Available online at www.sciencedirect.com

SCIENCE DIRECT®

Journal of Magnetic Resonance 167 (2004) 291–297

JMR
Journal of Magnetic Resonance

www.elsevier.com/locate/jmr

Communication

Cascaded z-filters for efficient single-scan suppression of zero-quantum coherence

Kristin E. Cano,[a] Michael J. Thrippleton,[b] James Keeler,[b] and A.J. Shaka[a,*]

[a] Department of Chemistry, University of California, Irvine, CA 92697-2025, USA
[b] Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK

Received 18 September 2003

Abstract

A simple and robust method to suppress zero-quantum coherence (ZQC) in NMR experiments, in a single scan and with very high suppression ratio, is described. It is an appreciable improvement on a previous technique by Thrippleton and Keeler [Angew. Chem. Int. Ed. 42 (2003) 3938]. The method, called a *z-filter cascade*, preserves longitudinal, or z-magnetization, with high efficiency. Losses depend mostly on T_1 relaxation but not T_2 relaxation mechanisms. At the same time, suppression of ZQC can be essentially complete in a single scan. The time duration of the z-filter cascade scales inversely to representative chemical shift differences between the coupled spins, and is typically a few tens of milliseconds. The high efficiency of the zero-quantum suppression and excellent retention of the desired z-magnetization, in a single scan without resort to phase cycling or difference spectroscopy, makes the z-filter cascade a useful new pulse sequence building block for a whole range of NMR experiments. In cases where unwanted residual ZQC may have previously contributed to baseline "t_1-noise" in two-dimensional NMR spectra, the z-filter cascade can deliver a noteworthy improvement in spectral quality.

Keywords: Zero-quantum coherence; Artifact suppression; Pulsed field gradient; Broadband inversion pulse; z-filter; NOESY

1. Introduction

Control of the detected nuclear spin magnetization produced by an NMR pulse sequence is essential to isolate the desired signal while rejecting other potentially interfering signals. Usually such selection schemes are framed in terms of coherence transfer pathways [1]. Phases of individual radiofrequency (RF) pulses, or groups of pulses, are systematically incremented in concert with the receiver reference phase to select only magnetization passing through the desired pathway, a technique known as phase cycling [2,3]. Fundamentally, selection by phase cycling relies on the transformation properties of different orders of coherence under rotations around the z-axis, mth-order coherence accruing a phase factor $\exp(-\mathrm{i}m\varphi)$ if all preceding RF pulses are shifted in-phase by an amount φ [4]. By making the phase factor a function of a spatial z-coordinate, using a pulsed field gradient (PFG), unwanted signals can be efficiently dephased. High suppression ratios result, as $\int \exp(-\mathrm{i}m\varphi(z))\,\mathrm{d}z \to 0$ when $\varphi(z)$ is a strong function of z over the active sample volume [5,6].

For the special case $m = 0$, however, no dephasing is possible. That is, z-magnetization, represented by an operator I_z, and zero-quantum coherence (ZQC) between two coupled spins, represented by product operator combinations, e.g., $ZQ_y = I_{1x}I_{2y} - I_{1y}I_{2x}$ and $ZQ_x = I_{1x}I_{2x} + I_{1y}I_{2y}$, cannot be directly influenced by PFGs. In most experiments the ZQC is unwanted, for example in NOESY [7] or TOCSY [8] experiments, and leads to either lineshape distortions or outright artifacts. The earliest methods to attenuate ZQC in NOESY experiments exploited the evolution of ZQC during a delay, the latter being present naturally as the mixing time τ_m. By varying τ_m systematically [9], or including a 180° pulse at a variable position [10], and summing the transients, some cancellation of the ZQC was achieved. Pure z-magnetization does not evolve in-phase during a delay, and so adds constructively.

---

[*] Corresponding author. Fax: 1-949-824-3168.
E-mail address: ajshaka@uci.edu (A.J. Shaka).

1090-7807/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.jmr.2003.12.017

Better methods of destroying ZQC were developed subsequently. By combining a strong RF field with a slowly switched PFG, for example one with a trapezoidal temporal profile, some significant dephasing of ZQC could be obtained in a single scan [11]. Deliberate off-resonance spin locking for brief periods can also attenuate ZQC by the natural inhomogeneity of the RF field B_1 [12–14]. To avoid losses of z-magnetization, the spin-locking field is switched on and off adiabatically. Both these methods are somewhat susceptible to T_2 losses or, more accurately, off-resonance $T_{1\rho}$ losses, during the filtering period. They are also somewhat system dependent, making their setup, optimization, and routine use less than completely transparent. For example, probe design continues to emphasize improvements in RF homogeneity. But improved B_1 homogeneity lengthens the minimum dephasing time for ZQC in the methods relying on off-resonance spin locking.

Very recently, Thrippleton and Keeler [15] proposed a superior method of ZQC elimination. An applied PFG is *maintained throughout* the filtering interval, while a frequency-modulated inversion pulse with a linear frequency sweep inverts the very wide gradient-broadened lineshape in a sequential manner. Effectively, a spatial average over all possible 180° pulse positions during the filtering interval is achieved once the imposed gradient is removed. It is as if a continuum of experiments, in which the 180° pulse were rastered across every possible time position, were performed and then summed, with the significant difference that only a single scan is actually required. The purpose of this Communication is to show that cascading a few such filters in series leads to extremely high suppression ratios. As with excitation sculpting [16,17], the key is that the attainable suppression ratio becomes the *product* of the individual filters, allowing efficient and complete ZQC suppression. In addition, an even number of filters in the cascade will return, e.g., H_2O magnetization to the +z-axis. This makes it possible to include a two-stage z-filter cascade into many pulse sequences with no other changes to the pulse sequence.

2. Results

2.1. Theory

Consider a pair of coupled spins of chemical shift difference Ω_{ZQ} (rad/s) prepared in some initial state represented by a density operator $\sigma(0)$ that can be expanded in terms of populations, as well as zero-, single-, and double-quantum coherence

$$\sigma(0) = \sigma_{\mathrm{p}}(0) + \sigma^{(0)}(0) + \sigma^{(\pm1)}(0) + \sigma^{(\pm2)}(0) \tag{1}$$

of which only the first two terms will survive a conventional PFG. We first apply a strong field gradient along the z-axis of the NMR sample tube, and make the simplifying assumption that coupling to the receiver coil is independent of z, which we scale to be in the unit interval. The gradient is strong in the sense that $\gamma G_z z|_{z=0}^{z=1} \gg \Omega_{ZQ}$, i.e., the linewidth in the presence of the gradient is much larger than the chemical shift difference. A frequency-swept pulse of duration τ_{p} is applied that just inverts the entire broadened line, but essentially no larger range. If the sweep rate is rapid enough that spins differing in frequency by Ω_{ZQ} or less are inverted nearly synchronously, then the spatial variable z is simply transformed effectively to a time variable t that measures the time of spin inversion, layer by layer, along the length of the sample tube. If the inversion pulse is correctly designed, then we may assume at position $z = 0$, $t \approx 0$ and at $z = 1$, $t \approx \tau_{\mathrm{p}}$. If we assume in addition that the instant of inversion is linear across the whole spatial range, then

$$t = z\tau_{\mathrm{p}}. \tag{2}$$

During the filtering time τ_{p} ZQC in each layer evolves, is inverted, and then evolves for the remainder of the time period. If the initial state is ZQ_y and inversion occurs at time t, then the net evolution is simply

$$\begin{aligned} ZQ_y \xrightarrow{t-180^\circ-(\tau_{\mathrm{p}}-t)} & -ZQ_y \cos\left(\Omega_{ZQ}(2t-\tau_{\mathrm{p}})\right) \\ & + ZQ_x \sin\left(\Omega_{ZQ}(2t-\tau_{\mathrm{p}})\right), \end{aligned} \tag{3}$$

which, using Eq. (2), can be written

$$\begin{aligned} ZQ_y(\tau_{\mathrm{p}}) = & -ZQ_y \cos\left(\Omega_{ZQ}\tau_{\mathrm{p}}(2z-1)\right) \\ & + ZQ_x \sin\left(\Omega_{ZQ}\tau_{\mathrm{p}}(2z-1)\right). \end{aligned} \tag{4}$$

Integration over z then gives the normalized attenuation factors for a z-gradient z-filter of duration τ_{p}:

$$\begin{aligned} A_z(ZQ_y \to ZQ_y) &= \frac{-\int_0^1 \cos\left(\Omega_{ZQ}\tau_{\mathrm{p}}(2z-1)\right)\mathrm{d}z}{-\int_0^1 \mathrm{d}z} \\ &= \frac{\sin\left(\Omega_{ZQ}\tau_{\mathrm{p}}\right)}{\Omega_{ZQ}\tau_{\mathrm{p}}}, \end{aligned} \tag{5}$$

$$A_z(ZQ_y \to ZQ_x) = \frac{\int_0^1 \sin\left(\Omega_{ZQ}\tau_{\mathrm{p}}(2z-1)\right)\mathrm{d}z}{\int_0^1 \mathrm{d}z} = 0. \tag{6}$$

Analogous equations result when starting with the initial state ZQ_x, so that the overall attenuation of ZQC by the filter is the sinc-function of Eq. (5). For any duration that is large compared with the inverse of the zero-quantum frequency, significant attenuation is achieved, although to guarantee an n:1 suppression ratio requires $\tau_{\mathrm{p}} > n/\Omega_{ZQ}$ implying a filter time of greater than 100 ms to attenuate ZQC by a factor of 100 if the two spins have a shift difference of 100 Hz, or 0.2 ppm in a proton spectrum at 500 MHz.

Fortunately, there are two other spatial directions that can be used for filtering. Assuming a perfect circular crosssection for the NMR tube, and following through as above, we find attenuation factors for x- or y-gradient z-filters to be

$$A_x(ZQ_y \to ZQ_y) = \frac{-\int_0^1 \sqrt{1-(2x-1)^2}\cos\left(\Omega_{ZQ}\tau_p(2x-1)\right)\mathrm{d}x}{-\int_0^1 \sqrt{1-(2x-1)^2}\,\mathrm{d}x}, \tag{7}$$

$$A_x(ZQ_y \to ZQ_x) = 0 \tag{8}$$

and analogous equations with an initial ZQ_x state. The integral in Eq. (7) does not admit an elementary functional form, but can be evaluated by numerical integration over x as a function of the zero-quantum frequency. However, it is fairly straightforward to see that the resulting attenuation profile will be somewhat broader near zero frequency, and decay somewhat more rapidly for higher zero-quantum frequencies, than A_z. Much as apodizing a truncated FID attenuates sinc-function oscillations in an FT spectrum but results in line broadening, so the natural spatial apodization of the sample tube leads to an initially somewhat broader but ultimately more rapidly decaying attenuation factor for the unwanted ZQC.

Clearly, by applying k such independent filters in series as a 'z-filter cascade' the ultimate attenuation of ZQC is the product of the individual filters. As the kth filter can have an adjustable duration τ_{pk} and simultaneous PFGs along more than one spatial direction may be imposed in concert, there is considerable flexibility in the design of these z-filter cascades. In particular, high suppression ratios for spins with sufficiently large chemical shift difference can be achieved with a short duration cascade. This observation, which parallels the approach taken in excitation sculpting [16,17], is our central result. Fig. 1 shows how the predicted attenuation of ZQC can be built up using a couple of filtering stages.

2.2. Practical considerations

Fig. 2 shows how multiplet distortions from ZQC, initially prepared in significant amounts in a 4-spin system, are systematically removed by application of z-filter cascades. Three stages of filtering along the three spatial directions leads to pure, undistorted absorption-mode spectra using 15 ms for each filter, a total of 45 ms duration. The filtered spectra are far superior for measurement of coupling constants and for integration of peak intensities. For convenience, a constant-amplitude broadband inversion pulse (BIP) [18] was used to achieve the inversion across the spatial profile. The BIP inverts the spins in a fashion that is close to linear in frequency, except at the beginning and end of the pulse, where there is a slight rise- or fall-time delay. The exact performance of the inversion pulse appears not to be critical as long as it is compensated for B_1 inhomogeneity, is of the correct duration, and inverts the bandwidth of interest but no larger range.

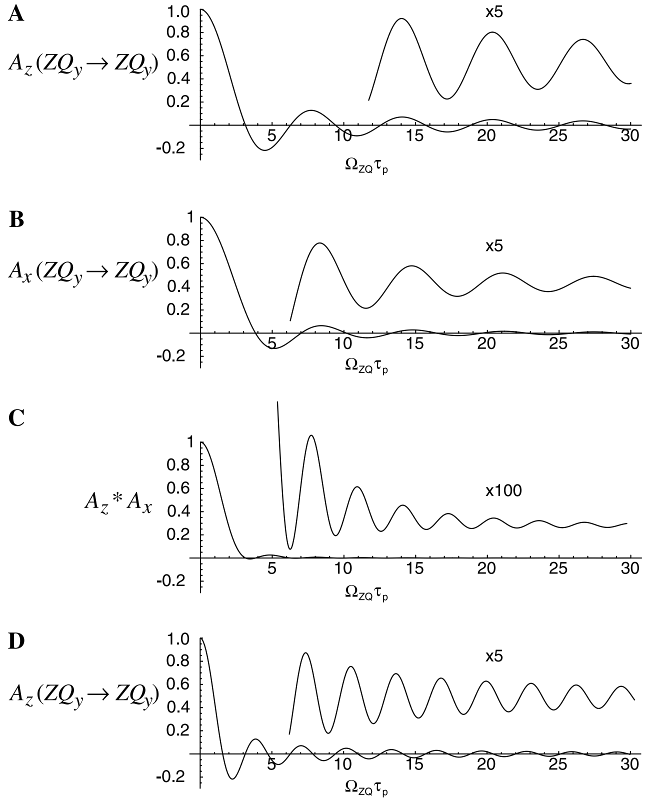

Fig. 1. Plots of calculated attenuation factors for zero-quantum coherence of frequency Ω_{ZQ} and filter time τ_p, as described in the text. (A) Idealized attenuation using a z-gradient; (B) corresponding idealized attenuation using an x- (or y-) gradient; (c) idealized attenuation expected by combining the two filters in series, giving a filter time $2\tau_p$; (D) attenuation using a z-gradient of time $2\tau_p$. The insets show the attenuation curves with either 5× or 100× expansion of the vertical scale, for clarity. When high suppression ratios are needed, the product of the filters is more effective than a single filter.

Fig. 3 shows a comparison with the *length* of the z-filter kept at 30 ms. A single z-filter of 30 ms leads to significant attenuation of the antiphase multiplet components, as shown in the second trace. However, two 15 ms filters is somewhat superior to the single 30 ms filter with respect to the multiplet intensity variations. Depending on the chemical shift difference of the spins, the level of ZQC suppression required, and any relaxation losses, multiple filters may be more or less desirable than a single filter. For small molecule NMR, multiple filters will generally be preferred.

While the transverse gradients give a sharper high-frequency ZQC cutoff, instrumental limitations may impose some bounds on their use, as maximum gradient strength is smaller than that along the z-axis. Combined with the much smaller transverse spatial sample extent, it may not be possible to establish a strong enough

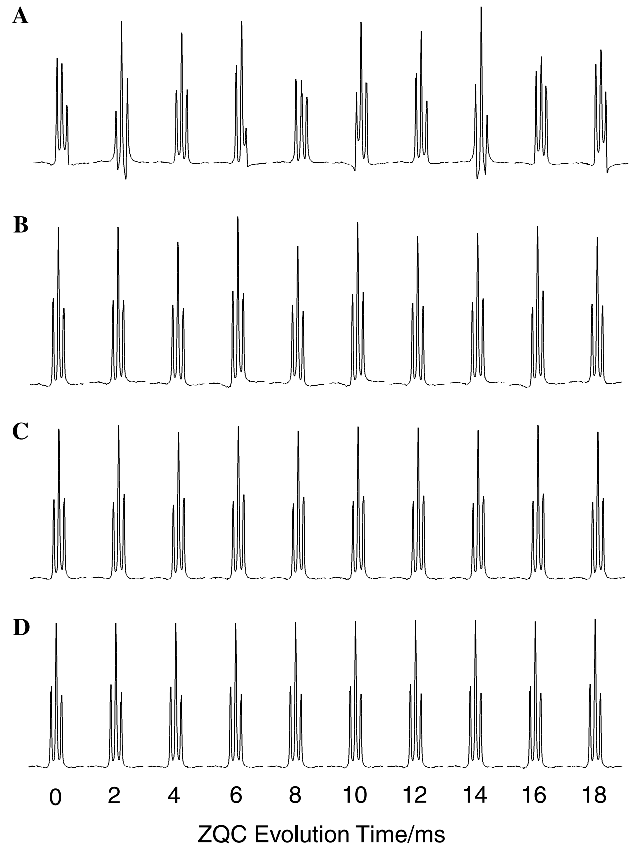

Fig. 2. Experimental demonstration of the ZQC attenuation using a z-filter cascade. A preparation sequence with $90°_x - \tau - 90°_x$ with $\tau = 30$ ms was used to prepare substantial amounts of ZQC in a system of four mutually coupled spins in the phenanthridinone molecule (shown in Fig. 4). A strong PFG then dispersed all coherence except ZQC and z-magnetization. This was then allowed to evolve for a variable time, stepped in 2 ms increments, and then filtered before detection. (A) No z-filter; (b) a single z-gradient z-filter of 15 ms; (c) a z-filter cascade using two 15 ms stages, one along z and the other along x; (d) a z-filter cascade using three 15 ms stages, one along each principal spatial direction. The **H5** triplet is shown (see Fig. 4). Chemical shift differences to **H6** ($J \sim 7$ Hz), **H4** ($J \sim 7$ Hz), and **H3** ($J < 2$ Hz) at 500 MHz are 319.7, −104.7, and 233.4 Hz, respectively. The progressive removal of multiplet intensity and phase distortions shows how the z-filter cascade progressively eliminates zero-quantum coherence in complex spin systems.

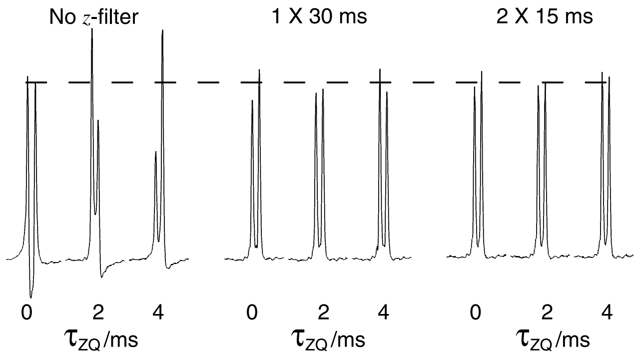

Fig. 3. Comparison of the ZQC suppression that can be achieved with a 30 ms filter. Prepared ZQC, as in Fig. 2, is allowed to evolve for the time shown, filtered, and then interrogated with a 90° read pulse. Left: no z-filter. Center: a single z-gradient z-filter of 30 ms; right: a z-filter cascade using two 15 ms stages, one along z and the other along x. The **H6** doublet of the phenanthridinone molecule (see Fig. 4) is shown. The dashed line is meant to guide the eye. The two shorter z-filters result in better phase and amplitude characteristics. The 7 Hz coupling is to **H5**, which has a 319.7 Hz chemical shift separation from **H6**. This gives $\Omega_{ZQ}\tau_p = 60$ rad for the single filter, and 30 rad for the cascade.

persistent gradient to dominate the entire 10 ppm proton shift range for the desired time of the filter. Modern PFGs are designed foremost for rapid rise- and fall-times and for rather shorter duration, in the 100 μs to 2 ms range. On our Varian UnityPlus 500 MHz spectrometer with a 5 mm HCN triple resonance triax probe, a transverse gradient strength giving a 12 kHz linewidth can be safely employed for 20 ms, whereas a z-gradient giving a 65 kHz linewidth can be employed for more than 30 ms. Smaller gradients give somewhat less efficient filtering, as the sweep across the spatial pattern is completed proportionately more rapidly for each resonance, shortening the effective filter time. For example, if a 12 kHz linewidth from a static transverse gradient is superimposed on the 5 kHz proton chemical shift range, an inversion pulse covering about 17 kHz is required. As any given resonance is only 12 kHz wide, the effective filtering time is reduce to $12\tau_p/17$. In addition, the two coupling partners may not be inverted quite synchronously, requiring a more involved analysis than presented above. In the example above, protons at 0 and 10 ppm (on the same molecule) would be inverted with a lag time of $5\tau_p/17$. Non-synchronous inversion is only probably of concern in NOESY applications, where it would diminish cross-peak intensity for spins with large chemical shift difference. While a correction could be made, the loss of sensitivity is a drawback. In the limit of large enough gradients, all these concerns evaporate.

With this in mind we can offer some suggestions. In practical terms, combining a longer duration z-filter, using a z-gradient, with a somewhat shorter one, using a transverse gradient, gives good results even if theoretically not quite optimum. An alternative is to use the largest transverse gradient feasible, and then *simultaneously* impose a z-gradient to achieve the required linewidth. In practice, 5–10 times the chemical shift range of interest appears to be more than sufficient. Note that spins with larger shift difference have larger zero-quantum frequencies, and so require a shorter filtering time. This makes larger gradients and faster frequency sweeps possible, so that non-synchronous inversion is not an issue.

During a two-dimensional NOESY sequence, the mixing time usually provides plenty of time to apply a highly selective two-stage z-filter cascade. Such a filter will return water magnetization to the +z-axis if it initially resided there, and the continuous presence of the PFGs will inhibit radiation damping, so that the

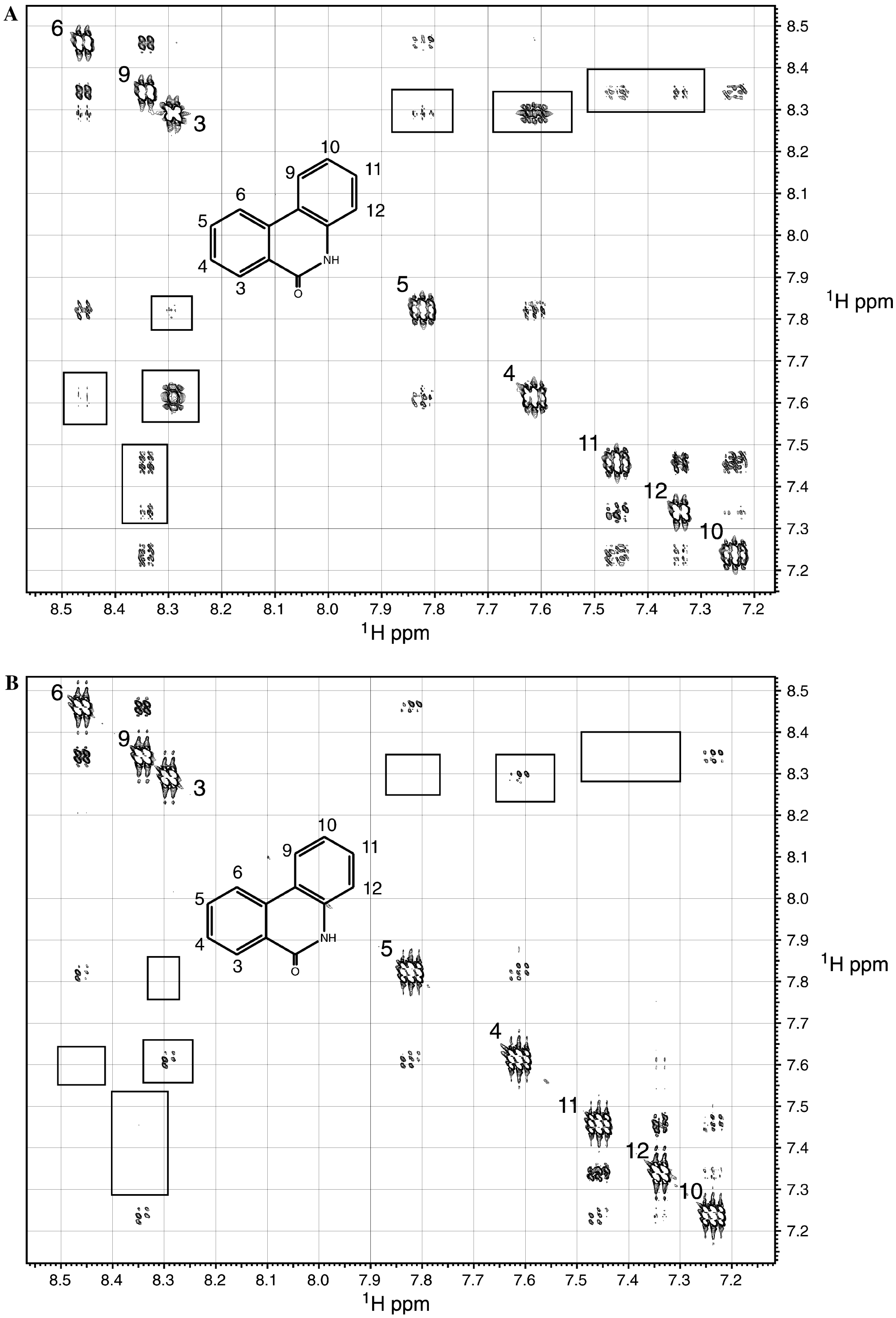

Fig. 4. Experimental demonstration of a z-filter cascade for 2D NOESY applied to a small molecule, shown in the insets, with relatively weak positive NOEs. The proton assignment follows the numbering on the structure. All data were processed using NMRPipe, and both positive and negative contours are drawn down to a level just above the t_1-noise in the spectra. (A) Conventional 2D NOESY, using a strong PFG during the $\tau_m = 550$ ms mixing time to disperse all coherence except ZQC. There are spurious cross-peaks between coupled spins, a couple of which are indicated by boxed areas. Some of these may also have NOEs, making assignment more difficult. (B) Using a two-stage z-filter cascade during the mixing time. A 30 ms BIP over a 65 kHz bandwidth was used in the presence of a z-gradient that produced this linewidth, and a 20 ms BIP over 12 kHz was used with a y-gradient. Here the ZQC has been effectively removed except between protons **10** and **12**, which are too close in chemical shift for this length filter. The two-stage filtered spectrum allows easier identification of the weak NOEs, which are less than 1%. The two-stage z-filter cascade, applied with the specific timing $0.3\tau_m$–filter–$0.5\tau_m$–filter–$0.2\tau_m$ also inhibits unwanted T_1 relaxation during the mixing time [19], lessening the tendency for axial peak breakthrough, and lessening t_1-noise.

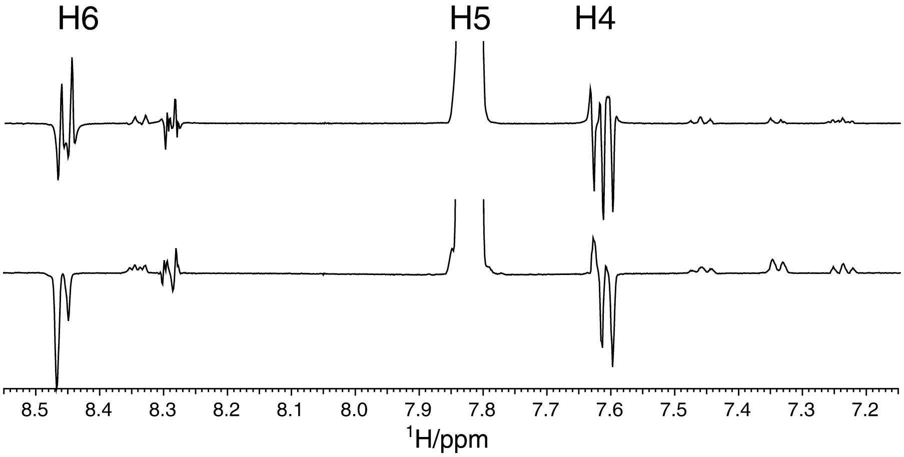

Fig. 5. Traces extracted from the 2D spectra shown in Fig. 4, through the **H5** multiplet. Top: conventional NOESY. Bottom: NOESY with *z*-filter cascade. The phase distortion in the top trace is from unwanted ZQC, and has been removed in the bottom trace on **H4** and **H6**. The absorptive antiphase components that remain originate from **H5** itself which, because the spin states are slightly mixed, contributes intensity outside what would normally be considered the **H5** multiplet. These contributions are very small, but not insignificant compared with the weak NOEs in this small molecule. As far as we are aware, this observation seems to have been overlooked, possibly because all multiplet distortions were ascribed to residual ZQC.

behavior of the strong water signal will be completely predictable. For small-molecule NOESY the mixing time may be hundreds of milliseconds and the problem is not so much the filter design as the ability to apply and maintain strong gradients for the requisite period. Fig. 4 shows a typical result for small-molecule NOESY, in which the filters were selected to simultaneously remove all but the lowest frequency ZQC, and simultaneously suppress auto relaxation that leads to axial peaks [19], the latter being removed by phase cycling but contributing to "t_1-noise" nevertheless. A high quality spectrum is obtained, free from unwanted ZQC and with rather less t_1-noise. Thus, although the NOESY cross-peaks are quite weak, clear and unambiguous assignments can be made with confidence. A representative comparative trace through proton **H5** is shown in Fig. 5. The large antiphase contributions to **H3** and **H6** have been removed, the residual absorptive antiphase pattern reflecting departure from first-order spin-spin coupling and arising from **H5** magnetization, i.e., the diagonal peak on the trace. Although the mixing of product states is only a few percent, the small NOEs make this contribution noticeable, as in the case of carefully conducted 1D transient NOE experiments [20].

3. Conclusions

We have demonstrated an efficient, robust method to achieve very high levels of zero-quantum suppression while simultaneously retaining desired *z*-magnetization with negligible loss. The *z*-filter cascade is applicable to a whole range of experiments, and suggests that other possibilities to improve NMR spectra can be developed along the same lines of thought.

Acknowledgments

This work was supported by the National Science Foundation. M.J.T. thanks the EPSRC, Proctor and Gamble, and the Newton Trust for support.

References

[1] G. Bodenhausen, H. Kogler, R.R. Ernst, Selection of coherence-transfer pathways in NMR pulse experiments, J. Magn. Reson. 58 (1984) 370–388.

[2] D.E. Demco, P. Van Hecke, J.S. Waugh, Phase-shifted pulse sequences for measurement of spin–lattice relaxation in complex systems, J. Magn. Reson. 16 (1974) 467–470.

[3] G. Bodenhausen, R. Freeman, D.L. Turner, Suppression of artifacts in two-dimensional *J* spectroscopy, J. Magn. Reson. 27 (1977) 511–514.

[4] G. Drobny, A. Pines, S. Sinton, D.P. Weitekamp, D. Wemmer, Fourier transform multiple quantum nuclear magnetic resonance, Faraday Symp. Chem. Soc. 13 (1978) 49–55.

[5] P. Barker, R. Freeman, Pulsed field gradients in NMR. An alternative to phase cycling, J. Magn. Reson. 64 (1985) 334–338.

[6] R.E. Hurd, Gradient-enhanced spectroscopy, J. Magn. Reson. 87 (1990) 422–428.

[7] J. Jeener, B.H. Meier, P. Bachmann, R.R Ernst, Investigation of exchange processes by two-dimensional NMR spectroscopy, J. Chem. Phys. 71 (1979) 4546–4546.

[8] L. Braunschweiler, R.R. Ernst, Coherence transfer by isotropic mixing: application to proton correlation spectroscopy, J. Magn. Reson. 53 (1983) 521–528.

[9] S. Macura, K. Wüthrich, R.R. Ernst, Separation and suppression of coherent transfer effects in two-dimensional NOE and chemical exchange spectroscopy, J. Magn. Reson. 46 (1982) 269–282.

[10] M. Rance, G. Bodenhausen, G. Wagner, K. Wuthrich, R.R. Ernst, A systematic approach to the suppression of *J* cross peaks in 2D exchange and 2D NOE spectroscopy, J. Magn. Reson. 62 (1985) 497–510.

[11] A.L. Davis, G. Estcourt, J. Keeler, E.D. Laue, J.J. Titman, Improvement of *z* filters and purging pulses by the use of

zero-quantum dephasing in inhomogeneous B_1 or B_0 fields, J. Magn. Reson. A 105 (1993) 167–183.

[12] J.J. Titman, A.L. Davis, E.D. Laue, J. Keeler, Selection of coherence transfer pathways using inhomogeneous adiabatic pulses. Removal of zero-quantum coherence, J. Magn. Reson. 89 (1990) 176–183.

[13] G. Estcourt, A.L. Davis, J. Keeler, Suppression of zero-quantum interference in two-dimensional *z*-COSY spectra, J. Magn. Reson. 96 (1992) 191–198.

[14] G. Mackin, A.J. Shaka, Phase-sensitive two-dimensional HMQC and HMQC-TOCSY spectra obtained using double pulsed-field-gradient spin echoes, J. Magn. Reson. A 118 (1996) 247–255.

[15] M.J. Thrippleton, J. Keeler, Elimination of zero-quantum interference in two-dimensional NMR spectra, Angew. Chem. Int. Ed. 42 (2003) 3938–3941.

[16] T.-L. Hwang, A.J. Shaka, Water suppression that works. Excitation sculpting using arbitrary waveforms and pulsed field gradients, J. Magn. Reson. A 112 (1995) 275–279.

[17] K. Stott, J. Stonehouse, J. Keeler, T.-L. Hwang, A.J. Shaka, Excitation sculpting in high-resolution nuclear magnetic resonance spectroscopy—application to selective NOE experiments, J. Am. Chem. Soc. 117 (1995) 4199–4200.

[18] M.A. Smith, H. Hu, A.J. Shaka, Improved broadband inversion performance for NMR in liquids, J. Magn. Reson. 151 (2001) 269–283.

[19] K. Stott, J. Keeler, Q.N. Van, A.J. Shaka, One-dimensional NOE experiments using pulsed field gradients, J. Magn. Reson. 125 (1997) 302–324.

[20] A.J. Shaka, European Small Molecule Meeting, MSD Terlings Park, UK, 2001.