Two-Dimensional NMR Spectroscopy

Elimination of Zero-Quantum Interference in Two-Dimensional NMR Spectra**

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High-resolution NMR experiments often contain periods during which the magnetization is placed along the z-axis. For example, the magnetization must be along the z-axis during the mixing time in a NOESY experiment so that cross-relaxation can take place. Either phase cycling or field gradient pulses are used to ensure that only the wanted z-magnetization ends up contributing to the spectrum. However, neither of these methods can distinguish between z-magnetization and homonuclear zero-quantum coherence, which is invariably present. The zero-quantum coherence gives rise to anti-phase dispersive components in the spectra, thereby reducing the effective resolution, introducing misleading correlations, and obscuring wanted features. Over the years a number of methods have been devised to suppress these zero-quantum contributions,[1–5] but it is fair to say that none of these methods have proved entirely satisfactory. Herein we present a new method for suppressing zero-quantum coherence; the method is widely applicable, does not extend the duration of the experiment significantly, and can be implemented easily on any modern spectrometer.

Our new method of zero-quantum suppression involves applying simultaneously a swept-frequency 180° pulse and a gradient. Figure 1a shows how this combination can be introduced into the NOESY pulse sequence. The way in which this swept-pulse/gradient pair works can be envisaged in the following way. The application of the gradient (along the z-axis) results in the Larmor frequency becoming a function of position in the NMR tube. The swept-frequency 180° pulse will therefore flip the spins at different positions in the sample at different times. Thus, the top of the sample might experience the 180° pulse at the start of the sweep, the middle of the sample at time $\tau_f/2$, and the bottom at time τ_f, where τ_f is the duration of the sweep. In general, the 180° pulse occurs at time $\alpha\tau_f$, where α is 0 at the top of the sample and 1 at the bottom.

The 180° pulse forms a spin echo which refocuses the evolution of the zero-quantum coherence over a time $2\alpha\tau_f$; however, for the remainder of the time, $(1–2\alpha)\tau_f$, the zero-quantum continues to evolve. The result is that in different parts of the sample the zero-quantum has evolved for different times, and so has acquired a different phase. If the range of these phases across the sample is large enough, the net result will be cancelation of the zero-quantum coherence.

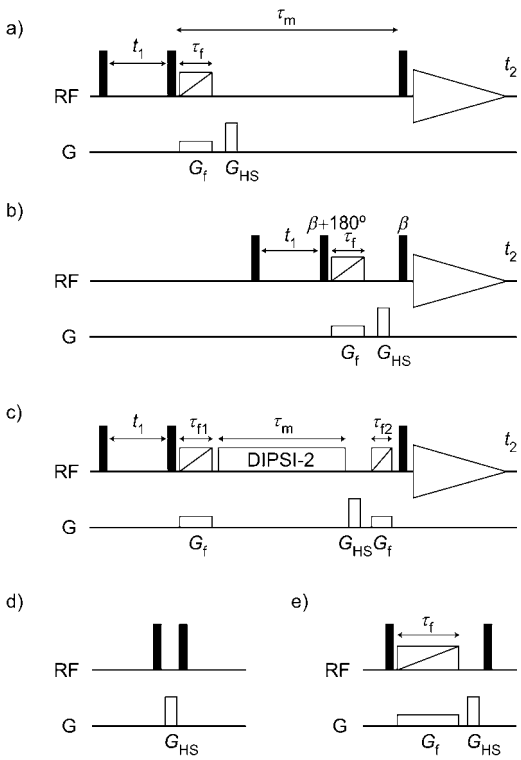

Figure 1. Pulse sequence timing diagrams for the a) NOESY, b) z-COSY, and c) TOCSY experiments incorporating swept-pulse/gradient pairs for the dephasing of zero-quantum coherence. Radiofrequency pulses are shown on the line marked RF. Unless otherwise specified, filled-in rectangles represent pulses of flip angle 90° and phase x, and swept-frequency 180° pulses are indicated by an open box containing a diagonal line. Gradient pulses are shown on the line marked G. G_{HS} denotes a homospoil gradient pulse. The z-filter pulse sequence element shown in (d) results in the selection of in-phase magnetization along the y-axis. Zero-quantum suppression can be introduced into this element as shown in (e); this modified z-filter appears in sequences (a)–(c). We note that the swept-frequency pulse may be considered as part of the mixing time in the NOESY pulse sequence (a).

A simple calculation (see Supporting Information) shows that the degree of attenuation A of the zero-quantum depends on both its frequency, Ω_{ZQ} (in rad s^{-1}), and the length of the swept-pulse/gradient pair, τ_f, [Eq. (1)]:

$$A = \frac{\sin \Omega_{ZQ}\,\tau_f}{\Omega_{ZQ}\,\tau_f} \qquad (1)$$

Ω_{ZQ} is simply $(\Omega_1 - \Omega_2)$, where Ω_1 and Ω_2 are the frequencies of the two spins involved. If the oscillations produced by the sine term are ignored, the zero-quantum is attenuated by a factor $1/\Omega_{ZQ}\tau_f$. For example, for a zero-quantum frequency of 500 Hz (namely, with two spins separated by 1 ppm at 500 MHz) and a τ_f value of 30 ms, the attenuation factor will be 0.01, that is, the zero-quantum coherence is reduced to 1% of its original size.

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The key feature of our new method is that suppression of zero-quantum coherence is achieved in a single scan; no repetition of the experiment is necessary. This represents a very significant improvement over the usual method of zero-quantum suppression, which is to repeat the experiment for a range of mixing times of the order of $2\pi/\Omega_{ZQ}$.[1] Not only does this greatly extend the minimum required experiment time, but it is also difficult to choose a small set of mixing-time values which give adequate suppression over a range of zero-quantum frequencies. The alternative approach of varying the mixing time randomly from one t_1 increment to the next merely transforms the unwanted signals into t_1 noise.[1]

In a previous study we showed how spin-locking in the presence of an inhomogeneous B_0 or B_1 field can also result in the dephasing of zero-quantum coherence.[4] However, the technique proposed herein is far superior to our earlier work in that the new approach is simpler to implement, leads to faster dephasing of the zero-quantum coherence, and does not suffer from additional complications, such as unwanted TOCSY-type transfer.

Figure 2 compares part of the NOESY spectrum of strychnine recorded with and without the new zero-quantum suppression method; a short mixing time τ_m of 400 ms has been used, as it is under these conditions that the anti-phase contributions are most troublesome. Figure 2a shows a spectrum recorded without zero-quantum suppression; the significant phase distortions on both the diagonal- and cross-peak multiplets arise from the zero-quantum coherence present during the mixing time. Figure 2a′ shows a spectrum recorded using the new zero-quantum suppression method: the anti-phase dispersive contributions are removed, which leaves all of the peaks in pure absorption—the improvement is dramatic (the remaining intensity distortions result from a combination of strong coupling and flip-angle effects). Clearly, one-dimensional NOE experiments, such as the double-pulsed field gradient spin echo (DPFGSE) NOE, which also require zero-quantum suppression, will benefit from the same approach.

There are other experiments in which the new zero-quantum suppression method can be used to good effect; here we consider just two: z-COSY and TOCSY. The z-COSY experiment is similar to NOESY except that the two pulses bracketing the mixing period have small flip angles, typically 20°.[6] In the resulting spectrum both the cross- and diagonal-peak multiplets have absorption-mode line shapes and, most importantly, the multiplets are "reduced", thus making it possible to measure both the size and relative sign of passive couplings.

Effective suppression of zero-quantum coherences that are present between the two small flip angle pulses is crucial to the success of the z-COSY experiment; the pulse sequence in Figure 1b shows how this can be achieved by using the new method. Figure 2b shows part of the z-COSY spectrum of strychnine recorded without zero-quantum suppression; significant dispersive contributions obscure the fine structure of the multiplets. Zero-quantum suppression gives the dramatic improvement shown in Figure 2b′: the line shapes are absorptive and the reduced multiplets can now clearly be seen.

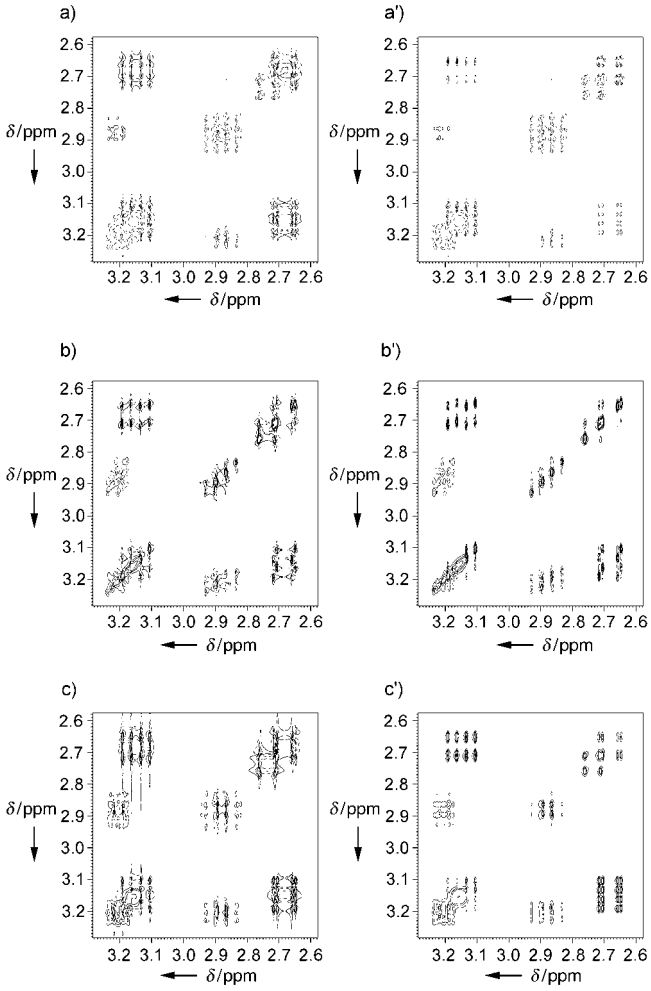

Figure 2. Comparison of spectra recorded without zero-quantum suppression (left) and with (right) the new swept-pulse/gradient method for the suppression of zero-quantum coherence; a, a′): NOESY spectra (mixing time 400 ms), b, b′): z-COSY spectra, and c, c′): TOCSY spectra (mixing time 23 ms). The same region of the spectrum of strychnine is shown in each case. The spectra on the left all show dispersive anti-phase contributions arising from zero-quantum coherence present during the mixing time. In the spectra shown on the right, these contributions have been removed by the new dephasing method; as a result, the spectra show pure absorption line shapes and are thus much easier to interpret. Positive contours are indicated by full lines, negative contours by dashed lines; the F_1-axis is vertical.

Finally, we consider TOCSY (total correlation spectroscopy) experiments.[7] Again, the pulse sequence is similar to that used in NOESY experiments except that an isotropic mixing sequence (such as DIPSI-2) is applied during the mixing time. Such a sequence results in the interchange of z-magnetization between coupled spins via a state of zero-quantum coherence. To obtain spectra with pure phase, it is necessary to dephase zero-quantum coherence present both before and after the period of isotropic mixing; a suitable pulse sequence is shown in Figure 1c. Note that to avoid the zero-quantum coherence dephased by the first swept-pulse/gradient pair being rephased in the second, different durations (τ_{f1} and τ_{f2}) must be used.

Figure 2c shows part of the TOCSY spectrum of strychnine recorded without any suppression of the zero-quantum contributions; the presence of anti-phase dispersive contributions is clear, both for cross- and diagonal-peak multiplets. In contrast, the spectrum shown in Figure 2c′, recorded using the new zero-quantum suppression method, has multiplets that are both in-phase and with absorption-mode line shapes.

In fact, all of these experiments can be thought of as utilizing a z-filter, which is the pulse sequence element shown in Figure 1d; it is used to select one in-phase component of transverse magnetization by rotating it, temporarily, on to the z-axis. Figure 1e shows how the z-filter element can be modified to include our new method for zero-quantum suppression.

We are confident that the method presented here is also applicable to NMR spectroscopic analysis of biological macromolecules. Much shorter NOESY mixing times are typically used for such molecules, and the duration of the swept-frequency pulse will limit the shortest mixing-time available. The sweep will also need to be kept short in other z-filtered experiments to minimize relaxation losses. However, while a somewhat generous τ_f value of 50 ms was mainly used in this work, significantly shorter sweeps are likely to be feasible in biological applications. This is because such experiments are routinely performed at higher spectrometer frequencies than the 300 MHz used here; since the value of Ω_{ZQ} increases in proportion to field strength, the τ_f value can be reduced by the same factor. Furthermore, as the method does not use gradients for defocusing and refocusing, it is no more sensitive to the effects of diffusion and convection than the conventional experiments.

In conclusion, we have introduced a new method which, in a single scan, results in excellent suppression of zero-quantum coherence, thus leading to spectra with excellent phase properties. Furthermore, it is simple to implement on any modern spectrometer and we believe that its adoption will see an improvement in many NMR experiments. The key idea of generating spatially dependent evolution clearly has other applications in high-resolution NMR spectroscopy.[8,9]

Experimental Section

All experiments were performed at 300 MHz on a Bruker DRX300 spectrometer using a sample containing strychnine (11 mg) dissolved in $CDCl_3$ (1 mL). All two-dimensional spectra were recorded with two scans per increment and a simple two-step phase cycle in which the phase of the first pulse and receiver were simultaneously changed by 180°; coherence transfer pathway selection was completed using a homospoil gradient pulse of strength 50% of the maximum (60 G cm^{-1}) with a duration of 6–8 ms; frequency discrimination was achieved in the t_1/F_1 dimension using TPPI. The spectral width in each dimension was 2216 Hz, the acquisition time in t_2 was 1.8 s, and 1024 increments of t_1 were recorded to a maximum value of 0.23 s. Gaussian multiplication was used in processing the t_1/F_1 dimension to reduce truncation artifacts.

The swept-frequency pulses were adiabatic 180° CHIRP pulses;[10] the frequency was swept through 20 kHz in $\tau_f = 50$ ms (except for the TOCSY pulse sequence, where the frequency was swept through 20 kHz in $\tau_{f1} = 50$ ms for the first sweep and in $\tau_{f2} = 30$ ms for the second sweep); the strength of the radiofrequency field was constant at 1 kHz, except during the first and final tenths of the pulse, when the field was smoothed to zero according to a sine function. The gradient strength G_f was 4% of the maximum. Recommendations for the determination of the parameters for the swept-pulse and gradient are given in the Supporting Information.

The comparison experiments without zero-quantum suppression were run as described, but with the G_f value and the radiofrequency field strength for the swept-frequency pulses set to zero. In the conventional z-COSY experiment two pulses of flip angle β were used. However, in the version with zero-quantum suppression the first of these was increased to $\beta + 180°$ to compensate for the 180° sweep, which would otherwise alter the multiplet structures. A value of $\beta = 20°$ was used in this work.

To reduce line-shape distortions resulting from eddy currents, it may be beneficial to include a short delay between the last gradient pulse and the last radiofrequency pulse. Delays of 43.5 ms for the z-COSY experiment and 20 ms for the TOCSY experiment were used, although these values will depend on the performance of the spectrometer used.

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