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Superior Pulse Schemes for Spin-Echo and Other Two-Dimensional Homonuclear Correlated Spectroscopies

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Pulse schemes are suggested which remove the antiphase intensity character from the multiplets of cross peaks and introduce antiphase intensity character into the multiplets of diagonal peaks in spin-echo correlated 2D spectroscopy (SECSY) and in relay coherence transfer 2D spectroscopy (RCOSY). These are suggested with the aim of enhancing the intensity of cross peaks in situations of limited digital resolution.

Two-dimensional NMR spectroscopy has had a revolutionary effect on the practice of NMR in biological systems. Essentially total resonance assignments have become possible (*1–6*) with the help of correlated two-dimensional spectroscopy (COSY) (*7*), spin-echo correlated spectroscopy (SECSY) (*8*), nuclear Overhauser effect spectroscopy (NOESY) (*9*), relayed coherence transfer spectroscopy (RCOSY) (*10–12*), and multiple-quantum transition (MQT) spectroscopies (*13–17*). COSY and SECSY, which yield information on resonances which are spin coupled, have proved to be extremely useful and have become widely used experiments for resonance assignments in many systems. In biomolecules, however, these experiments require samples having reasonably good concentration of biomolecules and several hours of data accumulation.

We have recently pointed out that the COSY experiment can be very much improved by addition of fixed time delays in the evolution and detection periods (*18*). These delays remove antiphase character from the multiplets of the cross peaks and introduce antiphase character into the multiplets of the diagonal peaks, achieving many improvements in the COSY spectrum. The scheme, named SUPER COSY, drastically enhances the sensitivity of detection of the cross peaks under conditions of limited digital resolution, reduces the dynamic range of diagonal to cross peaks, and allows J tuning of the COSY spectra for detection of particular couplings (*19*). In this paper we describe similar improvements in the other schemes used for homonuclear 2D correlation, namely SECSY and RCOSY.

SECSY EXPERIMENTS

The experimental schemes for SECSY and two versions of SUPER SECSY are given in Fig. 1. Standard phase-cycling procedure needed for cancellation of P peaks

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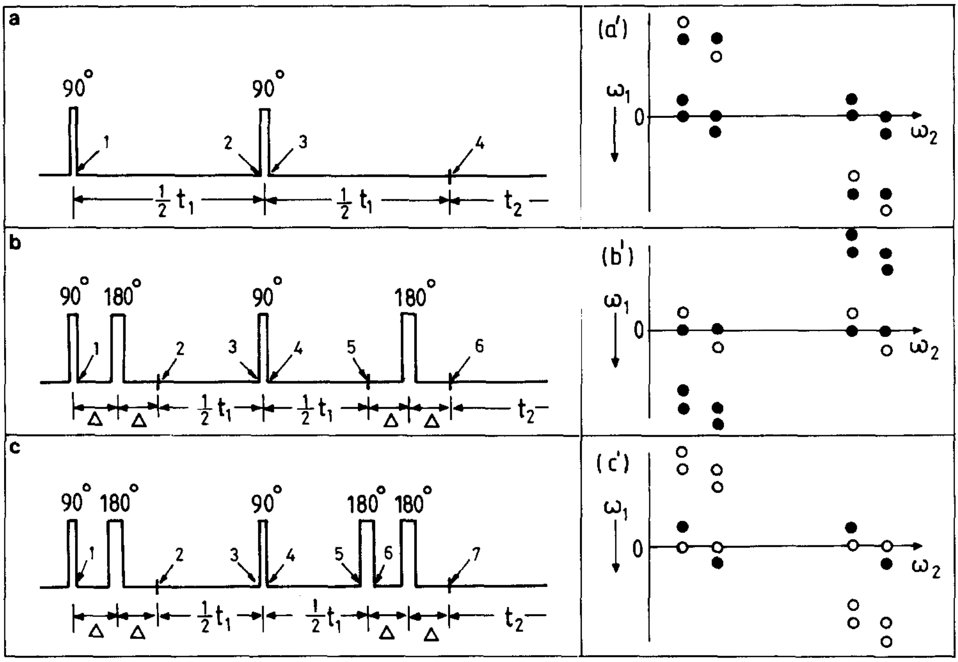


FIG. 1. The pulse schemes for (a) SECSY, (b) SUPER SECSY1, and (c) SUPER SECSY2 along with their schematic spectra for an AX spin system in (a'), (b'), and (c'), respectively. In (a'), (b'), and (c'), each circle is of equal absolute intensity with filled symbols representing negative intensity.

(viz., $x, x, +; x, y, -$) (*20,* p. 217) is adopted in each case. Using the product operator formalism of Sørensen *et al.* (*21*) the density operators in SECSY, SUPER SECSY1, and SUPER SECSY2 have been calculated for two weakly coupled spins k and l (each spin $\frac{1}{2}$) and are given below.

SECSY. The observable part of the density operator at the beginning of period t_2, after coaddition of the two phase cycled experiments for cancellation of P peaks, is given by

$$\begin{aligned}\sigma_4 = {} & \tfrac{1}{2}(I_{ky} + I_{ly})(1 + \cos(\tfrac{1}{2}Jt_1)) - (I_{kx}I_{lz} + I_{lx}I_{kz})\sin(\tfrac{1}{2}Jt_1) + \tfrac{1}{2}(I_{kz} - \tfrac{1}{2})[I_{ly}\cos(\Omega_1 t_1) \\ & + I_{lx}\sin(\Omega_1 t_1)] - \tfrac{1}{2}(I_{kz} + \tfrac{1}{2})[I_{ly}\cos(\Omega_2 t_1) + I_{lx}\sin(\Omega_2 t_1)] + \tfrac{1}{2}I_{lx}\sin[\tfrac{1}{2}(\Omega_k - \Omega_l)t_1] \\ & + \tfrac{1}{2}I_{ly}\cos[\tfrac{1}{2}(\Omega_k - \Omega_l)t_1] + \tfrac{1}{2}(I_{lz} - \tfrac{1}{2})[I_{ky}\cos(\Omega_2 t_1) - I_{kx}\sin(\Omega_2 t_1)] \\ & - \tfrac{1}{2}(I_{lz} + \tfrac{1}{2})[I_{ky}\cos(\Omega_1 t_1) - I_{kx}\sin(\Omega_1 t_1)] - \tfrac{1}{2}I_{kx}\sin[\tfrac{1}{2}(\Omega_k - \Omega_l)t_1] \\ & + \tfrac{1}{2}I_{ky}\cos[\tfrac{1}{2}(\Omega_k - \Omega_l)t_1] \qquad [1]\end{aligned}$$

where

$$\Omega_1 = \tfrac{1}{2}((\Omega_k - \Omega_l) + J)$$

$$\Omega_2 = \tfrac{1}{2}((\Omega_k - \Omega_l) - J).$$

The terms containing I_{lx} and I_{ly} give peaks at $\omega_2 = \Omega_l \pm \frac{1}{2}J$, while the terms containing I_{kx} or I_{ky} give peaks at $\omega_2 = \Omega_k \pm \frac{1}{2}J$. The ω_1 frequencies of the various peaks are 0, $(\pm\frac{1}{2}J)$, $(\pm\frac{1}{2}(\Omega_k - \Omega_l))$, and $(\pm\frac{1}{2}(\Omega_k - \Omega_l) \pm \frac{1}{2}J)$.

From the SECSY spectrum of AX, Fig. 1a′, it is seen that the multiplets of diagonal and auto peaks at $\omega_1 = 0$ and $\pm\frac{1}{2}J$, have in-phase character while the multiplets of cross peaks at $[\pm\frac{1}{2}(\Omega_k - \Omega_l)]$ and $[\pm\frac{1}{2}(\Omega_k - \Omega_l) \pm \frac{1}{2}J]$ have antiphase character. This results in a SECSY spectrum of an AX spin system, under limited digital resolution, of the type shown in Fig. 2a. From this spectrum it is seen that the diagonal peaks are quite large and the cross peaks are quite small in intensity, due to cancellation of antiphase components of the cross peaks. This is typically what is obtained in the SECSY spectra of biomolecules (*8*).

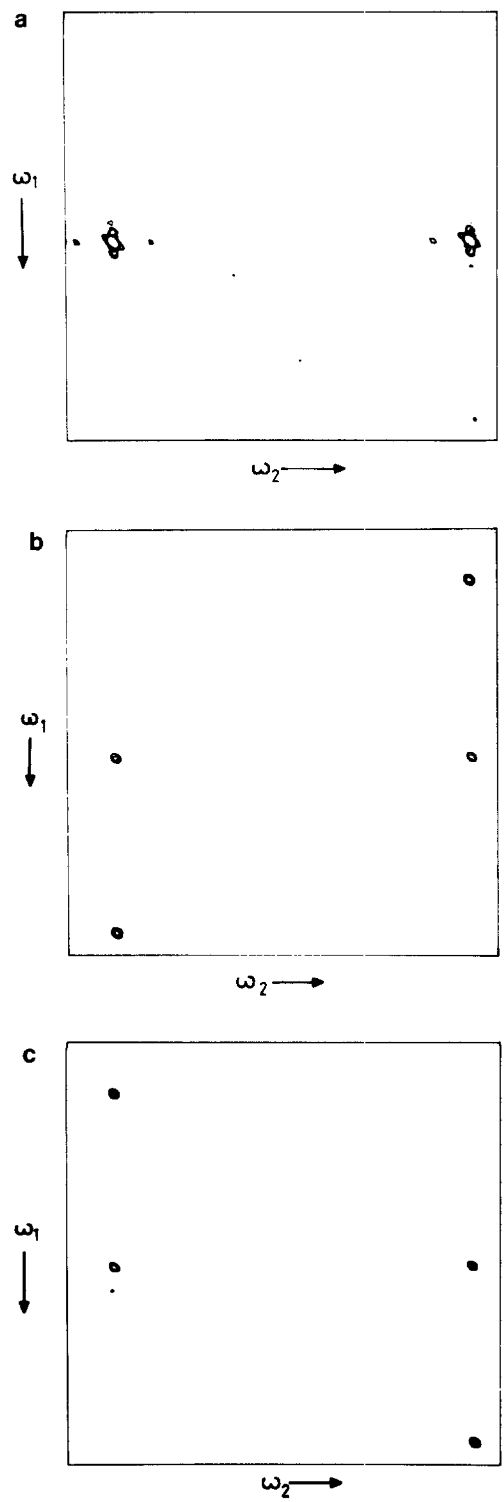
SUPER SECSY1. The observable part of the density operator at the beginning of period t_2, for $\Delta = 1/(4J)$ (Fig. 1b), and after coaddition of two phase-cycled experiments for cancellation of *P* peaks, is given by

$$\begin{aligned}\sigma_6 = {} & \tfrac{1}{2}(I_{ky} + I_{ly})(1 - \cos(\tfrac{1}{2}Jt_1)) + (I_{kx}I_{lz} + I_{lx}I_{kz})\sin(\tfrac{1}{2}Jt_1) + \tfrac{1}{2}(I_{kz} + \tfrac{1}{2})[I_{ly}\cos(\Omega_1 t_1) \\ & - I_{lx}\sin(\Omega_1 t_1)] - \tfrac{1}{2}(I_{kz} - \tfrac{1}{2})[I_{ly}\cos(\Omega_2 t_1) - I_{lx}\sin(\Omega_2 t_1)] - \tfrac{1}{2}I_{lx}\sin[\tfrac{1}{2}(\Omega_k - \Omega_l)t_1] \\ & + \tfrac{1}{2}I_{ly}\cos[\tfrac{1}{2}(\Omega_k - \Omega_l)t_1] + \tfrac{1}{2}(I_{lz} + \tfrac{1}{2})[I_{ky}\cos(\Omega_2 t_1) + I_{kx}\sin(\Omega_2 t_1)] \\ & - \tfrac{1}{2}(I_{lz} - \tfrac{1}{2})[I_{ky}\cos(\Omega_1 t_1) + I_{kx}\sin(\Omega_1 t_1)] + \tfrac{1}{2}I_{kx}\sin[\tfrac{1}{2}(\Omega_k - \Omega_l)t_1] \\ & + \tfrac{1}{2}I_{ky}\cos[\tfrac{1}{2}(\Omega_k - \Omega_l)t_1]. \qquad [2]\end{aligned}$$

This scheme results in antiphase character in multiplets of the diagonal and auto peaks and in-phase character in the multiplets of the cross peaks, Fig. 1b′. The experimental spectrum of Fig. 2b, obtained under conditions identical to the spectrum of Fig. 2a, shows the dramatic improvement in the intensities of the cross peaks and a reduction in the intensities of the diagonal peaks. It is clear that this is a far improved spectrum and points to the power of the proposed method. Increase in the intensity of cross peaks reduces the signal averaging time required for obtaining a 2D SECSY spectrum or allows use of lower sample concentrations. Reduction in intensity of diagonal and the choice of appropriate delay Δ allows optimization of the SECSY spectrum for a particular long or short-range coupling.

The crosspeaks in the SECSY spectra obtained from this scheme appear at 45° angle from the ω_2 axis, Figs. 1b′ and 2b, as opposed to the 135° angle in conventional SECSY, Figs. 1a′ and 2a. While this in itself presents no limitation in the interpretation of the cross peaks, it is possible to obtain the 135° tilted spectrum using the following scheme of SUPER SECSY2. The multiplets within the cross peaks and the diagonal peaks appear with a tilt of 135° in all the three schemes (Figs. 1 and 2).

SUPER SECSY2. From this scheme, Fig. 1c in which an extra 180° pulse has been added at the end of period t_1, the observable part of the density operator at the beginning of period t_2, after coaddition of two phase-cycled experiments for cancellation of *P* peaks, and for $\Delta = 1/(4J)$, is given by


a
ω_1
ω_2
b
ω_1
ω_2
c
ω_1
ω_2

$$\sigma_7 = -\tfrac{1}{2}(I_{ky} + I_{ly})(1 - \cos(\tfrac{1}{2}Jt_1)) - (I_{kx}I_{lz} + I_{lx}I_{kz})\sin(\tfrac{1}{2}Jt_1) + \tfrac{1}{2}(I_{kz} - \tfrac{1}{2})[I_{ly}\cos(\Omega_1 t_1) + I_{lx}\sin(\Omega_1 t_1)] - \tfrac{1}{2}(I_{kz} + \tfrac{1}{2})[I_{ly}\cos(\Omega_2 t_1) + I_{lx}\sin(\Omega_2 t_1)] - \tfrac{1}{2}I_{lx}\sin[\tfrac{1}{2}(\Omega_k - \Omega_l)t_1] - \tfrac{1}{2}I_{ly}\cos[\tfrac{1}{2}(\Omega_k - \Omega_l)t_1] + \tfrac{1}{2}(I_{lz} - \tfrac{1}{2})[I_{ky}\cos(\Omega_2 t_1) - I_{kx}\sin(\Omega_2 t_1)] - \tfrac{1}{2}(I_{lz} + \tfrac{1}{2})[I_{ky}\cos(\Omega_1 t_1) - I_{kx}\sin(\Omega_1 t_1)] + \tfrac{1}{2}I_{kx}\sin[\tfrac{1}{2}(\Omega_k - \Omega_l)t_1] - \tfrac{1}{2}I_{ky}\cos[\tfrac{1}{2}(\Omega_k - \Omega_l)t_1]. \quad [3]$$

The resulting spectra, Figs. 1c′ and 2c, have the look of the conventional SECSY and the improvements of SUPER SECSY.

Dipeptide Lys-Phe. Portions of SECSY and the SUPER SECSY2 spectra of the dipeptide Lys-Phe are given in Fig. 3, and show the improvements in the SECSY spectra. The diagonal peaks are very much reduced in intensity and several cross peaks have intensities larger than the corresponding diagonal peaks. Assignments of some of the resonances are also indicated in Fig. 3 (*22*).

RELAYED COSY EXPERIMENTS

RCOSY. The aim of relayed coherence transfer experiment, Fig. 4a, is to obtain cross peaks between uncoupled spins k and m, via their mutual couplings to a third spin l. The portion of the density operator which describes such a cross peak is, at the beginning of period t_2, given by (*21*):

$$\sigma_5^{(k\to l\to m)} = -2I_{lz}I_{my}\sin(\Omega_k t_1)\sin(\tfrac{1}{2}(J_{kl}t_1))\sin(J_{kl}\tau)\sin(J_{lm}\tau). \quad [4]$$

This term describes a cross-peak multiplet at $\omega_1 = \Omega_k$ and $\omega_2 = \Omega_m$ with antiphase doublet structure in both dimensions and with an amplitude determined by the function $\sin(J_{kl}\tau)\sin(J_{lm}\tau)$.

The experimental scheme for SUPER RCOSY is shown in Fig. 4b, and the relevant part of the density operator at the beginning of period t_2, for $\Delta_1 = 1/(4J_{kl})$ and $\Delta_2 = 1/(4J_{lm})$ is given by

$$\sigma_7^{(k\to l\to m)} = I_{mx}\sin(\Omega_k t_1)\cos(\tfrac{1}{2}(J_{kl}t_1))\sin(J_{kl}\tau)\sin(J_{lm}\tau) \quad [5]$$

which has in-phase multiplet structure in both dimensions. However, the cross peak at $\omega_1 = \Omega_m$ and $\omega_2 = \Omega_k$ requires $\Delta_1 = 1/(4J_{lm})$ and $\Delta_2 = 1/(4J_{kl})$ for in-phase multiplet structure in both dimensions. For simultaneous improvement of both cross peaks an intermediate value for Δ_1 and Δ_2 may be used.

DISCUSSION

In all the SUPER schemes mentioned in this paper, as well as in earlier communications of SUPER COSY (*18, 19*), the 180° pulses in the middle of Δ

FIG. 2. Absolute intensity experimental 2D spectra of nonexchangeable protons of uracil dissolved in D_2O forming an AX spin system with $J = 8.0$ Hz and $\delta = 1.75$ ppm; (a) SECSY, (b) SUPER SECSY1, and (c) SUPER SECSY2. The matrix used for time domain data in each case was 256 × 512 yielding a digital resolution of 4.7 Hz/point in ω_1 and 5.85 Hz/point in ω_2 domains. The delay parameter $\Delta = 31$ ms was used in (b) and (c). The accumulation time in all the three cases was identical and equal to ~30 min. Quadrature detection with quadrature phase and cancellation of P and axial peaks (*20*) was used in each case. However, for (c), the receiver phase was cycled such that the data was alternately added and subtracted. The spectra were recorded on a Bruker AM-500 spectrometer.

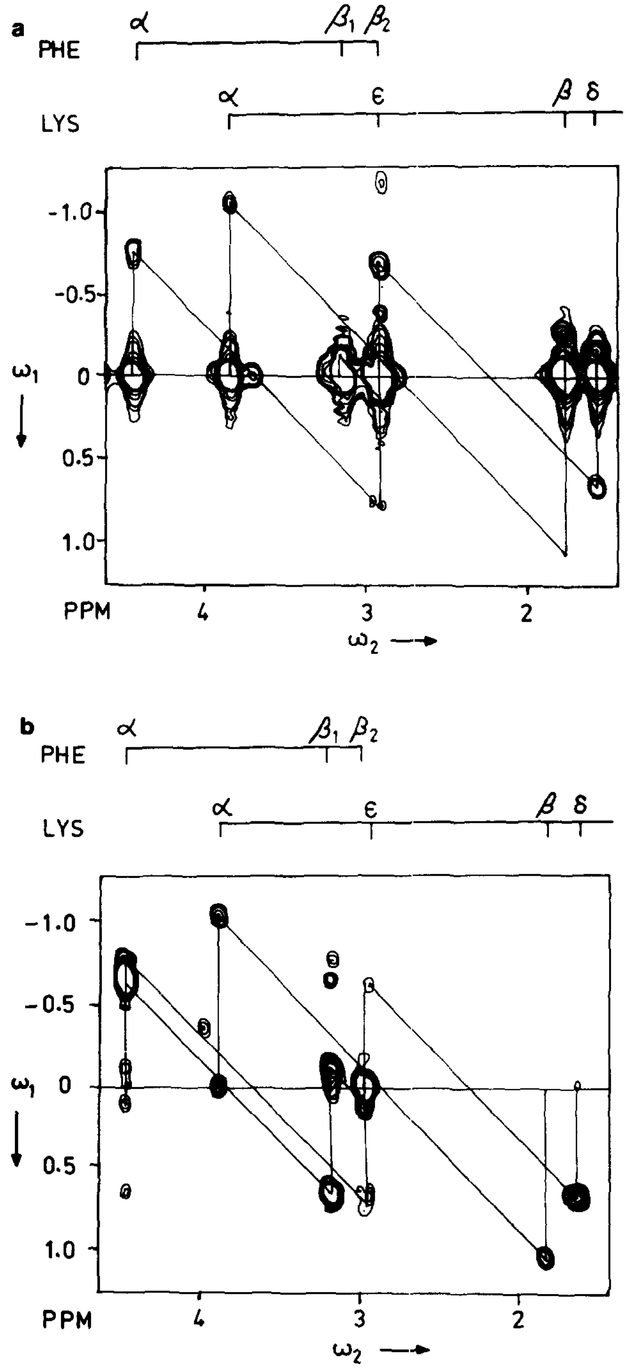


FIG. 3. Portions of (a) SECSY and (b) SUPER SECSY2 absolute intensity spectra of dipeptide Lys-Phe recorded under identical data accumulation and processing conditions. Data matrix used for time domain data was 128 × 512 (512 in t_2 domain) and 256 × 512 for 2D Fourier transformation, yielding a digital resolution of 15.9 Hz/point in both ω_1 and ω_2 dimensions. The delay parameter Δ = 40 ms, was used in (b). Some of the resonance assignments are indicated at the top.

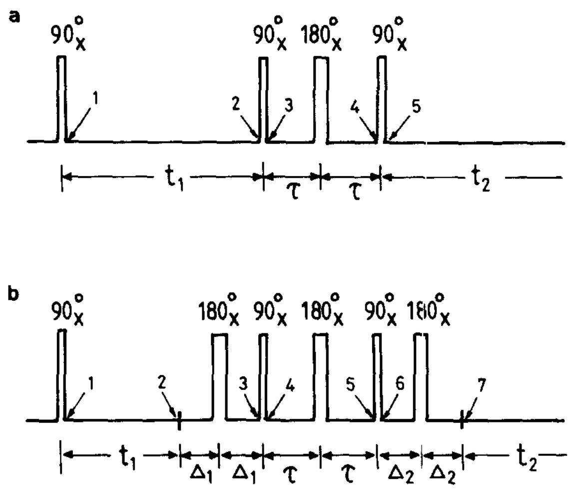


FIG. 4. The pulse schemes for (a) RCOSY and (b) SUPER RCOSY.

delays, make the SUPER schemes independent of frequency offsets. Elimination of these 180° pulses, will still bring the multiplets of each cross peak in-phase and diagonal peaks antiphase, but will lead to a frequency dependent absolute phase of each peak. When 2D spectra are desired in absolute-intensity mode, these additional 180° pulses can be eliminated, retaining the Δ delays.

A common limitation of all the SUPER schemes is that during the Δ delay periods there is a certain loss of magnetization due to transverse relaxation, T_2. This may, in some cases, require use of Δ smaller than optimum for in-phase multiplet structure.

In summary, the SUPER SECSY and SUPER RCOSY schemes achieve several improvements over conventional schemes under limited digital resolution conditions, such as in bilogical systems. The main advantages are better cross-peak intensities, reducing the accumulation time or sample concentration; reducing diagonal peak intensities, allowing detection of cross peaks near to the diagonal; and J tuning of correlated spectroscopy.

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