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Use of 45° Pulse Pair as a Filter for Pure-Phase Two-Dimensional NMR Spectroscopy

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Conventionally two-dimensional NMR spectra are recorded in the absolute-intensity mode (*1–4*). It has recently been demonstrated that absorption-mode 2D spectra have much higher resolution and are the preferred mode of presentation, especially for 2D spectra of biomolecules (*5–7*). Indeed, any experimental scheme which yields phase-mixed lineshapes is subject to modification to yield pure-phase spectra, even at the expense of intensity and anomalous multiplet structure (*8–10*). For this purpose two types of filters are already known: the z filter (*9, 10*) and the purging pulse (*8, 10*). In this note, we propose a 45° pulse pair as a filter for obtaining pure-phase 2D spectra, mainly for experiments in which the above filters do not yield pure-phase spectra.

In any two-dimensional experiment, one can have at the start of the detection period, detectable operator products such as (*11*)

$$\begin{aligned} &I_{kx}, 2I_{kx}I_{lz}, 4I_{kx}I_{lz}I_{mz}, \cdots \\ &I_{ky}, 2I_{ky}I_{lz}, 4I_{ky}I_{lz}I_{mz}, \cdots \end{aligned} \quad [1]$$

undetectable terms, such as

$$\begin{aligned} &I_{kz}, 2I_{kz}I_{lz}, 4I_{kz}I_{lz}I_{mz} \cdots \\ &2I_{kx}I_{lx}, 4I_{kx}I_{lx}I_{mz}, \cdots \\ &2I_{kx}I_{ly}, 4I_{kx}I_{ly}I_{mz}, \cdots \\ &2I_{ky}I_{ly}, 4I_{ky}I_{ly}I_{mz}, \cdots \end{aligned} \quad [2]$$

and higher order undetectable coherences.

A $(\pi/2)_x$ purging pulse retains detectable terms such as I_{kx}, converts single operator terms such as I_{kz} into I_{ky}, converts higher order terms such as $I_{ky}I_{lz}$ and $I_{ky}I_{ly}I_{mz}$, respectively, into $I_{kz}I_{ly}$ and $I_{kz}I_{lz}I_{my}$, etc., and makes all other terms undetectable. With such a pulse it is thus possible to retain only a few terms and obtain pure-phase spectra, such as in INEPT$^+$ and DEPT^{++} (*8, 10*). A z filter on the other hand uses a $(\pi/2)$ pulse pair, retaining only the single operator terms I_{kx} or I_{ky} and removes all other terms. Such a filter clearly discriminates the antiphase magnetization from the in-phase magnetization and has been used for obtaining pure phase SECSY and MQT spectra (*9, 10, 12*).

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The proposed $(\pi/4)$ pulse-pair filter converts x or y observable and some nonobservable terms into observable *single phase* terms. For example, the observable terms for $(\pi/4)_x$–τ–$(\pi/4)_x$ with τ jitter are

$$\begin{aligned}
I_{ky} &\rightarrow -\tfrac{1}{2}I_{ky} \\
I_{ky}I_{lz} &\rightarrow -\tfrac{1}{4}(I_{kz}I_{ly}+I_{ky}I_{lz}) \\
I_{ky}I_{lz}I_{mz} &\rightarrow -\tfrac{1}{8}(I_{ky}I_{lz}I_{mz}+I_{ly}I_{kz}I_{mz}+I_{my}I_{kz}I_{lz}) \\
I_{kz} &\rightarrow -\tfrac{1}{2}I_{ky} \\
I_{kz}I_{lz} &\rightarrow -\tfrac{1}{4}(I_{kz}I_{ly}+I_{ky}I_{lz}) \\
I_{kz}I_{lz}I_{mz} &\rightarrow -\tfrac{1}{8}(I_{ky}I_{lz}I_{mz}+I_{ly}I_{kz}I_{mz}+I_{my}I_{kz}I_{lz}) \\
I_{ky}I_{ly} &\rightarrow -\tfrac{1}{4}(I_{kz}I_{ly}+I_{ky}I_{lz}).
\end{aligned} \qquad [3]$$

Changing the phase of the first pulse from x to y retains I_{kx} terms instead of I_{ky} while changing the phase of the second pulse to y converts all the above into I_{kx} terms. During the τ period all terms having x or y operators are removed by coadding experiments with several values of τ (*9*).

Double-quantum filtered COSY (DQFC) yields pure-phase 2D spectra, even under conditions of strong coupling, for two-spin systems (*13*). Higher order spin systems give rise to mixed phases of diagonal and auto peaks even under weak coupling limit (*10*). For example, for three weakly coupled spins the diagonal terms contain the desirable two-operator terms such as $2I_{kx}I_{lz}$ and undesirable three-operator terms such as $4I_{ky}I_{lz}I_{mz}$ which mix the phases. Higher order spin systems yield even higher order terms such as $8I_{ky}I_{lz}I_{mz}I_{nz}$ (*10*). The proposed $(\pi/4)$ pulse-pair filter eliminates all 90° out-of-phase terms yielding pure-phase spectra.

The detectable part of the density operator in the DQFC experiment at the start of the detection period for a three-spin system (Fig. 1a) is given by (*10*)

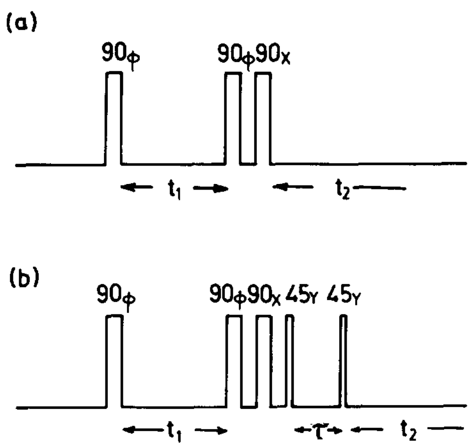

FIG. 1. Pulse sequences for (a) double-quantum-filtered COSY (DQFC) (*4*) and (b) DQFC with $\pi/4$ pulse-pair filter.

$$\sigma^{\mathrm{cross}} = -\tfrac{1}{2}C_k\{S_{kl}C_{km}2I_{kz}I_{lx} + C_{kl}S_{km}2I_{kz}I_{mx}\},$$

$$\sigma^{\mathrm{dia}} = -\tfrac{1}{2}C_k\{S_{kl}C_{km}2I_{kx}I_{lz} + C_{kl}S_{km}2I_{kx}I_{mz} - S_{kl}S_{km}4I_{ky}I_{lz}I_{mz}\}, \quad [4]$$

and with the $(\pi/4)_y$–τ–$(\pi/4)_y$ filter, (Fig. 1b), is given by,

$$\sigma^{\mathrm{cross}} = \tfrac{1}{4}C_k\{S_{kl}C_{km}2I_{kz}I_{lx} + C_{kl}S_{km}2I_{kz}I_{mx}\},$$

$$\sigma^{\mathrm{dia}} = \tfrac{1}{4}C_k\{S_{kl}C_{km}2I_{kx}I_{lz} + C_{kl}S_{km}2I_{kx}I_{mz}\}. \quad [5]$$

Here $C_k = \cos(\omega_k t_1)$, $C_{km} = \cos(\pi J_{km} t_1)$, and $S_{km} = \sin(\pi J_{km} t_1)$. The three-operator term containing I_y, responsible for mixed phases in both ω_1 and ω_2 domains, is eliminated giving pure-phase spectra under phase-sensitive Fourier transformation. The intensities are reduced by a factor of 2 compared to DQFC. Spin–spin relaxation does not attenuate the retained signal, since it is converted into a longitudinal magnetization and is subject only to spin–lattice relaxation during τ.

The experimental verification of the 45° pulse-pair filter is carried out using one-dimensional versions of Schemes a and b of Fig. 1, by converting the t_1 period into a fixed time interval τ_J. The results are compared with normal one-dimensional spectrum (Fig. 2). It is seen that the DQ filtered spectrum (Fig. 2B) has mixed phases, while the 45° pulse-pair filtered spectrum has pure phases (Fig. 2C), confirming the elimination of the three-spin-operator term of Eq. [4].

The experimental scheme for spin-echo correlated spectroscopy (SECSY) in its conventional form yields mixed phases (*2*). Sørensen *et al.* have recently suggested use of the *z* filter for obtaining pure-absorption-mode SECSY spectrum, which for an AX

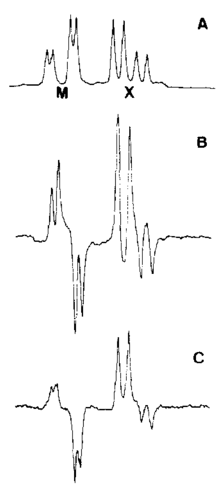

FIG. 2. The (A) normal, (B) double-quantum filtered, and (C) double-quantum +45° pulse-pair-filtered one-dimensional spectra of the β protons of asparagine. The two β protons form the MX part of an AMX spin system with A being the α proton. The coupling constants and 500 MHz chemical shifts are $|J_{AM}| = 3.9$ Hz, $|J_{AX}| = 7.9$ Hz, $|J_{MX}| = 17.0$ Hz, $\delta_{AM} = 526.0$ Hz, and $\delta_{MX} = 49.7$ Hz. The τ_J was 38 ms which yielded maximum amplitude for the MX double-quantum coherence. τ was varied from 1 to 28 ms in steps of 1 ms. Spectrum C was multiplied with a minus sign for easy comparison with (B) (Eqs. [4] and [5]).

spin system yields an unconventional 1:2:1 intensity distribution (*9, 10*). Use of the $(\pi/4)_x-\tau-(\pi/4)_x$ pulse pair also yields pure-absorption-mode SECSY spectrum with a similar intensity pattern for an AX spin system, except that every peak has half the intensity. However, the intensity distribution for higher order spin systems is different for SECSY with a z filter and for SECSY with a $\pi/4$ pulse-pair filter.

It may be pointed out that $\pi/4$ pulse pairs have also been used in exchange and NOE studies in which exchange of two-spin longitudinal order is monitored as a function of mixing time (*14*). The use of a $\pi/4$ pulse pair as a filter as suggested here is for a different purpose and is aimed at retaining some terms and removing others, to yield pure-phase spectra.

The $(\pi/4)$ pulse-pair filter throws away significant intensity in the 2D spectrum and has to be evaluated in the light of the need for pure-phase spectra.

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REFERENCES

1. W. P. Aue, E. Bartholdi, and R. R. Ernst, *J. Chem. Phys.* **64,** 2229 (1976).
2. K. Nagayama, Anil Kumar, K. Wüthrich, and R. R. Ernst, *J. Magn. Reson.* **40,** 321 (1980).
3. U. Piantini, O. W. Sørensen, and R. R. Ernst, *J. Am. Chem. Soc.* **104,** 6800 (1982).
4. G. Wagner, Anil Kumar, and K. Wüthrich, *Eur. J. Biochem.* **114,** 375 (1981).
5. D. J. States, R. A. Haberkorn, and D. J. Ruben, *J. Magn. Reson.* **48,** 286 (1982).
6. D. Marion and K. Wüthrich, *Biochem. Biophy. Res. Commun.* **113,** 967 (1983).
7. H. Widmer, G. Wagner, and K. Wüthrich, Proceedings of XXII Congress Ampere, Zürich, Switzerland, 1984, p. 498.
8. O. W. Sørensen and R. R. Ernst, *J. Magn. Reson.* **51,** 477 (1983).
9. O. W. Sørensen, M. Rance, and R. R. Ernst, *J. Magn. Reson.* **56,** 527 (1984).
10. O. W. Sørensen, Thesis, E.T.H. Zürich, Switzerland, 1984.
11. O. W. Sørensen, G. W. Eich, M. H. Levitt, G. Bodenhausen, and R. R. Ernst, *Prog. Nucl. Magn. Reson. Spectrosc.* **16,** 163 (1983).
12. M. Rance, O. W. Sørensen, W. Lupin, H. Kogler, K. Wüthrich, and R. R. Ernst, *J. Magn. Reson.* **61,** 67 (1985).
13. M. A. Thomas and Anil Kumar, *J. Magn. Reson.* **61,** 540 (1985).
14. G. Bodenhausen, G. Wagner, M. Rance, O. W. Sørensen, K. Wüthrich, and R. R. Ernst, *J. Magn. Reson.* **59,** 542 (1984).