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 $(11)$

$$I_{xx}, 2I_{kx}I_{lz}, 4I_{kx}I_{lz}I_{mz}, \cdots$$

$$I_{ky}, 2I_{ky}I_{lz}, 4I_{ky}I_{lz}I_{mz}, \cdots$$

undetectable terms, such as

$$I_{zz}, 2I_{kz}I_{lz}, 4I_{kz}I_{lz}I_{mz}, \cdots$$

$$2I_{kz}I_{lz}, 4I_{kz}I_{lz}I_{mz}, \cdots$$

$$2I_{kz}I_{lz}, 4I_{kz}I_{lz}I_{mz}, \cdots$$

and higher order undetectable coherences.

A $(\pi/2)_x$ purging pulse retains detectable terms such as $I_{kx}$, converts single operator terms such as $I_{kx}$ into $I_{ky}$, converts higher order terms such as $I_{ky}I_{lz}$ and $I_{kx}I_{lz}I_{mz}$, respectively, into $I_{kz}I_{lz}$ and $I_{kz}I_{lz}I_{mz}$, 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).
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 \((\pi/4)_x-\tau-(\pi/4)_x\) with \(\tau\) jitter are

\[
\begin{align*}
I_{ky} & \rightarrow -\frac{1}{2} I_{ky} \\
I_{ky}I_{z} & \rightarrow -\frac{1}{4}(I_{kz}I_{ly} + I_{ky}I_{lz}) \\
I_{ky}I_{lz}I_{mz} & \rightarrow -\frac{1}{8}(I_{kz}I_{ly}I_{mz} + I_{ly}I_{kz}I_{mz} + I_{my}I_{kz}I_{lz}) \\
I_{kz} & \rightarrow -\frac{1}{2} I_{ky} \\
I_{kz}I_{ly} & \rightarrow -\frac{1}{4}(I_{kz}I_{ly} + I_{ky}I_{lz}) \\
I_{kz}I_{lz}I_{mz} & \rightarrow -\frac{1}{8}(I_{kz}I_{ly}I_{mz} + I_{ly}I_{kz}I_{mz} + I_{my}I_{kz}I_{lz}) \\
I_{ky}I_{ly} & \rightarrow -\frac{1}{4}(I_{kz}I_{ly} + I_{ky}I_{lz}).
\end{align*}
\]

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 \(\tau\) period all terms having \(x\) or \(y\) operators are removed by coadding experiments with several values of \(\tau\) (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 \(8I_{ky}I_{lz}I_{mz}I_{n}\) (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)

\[
\begin{align*}
(\text{a}) & \\
\text{FIG. 1. Pulse sequences for (a) double-quantum-filtered COSY (DQFC) (4) and (b) DQFC with } \pi/4 \text{ pulse-pair filter.}
\end{align*}
\]
\[
\sigma^{\text{cross}} = -\frac{1}{2} C_k \{ S_k C_{k\ell} 2 I_{k\ell} I_{\ell x} + C_{k\ell} S_{k\ell} 2 I_{k\ell} I_{m\ell} \},
\]

\[
\sigma^{\text{dia}} = -\frac{1}{2} C_k \{ S_k C_{k\ell} 2 I_{k\ell} I_{\ell z} + C_{k\ell} S_{k\ell} 2 I_{k\ell} I_{m\ell} - S_k S_{k\ell} 4 I_{k\ell} I_{m\ell} I_{m\ell} \},
\]  \[\text{[4]}\]

and with the \((\pi/4)_x - \tau - (\pi/4)_y\) filter, (Fig. 1b), is given by,

\[
\sigma^{\text{cross}} = \frac{1}{4} C_k \{ S_k C_{k\ell} 2 I_{k\ell} I_{\ell x} + C_{k\ell} S_{k\ell} 2 I_{k\ell} I_{m\ell} \},
\]

\[
\sigma^{\text{dia}} = \frac{1}{4} C_k \{ S_k C_{k\ell} 2 I_{k\ell} I_{\ell z} + C_{k\ell} S_{k\ell} 2 I_{k\ell} I_{m\ell} \}. \]  \[\text{[5]}\]

Here \(C_k = \cos(\omega_k t_1)\), \(C_{k\ell} = \cos(\pi J_{k\ell} t_1)\), and \(S_{k\ell} = \sin(\pi J_{k\ell} t_1)\). The three-operator term containing \(I_{\ell y}\), responsible for mixed phases in both \(\omega_1\) and \(\omega_2\) 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 \(\tau\).

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 \(\tau_1\). 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 \(\beta\) protons of asparagine. The two \(\beta\) protons form the MX part of an AMX spin system with A being the \(\alpha\) proton. The coupling constants and 500 MHz chemical shifts are \(\delta_{AM} = 3.9\text{ Hz}, \delta_{AX} = 7.9\text{ Hz}, \delta_{MX} = 17.0\text{ Hz}, \delta_{AM} = 526.0\text{ Hz}, \) and \(\delta_{MX} = 49.7\text{ Hz}.\) The \(\tau_1\) was 38 ms which yielded maximum amplitude for the MX double-quantum coherence. \(\tau_1\) 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]).](image-url)
spin system yields an unconventional $1:2:1$ intensity distribution \((9, 10)\). Use of the \((\pi/4)\tau-(\pi/4)\) 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