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Removal of zero-quantum peaks from 1D selective TOCSY and NOESY spectra

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Abstract

Selective 1D TOCSY and NOESY experiments are widely used for structure determination. However, they often give distorted peak shapes owing to coherence transfer though zero-quantum coherence (ZQ) which cannot be suppressed by conventional phase cycling or pulse-field gradients. This paper demonstrates that ZQ contributions can be removed from selective 1D spectra by introducing a ZQ evolution time, as previously demonstrated for 2D NOESY spectra by Wang et al. [A three-dimensional method for the separation of zero-quantum coherence and NOE in NOESY spectra, J. Magn. Reson. A 102 (1993) 116–121]. This approach is simple to implement and robust, and is not demanding of spectrometer hardware. Using a new approach to phase cycling described here, spectra can be acquired in a similar time to spectra without a ZQ evolution time.

Keywords: Zero-quantum coherence; Artifact suppression; Z-filter; DPFGSE; Phase cycling

1. Introduction

Multiple-pulse NMR experiments are an essential part of chemical analysis. In almost all NMR experiments, magnetization can be transferred through a range of pathways so selecting the pathway of interest is essential if the experiment is to give the information that was intended. There are two commonly recognised methods of selecting coherence transfer pathways. The first is phase cycling, where multiple FIDs are recorded with systematic changes in the phases of radiofrequency (RF) pulses and the receiver [2]. Phases are chosen so that signals from the pathway of interest add constructively while undesired pathways cancel out. The second method is pulsed-field gradients [3]. These use spatial encoding of phase information to select for transfer between coherences within an individual FID. A less commonly recognised method of selecting for a particular coherence transfer pathway is the use of evolution dimensions. In an evolution time, the coherence of interest is separated from any coherences which do not evolve in chemical shift during that evolution time; the undesired coherences have a zero frequency so will not overlap with signals from the coherence of interest. Removal of signals out of the spectrum in this way is an important contributor to the quality of HSQC, HMQC, and INADEQUATE spectra.

Z-filters pose a key problem for both phase cycling and PFG coherence selection. This is because longitudinal (z) magnetization and ZQ both have coherence order zero, so behave identically. The presence of ZQ leads to distorted peak shapes in experiments which include a z-filter, such as NOESY and TOCSY. For these reasons, a range of methods of suppressing ZQ have been proposed. These include the combination of adiabatic pulses and PFGs [4,5] spin-locks and PFGs [6], and off-resonance spin-locks alone [6]. Although these methods are elegant and can provide suppression within a single FID, they have disadvantages. They are complex to implement, require extra calibrations and are demanding on spectrometer hardware because they use gradients and pulses lasting tens of milliseconds.

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In this paper, I highlight the advantages of an alternative method of removing ZQ from NOESY and TOCSY spectra. It is based on methods of suppressing ZQ developed in the 1980's, which separate ZQ from longitudinal magnetization by chemical shift evolution. During a z-filter, longitudinal magnetization does not undergo chemical shift evolution while ZQ does. Wang et al. proposed to exploit this by introducing a ZQ evolution delay into the z-filter. A 180° pulse is placed in the middle of the z-filter and is then moved through it to give an evolution time which can be Fourier-transformed. Longitudinal magnetization will not evolve so will be at zero frequency, while ZQ will evolve during this delay, so will be displaced away from zero frequency. This approach is the exact opposite to the displacement of unwanted signals to the spectral edge in HSQC, HMQC, and INADEQUATE spectra mentioned above.

The original demonstration of this approach was in a 2D NOE spectrum of a biomolecule. Because the ZQ evolution dimension was in addition to the indirect proton dimension, there was a 12-fold increase in experiment length and data set size. This is probably why it has never entered routine use. However, it is ideally suited to selective 1D experiments because they can be acquired rapidly. This makes increases in experiment length acceptable.

One possible implementation of a ZQ evolution dimension is shown by the selective TOCSY sequence in Fig. 1. Selective excitation is achieved using Excitation Sculpting [7] and this is followed by TOCSY mixing and the z-filter. A 180° pulse is placed in the middle of the z-filter delay and moved through it to give a constant-time evolution delay. The z-filter is followed by an excitation pulse and an optional Hahn echo to improve baseline.

Previous work shows that as few as 12 ZQ increments effectively remove ZQ peaks from spectra [1]. This leads to a minimum 12-fold increase in experiment time if the complete phase-cycle of the 1D experiment is repeated for each increment. However, this can be greatly reduced by recording only one scan per ZQ increment, and phase cycling between the increments. For example, applying this to the pulse sequence in Fig. 1, the first ZQ increment is recorded with all pulse phases x and the receiver phase x, the second increment is then acquired with $\Phi_1 = -y$ and the receiver $-x$, the third with $\Phi_1 = -x$ and the receiver x, and the fourth increment with $\Phi_1 = -y$ and the receiver $-x$. Other pulses are then cycled in subsequent increments.

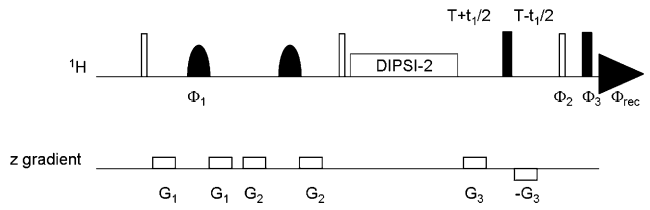

Fig. 1. Pulse-sequence for a selective 1D TOCSY experiment incorporating a ZQ evolution time. Unfilled bars represent 90° pulses, filled bars represent 180° pulses, and filled shapes are shaped 180° pulses. The first four pulses are a DPFGSE sequence which is followed by TOCSY mixing. The z-filter (duration $2T = 16$ ms) contains the constant-time ZQ dimension (t_1). It is followed by a Hahn echo, to give a flat baseline and zero linear phase correction in the direct dimension [13]. The phase cycling is Φ_1 $(x,y,-x,-y)$, Φ_2 $(8x,8y)$, Φ_3 $(4x,4-x,4y,4-y)$, and Φ_{rec} $(x,-x,x,-x,x,-x,x,-x,y,-y,y,-y,y,-y,y,-y)$. Z-gradient amplitudes and durations were G_1 15.2 G/cm for 0.5 ms, G_2 6.5 G/cm for 0.5 ms, and G_3 5.4 G/cm for 1.0 ms.

Changing the pulse phases and receiver phase in this way ensures that magnetization from the desired coherence transfer pathway experiences no net phase change between ZQ increments. Therefore, it remains at zero frequency. In contrast, unwanted coherence transfers undergo phase changes so are displaced away from zero frequency and appear as peaks elsewhere in the ZQ dimension. This approach makes it possible to acquire a single scan for each ZQ increment. The 2D ZQ-removed experiment shown here can be acquired in 16 scans, which takes approximately 45 s in total. The 1D experiment without ZQ removal requires 4 scans for acceptable spectra, so takes a minimum of approximately 15 s. This small increase in experiment time is barely of significance for most applications. If sensitivity is limited by sample concentration and it is necessary to acquire more than 16 scans for the desired sensitivity, then the two approaches will take exactly the same time.

2. Results

The effectiveness of this approach is demonstrated by 1D selective TOCSY spectra acquired on a sample of menthol. As shown by Fig. 2A, selective excitation of the isopropyl methine proton followed by TOCSY mixing generates significant amounts of ZQ, leading to distorted peak shapes. Fig. 2B shows that introducing a ZQ evolution time eliminates the ZQ contribution from the TOCSY spectrum and dramatically improves line shape. The spectrum in Fig. 2B was acquired using 4 scans per t_1 increment for 32 increments. As described in Section 1, experiment time can be minimised by phase-cycling pulses simultaneously with incrementation of t_1. Fig. 2C shows that this gives spectra of similar quality to that obtained by completing a 4-step phase cycle for each t_1 increment. Fig. 2D demonstrates that reducing the number of t_1 increments from 32 to 16 does not compromise the effectiveness of ZQ removal. A 20-fold vertical expansion of the spectra (Fig. 3) reveals that using only 1 scan per t_1 increment does cause a small phase distortion, while reducing the number of increments introduces some minor baseline distortions. These do not hinder interpretation of the data in any way.

Fig. 4 shows the 2D spectrum used to produce Fig. 2B. ZQ cross-peaks are clearly visible in the ZQ dimension, with the TOCSY spectrum at zero frequency.

For the method described in this paper to remove a ZQ peak, it has to be resolved in the ZQ dimension from the spectrum at zero frequency. In the example shown in Fig. 2, the ZQ peaks are well resolved from zero frequency,

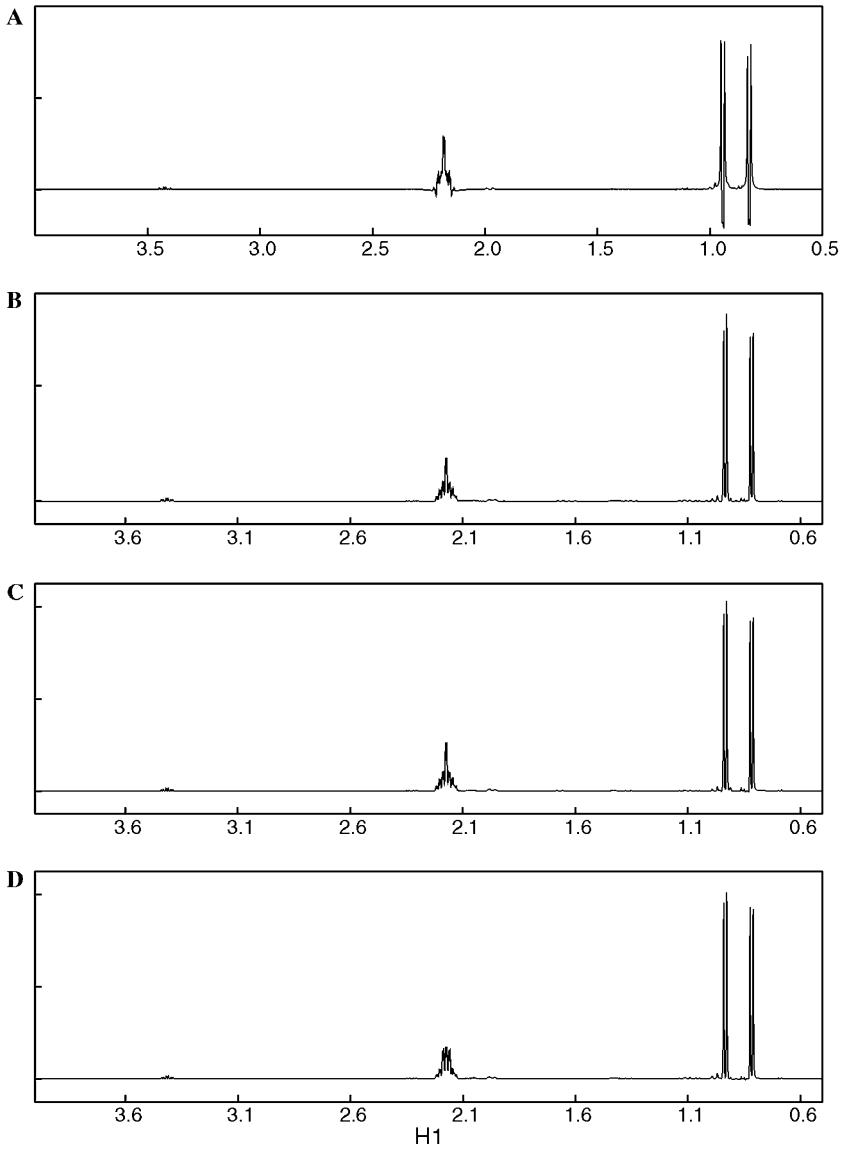

Fig. 2. Selective TOCSY spectra of Menthol dissolved in deuterochloroform. The isopropyl methine proton was selected using a Gaussian 180° pulse. (A) 1D experiment with 32 scans (t_1 not incremented); (B) 1D trace at zero frequency from a 2D experiment with 32 increments in the t_1 dimension and 4 scans per increment; (C) 1D trace at zero frequency from a 2D experiment with 32 increments in the t_1 dimension and 1 scan per increment with phase cycling between increments; (D) 1D trace at zero frequency from a 2D experiment with 16 increments in the t_1 dimension and 1 scan per increment with phase cycling between increments.

so the range of ZQ frequencies that can be removed was investigated by simulation using the acquisition and processing parameters of the spectrum shown in Fig. 2B. The simulation showed that a ZQ peak at 90 Hz ($1.5 \times$ digital resolution) can be resolved from a peak at 0 Hz of the same intensity; less than 9% of the signal of the ZQ peak is observed at 0 Hz. For a ZQ peak at 150 Hz ($2.5 \times$ digital resolution), the contribution is less than 3%. This suggests that suppression is highly effective in the region 150–1850 Hz and is acceptable in the region 90–1910 Hz.

3. Discussion

The quality of spectra in Fig. 2 demonstrates how well ZQ contributions are removed from selective spectra by introducing a ZQ evolution time. Although all spectra shown are selective TOCSY spectra, the approach can be incorporated into selective NOESY spectra, and any other experiment which requires a z-filter. Compared with other approaches for suppressing ZQ, it is simple to implement, and requires no special calibrations.

A ZQ evolution time will not remove artefacts caused by strong coupling (ZQ frequencies approaching zero), but this applies to all the other methods of ZQ suppression. The range of ZQ frequencies removed by a ZQ evolution time depends on the digital resolution of the ZQ dimension and the apodization function applied during processing. Simulations based on the experimental conditions used here suggest that the ZQ evolution time results in 90% suppression of a peak with a ZQ frequency of $1.5 \times$ digital

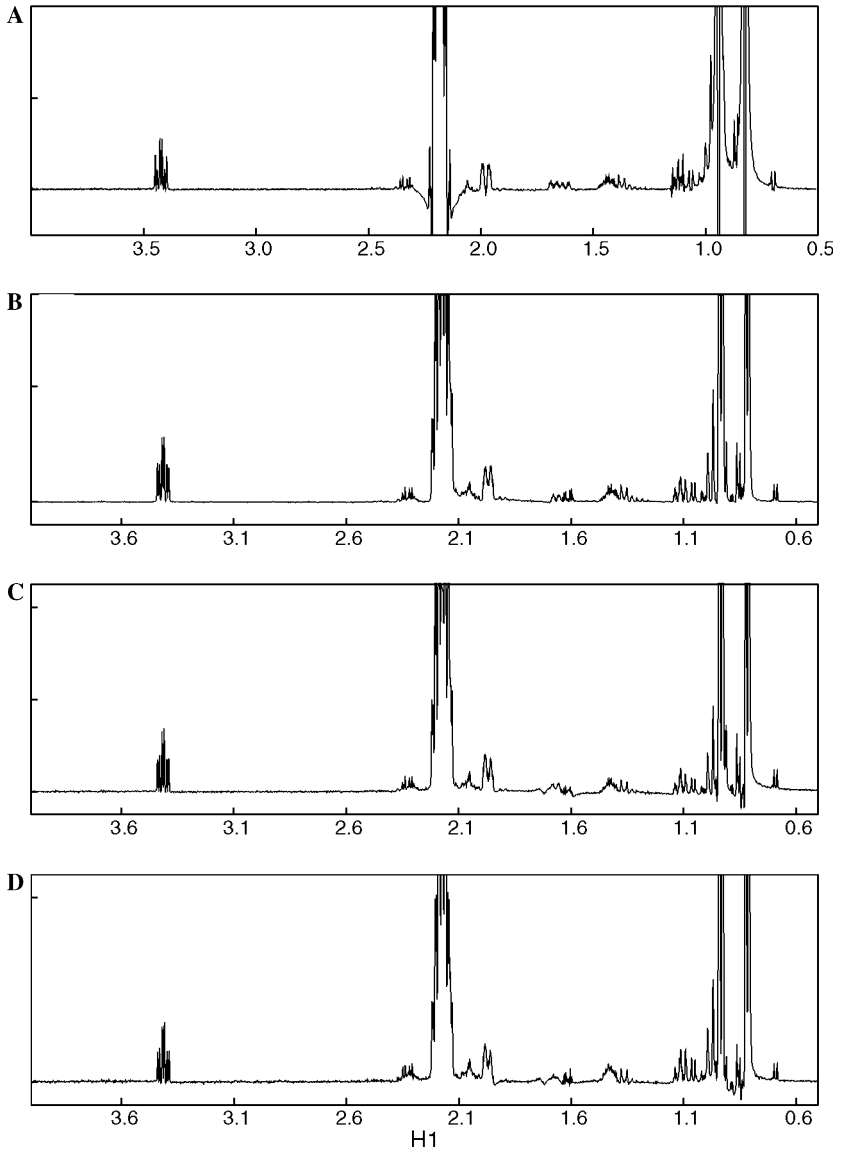

Fig. 3. Twentyfold expansion of the spectra shown in Fig. 2. The weak peaks have intensities less than 1% of the peaks on interest. They are present in all spectra and appear to arise from long range TOCSY transfer and imperfect selection by the shaped 180° pulses.

resolution. This compares well with the suppression achieved by other methods.

There is the possibility that an undesired ZQ could have a frequency close to a multiple of the spectral width in the ZQ dimension. This will result in overlap with the spectrum at zero frequency due to folding. If this is observed, the spectrum would have to be repeated with a different spectral width, but it is worth noting that the range of possible ZQ frequencies for a spin system can be predicted in advance based on the chemical shifts of protons which may be directly coupled.

The main criticism of the approach is that it requires multiple increments. This paper demonstrates that this can be minimised by phase-cycling pulses between rather than within increments. Fig. 2 shows that high-quality spectra can be obtained using only 16 scans. This is acceptable for almost all applications.

A second possible criticism is that the evolution time lengthens the z-filter, leading to losses through T_1 and magnetization transfer through the NOE. With small organic molecules, these effects are likely to be trivial. Also, these problems will be more significant with the alternative approaches to ZQ suppression, which either require a longer z-filter or a spin-lock leading to $T_{1\rho}$ relaxation and ROE transfer.

A final possible criticism of the approach is that it leads to a reduction in signal-to-noise owing to the finite line-width in the ZQ evolution time. This has never been an issue within our laboratory; as shown by Fig. 2, the removal of ZQ components more than compensates for the decrease in sensitivity due to line-width. If it were an issue, it could be eliminated using alternative transformation methods such as Linear Prediction [8], Covariance NMR [9] or Principal Component Analysis.

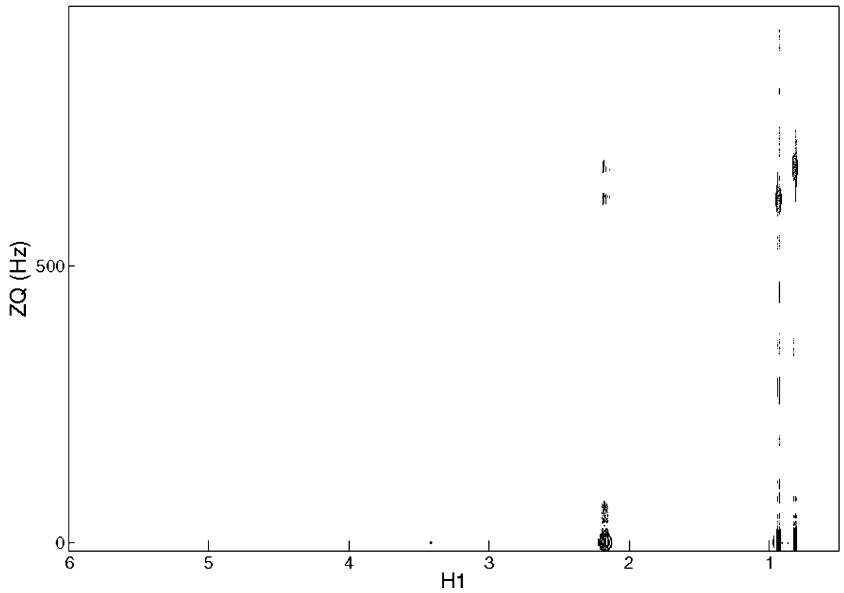

Fig. 4. The 2D spectrum resulting from a 2D experiment with 32 increments in the t_1 dimension and 4 scans per increment. The peaks resulting from ZQ are displaced away from zero frequency.

The concept of an evolution time with phase cycling for separating out unwanted magnetization can be applied more widely; it can be used in any situation where the magnetization of interest is not evolving with chemical shift, either because it is refocused or because it is longitudinal. I am currently investigating other experiments to see whether this approach does produce improved spectra.

4. Conclusions

This paper demonstrates a simple approach to remove ZQ contributions from selective 1D experiments. It requires no special hardware, no extra calibrations and can be implemented within any z-filter delay with little decrease in sensitivity.

5. Experimental

All spectra were acquired at 298 K on a Varian Unity Inova NMR spectrometer operating at 500.06 MHz fitted with a 5 mm H{CN} probe. The selective 11 ms 180° pulse had a Gaussian shape (truncated at 1% maximum intensity) and was applied with an RF power of 0.108 kHz. The 46 ms TOCSY mixing period used a DIPSI-2 sequence and an RF power of 4.34 kHz. In the acquisition dimension, 8192 complex points were acquired with a spectral width of 11 kHz. The ZQ evolution time had a spectral width of 2 kHz and either 16 or 32 real increments were acquired.

Spectra were transformed using the program NMRPipe [10] and analysed using the program NMRView [11]. The FID was apodized with a 90° shifted sine-bell function and zero-filled once before Fourier transformation. The indirect dimension was extended doubled in size by mirror-image linear prediction [12], apodized with a 90° shifted sine-bell function and zero-filled once before Fourier transformation.

The effectiveness of ZQ removal was simulated using the program Mathematica 4.1 (Wolfram Research). Simulated FIDs contained two signals of equal intensity without any exponential decay (ZQ evolves during a constant-time so there are no significant sources of signal decay). One was placed at 0 Hz, and the other was varied over the range 0–200 Hz. The resulting FIDs were apodized and zero-filled as described above and then Fourier-transformed.

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