Recent Developments in Homocorrelated Two-dimensional NMR Spectroscopy

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I. Introduction

A. Background and Scope

Little would have Professor Jeener realized that the rather esoteric looking experimental trick he was about to describe in a summer school in 1971 (1), would develop into a powerful technique, transform the practice of NMR from one to two frequency dimensions and revolutionize the application of NMR to biomolecules. Since then, the conventional double resonance techniques, where transitions are selectively perturbed one by one, have almost been given up in favor of the more versatile, non-selective, large information bearing and almost easier to do experiments such as COSY, NOESY and their unending modifications.

The large number of developments in the field of two-dimensional NMR have been reviewed in sev-
eral articles and books, only a partial list of which is given herein (2-27). Particular attention may be drawn to three very recent books, two on techniques (24,25) and one on applications of two-dimensional NMR to biological systems (23). These cover a large portion of the subject and review the latest developments in the techniques, until their submission to the press. However, the field continues to grow at a rapid pace necessitating periodic reviews of various developments. The present article aims to describe recent developments in the experimental techniques of homonuclear shift correlation by coherent coupling (COSY) experiments. The article covers the SUPER schemes, the scaling experiments, bilinear and E.COSY schemes, pulse pair filtering, coherence transfer via longitudinal spin order, Z-COSY and rotating frame experiments.

B. Earlier Developments (24)

The original two pulse scheme of Jeener, which now has the well known acronym—"COSY", uses the sequence (90° - t1 - 90° - t2) (1,2). This yields a spectrum which is a function of two frequency variables F1 and F2, the diagonal of which reflects the one-dimensional NMR spectrum and the cross-peaks identify spin-spin coupling between corresponding diagonal peaks. It does so non-selectively and yields a large amount of information on directly coupled resonances. A variant, known as spin-echo correlated spectroscopy (SECSY: 90° - t1/2 - 90° - t1/2 - t2) also yields essentially the same information, with half the data size, but is less popular mainly due to the coupling information being displayed along a skew axis, the folding of cross-peaks in case of coupled resonances being more than half the spectral width away, and mixed phases of lines (28). Remote connectivities can be delineated by Relay COSY (90° - t1 -90° - τ - 90° - t2) or by multiple quantum transition (MQT) spectroscopy (2,29-32). Additional relay steps have been introduced in Relay COSY experiments (33) which matured into Total Correlation Spectroscopy (TOCSY) using Isotropic Mixing (34).

The multiple-quantum transition spectroscopy can be reduced to double quantum spectroscopy cancelling other orders, by co-adding several experiments with different phases (32,35). Multiple quantum transition spectroscopy requires a larger frequency scale along the F1 axis compared to the F2 axis leading to rectangular data matrices. This is reduced to conventional square matrices with single quantum transitions along both F1 and F2 being correlated, but the coherence transfer being restricted through a known multiple quantum order - the so called multiple-quantum-filtered COSY; the most popular and powerful of these being the double-quantum-filtered COSY (DQFC) (36). Higher order filtering is also used but leads to loss of total signal intensity and requires less than 90° phase shifters (36).

COSY experiments performed with a variable flip-angle of the second pulse (the mixing pulse) leads to selectivity in the coherence-transfer process and can identify connected and unconnected transitions (COSY-45), which lead to simplification of the spectrum and information on the relative signs of coupling constants (37). Phase purity of 2D spectra plays a crucial role, since it allows the possibility of recording pure absorption mode spectra, which have inherently higher resolution and a better signal to noise ratio. For this purpose phase cycling procedures have been utilized (38,39) and additional pulses, namely purging pulses and Z-filters, have been developed (40,41).

II. Recent Developments

A. Enhancing Cross-peak Intensity in COSY

1. SUPER Schemes

The cross-peak in a COSY or SECSY experiment is a multiplet consisting of a minimum of 4 peaks which have antiparallel (antiphase) intensity character in both F1 and F2 dimensions (Figure 1). Remote connectivities can be delineated by Relay COSY (90° - t1 -90° - τ - 90° - t2) or by multiple quantum transition (MQT) spectroscopy (2,29-32). Additional relay steps have been introduced in Relay COSY experiments (33) which matured into Total Correlation Spectroscopy (TOCSY) using Isotropic Mixing (34).

The multiple-quantum transition spectroscopy can be reduced to double quantum spectroscopy cancelling other orders, by co-adding several experiments with different phases (32,35). Multiple quantum transition spectroscopy requires a larger frequency scale along the F1 axis compared to the F2
Figure 1. Pulse schemes for two dimensional correlation experiments along with their schematic spectra for an AX spin system with $\Delta_1 = \Delta_2 = 1/(4J)$. (A) COSY (B) SUPER COSY (C) SECSY (D) SUPER SECSY 1 (E) SUPER SECSY 2.
Figure 2. (A) COSY and (B) SUPER COSY proton spectra of decapeptide LHRH recorded at 500 MHz. The two spectra were recorded under identical sample, data set and processing conditions. The digital resolution was 23.3 Hz/point in both dimensions the total accumulation time of 20 minutes for each case. The delay used in SUPER COSY was $\Delta_1 = \Delta_2 = 40$ ms (from ref. 42).

Figure 3. Absolute intensity 500 MHz experimental 2D spectra of AX spin system of nonexchangeable protons of uracil. (A) SECSY (B) SUPER SECSY 1 (C) SUPER SECSY 2. The digital resolution was 4.7 Hz/point in $\omega_1$ and 5.85 Hz/point in the $\omega_2$ dimensions. The delay parameter $\Delta_1 = \Delta_2 = 31$ ms was used in (B) and (C). Accumulation time in each experiment was $\sim 30$ m. (From ref. 47).
(42-47). The SUPER schemes incorporate appropriate delays, with a π pulse in the middle of the delay. These delays bring the antiparallel intensity components into parallel configuration and allow the COSY and SECSY experiments to be performed under limited digital resolution condition with significantly reduced experimental time (Figure 1). The magnitude of the delay depends on the spin-system and coupling values. For example for an AX spin system (each with spin 1/2), the delay $\Delta = 1/(4J)$ is required to bring the antiparallel components of the cross-peak into parallel configuration. Additionally, the diagonal peaks, which were in a parallel intensity configuration, acquire antiparallel (or antiphase) character and appear with reduced intensity under the low digital resolution condition. The pulse in the middle of the delay refocuses field inhomogeneity and removes the off-set dependence from the precession frequency of the spins during the delays. Figures 2 and 3 contain experimental results on SUPER COSY and SUPER SECSY respectively, clearly demonstrating the enhanced signal intensity of cross-peaks and reduced signal intensity of diagonal peaks in experiments utilizing limited digital resolution.

**Optimization of SUPER COSY**

A limitation of SUPER schemes is the loss of signal intensity during the Δ delays due to spin-spin ($T_2$) relaxation. During the delay the antiparallel components precess to become parallel and also decay due to $T_2$ relaxation, and therefore the optimum signal intensity is obtained for values of the delay smaller than the delay $\Delta_0$ needed for complete conversion of antiparallel components into parallel. The optimum delay $\Delta_{opt}$ for maximum signal intensity is a function of the spin-spin coupling J, digital resolution (Hz/point) and $T_2$. In the SUPER COSY scheme ($90°-t_1-\Delta_1-180°-\Delta_1-90°-\Delta_2-180°-\Delta_2-t_2(\text{Acq})$) the cross-peak intensity immediately after the second 90° pulse is obtained from the density operator (for a weakly coupled two spin system) given by

$$\sigma(t + 2\Delta_1) = -2I_{k2}I_{k5}[(\cos(\pi J t_1)) \sin(2\pi J \Delta_1)]
+ \sin(\pi J t_1) \cos(2\pi J \Delta_1) \sin(\omega_k t_1) \exp[-(t_1+2\Delta_1)/T_2].$$

Without delays in the $t_2$ dimension, the cross-peak multiplet is in antiparallel character with respect to $t_2$ and consists of two terms, one of amplitude $\sin(2\pi J \Delta_1) \exp(-2\Delta_2/T_2)$ which is in parallel intensity configuration with respect to $t_2$ and the other of amplitude $\cos(2\pi J \Delta_1) \exp(-2\Delta_2/T_2)$ which is in antiparallel intensity configuration with respect to $t_1$. For obtaining the optimum value of $\Delta_1$, the delay in the $t_1$ dimension, the optimum cross-peak intensity is obtained from eqn. 1 by finding the optimum $\sigma$ as a function of $\Delta_1$ for a given set of parameters J, $T_2$ and $(t_1)_{\text{max}}$. For example, for a fully resolved multiplet in the $t_1$ dimension, $(t_1)_{\text{max}} > > 1/J$ and $(\Delta_1)_{\text{opt}} \rightarrow 0$, while for a severely overlapped multiplet $(\Delta_1)_{\text{opt}}$ is given by the optimum value of $\sin(2\pi J \Delta_1) \exp(-2\Delta_2/T_2)$ as in the case of decoupling in the $t_2$ dimension or unresolved long range couplings (6,24). For a partially resolved multiplet both terms of eqn. 1 need to be retained. This optimum value has recently been calculated in a closed form for a two spin system and the results are plotted in Figure 4 as a function of $(\Delta_1)_{\text{opt}}/\Delta_0$ versus $J/(\text{Hz/point})$ for several values of $J/(\text{line width})$, $(48)$. A general conclusion of this work is that the optimum delay $\Delta_{opt}$ is less than $\Delta_0$ and decreases as either digital resolution or linewidth increase. In a given realistic experimental situation, the sample usually consists of several types of spin systems with varying spin patterns and coupling values; there is therefore no one optimum delay. The curves of Figure 4 provide the guidelines for choosing a compromise value. It may also be emphasised that increasing digital resolution in the $t_2$ dimension does not require extra experimental time as data can be collected during the relaxation delay between experiments. Therefore one may choose higher digital resolution during $t_2$ compared to $t_1$, and as a consequence smaller delays during $t_2$ compared to $t_1$, the optimum in each case being given by the curves of Figure 4. SUPER schemes often lead to mixed phase lineshapes and pulse schemes have also been suggested for obtaining pure phase SUPER COSY and SUPER SECSY spectra (47,48).

SUPER COSY may be tuned to optimize the delay for a particular coupling value and has proven useful in obtaining 4 and 5 bond couplings (46,49,50). Enhanced cross-peak intensity and reduced diagonal peak intensity have proved useful in resonance assignments in biological systems (51).
Several applications of SUPER COSY have recently been reported in studies of ferrocenophanes (52) and polymers (53).

2. Scaling Experiments

The idea of increasing the cross-peak intensity in COSY experiment without significant increase of the total experimental time has also led to several scaling experiments. The overlap of antiparallel components of cross-peak multiplets can be reduced by the idea of J-scaling, especially in the F1-dimension (54,55). This is achieved by adding an additional precession period during which chemical shift does not evolve, but the J coupling does. The additional period is designated as 2kt1, (Figure 5A), resulting in the scaling of J to J' = J (1 + 2k). This leads to a separation of antiparallel multiplet components, reducing their mutual cancellation and enhancement of cross-peak intensity (Figure 6B). While this scheme does increase the cross-peak intensity in a COSY experiment, it leads to an overlap of different cross-peak multiplets, reducing the resolution of different cross-peaks in the 2D spectrum.

The resolution in the spectrum can be enhanced by ω1-scaling, named COSS, for which two schemes have been suggested (56,57). The first of these, (Figure 5B), has a fixed delay added to the t1 period.

Figure 4 Plot of Δopt/Δ0 versus J/(Hz/point) for various values of J/(Linewidth) calculated for an AX spin system in SUPER COSY1 (90° – t1 – Δ – 180° – Δ – 90° – t2) (from ref. 48).
of which only a part ($k_t$) is utilized for chemical shift evolution (56). During the remaining part of the delay, chemical shift evolution is focussed by use of a $180^\circ$ pulse in the middle of the $(\Delta-k_t)$ part. While $J$ evolution takes place during the entire $\Delta$ period, it is not Fourier analysed since $\Delta$ is fixed and therefore there is no $J$-scaling in the experiment. However, the various multiplet components are having differential precession and the antiparallel components are coming in parallel character and vice versa. The optimum delay $\Delta$ should then be chosen according to consideration of Figure 4 and scaling considerations. The minimum delay in this scheme is $\Delta_{\text{min}} = k(t_1)_{\text{max}}$. In the second COSS scheme (Figure 5C), the interval between two $90^\circ$ pulses is designated as $(\Delta - t_1)$ and is decremented as $t_1$ increases, leading to resolution enhancement as well as $\omega_1$-scaling (57). The minimum delay in this

Figure 5. Pulse schemes for scaled COSY experiments: (A) J-scaling (B) $\omega_1$ scaling; COSS-I (C) $\omega_1$ scaling; COSS-II (d) S. COSY.
Figure 6. Portions of experimental two-dimensional NMR spectra of a mixture of oligonucleotides recorded using (A) Normal COSY (B) J-scaled COSY with J values scaled by a factor of 2 (C) COSS-I with chemical shifts scaled by 2, $t_1^{\text{max}} = 15.9$ ms and $\Delta = 15.9$ ms (D) COSS-II with chemical shift scaled by 2, $t_1^{\text{max}} = 15.9$ m sec and $\Delta = 71.4$ m sec (E) S. COSY with chemical shift scaled by 2 and J by 0.6 (from refs. 57 and 58).
scheme is \( \Delta_{\text{min}} = (2 + k)(t_1)_{\text{max}} \). In both schemes the chemical shift is scaled as \( \delta' = (1 + k)\delta \) while the \( J \) remains unscaled. These experiments lead to improved resolution in the 2D spectra (Figure 5C,D).

The \( \omega_1 \)-scaling and the \( J \)-scaling schemes have been combined into one scheme, named S.COSY, which allows independent scaling of \( \delta \) and \( J \), resulting in better resolution and cross-peak intensity (Figure 5D and 6E), (58). In this scheme the interval between the two 90° pulses is \( (\Delta - \gamma t_1) \) resulting in \( \delta' = (1 + k)\delta = \alpha \delta \) and \( J' = \gamma J \). In this scheme \( \alpha \) and \( \gamma \) can be independently chosen. For \( \gamma = 1 \) this scheme reduces to COSS-II (Figure 5C). For \( \gamma = 0 \) this scheme yields an \( \omega_1 \) decoupled shift scaled spectrum. The S.COSY scheme yields significantly improved COSY spectra (Figure 6E).

A \( J \)-scaling scheme has also been proposed for the SECSY experiment (56,59). An attractive feature of scaling in SECSY is that it is sufficient to carry it out in the \( F_1 \) dimensions since the cross-peak multiplet pattern yields a parallel intensity character for near neighbor lines in the \( F_2 \) dimension (56,59). Earlier, another scaling scheme for COSY had been proposed in which the shifts are scaled by \( (k-2) \) and the \( J \) values by a factor \( k \) (60).

An important consideration in the scaling experiments is the accompanying scaling of linewidths in the spectrum. Linewidths are scaled to a lower level than the \( \delta \) and \( J \), resulting in enhanced resolution. A detailed discussion on the resolution and sensitivity of the various scaling experiments has recently been given (57).

### B. Restricting Coherence Transfer in COSY

The COSY spectrum of a coupled spin system, under complete resolution of the multiplet structure, gives in general, a large number of cross-peaks and often it is desirable to simplify the spectrum by restricting the coherence transfer to a limited number of transitions. Early efforts in this direction were restricted to changing the flip angle of the coherence transfer pulse – smaller flip angles reducing the intensities of the cross-peak between transitions which do not share a common energy level (the so-called unconnected transitions in 2D spectroscopy) – thereby giving a simpler spectrum and information on relative signs of coupling constants (6-8,24). Recently, this idea has been further extended and several pulse sequences suggested for achieving better selectivity in the coherence transfer pathways.

#### 1. Bilinear COSY (61)

Figure 7A contains the normal COSY spectrum of a three coupled spin system AMX (each spin 1/2) and shows cross-peaks from one to every 12 transitions in the spectrum yielding all the possible 288 peaks in the COSY spectrum including peaks in the +\( F_1 \) (P peaks) and -\( F_1 \) (N peaks) domains. This spectrum is obtained with a non-selective 90° mixing pulse. Figure 7B contains the COSY spectrum of the same spin system obtained by using the Bilinear Rotation (BIRD) sequence \( [(\pi/2)x - \gamma/2 - (\pi)x - \gamma/2 - (\pi/2)x] \) instead of a single \( (\pi/2)x \) pulse (61). In weakly coupled spins the coherence transfer via Bilinear rotation restricts coherence transfer to certain coherences. In order to describe these selection rules, define parity numbers to the eigenstate \( |r> \) as

\[
\Pi_{1...N}^\delta = 2^{N-1} \prod_{k=1}^{N} m_k(r) \tag{2}
\]

and for coherences between states \( |r> \) and \( |s> \) as

\[
\Pi_{1...N}^s = \Pi_{1...N}^\delta - \Pi_{1...N}^\delta \tag{3}
\]

where \( m_k(r) \) is the magnetic quantum number of spin \( k \) in the eigenstate \( |r> \) of \( N \) weakly coupled spins. For a coupled spin-system consisting each of spin \( 1/2 \), \( \Pi_{1...N}^s = +1/2 \) or \( -1/2 \). All even quantum coherences have \( \Pi_{1...N}^s = 0 \) and all odd quantum coherences have \( \Pi_{1...N}^s = 1 \) or \( -1 \). The above given bilinear rotation sequence preserves the number of the coherences during the coherence transfer process. In a single-quantum to single-quantum correlation experiment (COSY) the bilinear mixing, therefore, gives only half the number of cross-peaks compared to a general \( (\pi/2)x \) pulse. For example in the three coupled spin 1/2 system described above, for the eigenstate \( \alpha\alpha\alpha \), \( \Pi_{1...N}^s = +1/2 \), while for \( \alpha\alpha\beta \) it is \( -1/2 \). Therefore \( \Pi_{1...N}^s = +1 \) while \( \Pi_{1...N}^r = -1 \) and a coherence transfer from \( (rs) \) to \( (rs) \) is allowed while \( (rs) \) to \( (sr) \) is forbidden. This means that the diagonal peak will appear only in the +\( F_1 \) domain (P peaks or +1 quantum quadrant) and not in the -\( F_1 \) domain (N peaks or +1 quantum quadrant). The 144 peaks that appear in the two-dimensional multiplets resemble noughts and crosses (Figure 7 (II))
Figure 7. (I) Absolute value two-dimensional spectrum of the protons of 1,2-dibromopropionic acid forming an AMX spin system obtained by using a 90° mixing pulse. The top half of spectrum contains (-1) quantum peaks and bottom half the (+1) quantum peaks. Expanded plots of three (+1) quantum regions marked a, b and c are also shown. All sixteen peaks in each multiplet are clearly seen in the expansions. (II) Same as (I) except the spectrum has been obtained by using a bilinear mixing sequence instead of 90° mixing pulse (61) with $\tau = 50$ ms. The "noughts" and "crosses" are clearly seen in the spectrum. (III) Same as (II) but with $\tau = 5$ ms. The number of peaks is further reduced in this experiment (from ref. 61).
This form of cross-peak pattern allows determination of relative signs of coupling constants by inspection. For the +1 quantum peaks (N peaks) in a three spin AMX system, a “Nought” for AM cross-peak indicates identical sign for $J_{AX}$ and $J_{MX}$ while a “Cross” indicates opposite sign. The role of “Noughts” and “Crosses” is reversed in -1 quantum peaks (P peaks). According to Figure 7 (II) $J_{AM}$ and $J_{AX}$ have the same sign opposite to that of $J_{MX}$. These experiments differ from small flip angle COSY in the type of selected peaks and in having zero intensity of rejected peaks assuming ideal pulses and weak coupling.

The above spectrum was recorded for $\tau = 50$ ms. Reduction of $\tau$ to 5 ms, further restricts the coherence transfer and only 72 peaks are observed in the AMX spectrum Figure 7 (III) (61). In this case only the linear term in the mixing operator is retained leading to a more restricted selection rule, viz. for the cross-peak between spins j and k, a reduced parity number

$$\Pi_{j,k}^{P} = \Pi_{j,k}^{P} - \Pi_{j,k}^{’}$$

is conserved. Here $\Pi_{j,k}^{P} = 2m_{j}m_{k}$, the product involving the magnetic quantum numbers of the involved spins in each eigenstate rather than all the spins. For example, for both the coherences $\alpha\alpha\alpha \rightarrow \beta\alpha\alpha$ and $\alpha\alpha\alpha \rightarrow \alpha\beta\alpha$ $\Pi_{AM} = +1$ and the AM cross peak between them is allowed, while the coherence $\beta\alpha\beta \rightarrow \beta\beta\beta$ has $\Pi_{AM} = -1$ and the cross-peak between this and $\alpha\alpha\alpha \rightarrow \beta\alpha\alpha$ is not allowed. Both cross-peaks will be allowed in bilinear mixing using a longer mixing time experiment since $\Pi_{AX}$ for the above 3 coherences is +1. In the short mixing time limit experiments (2$\pi\tau < 1$), the two-dimensional multiplet contains only 4 peaks (instead of 16 in COSY and 8 in bilinear COSY) of significant intensity and the +1 quantum quadrant (N peaks) contains no diagonal peaks (61).

It may be pointed out that since in these experiments, utilizing bilinear mixing operators, the N and P peak quadrants have non-identical intensities, the resulting spectra do not have pure phases. On the otherhand, with selection of +1 quantum peaks (N-peaks) by phase cycling, the bilinear COSY provides a spectrum having information similar to small flip angle COSY, but without diagonal and auto peaks and with a simplified cross-peak pattern allowing higher resolution and yielding relative signs of coupling constants by inspection. For longer mixing times the bilinear COSY acts like a Relay COSY, but with the selection rule still operative (61).

2. E. COSY

This method, which basically combines several experiments with different flip angle of the mixing pulse, results in coherence transfer exclusively to connected (or unconnected) transitions of weakly coupled spins (“exclusive” hence E.COSY). The experiment uses the sequence $[(\pi/2)_{x} - t_{1} - \beta(t_{2}) - (\pi/2)_{x} - t_{2}]$ or a more accurate but equivalent sequence $[(\pi/2)_{y} - t_{1} - (\pi/2)_{y}(\pi/2)_{y} - t_{1} - (\pi/2)_{y}(\pi/2)_{y} - t_{2}]$ where the variable angle $\beta$ appears as a phase shift (62). The latter sequence is basically a sequence for multiple-quantum (MQ) filtering and the E.COSY experiment can also be visualized as a combination of several orders of MQ filtered COSY. A combination of p-quantum filtered COSY where $p = 2, 3, 4...$ in the ratio 1:2:4... results in a cancellation of unconnected peaks in the 2D spectrum and retention of connected peaks which then appear in pure phase (62). For a weakly coupled three spin system, combination of two and three quantum filtered spectra in the intensity ratio 1:2 results in efficient cancellation of unconnected transitions.

The optimum sensitivity for E.COSY is achieved by the following linear combination of phase-shifted experiments $[4(0°) - 3(60°) + 1(120°) + 1(240°) - 3(300°)]$, where the angles in the brackets give $\beta$ and the numbers outside are the weights for each experiment (61). Enhanced resolution of the E.COSY spectrum allows determination of the magnitudes of $J$-couplings and the information on connectivity of transitions yields their relative signs. Detailed analysis of the E.COSY experiment has been recently given with generalization to multiple spin systems and optimization of pulse angles (63).

Another method, named primitive E.COSY (P.E.COSY), for obtaining spectra which have information content similar to E.COSY, has recently been suggested (64). This method co-adds experiments given by (i) $(\pi/2)_{y} - t_{1} - (35)^{2}\phi_{2} - t_{2}$ (ii) $(\pi/2)_{y} - t_{1} - (35)^{2}\phi_{3} - t_{2}$ and (iii) $(\pi/2)_{y} - t_{1} - (0) - t_{2}$ with $\phi_{1} = 0°, 0°, 90°, 90°, 180°, 180°, 270°, 270°; \phi_{2} = 0°, 180°, 90°, 270°, 180°, 0°, 270°, 90°; \phi_{3} = 90°, 270°, 180°, 0°, 270°, 90°, 90°, 0°$ and $\phi_{\text{receiver}} = \phi_{1}$. The primitive E.COSY experiment can be improved and simplified by the use of an Al-
ternate scheme (A.E.COSY) (65). The A.E.COSY needs no phase cycling and utilizes only four experiments \[+(0°), -(60°), -(90°), +(120°)\] where the angle \(\beta\) is given in brackets and the sign outside indicates addition or subtraction of the experiments of the sequence \([90° - t_1 - \beta - t_2]\). The ratio of connected to unconnected peak intensity in a three spin system AMX is 7 in A.E.COSY compared to 5.8 in COSY-45, 10 in P.E.COSY and infinity in E.COSY. All the E.COSY schemes yield cross peaks in pure phase, but the diagonal peaks have mixed phases. This phase mixing is less severe in E.COSY and A.E.COSY compared to P.E.COSY (65). Figure 8 shows the result of the A.E.COSY experiment in a three spin system and clearly demonstrates discrimination between connected and unconnected transitions and pure phase on cross-peaks (65).

C. Phase Purification of MQ-filtered COSY and Coherence transfer via longitudinal spin-order

Multiple-quantum filtered COSY in which the magnetization transfer is filtered through a well defined multiple-quantum coherence order has several advantages, such as simplification of spectra, reduced dynamic range of intensities and pure phase 2D spectra (23,24). The N-quantum filtered COSY of N coupled spins (each of spin 1/2) has pure phase for all the peaks even under conditions of strong couplings (66). However, the diagonal peak multiplets of p-quantum filtered COSY (for \(p < N\)) has mixed phase even under conditions of weak couplings (67). Pure phase absorption mode spectra have inherently higher resolution and are much desired even if they have less signal intensity.

1. 45° Pulse-pair Filter

A 45° pulse pair has recently been suggested for obtaining pure phase \(p\)-quantum filtered COSY (68). The first 45° pulse converts transverse coherences partially into multipin longitudinal order and the second 45° pulse partially converts these into detectable magnetization. The transverse coherences during the interval between the two 45° pulses are cancelled by random variation of the interval and co-addition of resulting signals. The 45° pulse-pair filter converts some observable and non-observable terms into detectable terms of a single phase. For
example the observable terms for \((\pi/4)_y - \tau - (\pi/4)_y\) with \(\tau\)-jitter are (68):

\[
\begin{align*}
I_{kz} &\to -(1/2)I_{kz}; \\
I_{kz}I_{lz} &\to -(1/4)(I_{kz}I_{lz} + I_{kz}I_{lz}) \\
I_{kz}I_{lz}I_{mz} &\to -(1/8)I_{kz}I_{lz}I_{mz} + I_{kz}I_{lz}I_{mz} \\
+I_{kz}I_{lz}I_{mz}) \\
I_{kz} &\to -(1/2)I_{kz} \\
I_{kz}I_{lz} &\to -(1/4)(I_{kz}I_{lz} + I_{kz}I_{lz}); \\
I_{kz}I_{lz} &\to -(1/4)(I_{kz}I_{lz} + I_{kz}I_{lz}) \\
+I_{kz}I_{lz}I_{mz})
\end{align*}
\]

etc. (5)

All terms having \(I_y\) operator terms are eliminated by the \(\tau\)-zitter, giving rise to pure phase spectra.

The detectable part of the signal in the double-quantum-filtered-COSY (DQFC) experiment at the start of the detection period \(t_2\), for a three spin system (each of spin 1/2) (Figure 9A) is given by

\[
\begin{align*}
\sigma^\text{cross} &= -(1/2)C_k\{S_{kl}C_{km}2I_{kz}I_{lz} + C_{kl}S_{km}2I_{kz}I_{mz}\} \\
\sigma^\text{dia} &= -(1/2)C_k\{S_{kl}C_{km}2I_{kz}I_{lz} + C_{kl}S_{km}2I_{kz}I_{mz}\} \\
&-S_{kl}S_{km}4I_{ky}I_{lz}I_{mz}\}
\end{align*}
\]

where \(C_k = \sin(\omega_k t_1); C_{ij} = \sin(2\pi I_{ij} t_1); S_{ij} = \cos(2\pi I_{ij} t_1)\). The \((\pi/4)_y - \tau - (\pi/4)_y\) filter, Figure 9B, removes the mixed phase terms \(S_{kl}S_{km}4I_{ky}I_{lz}I_{mz}\) and multiplies the remaining terms by \(-(1/2)\), yielding pure phase spectra of reduced intensity (68).

2. Generalized Pulse Pair Filtering

The 45° pulse-pair-filter has recently been generalized as a \((\alpha_y - \tau - \beta_y)\) filter for arbitrary filtering of various product operator terms (69). In a coupled N spin 1/2 system, the possible product operator terms consist of 1, 2...n (n \(\leq N\)) spin operator terms, \(p\) of which are transverse and \(h\) longitudinal operators with \(p + h = n\). If the first pulse of the pair has phase \(y\), then only terms which do not have \(y\) spin operators are retained. The retained terms are, for example, \(I_{kz}, I_{kz}, I_{kz}I_{lz}, I_{kz}I_{lz}, I_{kz}I_{lz}I_{mz}, I_{qz}, etc.\)

The flip angle dependence of conversion of these terms into longitudinal spin-order by an \(\alpha\)-pulse is given by \((\sin \alpha)^p (\cos \beta)^h\) and their conversion into detectable magnetization by the \(\beta\) pulse is given by \((\sin \beta) (\cos \beta)^{n-1}\). The optimum detection of various terms through the generalized \((\alpha_y - \tau - \beta_y)\) filter is given in Table I.

The single-spin transverse order \(I_{kz}\) is optimally detected by the use of a 90° pulse-pair; the so called \(z\)-filter (41). The optimum filtering of antiparallel (antiphase) magnetization \(I_{kz}I_{lz}I_{mz}\) resulting from triple-quantum filtering is optimally detected by a 35.3° pulse pair, etc. The two-spin order \(2I_{kz}I_{lz}\) is optimally filtered by a 90° - \(\tau - 45°\) pulse pair, while antiparallel two-spin order \(4I_{kz}I_{lz}I_{mz}\) is optimally filtered by a \((54.7° - \tau - 35.3°)\) pulse pair (69).

3. Coherence Transfer via Longitudinal Spin-Order (CLOSY)

The above method of filtering suggests an alternate method of coherence transfer via longitudinal spin-order. Prior to this, experiments such as COSY, Relay COSY, MQT, MQFC, etc. all utilize transfer of coherence via transverse spin-order. The method of coherence transfer via transverse spin-order often leads to mixed phase line shapes in 2D spectroscopy. The method of coherence transfer via an intermediate state of longitudinal spin order leads to pure phase 2D spectrum irrespective of the strength of coupling (weak or strong) and number of coupled spins. The method utilizes pulse-pair and needs no phase cycling. Phase purity of the method is insensitive to pulse imperfections (r.f. inhomogeneity, off-set effects and pulse- width errors) which only lead to intensity anomaly (69).

The pulse scheme for coherence transfer via longitudinal two-spin-order (CLOSY 2) is \([90° - \tau - 45°\} - 180° - 45° - \tau - 45°\} - t_2\], where the \(\{180°\}\) pulse immediately after the first 45° pulse is applied in alternate scans, for same value of \(t_1\), and the two experiments co-added, Figure 9C (69).

The 90° pulse creates single quantum coherences, which are frequency labelled during \(t_1\) period and develop antiparallel magnetization due to J-coupling evolution. The first 45° pulse converts part of these into longitudinal two-spin order and the remaining transverse components are cancelled during the \(\tau\) period by random variation of \(\tau\) during different scans of the two-dimensional experiment. The second 45° pulse converts part of the longitudinal two-spin order into observable magnetization which is detected during \(t_2\). The 180° pulse eliminates longitudinal order involving odd number of
spins. The detectable part of the density operator, at the start of the $t_2$ period for 3 weakly coupled spins (each of spin $1/2$) resulting from the $k$ spin transverse magnetization during $t_1$ period is given as
\[
\sigma_{\text{dia}} = (1/4)S_k[C_{kl}S_{km}2I_{kz}I_{mx} + C_{km}S_{kl}2I_{kz}I_{lx}]
\]
\[
\sigma_{\text{cross}} = (1/4)S_k[C_{kl}S_{km}2I_{kz}I_{mx} + C_{km}S_{kl}2I_{kz}I_{lx}]
\] (7)

Figure 9. Pulse schemes for (A) Multiple-quantum filtered COSY (MQFC) (B) MQFC + 45° pulse pair filter (C) Coherence transfer by longitudinal 2 spin order (CLOSY 2).

Comparison of eqn. 7 with eqn. 6 shows that in this experiment the resulting spectrum is in pure phase and the signal intensity is identical to double-quantum-filtered COSY with 45° pulse-pair-filtering. This has been achieved here (Figure 10) with simpler pulse scheme which needs no phase shifters (69).

In higher order spin systems (for $N = 4$ or 5) the 4 spin-order will also give detectable signal in the above CLOSY 2 scheme. The 4 spin order terms can be largely eliminated by subtracting from CLOSY 2, the following two experiments $[90° - t - 15° \{180° - t\}] - (\tau - 45° - t)$, where again the $\{180°\}$ pulse is applied in alternate scans. This results in a reduction of
Table I. The optimum flip angle for creation of longitudinal spin order by the $\alpha_y$ pulse and detection by the $\beta_y$ pulse of various spin operator terms by the pulse pair ($\alpha_y - \tau - \beta_y$) (69).

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Spin Operator Terms</th>
<th>$p$</th>
<th>$h$</th>
<th>$\alpha^0$</th>
<th>$\beta^0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$I_{kz}$</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>$I_{kx}$</td>
<td>1</td>
<td>0</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>$2I_{kz}I_{lx}$</td>
<td>1</td>
<td>1</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>4</td>
<td>$4I_{kz}I_{lz}I_{mx}$</td>
<td>1</td>
<td>2</td>
<td>35.3</td>
<td>35.3</td>
</tr>
<tr>
<td>5</td>
<td>$8I_{kx}I_{lz}I_{mx}I_{qz}$</td>
<td>1</td>
<td>3</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>$2I_{kz}I_{lx}$</td>
<td>2</td>
<td>0</td>
<td>90</td>
<td>45</td>
</tr>
<tr>
<td>7</td>
<td>$4I_{kx}I_{lz}I_{mx}$</td>
<td>2</td>
<td>1</td>
<td>54.7</td>
<td>35.3</td>
</tr>
<tr>
<td>8</td>
<td>$8I_{kx}I_{lz}I_{mx}I_{qz}$</td>
<td>2</td>
<td>2</td>
<td>45</td>
<td>30</td>
</tr>
<tr>
<td>9</td>
<td>$4I_{kx}I_{lz}I_{mx}$</td>
<td>3</td>
<td>0</td>
<td>90</td>
<td>35.3</td>
</tr>
<tr>
<td>10</td>
<td>$8I_{kx}I_{lz}I_{mx}I_{qz}$</td>
<td>3</td>
<td>1</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>11</td>
<td>$8I_{kx}I_{lz}I_{mx}I_{qz}$</td>
<td>4</td>
<td>0</td>
<td>90</td>
<td>30</td>
</tr>
</tbody>
</table>

Figure 10. Diagonal and cross-peak regions of the (A) COSY (B) CLOSY 2 spectra of the ABX spin system of protons of asparagine recorded at 270° MHz. The digital resolution was 0.8 Hz/point in both dimensions and in (B) $\tau$ was varied from 1 ms to 28 ms in steps of 1 ms in the 256 $t_1$ experiments (from ref. 69).
2-spin-operator terms by a factor of 4 while the 4-spin-operator terms are reduced by a factor of 16 (69). For spin systems with \( N > 5 \), it is suggested that the method of multiple quantum filtering with \((\alpha - \tau - \beta)\) filter be utilized in a manner indicated in Table I.

4. Correlation of Connected Transitions; Z-COSY

The above method [CLOSY 2; 90° - \( t_1 \) - 45° \( \{180° \} - \tau - 45° \) - \( t_2 \)] yields identical intensities for connected and unconnected transitions of the 2D spectrum. In order to enhance the information content of the experiment, in a manner similar to E.COSY, the angles \( \alpha \) and \( \beta \) of the pulses can be changed which lead to selective reduction in the intensity of either connected or unconnected transitions. From Table I, it is seen that optimum filtering of terms \( 2I_{xz}I_{xz}, 4I_{xz}I_{xz}I_{ixz} \) and \( 8I_{xz}I_{xz}I_{ixz}I_{ixz} \) need respectively 45°, 35.3° and 30° pulse pairs (having \( \alpha = \beta \)), yielding an intensity ratio of 1.0, 0.6 and 0.4 respectively for the double, triple and four quantum filtering. However, as in E.COSY, these should be combined in the ratio 1:1:1 for ideal suppression of unconnected transitions. The intensities for these terms are given by \((\sin \alpha)^{p+1}(\cos \alpha)^{2n-\alpha}\) where \( p \) is the number of transverse and \( n \) the number of total spin operators. The ratio 1:1:1 is obtained for \( \alpha \rightarrow 0 \), which causes no coherence transfer. A compromise between the ratio of connected to unconnected intensity, with finite intensity of connected transitions in these experiments (named Z-COSY (70)) is reached with the use of a small flip angle \( \alpha \). The intensity ratio of connected to unconnected transitions for a three spin system is 64 for \( \alpha = 10° \) and 6 for \( \alpha = 30° \) (70). The latter is about the same as in COSY-45° (37). The signal intensity of Z-COSY versus COSY-45° has been given as (70).

\[
\frac{(S)_{Z-COSY}}{(S)_{COSY}} = \frac{\sin^2 \alpha \cos^2(n-1)(\alpha/2)}{\sin^2 \beta \cos^2(n-1)(\beta/2)}
\]

Compared to COSY-45, this ratio varies from 0.07 to 0.09 for \( N = 2 \) to 4 for \( \alpha = 10° \) and from 0.43 to 0.45 for \( N = 2 \) to 4 for \( \alpha = 30° \). The Z-COSY experiment \((90° - t_1 - \alpha - \tau - \alpha)\) utilized for a high connected to unconnected intensity ratio has poor signal intensity compared to COSY-45, but has pure phases (70).

The intensity of unconnected transitions can be increased and that of connected transitions decreased by a complementary experiment named Anti Z-COSY which uses the sequence 270° - \( t_1 \) - \((\alpha + \beta) - \tau - \alpha\), with \( \alpha = 10° \) to 30° (70). Application of Z-COSY and Anti Z-COSY for pattern recognition has been recently given (71).

To supplement the cancellation of transverse components during the randomly variable \( \tau \) period, the last pulse (the \( \beta \) pulse, is phase cycled along with the receiver phase in 90° increments (causing cancellation of all orders except \( p = \pm 4k \), where \( k = 0, 1, 2,... \)) (70). The \( \tau \)-jitter is then mainly utilized for cancellation of zero-quantum coherences. It may further be pointed out that during the \( \tau \) interval there can be transfer of magnetization from one spin to another via relaxation. To avoid any significant transfer by relaxation the \( \tau \) period should be kept small (< 20 - 30 ms).

**Phase Purity Under Strong Coupling**

It is a general property of the method of coherence transfer via longitudinal spin order, in which the transverse components are eliminated by \( \tau \)-jitter, that the resulting spectra are all in pure phase, irrespective of the strength of coupling and number of coupled spins (69). The phase of the first pulse of the pair determines the transverse component retained. For example, if the first pulse is an \( x \) pulse, all spin operators having \( I_x \) operators are eliminated and only \( I_y \) and \( I_z \) operators and their products are retained. This results in pure phase spectra irrespective of the number of coupled spins. To prove that strong coupling does not mix phases, consider the density operator at time \( t_2 \) in the experiment \((90° - t_1 - \alpha - \tau - \beta - t_2)\). It is given by

\[
\sigma(t_1, t_2) = e^{-iHt_2}[P_Y(\beta)]e^{-iH\tau}[P_Y(\alpha)]e^{-iHt_1} \times I_x e^{iHt_1}[P_Y(\alpha)]^{-1} e^{iH\tau}[P_Y(\beta)]^{-1} e^{iHt_2}
\]

Of this only the diagonal part is projected out at the end of \( \tau \) period in these experiments. That is

\[
\sigma(t_1, \tau) = e^{-iH\tau}[P_Y(\alpha)]e^{-iHt_1} I_x e^{iHt_1}[P_Y(\alpha)]^{-1} e^{iH\tau}
\]

is purely diagonal. Taking the matrix elements in the eigenbase of \( H \), the signal at the end of the \( t_2 \) period is given by

\[
S(t_1, \tau, t_2) = \sum_{a,b,c,d} Z_{abcd} e^{i\omega_{ab}t_2} e^{-i\omega_{cd}t_1}
\]
where,

$$Z_{abcd} = \sum_e (I_e)_{ab} [P_Y(\beta)]_{be} [P_Y(\alpha)]_{ec} (I_e)_{cd}$$

$$\times [P_Y(\alpha)]_{de}^{-1} [P_Y(\beta)]_{ea}^{-1}$$

(12)

The amplitude in the $-\omega_1$ domain is given by

$$Z_{abcd} = \sum_e (I_e)_{ab} [P_Y(\beta)]_{be} [P_Y(\alpha)]_{ec} (I_e)_{dc}$$

$$\times [P_Y(\alpha)]_{le}^{-1} [P_Y(\beta)]_{ea}^{-1}$$

(13)

Therefore this experiment yields pure phase spectra, irrespective of the coupling strength, since the above calculation is in the eigenbase.

Recently detailed analysis of strong-coupling patterns has been reported in experiments utilizing pulse-pair filtering via longitudinal spin-order (72).

D. Coherence Transfer in the Rotating Frame

The conventional COSY experiment yields cross-peaks only between directly coupled spins. A Relay COSY relays the coherence over one additional step of coupling and multiple relay leads to a total correlation of coupling networks (TOCSY) and to the ideas of isotropic mixing (24,34). These are achieved by application of 180° pulses at rapid intervals of 2$T$. The mixing period, consisting of $(r-180°-r)n$, can also be replaced by a continuous radiofrequency field which leads to correlation of spins in the rotating frame through J coupling. If the effective fields in the rotating frame of the two coupled spins, are nearly identical in magnitude, then as in the case of solid state NMR, they can exchange magnetization through what is now popularly known as homonuclear Hartmann-Hahn cross-polarization (HOHAHA) (73,74). This has proved to be an extremely powerful method for correlation of spins across complete coupling networks.

The experimental scheme consists of a non-selective 90° pulse followed by the evolution period $t_1$, a mixing period consisting of continuous radiofrequency (r.f.) of duration and amplitude $B_1$, and the detection period $t_2$ (Figure 11A)) (73,74).

The continuous r.f. applied in the middle of the spectrum, spin-locks part of the magnetization of each spin along their respective effective fields. The effective fields are given by $(\nu^2 + \nu_A^2)^{1/2}$ and $(\nu^2 + \nu_B^2)^{1/2}$ for spins A and B for which the offsets are respectively $\nu_A$ and $\nu_B$ from the carrier frequency of the spin-locking field and $\nu$ is the amplitude of the spin locking field expressed in frequency units. If $\nu >> \nu_A, \nu_B$, the effective fields are nearly identical, causing oscillatory transfer of magnetization between the two spins at a frequency given by J, the coupling parameter between the two spins. Since in the rotating frame the quantization fields are much less than $B_0 (\nu << B_0)$, the chemical shifts are correspondingly scaled down, making the spin systems behave as if they are very strongly or isotropically coupled having little chemical shift between them. If there is more than one coupling value in the spin system, the oscillatory transfer is a complicated function. The oscillatory transfer is often damped due to r.f. inhomogeneity and relaxation. The rate of transfer is dependent on the difference in the effective fields and the strength of coupling.

The theory for these transfers has been discussed in the context of heteronuclear Hartmann-Hahn cross-polarization in liquids (75,76) and the same applies in the homonuclear case discussed here. An effective magnetization transfer takes place between protons A and B, if $|(\nu_A - \nu_B)/2| < |J_{AB}|$ at a rate that depends on $J_{AB}$ and $(|\nu_A| - |\nu_B|)$. This requires large r.f. field $\nu$, for an effective transfer over spins which are J coupled but have shift differences from the carrier of varying amount such that the above inequality is satisfied.

The restriction imposed by the above inequality can be largely overcome by periodically alternating the phase of the applied r.f. at a rate $1/T_m$ [Figure 11B]. To prevent rotary echo effects, the total durations that the r.f. fields are applied along +x and -x axis are made different. The effective magnetization transfer takes place at a rate given by $J_{AB}$ provided (73,74),

$$T_m(\nu_A^2 - \nu_B^2) < \nu$$

(14)

With this modification the requirement of large r.f. fields is largely overcome and efficient transfers are possible between all coupled spins. In this technique a major advantage is that net magnetization transfers take place in contrast to COSY; as a result there is no cancellation of cross-peak intensity due to limited resolution. Net magnetization transfer has the additional advantage that the magnetization can be
Figure 11. Experimental schemes for cross-correlation in rotating frame using: (A) spin-lock, (B) phase alternated spin-lock, (C) MLEV-17 sequence, (D) $(\beta - \tau)$ sequence.

Further relayed to additional coupled spins and as a result cross-peaks are obtained between complete networks of coupled spins (73). A further advantage of the technique is that the peaks are largely in pure phase yielding high resolution in crowded regions. The absorptive part is in parallel intensity configuration while the dispersive part is in antiparallel intensity configuration, partially cancelling the dispersive character (73).

For efficient coherence transfer, the chemical shift between coupled resonances should be either absent or equal and opposite from the carrier. This aspect has been earlier utilized in schemes such as TOCSY, which use one of several sequences of rapid
r.f. pulses during the mixing period, so that effectively the chemical shift is removed from the Hamiltonian. The spins achieve the state of isotropic mixing, yield net coherence transfers and cross correlation over complete networks of coupled spins. The TOCSY 2D spectra have pure absorption mode signals with peaks of positive intensity (34). Recently another method has been suggested in which the mixing period is based on the composite pulse decoupling cycle MLEV-16 (77,78). In the MLEV-16 sequence (ABBA, BBAA, BAAB, AABB where A is a composite 180° rotation about the x axis (90°_y, -180°_x, 90°_y) and B is the inverse of A (90°_y, -180°_x, 90°_y)) an extra 180° pulse has been added at the end, to make it less sensitive to pulse imperfections and this sequence is named MLEV-17 (78). The mixing period consists of an integral number of MLEV-17 sequences, preceded and followed by short spin locking pulses (5 ms) along x axis, (Figure 11 (c)). These two short spin-locking pulses serve to diffuse any magnetization not parallel to the x axis to ensure that the 2D spectrum can be phased to pure absorption. This method again provides net magnetization transfer over a substantial spectral width with only limited r.f. power and has the added advantage that the apparent decay constant of the spin-locked magnetization is prolonged by about a factor of two over the continual r.f. T1p (78).

It should be pointed out that the experiments involving spin-lock of magnetization in the rotating frame give, in addition to a coherence transfer, transfer of magnetization from one spin to another through relaxation, the so called rotating frame NOE (79). However, the NOE transfer is usually small for the short mixing times (< 50 ms) used for coherence correlation. Furthermore the NOE peaks have a sign opposite to the Hartmann-Hahn cross-polarization peaks which have the same sign as the diagonal peaks. (72,73,80). Recently a method has also been suggested, which uses β angle pulses with a short interval τ between them for the mixing period (Figure 11D), such that the rotating frame NOE is insensitive to the (β/τ) ratio, while the J-coupling cross peaks are maximum for β = 180° and absent for β = 35° (81).

These various techniques of coherence transfer in the rotating frame have become extremely popular in recent years and have been put to wide usage for total correlation of coupled spins in biological and other complex spin systems, yielding total resonance assignments (82-85). An example is given in Figure 12 (73).

III. Conclusions

The original two pulse COSY experiment is still the first experiment carried out for resonance assignments in any system. This is then supplemented for enhanced sensitivity by SUPER COSY and scaling experiments, for enhanced resolution by scaling and multiple quantum filtering experiments, and for additional information on connected transitions and signs of coupling constants by COSY-45, E.COSY, Z-COSY and bilinear COSY experiments. For pure-phase spectroscopy use of pulse-pair filters and coherence transfer via longitudinal spin order has been suggested which enhances the applications of COSY. Information on complete network of coupled spins is obtained by relay COSY, multiple relay COSY, TOCSY and finally by use of rotating frame cross-correlation using HOHAHA and its several modifications.

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Notes Added In Proof

Since submission of this article several developments have been reported including two review articles, one on the techniques (86) and the other on applications of correlated spectroscopy to oriented molecules (87) and an International Conference, where many related developments and applications have been reported (88). Some practical aspects of E.COSY have been reported with special emphasis on the measurement of the spin-spin couplings with high degree of accuracy (89). The zero quantum coherences in the mixing period of Z-COSY have been shifted away in frequency by using τ_m = τ_0 + t_1 instead of τ_m-zitter, and the modified Z-COSY used for measurement of J-couplings
Several developments have been reported in coherence transfer in the rotating frame. Detailed theoretical analyses of homonuclear coherence transfer in the rotating frame have been reported (91-93). Suppression of J transfer in the rotating frame, in order to allow a quantitative estimate of incoherent NOE transfer, has been the subject of considerable attention (92). A modified pulse scheme to suppress J transfer (93) has been reported. Relay experiments in the rotating frame have been suggested in which the rotating frame NOE transfer and coherence transfer are combined to yield spectra which help in making the assignments (94). TOSCY has also been utilized for restoration of the magnetization of resonances which lie close to the saturated water resonance and which are spin-spin coupled to other resonances (95).

Frequency selective pulses have been suggested for selective $\omega_1$ decoupling in homocorrelated 2D spectroscopy (96). Obtaining pure phase spectra continues to draw attention and a combined use of COSS and S.COSY has been suggested for pure phase 2D spectroscopy (97). Zero quantum coherences lead to mixed phases in experiments utilizing coherence transfer in the rotating frame. Elimination of these have been suggested by addition of pseudo random delays before and after the rotating frame mixing (98).

In order to enhance the resolution of a particular region of the 2D COSY spectrum (zooming in), without a corresponding increase in the experimental time, selective experiments have been suggested. These include the use of selective pulses both for selective excitation and selective coherence transfer on one hand (14) and by pseudo correlation spectroscopy (named $\Psi$-COSY) on the other (100). The later is equivalent to stacking of several one-dimensional double resonance spectra in which the perturbing frequency is systematically incremented in fine steps over the frequency range of interest and the data is displayed as conventional two-dimensional spectra. An important innovation in $\Psi$-COSY is the use of a soft (selective) 90° pulse as the perturbing frequency, resulting in conventional COSY looks having cross-peaks in antiphase absorption and diagonal peaks in-phase dispersion (100). Uniform excitation of double quantum coher-
ences has been suggested by incrementing the preparation period in concert with the evolution period in a manner reminiscent of "accordion spectroscopy", giving rise to one-and-a-half quantum spectroscopy (101). The advantages of the techniques have also been outlined.

Symmetry in the coherence transfer process has been investigated in detail and conditions under which 2D spectra having peaks symmetric with respect to the diagonal have been described (102,103). It has been shown that in experiments using a single non-selective excitation pulse, symmetric 2D spectra are obtained whenever the mixing pulse sequence has time and phase reversal symmetry such that the mixing sequence is invariant under reversal of the order of pulses and simultaneous change in sign of all phases.

Application of P.E.COSY for measurement of J coupling constants in DNA fragments has been recently reported (104). Practicality of combining two correlated experiments (COSY with delayed COSY or COSY with relay COSY) into a single pulse sequence to reduce the total experimental time has also been demonstrated (105).

IV. References


