COHERENCE TRANSFER VIA LONGITUDINAL SPIN ORDER GENERALIZED PULSE PAIR FILTERING FOR PURE PHASE TWO-DIMENSIONAL NMR SPECTROSCOPY

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Received 13 March 1987

A generalized pulse pair has been suggested in which the longitudinal spin order is retained and the transverse components cancelled by random variation of the interval between pulses, in successive applications of the two-dimensional NMR algorithm. This method leads to pure phases and has been exploited to provide a simpler scheme for two-spin filtering and for pure phase spectroscopy in multiple-quantum-filtered two-dimensional NMR experiments.

1. Introduction

Two-dimensional (2D) NMR spectroscopy has been extremely successful in the study of biomolecules largely due to significant resonance assignments made possible by experiments which yield correlation information between various resonances. This has mainly been achieved by exploiting transfer of coherence (transverse spin order) from one resonance to another. Many of these experiments, however, yield spectra which have mixed absorption and dispersion components and require computation to give the absolute intensity 2D spectra with a consequent loss of resolution [1–3]. Pure phase absorption mode spectra have inherently higher resolution and have become highly desirable. For this purpose a variety of filters have recently been suggested requiring many pulses and complex phase cycling [4–8].

In this paper a completely different method of coherence tranfer, namely coherence transfer via longitudinal spin order is suggested, which yields pure-phase two- or more-spin-filtered correlated spectra, irrespective of the strength of the coupling and the number of coupled spins. The method utilizes a pulse pair and needs no phase cycling. The phase purity of the method is insensitive to pulse imperfections (rf inhomogeneity, off-set effects and pulse width errors), which only lead to intensity anomalies.

The suggested method of coherence transfer via longitudinal spin order (CLOSY) when applied to three- or more-spin filtering in N coupled spins, while yielding pure phase spectra, gives rise to anomalous intensity patterns and reduced signal intensity. Under such circumstances it is suggested that the usual techniques of multiple-quantum filtering [4] be utilized and the method of longitudinal spin order be used for cleaning the phase mixtures. Recently we suggested a 45° pulse pair as a filter to yield pure phase spectra in two-quantum filtering of third- or higher-order spin systems [8]. This pulse pair, which utilizes longitudinal spin order, is here generalized and its use as a filter for pure phase spectra in higher quantum filtering is proposed.

2. Coherence transfer via longitudinal two-spin order (CLOSY2)

The proposed pulse scheme is given in fig. 1a. The 90°_y pulse creates single quantum coherences which are frequency labelled during the t_1 period. The first 45° pulse converts part of these coherences into longitudinal spin order. The remaining transverse components present in the τ period are cancelled by

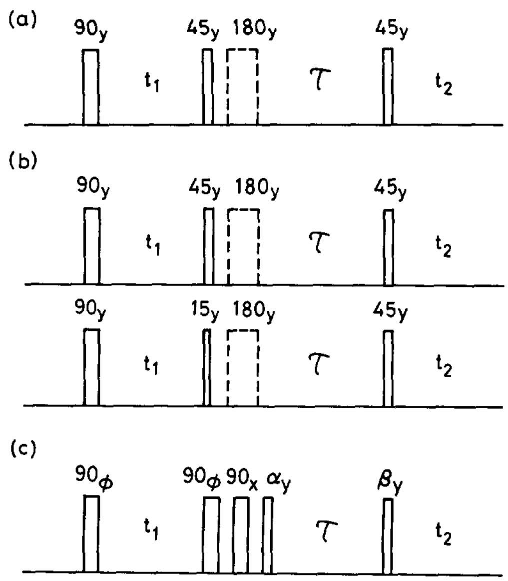

Fig. 1. (a) Pulse scheme for coherence transfer via longitudinal two-spin order (CLOSY2). The 180° pulse is used in alternate scans for identical values of t_1 and the two scans co-added to retain even longitudinal spin orders. (b). Scheme for suppression of longitudinal four-spin order CLOSY2. The 180° pulses are used in alternate scans and the scans are co-added. The 15° scans are subtracted from the 45° scans. (c). Scheme for obtaining pure phase multiple quantum filtered COSY spectra using generalized $(\alpha_{\iota}-\tau-\beta_{\iota})$ pulse pair filter. The 90_{ϕ} pulses are phase cycled to retain the desired multiple quantum coherence order [4].

varying τ during different scans of the two-dimensional experiment. The second 45° pulse converts part of the longitudinal spin order into observable magnetization which is detected during the t_2 period. A 180° pulse immediately following the first 45° pulse in alternate scans, on coaddition, eliminates longitudinal spin order involving an odd number of spins. The detectable part of the density operator at the start of the t_2 period for three weakly coupled spins, resulting from the k spin magnetization of the t_1 period, using the product operator formalism [9], is given by

$$\sigma^{\mathrm{dia}}=\tfrac{1}{4}[2I_{kx}(I_{mz}C_{kl}S_{km}+I_{lz}C_{km}S_{kl})]\sin(\omega_k t_1)\,,$$

$$\sigma^{\mathrm{cross}}=\tfrac{1}{4}[2I_{kz}(I_{mx}C_{kl}S_{km}+I_{lx}C_{km}S_{kl})]\sin(\omega_k t_1)\,. \tag{1}$$

The corresponding density operator for COSY is [10]

$$\sigma^{\mathrm{dia}}=I_{ky}C_{kl}C_{km}\sin(\omega_k t_1)\,,$$

$$\sigma^{\mathrm{cross}}=-[2I_{kz}(I_{mx}C_{kl}S_{km}+I_{lx}C_{km}S_{kl})]\sin(\omega_k t_1) \tag{2}$$

and for two-quantum-filtered COSY (DQFC) is [8,10]

$$\sigma^{\mathrm{dia}}=-\tfrac{1}{4}[2I_{kx}(I_{mz}C_{kl}S_{km}+I_{lz}C_{km}S_{kl}$$
$$-4I_{ky}I_{lz}I_{mz}S_{kl}S_{km})]\cos(\omega_k t_1)\,,$$

$$\sigma^{\mathrm{cross}}=-\tfrac{1}{4}[2I_{kz}(I_{mx}C_{kl}S_{km}+I_{lx}C_{km}S_{kl})]\cos(\omega_k t_1)\,, \tag{3}$$

where $C_{ij}=\cos(\pi J_{ij}t_1)$, $S_{ij}=\sin(\pi J_{ij}t_1)$.

It can be seen that the dispersive character of the diagonal peak of the COSY and the dispersive character of the diagonal peak of DQFC arising from the three-spin-operator term, are absent in eq. (1), resulting in a pure phase two-spin-filtered COSY spectrum. The signal intensity in eq. (1) is identical to that in 45° pulse pair filtered DQFC [8], but lower than COSY and DQFC without filtering (eqs. (2) and (3)). In fig. 2, the longitudinal two-spin-order filtered COSY spectrum of asparagine is compared with the conventional COSY spectrum, demonstrating the phase purification. The loss of signal intensity in CLOSY2 is well compensated by phase purification which allows plotting of lower contours, unhindered by dispersive tails.

In higher-order spin systems $(5\geqslant N>3)$ longitudinal four-spin order will also give rise to a detectable signal in the CLOSY2 experiment of fig. 1a. For a four-spin system the relevant part of the density operator changes from its form in eq. (1) to

$$\sigma^{\mathrm{dia}}=\tfrac{1}{4}[2I_{kx}(I_{rz}C_{kl}C_{km}S_{kr}+I_{mz}C_{kl}C_{kr}S_{km}$$
$$+I_{lz}C_{km}C_{kr}S_{kl})\sin(\omega_k t_1)$$
$$-\tfrac{1}{16}(8I_{kx}I_{lz}I_{mz}I_{rz}S_{kl}S_{km}S_{kr})\sin(\omega_k t_1)\,,$$

$$\sigma^{\mathrm{cross}}=\tfrac{1}{4}[2I_{kz}(I_{rx}C_{kl}C_{km}S_{kr}+I_{mx}C_{kl}C_{kr}S_{km}$$
$$+I_{lx}C_{km}C_{kr}S_{kl})]\sin(\omega_k t_1)$$
$$-\tfrac{1}{16}[8I_{kz}(I_{lz}I_{mz}I_{rx}+I_{lz}I_{mx}I_{rz}$$
$$+I_{lx}I_{mz}I_{rz})S_{kl}S_{km}S_{kr}]\sin(\omega_k t_1)\,. \tag{4}$$

Since all these signals occur through longitudinal spin order the four-spin operator terms have the same phase as the two-spin operator terms and the phase purity of the CLOSY2 spectrum is maintaned.

The four-spin operator terms of eq. (4) can be largely eliminated by the pulse sequence of fig. 1b. This modification reduces the two-spin operator terms of eq. (4) by a factor of 2 and the four-spin operator terms by a factor of 16.

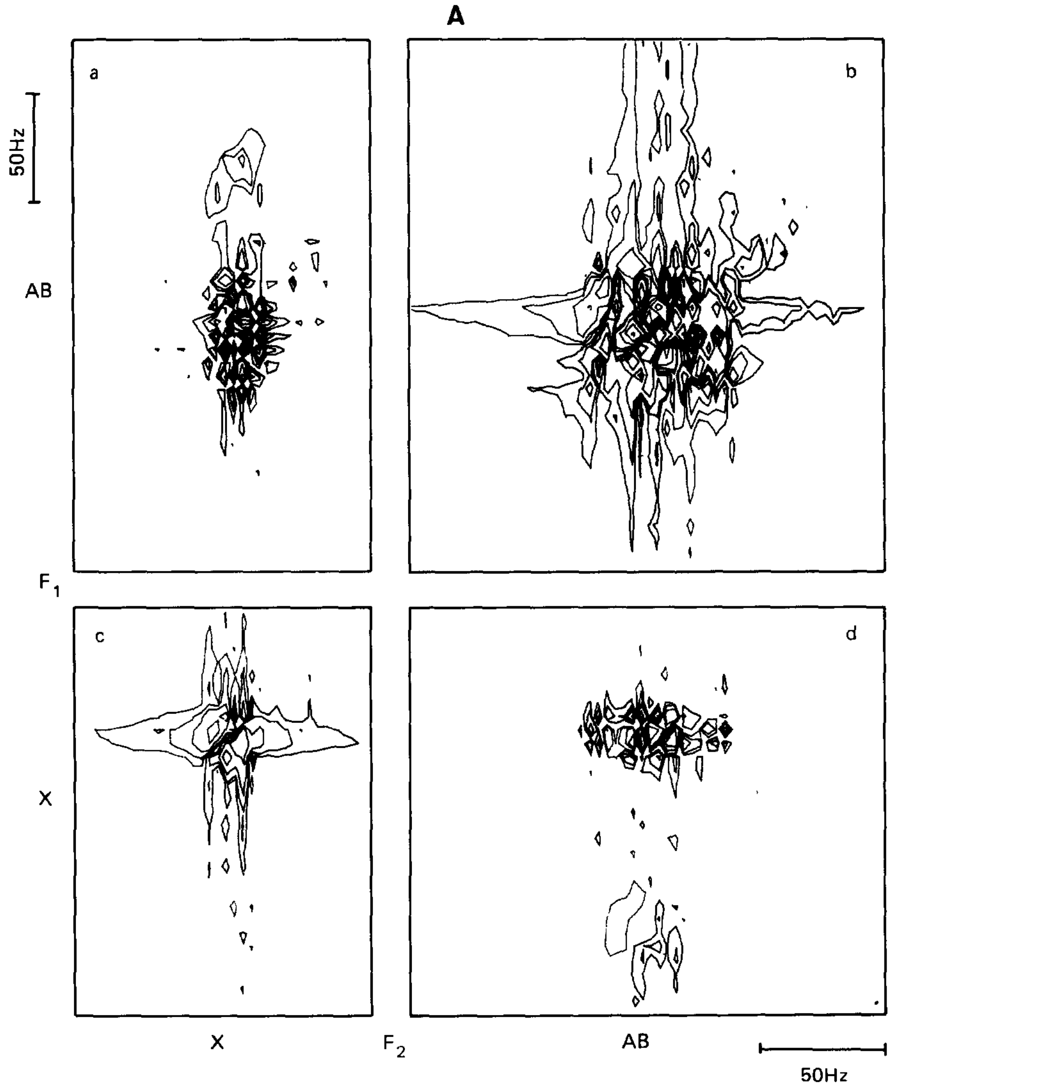

Fig. 2. Two-dimensional proton-correlated experiments on the ABX spin system of asparagine at 270 MHz. (A) Conventional COSY experiment and (B) longitudinal two-spin-order filtered COSY (fig. 1a). (a) Cross peak region of X with AB; (b) cross and diagonal peak regions of AB; (c) diagonal peak region of X and (d) cross peak region of AB with X. Light contours represent negative and dark contours positive intensities. The digital resolution was 0.8 Hz/point in both F_1 and F_2 dimensions. In (B) the τ period was varied from 1 to 28 ms in steps of 1 ms, in the 256 t_1 experiments. The parameters of the ABX spin system were. $\delta_A=0.0$ Hz, $\delta_B=26.6$ Hz, $\delta_X=310.1$ Hz, $J_{AB}=16.7$ Hz, $J_{AX}=7.8$ Hz and $J_{BX}=4.2$ Hz.

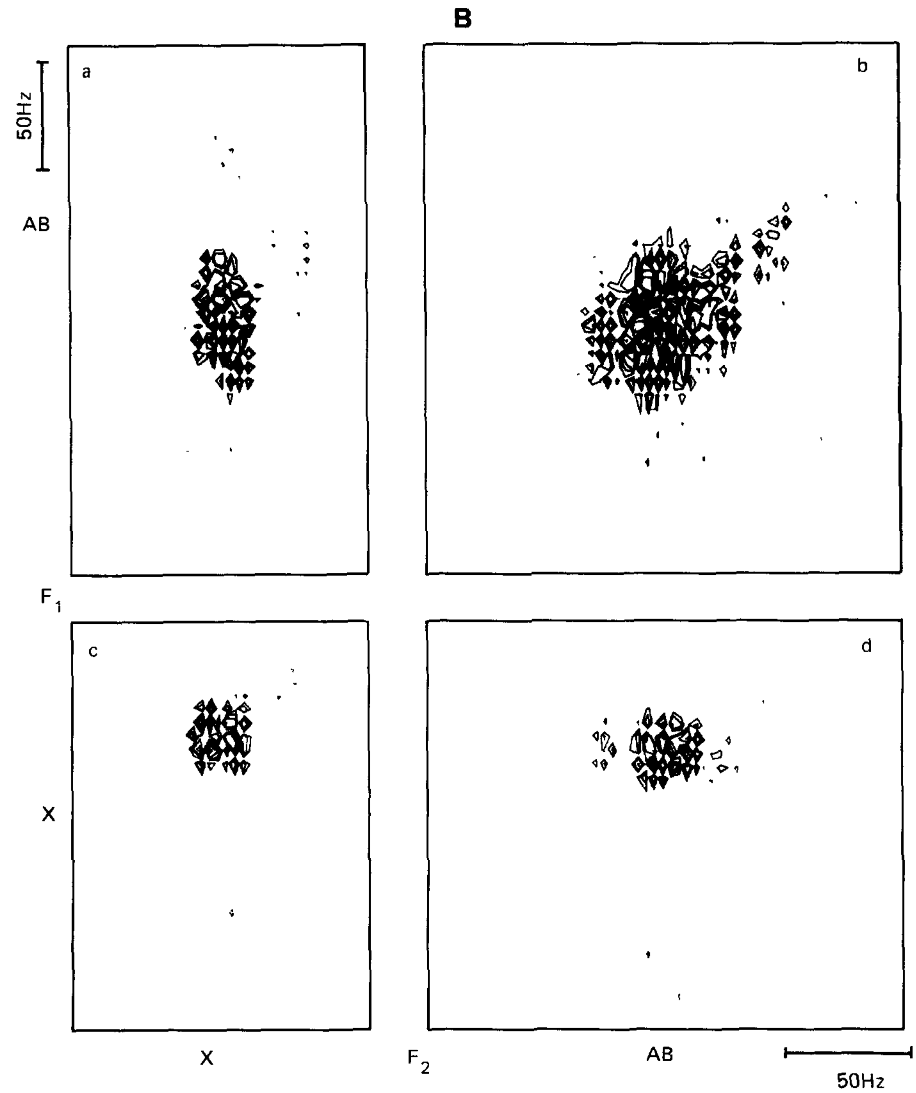

Fig. 2. Continued.

3. Generalized pulse-pair filtering

The above method of coherence transfer via longitudinal spin order leads to severe intensity anomalies when attempted for three- or more-spin filtering. However, it is possible to use this method as a phase filter for pure phase spectra in conventional spin-filtering techniques. A 45° pulse pair was earlier suggested by us for obtaining pure phase two-quantum-filtered COSY spectrum of three or more coupled spins [8]. This pulse pair filter is generalqed here for arbitrary order filtering.

In a coupled N-spin system the possible product operator terms consist of 1-,2-, ..., n- (with $n \leqslant N$) spin-order terms, p of which are transverse operators and h longitudinal such that $n=p+h$. For example $I_{ky}I_{ly}I_{mz}I_{qz}I_{rz}$ has $p=2$ and $h=3$. The pulse pair filter (fig. 1c), where y pulses have been utilized, retains only terms which do not have y spin operators. The retained terms are for example I_{kx}, I_{kz}, $I_{kx}I_{lz}I_{kz}I_{lx}$, $I_{kx}I_{lx}I_{mz}I_{qz}I_{rz}$, etc. The flip-angle dependence of the

Table 1
The optimum flip angles for creation of longitudinal order by the α_y pule and detection by the β_y pulse for various spin-operator terms.

Spin operator	p	h	Optimum flip angle	
			α (deg)	β (deg)
I_{kz}	0	1	0	90
I_{kx}	1	0	90	90
$2I_{kx}I_{lz}$	1	1	45	45
$4I_{kx}I_{lz}I_{mz}$	1	2	35.3	35.3
$8I_{kx}I_{lz}I_{mz}I_{qz}$	1	3	30	30
$16I_{kx}I_{lz}I_{mz}I_{qz}I_{rz}$	1	4	26.6	26.6
$2I_{kx}I_{lx}$	2	0	90	45
$4I_{kx}I_{lx}I_{mz}$	2	1	54.7	35.3
$8I_{kx}I_{lx}I_{mz}I_{qz}$	2	2	45	30
$16I_{kx}I_{lx}I_{mz}I_{qz}I_{rz}$	2	3	39.2	26.6
$4I_{kx}I_{lx}I_{mx}$	3	0	90	35.3
$8I_{kx}I_{lx}I_{mx}I_{qz}$	3	1	60	30
$16I_{kx}I_{lx}I_{mx}I_{qz}I_{rz}$	3	2	50.8	26.6
$8I_{kx}I_{lx}I_{mx}I_{qx}$	4	0	90	30
$16I_{kx}I_{lx}I_{mx}I_{qx}I_{rx}$	5	0	90	26.6

conversion of these terms into longitudinal spin order is given by $(\sin\alpha)^p(\cos\alpha)^h$. The conversion of the longitudinal orders into observable magnetization by the β pulse has a flip-angle dependence given by $(\sin\beta)(\cos\beta)^{n-1}$.

The optimum detection of various terms through a generalized α_y–τ–β_y filter is given in table 1. The single spin transverse order (I_{kx}) is optimally detected by the use of a 90°_y pulse pair, the so-called Z filter [6]. The optimum filtering of antiphase magnetization ($I_{kx}I_{lz}$) resulting from a two-quantum-filtered COSY requires a 45° pulse pair [8]. Doubly antiphase magnetization ($I_{kx}I_{lz}I_{mz}$) resulting from a three-quantum-filtered COSY, is optimally detected by use of 35.3° pulse pair. In general for obtaining a pure phase q-quantum-filtered COSY for $q<N$ a symmetrical pulse-pair filter is required whose optimum flip angle is obtained from the maximum value of $\sin\beta(\cos\beta)^{q-1}$. Terms such as I_{kx}, $I_{kx}I_{lx}$, $I_{kx}I_{lx}I_{mx}$, ..., are optimally filtered by a pulse pair having $\alpha=90°$ and a β for which $\sin\beta(\cos\beta)^{q-1}$ is optimum. Note that a q-quantum-filtered COSY for $q=N$ yields pure phase 2D spectrum, and these filters are needed only for $q<N$ [8,11]. It may further be noted that a general property of the method of coherence transfer via longitudinal spin order in which the transverse components are eliminated by τ jitter is that the resulting 2D spectra are all in a pure phase irrespective of the strength of the coupling and the number of coupled spins. The cancellation of transverse components during the τ period can be improved by using a phase cycling scheme identical to 2D NOE schemes [12,13] and the τ jitter is essentially used for eliminating the zero-quantum coherences.

The proposed CLOSY2 pulse scheme is identical to the pulse scheme given by Bodenhausen et al. [14] for NOE transfers via longitudinal two-spin order. In the present applications NOE transfers are small as the jitter time τ is restricted to small values (<30 ms). While this paper was under preparation, another paper from Oschikinat et al. has appeared which utilizes longitudinal spin orders mainly for obtaining information on connectivity of transitions in coupled-spin system [15].

4. Conclusions

Coherence transfer via longitudinal spin order, effected by a pulse pair, leads to pure phase 2D spectroscopy irrespective of the strength of coupling and the number of coupled spins. This has been exploited here in CLOSY2, a simpler scheme for two-spin filtering and for obtaining pure phase spectra in multiple-quantum-filtered COSY.

Acknowledgement

The use of the WH-270 FT NMR spectrometer of the Sophisticated Instruments Facility at Indian Institute of Science, Bangalore is gratefully acknowledged.

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