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z Filters for Purging Phase- or Multiplet-Distorted Spectra

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We propose in this communication a new filter, called a "z filter," which eliminates phase and multiplet distortions in the resulting one- or two-dimensional spectra when inserted into a NMR pulse sequence.

Many of the pulse sequences proposed for homonuclear or heteronuclear experiments in high-resolution liquid state spectroscopy lead to spectra with multiplet and phase anomalies which may be method-inherent or due to conditions imperfectly matched to the actual spectral parameters. In sequences with pulses separated by fixed time intervals, it is important to match these delays to the reciprocal of a selected value of the coupling constant, J. The consequences of a mismatch are manifold: (1) Some multiplets may be missing altogether, (2) multiplet intensity patterns may differ from those observed under ideal timing, (3) phase anomalies may appear caused by the presence of out-of-phase magnetization components.

In favorable cases, simple remedies can be applied to eliminate the listed spectral distortions. For example, the multiplet and phase anomalies caused by heteronuclear pulse techniques such as INEPT (*1, 2, 4*), DEPT (*3, 4*), and SEMUT (*5*) disappear when proton decoupling is applied during acquisition. For obtaining undistorted proton-coupled heteronuclear spectra, the insertion of a $\pi/2$ purging pulse has proved quite efficient (*4*). However, purging in homonuclear spectra is more delicate. Nevertheless, a $\pi/2$ pulse has been used successfully in the context of multiple-quantum filtering (*6, 7*) for cleaning spectra (*8, 9*). Purging by a single pulse hinges upon the variation of an extended time delay of the order of a reciprocal coupling constant, J. When transverse relaxation times are short, the delay will lead to a decay of the magnetization and to poor sensitivity. We will demonstrate in the following a more efficient purging technique which is less sensitive to transverse relaxation.

The proposed z-filter technique selects the in-phase magnetization along any desired direction in the xy plane. The procedure goes as follows: The transverse magnetization component to be selected is rotated by a $\pi/2$ pulse into z magnetization. The undesired components of the density operator are thereby transformed into coherences of various orders or into multiple spin order (*10*). In the course of a following free precession period, all components, except for the z magnetization I_{kz} and spin order terms $I_{kz} \cdot I_{lz} \ldots$, are oscillating and can be suppressed by coadding results of experiments with different precession times τ_z. A final $\pi/2$ pulse (e.g., of phase y) transforms the

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retained z components into transverse magnetization I_{kx} and into multiple-quantum coherence $I_{kx} \cdot I_{lx} \ldots$. The multiple-quantum coherence remains unobservable when no further pulses are applied before observation.

To improve the efficiency of filtering, it is advisable to phase cycle the first pulse of the filter (together with all previous pulses) or the second pulse (together with the following pulses and the receiver reference phase) to eliminate all coherence components except zero-quantum coherence which is suppressed by variation of τ_z. In most cases it is sufficient to use a four-step phase cycle with 90° increments equivalent to the CYCLOPS procedure (*11*). This also eliminates even spin order terms.

The name for this new filter is cast in analogy to multiple-quantum filters where the selected components are passed through different orders of multiple-quantum coherence.

A z filter as described works properly when inserted immediately before observation without any pulse following or when inserted after a single pulse followed by a free precession period. In the rare case where a z filter has to be applied in the middle of a longer pulse sequence, it is necessary to suppress the remaining multiple-quantum coherence exploiting the characteristic dependence on the flip angle of the filter pulses.

In more scholarly terms, we may understand a z filter as a diagonal part projector with respect to the transformed Hamiltonian $\mathscr{H}^{\mathrm{T}}$ rotated by the two $\pi/2$ pulses bracketing the filter period τ_z:

$$\mathscr{H}^{\mathrm{T}} = U^{-1}\mathscr{H}U \qquad [1]$$

with, e.g.,

$$U = \exp\left\{-i\frac{\pi}{2}F_y\right\}. \qquad [2]$$

The evolution during the z filter is effectively determined by $\mathscr{H}^{\mathrm{T}}$ which is obtained from $\mathscr{H}$ by replacing all z components by x components (assuming weak coupling). Variation of τ_z and coaddition of the results selects the diagonal part of the density operator with respect to $\mathscr{H}^{\mathrm{T}}$ and suppresses all terms which do not commute with $\mathscr{H}^{\mathrm{T}}$. This leaves, after filtering, terms of the form

$$B = \prod_k I_{kx}^{\nu_k}. \qquad [3]$$

For spin-1/2 nuclei ν_k is restricted to the values 0 and 1 while for higher spins also powers of I_{kx} may remain. The desired terms which lead to undistorted in-phase multiplets are the linear terms I_{kx}. The product terms are, as mentioned, unobservable or may be eliminated based on their characteristic flip-angle dependences. For both pulses the desired linear components display a sin β dependence whereas triple product terms have a $\sin^3 \beta$ dependence. Only in very rare cases must higher odd powers be considered.

For a Hamiltonian with N nondegenerate eigenvalues, there are just N independent operators, all of the form of Eq. [3]. However, in the presence of degenerate eigenvalues additional invariant terms may arise which can pass a z filter.

We should note that the z-filtering procedure is related to the elimination of coherence during the mixing process in NOESY experiments (*12*). Indeed, for sufficiently long τ_z there may be undesired magnetization transfer through cross-relaxation. Fortunately τ_z can usually be kept quite short as it compares with the inverse Larmor frequency difference of coupled nuclei, and cross-relaxation effects are normally negligible.

For illustration, we have inserted a *z* filter into the spin-echo multiplet selection technique of Campbell *et al.* (*13*) which is quite sensitive to the proper setting of the time interval τ (see Fig. 1). Setting $\tau = J^{-1}$ gives negative signals for those spins having an even number of coupling partners and positive signals for those with an odd number of coupling partners. An experimental test was carried out on an approximately equimolar mixture of isobutanol, isopropanol, 2-butanone, and 2-butanol, having a spread in the vicinal coupling constants from 6.1 to 7.4 Hz. The result of the simple spin-echo experiment is shown in Fig. 2a. The spectrum clearly exhibits phase and multiplet distortions arising from incomplete refocusing ($\tau \neq J^{-1}$). In Fig. 2b the spin-echo spectrum is presented obtained under the same conditions but with a *z* filter inserted prior to acquisition (Fig. 1b). Figure 2b demonstrates that the phase errors present in the spectrum of Fig. 2a have been removed by the *z* filter.

Having created a clean spin-echo-modified spectrum, we can now combine it with a standard spectrum obtained with a single $\pi/2$ pulse. Properly weighted addition, taking into account relaxation effects, should give a subspectrum of only those spins having an odd number of coupling partners while subtraction should produce a subspectrum of spins with an even number of coupling partners (*13*). Experimental spectra are presented in Fig. 3. Note the revelation of the low-frequency triplet in Fig. 3c which was obscured by an intense doublet in Fig. 2a.

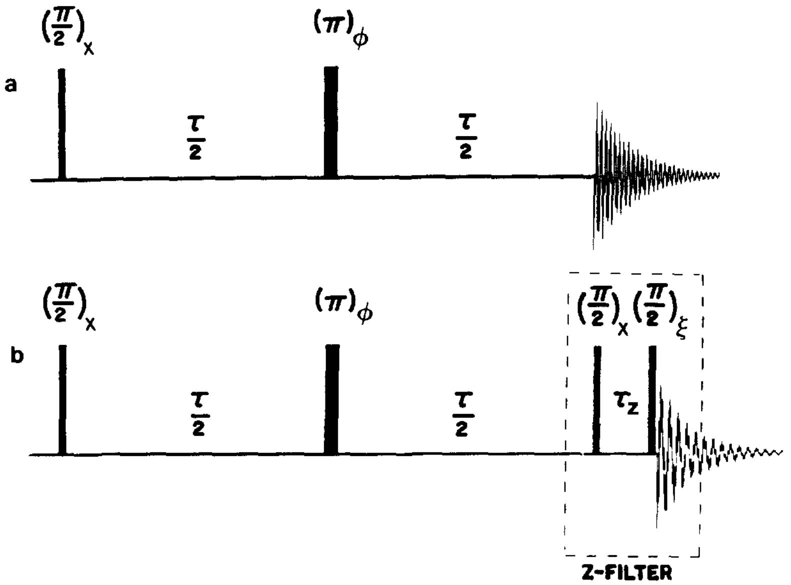

FIG. 1. Schemes for spin-echo multiplet selection techniques. (a) Simple spin-echo sequence; (b) spin-echo pulse sequence with a *z* filter inserted prior to acquisition to retain only the in-phase magnetization. The period τ_z is varied within a sequence of coadded experiments to average out the unwanted coherences. The final $\pi/2$ pulse and the receiver reference phase are taken through a CYCLOPS (*11*) cycle. Cycling of the π pulse according to EXORCYCLE (*16*) eliminates effects from a nonideal π pulse.

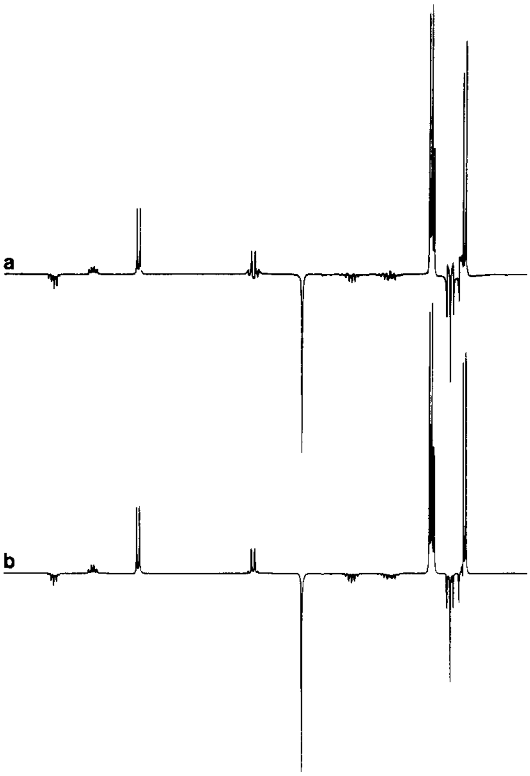

FIG. 2. (a) Spin-echo 300 MHz proton spectrum of an approximately equimolar mixture of isobutanol, isopropanol, 2-butanone, and 2-butanol, obtained by the sequence of Fig. 1a with τ = 156 msec. (b) Same as (a) but with a *z* filter inserted prior to acquisition (scheme of Fig. 1b). Ten τ_z values were used (0, 18, 36 . . . msec) for the *z* filter. Note the disappearance of dispersive contributions, e.g., for the negative triplet on the right. A Bruker CXP-300 spectrometer has been used. The spectra cover from 0.4 ppm on the right to 4.5 ppm on the left.

Although the separation for the mixture investigated here turned out quite well, less perfect performance may be encountered in practice when there is a large spread of *J* couplings (as in conjugated and aromatic systems) or if there is a large spread in relaxation times, because the subtractive elimination presupposes equally scaled responses of all multiplets.

Another application of the *z* filter is in connection with spin-echo-correlated 2D spectroscopy (SECSY) (*14*). The conventional SECSY sequence, $(\pi/2)_x$-$t_1/2$-$(\pi/2)_\phi$-$t_1/2$-acquisition, produces spectra with phase-twisted lineshapes consisting of a superposition of 2D absorption and 2D dispersion peaks due to the presence of both transverse magnetization components at the start of acquisition. Absolute-value presentation of SECSY spectra has therefore been customary.

A *z* filter inserted just prior to acquisition, as shown in Fig. 4, selects one of the in-phase magnetization components. As a consequence, all multiplets in the 2D spectrum will appear in-phase along the ω_2 dimension with the normal binomial intensity distribution. In the ω_1 domain, the multiplet patterns are quite different

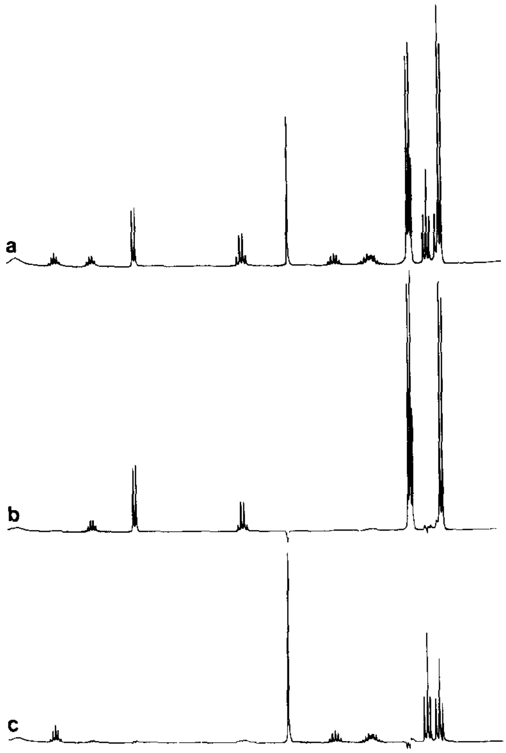

FIG. 3. (a) Normal single-pulse spectrum of the same mixture as in Fig. 2. Spectra obtained by combining the spectrum shown in (a) and the z-filtered spin-echo spectrum of Fig. 2b are shown in (b) and (c). Additive combination yields a spectrum exclusively of protons having an odd number of coupling partners (b) and subtractive combination results in a spectrum containing only those protons with an even number of coupling partners (c). Note the clear appearance of the low-frequency triplet in (c). The spectra cover from 0.4 ppm on the right to 4.5 ppm on the left.

from those known from 1D spectroscopy and from those characteristic for SECSY spectra: The signal $s_{kk}(t_1)$ giving rise to the diagonal peak of nucleus k is modulated in t_1 by the coupling between k and other nuclei m in the form

$$s_{kk}(t_1) \propto \prod_{m \neq k} \frac{1}{2}(1 + \cos \pi J_{km} t_1). \qquad [4]$$

This leads to an in-phase 1:2:1 triplet structure with splitting $J_{km}/2$ for each of the couplings J_{km} (instead of the usual doublet with splitting $J_{km}/2$ in SECSY). The cross-peak multiplets exhibit an antiphase structure, however, with the same absolute phase as the diagonal peaks in contrast to conventional SECSY where diagonal and cross-peaks are always 90° out of phase. The modulation function for the two cross-peaks between spins k and l is given by

$$s_{kl}(t_1) = \frac{1}{2}(1 - \cos \pi J_{kl} t_1)\Big(\prod_{m \neq k,l} \cos(\pi J_{lm} t_1/2)\Big)\Big(\prod_{n \neq k,l} \cos(\pi J_{kn} t_1/2)\Big). \qquad [5]$$

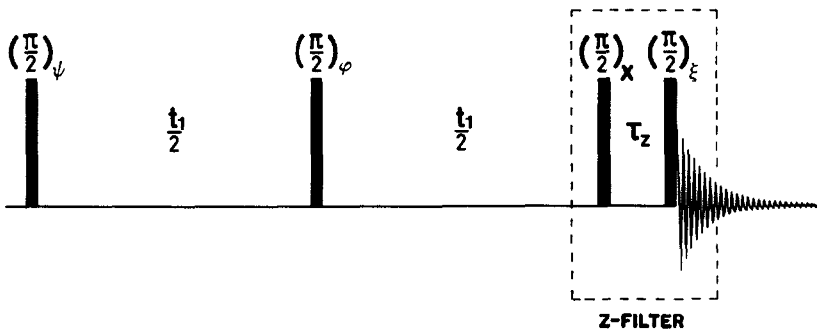

FIG. 4. Pulse sequence for the recording of phase-sensitive homonuclear correlation spectra in the SECSY representation. The z filter inserted between evolution and detection selects one of the quadrature components. The recommended phase cycling scheme is discussed in the text.

This leads to an antiphase −1:2:−1 triplet structure with splitting $J_{kl}/2$ for the J_{kl} coupling and in-phase doublet structure with splitting $J_{lm}/2$ or $J_{kn}/2$ for all other couplings involving spins k or l. The situation is identical in the analogous heteronuclear experiment (*17*) designed to trace out the connectivities of carbon atoms in the molecular skeleton. The absence of dispersive signal components is a significant advantage in comparison to SECSY where it is always necessary to apply strong resolution enhancement to suppress the long dispersive tails with the subsequent penalty in sensitivity.

The modified SECSY experiment shown in Fig. 4 was tested on a mixture of amino acids in D_2O including alanine and glutamate. A 16-step phase cycle was employed, consisting of cycling the $\pi/2$ mixing pulse through $\varphi = x, y, -x, -y$ as in the conventional SECSY experiment, with alternating add/subtract data accumulation; this four-step cycle was repeated four times with the last pulse of the z filter being cycled through $\xi = x, y, -x, -y$ and using CYCLOPS data routing in the computer. Good suppression of undesired components was obtained with only two values of τ_z, 10 and 30 msec. Quadrature detection in the ω_1 dimension has been achieved by time-proportional phase incrementation (TPPI) of the preparation pulse with Fourier transformation as described elsewhere (*15*).

In Fig. 5 is shown a section from the 2D spectrum parallel to the ω_1 axis which runs through one of the inner peaks of the alanine quartet and touches the C_α–H resonance of glutamate. Beside each peak in this ω_1 section is displayed the corresponding multiplet structure in the ω_2 dimension. At the top is the cross-peak multiplet due to coherence transfer from the alanine methyl group to the C_α–H proton. The expected in-phase quartet structure in the ω_2 dimension is clearly demonstrated, as is the antiphase triplet structure in the ω_1 dimension. The resonance in the center correlates the C_α–H resonance of glutamate with the C_β–H protons. At the bottom of Fig. 5 are the overlapping diagonal peaks for the alanine and glutamate C_α–H resonances. The pure absorption phase spectra obtained with this modified SECSY technique are useful in resolving multiplet structure, measuring coupling constants, and detecting cross-peaks close to the diagonal, while retaining the SECSY format of data presentation.

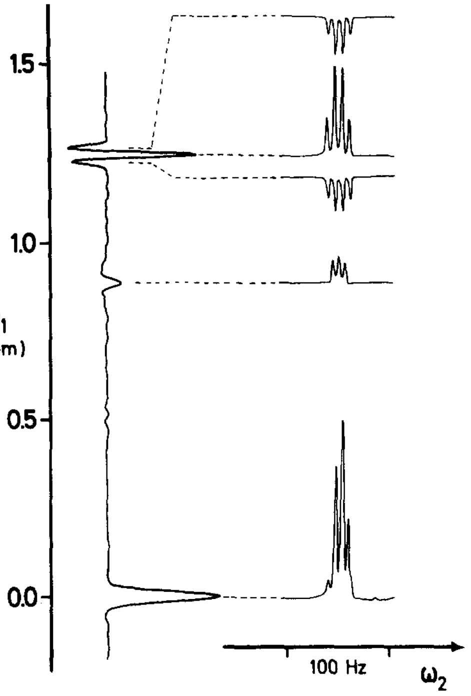

FIG. 5. Sections from the phase-sensitive 360 MHz proton 2D spectrum of an amino acid mixture in D_2O acquired using the modified SECSY pulse sequence shown in Fig. 4. Displayed vertically is a section parallel to the ω_1 axis through one of the inner peaks of the alanine quartet and touching the C_α–H resonance of glutamate. The corresponding multiplet structures in the ω_2 dimension are shown beside each of the peaks in the ω_1 section. The peak at $\omega_1 = 0$ (arbitrarily chosen to be 0 ppm), arising from nontransferred magnetization, represents in the ω_2 dimension the overlap of a quartet and a triplet from the C_α–H protons of alanine and glutamate, respectively. The cross-peak at ~0.9 ppm results from the glutamate α–β coupling while the cross-peak at ~1.25 ppm arises from the alanine α–β coupling. The spectrum has been recorded on a Bruker AM-360 spectrometer.

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REFERENCES

1. G. A. Morris and R. Freeman, *J. Am. Chem. Soc.* **101,** 760 (1979).
2. D. P. Burum and R. R. Ernst, *J. Magn. Reson.* **39,** 163 (1980).
3. D. M. Doddrell, D. T. Pegg, and M. R. Bendall, *J. Magn. Reson.* **48,** 323 (1982).
4. O. W. Sørensen and R. R. Ernst, *J. Magn. Reson.* **51,** 477 (1983).
5. H. Bildsøe, S. Dønstrup, H. J. Jakobsen, and O. W. Sørensen, *J. Magn. Reson.* **53,** 154 (1983).
6. U. Piantini, O. W. Sørensen, and R. R. Ernst, *J. Am. Chem. Soc.* **104,** 6800 (1982).
7. A. J. Shaka and R. Freeman, *J. Magn. Reson.* **51,** 169 (1983).
8. O. W. Sørensen, M. H. Levitt, and R. R. Ernst, *J. Magn. Reson.* **55,** 104 (1983).
9. H. Kessler, H. Oschkinat, O. W. Sørensen, H. Kogler, and R. R. Ernst, *J. Magn. Reson.* **55,** 329 (1983).

10. O. W. SØRENSEN, G. W. EICH, M. H. LEVITT, G. BODENHAUSEN, AND R. R. ERNST, *Progr. NMR Spectrosc.* **16**, 163 (1983).
11. D. I. HOULT AND R. E. RICHARDS, *Proc. Roy. Soc. London Ser. A* **344**, 311 (1975).
12. S. MACURA, Y. HUANG, D. SUTER, AND R. R. ERNST, *J. Magn. Reson.* **43**, 259 (1981).
13. I. D. CAMPBELL, C. M. DOBSON, R. J. P. WILLIAMS, AND P. E. WRIGHT, *FEBS Lett.* **57**, 96 (1975).
14. K. NAGAYAMA, K. WÜTHRICH, AND R. R. ERNST, *Biochem. Biophys. Res. Commun.* **90**, 305 (1979).
15. D. MARION AND K. WÜTHRICH, *Biochem. Biophys. Res. Commun.* **113**, 967 (1983).
16. G. BODENHAUSEN, R. FREEMAN, AND D. L. TURNER, *J. Magn. Reson.* **27**, 511 (1977).
17. O. W. SØRENSEN AND R. R. ERNST, *J. Magn. Reson.* **55**, 338 (1983).