulin (16.7 kDa) in 90% H$_2$O. The residual H$_2$O signal is responsible for the low-frequency component of the signal. To a good approximation, this low-frequency component of the FID can be calculated by averaging neighboring time-domain data points, which is equivalent to convolution with a rectangular function. The width of the rectangle corresponds to the number of time-domain data points that are averaged. This low-frequency component is then subtracted from the original signal (Fig. 1B). Better removal of the effect of high-frequency components to the calculated low-frequency signal can be obtained by convolution with an appropriately shaped (Gaussian or sine bell) function, i.e., by calculating a weighted average for each time-domain data point. Thus, the low-frequency component, $L(n)$, of the FID, $S(n)$, is calculated according to

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where $n$ is the number of the sampled data point, and $2K + 1$ is the width of the convolution window. The function, $f(k)$, defines the shape of the convolution window. In practice, we prefer using either a sine-bell-shaped \( f(k) = \cos(k\pi/(2K + 2)) \) or a Gaussian-shaped \( f(k) = \exp(-4k^2/K^2) \) window function. The low-frequency component, $L(n)$, is subtracted from the real signal, $S(n)$, prior to Fourier transformation. In practice, we typically use window functions with a width of 17–65 ($K = 8–32$) data points. The real and imaginary channels of the complex time-domain data are treated separately and independently.

The convolution procedure described above should not be used for the first $K$ and the last $K$ data points of the FID. Regular convolution of finite signals assumes periodicity of the signal (3) and in our case it would average the first with the last data points of the FID, a clearly undesirable feature. In this case, the Fourier transform of $L(n)$ would be equivalent to multiplication of the regular frequency-domain spectrum by a masking function that corresponds to the Fourier transform of $f(k)$, and apart from distorted resonance intensities, the spectrum would remain unchanged. In contrast, we calculate the first data points of $L(k)$ by linear extrapolation of its data points $L(K)$ and $L(K + M)$: $L(K - k) = L(K) + k[L(K) - L(K + M)]/M$. Similarly, the last $K$ data points are calculated by extrapolation of data points $N - K - M$ and $N - K$, where $N$ is the number of sampled data points.

Considering the well-known relation between convolution in the time domain and multiplication in the frequency domain, one may wonder whether our procedure could be performed in the frequency domain by multiplication with an appropriate window function that has a null at the solvent frequency. This is not the case. As pointed out above, the difference originates from the fact that our treatment is not a true cyclic convolution, which assumes periodicity of the time-domain signal (3), but instead, it uses linear extrapolation to calculate the data points near the ends of the time domain. An operation equivalent to our time-domain data treatment is not easily performed in the frequency domain.

The procedure is illustrated in Fig. 1. The regular free induction decay (Fig. 1A) contains a large low-frequency component, originating from the H$_2$O signal. This component is removed in Fig. 1B, after application of the modified deconvolution method described above, using the Gaussian function with $K = 8$ and extrapolation with $M = 16$. Figures 1C and 1D compare the spectra corresponding to the untreated and treated FID. As can be seen most clearly on the insets in Figs. 1C and 1D, the broad dispersive tail of the residual H$_2$O (Fig. 1C) has been completely removed in the spectrum of Fig. 1D, leaving a clean and straight baseline which does not need further frequency-domain manipulation, provided that the early data points of the FID are scaled correctly (4) or that linear prediction has been used to calculate them (5). The small “distortion” in the vicinity of the H$_2$O resonance in Fig. 1D is largely
Fig. 1. (A) The real part of the FID of a 1.5 mM sample of calmodulin in 90% H₂O, recorded with a jump-and-return sequence. (B) The corrected FID, using a Gaussian window with \( K = 8 \) and extrapolation with \( M = 16 \). (C) and (D) The Fourier transforms of (A) and (B), respectively. The insets in (C) and (D) illustrate that the baseline has been flattened by the time-domain correction procedure.

A real feature, caused by the inhomogeneous broadening of the H₂O resonance and the finite attenuation adjacent to the carrier frequency. When a Gaussian function is used as described above, the attenuation window in the frequency domain also has a Gaussian profile, with 50% attenuation at \( \pm 0.416 \text{SW}/K \) from the carrier frequency, where SW is the total spectral width.

The main application of our convolution method is found in the treatment of 2D and 3D data that have been recorded in H₂O solution. Frequency-domain baseline corrections are not always straightforward in these types of experiments since it may be difficult to define signal-free regions, necessary for defining the baseline. It also should be noted that the distortion introduced by the dispersive tails of H₂O causes a curved baseline that cannot be removed completely by linear baseline correction.
Our approach avoids these baseline distortions altogether and does not require any operator interaction for defining baseline positions. As an example, Fig. 2 compares the NOESY spectra of calmodulin obtained with a NOESY experiment that did not utilize any presaturation of the H2O but employed a 1-1 read pulse at the end of the mixing period (6). Frequency-domain baseline correction has been applied to the amide region of the regular spectrum (Fig. 2A); time-domain deconvolution but no frequency-domain manipulation has been used for the processing of spectrum B.
Of course, the method described here does not make H₂O suppression pulse schemes obsolete. To avoid overload in the RF receiver circuitry or in the ADC converter, suppression of the H₂O signal is needed before it reaches the receiver system. However, the dynamic range of modern NMR spectrometers is such that a 30-fold suppression of the H₂O resonance is often sufficient for obtaining ¹H NMR spectra with near optimal sensitivity. Such a modest level of suppression is easily obtained with very simple suppression schemes such as the 1-1 sequence. Further suppression is then achieved by the convolution method described above.

In the discussion above, we have assumed that the solvent signal is on-resonance. If the solvent signal is at the edge of the spectrum (at the Nyquist frequency), the same convolution procedure can be applied provided that the sign of even-numbered data points is changed prior to the convolution difference operation and changed back once completed. For complex data (with simultaneous acquisition of the real and imaginary channel) the solvent resonance frequency can be changed to any position, including the desired on-resonance position, by applying a linearly time-depen-
dent phase correction to the time-domain data (7, 8). After convolution difference, the frequencies can be shifted back to their original values by applying the opposite phase correction.

The present method has some similarity with the data-shift-accumulation (DSA) technique (9) which also treats time-domain data, with the main purpose of avoiding problems associated with limited computer word length. This DSA method also results in excellent suppression of the H₂O resonance, including its dispersive tails, and it gives a sinusoidal modulation of the resonance intensities and of the noise across the frequency-domain spectrum (9). Although correct intensities can be restored easily in the frequency domain, the DSA technique introduces a linearly frequency-dependent phase error which results in baseline problems in the 2D spectrum unless a special linear prediction algorithm is used (5).

In our laboratory, the convolution method described above has proven to be extremely valuable for the processing of 2D and 3D spectra, recorded in H₂O solution. Especially if only few scans are recorded per t₁ value (or per t₁/t₂ value for 3D) or when the water suppression level is not very reproducible for successive increments, the method results in a substantial improvement in spectral quality. The choice of the window function used is somewhat arbitrary: the Gaussian function has the advantage that the attenuation window in the frequency domain also has a Gaussian shape; the sine-bell-shaped window permits the use of smaller K values for the same half-width of the attenuation window, resulting in less error during the linear extrapolation of the first K data points. We have not found significant differences in spectral quality for data sets treated with a Gaussian or with a sine-bell window.

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