Non-Linear effects in Standard 2D NOE experiments in Coupled spin systems

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INTRODUCTION

The nuclear Overhauser effect (NOE) which monitors the transfer of magnetization from one spin to another, is critically dependent on the internuclear distance and has therefore become a powerful tool for elucidation of the structures of Biomolecules. Experimental methods for monitoring these effects often use radio frequency pulses which simultaneously excite and/or detect several spins at a time. If the spins are not coherently coupled (no J coupling), there are no non-linear effects of the pulses, except for a scaling factor. The non-linear effects in the presence of J-coupling for one-dimensional NOE experiments are well known(1,2). In this paper the non-linear effects in the 2D NOE (NOESY) experiment are analysed in detail.

The standard NOESY experiment uses the sequence $90^o - t_1 - 90^o - \tau_m - 90^o - t_2$, in which relaxation takes place during the mixing interval τ_m . The rate equations governing relaxation are exactly identical to the transient NOE experiment(3-5). It has been known that for uncoupled spins each cross-section in the NOESY experiment is equivalent to a 1D transient NOE experiment in which the peak corresponding to the diagonal peak is selectively inverted(1). When there are J-couplings present in the spin system, selective inversion has to be carefully defined. Recently, it has been shown that for small values of the second pulse ($90^o - t_1 - \alpha - \tau_m - 90^o - t_2$), any cross-section parallel to ω_2 at frequency $\omega_1 = \omega_a$ is equivalent to a 1D difference transient NOE experiment in which the transition at frequency ω_a is selectively inverted. This is true irrespective of the strength of the coupling(6,7). It has also been shown for weakly coupled spins that in the standard NOESY experiment, any cross-section parallel to ω_2 at $\omega_1 = \omega_a$, is equivalent to a 1D transient experiment in which, the whole multiplet of which ω_a is a part is non-selectively inverted. When the spins are strongly coupled the 90^o pulse distributes the perturbation over all the transitions of the strongly coupled network and the 2D NOE experiment is not equivalent to any standard transient 1D experiment. In addition, the third pulse in the NOESY experiment (the measuring pulse) measures the state of the spin system in a non-linear manner for finite angles. As a result it is shown here that in strongly coupled spin systems one can obtain 'cross-peaks' in the standard NOESY experiment without relaxation. The origin of these cross-peaks in terms of the non-linearity of the second and/or the third pulse is also discussed with the help of an ABX spin system.

Cross-correlations between pairwise dipolar relaxation and between dipolar and other mechanism of relaxation such as chemical shift anisotropy(CSA) are known to yield a multiplet effect in J-coupled spectra(7-11). A measurement of this effect in one and two dimensional spectra is carried out using small angle pulses. Recently Osckinat et al. have used small angles for the second and the third pulses in the NOESY experiment and have shown that in the initial rate approximation the effect of cross-correlations is present in all the multiplets of an AMX spin system(7). In their experiment the direct pumping effects and cross-correlation effects both give rise to multiplet effects. We propose here simple modifications which allows the direct pumping effects to be absent, with the cross-correlations exclusively exhibiting multiplet effects in weakly coupled spins.

A. STRONG COUPLING INDUCED CROSS-PEAKS IN NOESY

The signal in a NOESY experiment utilizing $90^o - t_1 - \alpha - \tau_m - \beta - t_2$ sequence in which only longitudinal magnetization is retained during τ_m period can be expressed as,

$$\begin{aligned} S(t_1,t_2) = \\ & Tr\{(F_x)exp(-iHt_2)exp(-i\beta F_x) \\ & [exp(-i\alpha F_x)exp(-iHt_1)exp(-i\tfrac{\pi}{2}F_y) \\ & \sigma_0 exp(i\tfrac{\pi}{2}F_y)exp(iHt_1)exp(i\alpha F_x)]' \\ & exp(\underline{W}\tau_m)exp(i\beta F_x)exp(iHt_2)\} \end{aligned} \quad (1)$$

where the prime indicates retention of only the diagonal elements of the density matrix after the α pulse, $\underline{W}$ is the matrix governing relaxation during τ_m period and σ_0 is the initial density matrix. If σ_0 is an equilibrium density matrix, then only single quantum coherences are created during t_1 period and since during period t_2 only single quantum coherences are detected, the above equation can be written as(7)

$$\begin{aligned} S(t_1,t_2) &= \sigma_{1\times M}\, P_{M\times N}(\beta_x) \\ & exp(W_{N\times N}\tau_m) \\ & P_{N\times M}(\alpha_x)\sigma_{M\times 1} \end{aligned} \quad (2)$$

$P_{M\times N}(\gamma_x)$ represents a matrix which transforms the N populations into M single quantum coherences by a pulse of angle γ_x . The N populations are arranged in descending order of energy while the M coherences represented by vectors $\sigma_{1\times M}$ and $\sigma_{M\times 1}$ are arranged in descending order of frequency. The matrix $P_{M\times N}(\gamma_x)$ can be re-expressed as,

$$P_{M\times N}(\gamma_x) = k\,(F_x)_{M\times M}\, P'_{M\times N}\,(\gamma) \quad (3)$$

where $k = i\sin(\gamma)$ and $(F_x)_{M\times M}$ is a diagonal matrix containing the matrix elements of the operator F_x arranged according to $\sigma_{1\times M}$. The matrix that transforms, M single quantum coherence to N populations is the transpose of the above matrix,

$$\begin{aligned} P_{N\times M}\,(\gamma_x) &= -P^T_{M\times N}(\gamma_x) \\ &= -k\, P'_{N\times M}\,(\gamma)(F_x)_{M\times M} \end{aligned} \quad (4)$$

The intensity of the peaks in the NOESY spectrum neglecting relaxation during τ_m period is given by,

$$\begin{aligned} S(w_1,w_2) &= |(F_x)_{M\times M}|^2\, P'_{M\times N}\,(\beta) \\ & P'_{N\times M}\,(\alpha)\,|(F_x)_{M\times M}|^2. \end{aligned} \quad (5)$$

AB spin system

In the strongly coupled two spin system(AB) if the coherences are arranged in a row vector in the sequence as, $\{+\beta\}$, $\{+\alpha\}$,$\{\beta+\}$, $\{\alpha+\}$ and the populations in a column vector in the sequence $\{\alpha\alpha\}$, $\{\alpha\beta\}$, $\{\beta\alpha\}$, $\{\beta\beta\}$ corresponding to a weakly coupled spin system, the matrix $P_{N\times M}(\gamma_x)$ is given by eq[6].

The initial state at the beginning of the mixing time ($\tau_m \implies 0$) in the 2D NOE experiment is calculated for a strongly coupled two spin system using the above analysis. For the general NOESY experiment ($90^o - t_1 - \alpha - \tau_m - \beta - t_2$) the results are given in Table.1. The cross-peaks in this experiment arise largely due to the unequal perturbation of the various transitions of the coupled spin system and will be present even in a weakly coupled spin system except when either α or $\beta = 90^o$. The results of the experiment when $\alpha = \beta = 90^o$ (Fig.1), show that there are cross-peaks present in the standard NOESY experiment even in the absence of relaxation due to strong coupling.

$$P_{N\times M}(\gamma) = k \begin{bmatrix} -S^2 & -C^2 & -S^2 & -C^2 \\ -C^2+ S^2(1-v^2) & -u^2S^2 & u^2S^2 & C^2- S^2(1-v^2) \\ v^2S^2 & C^2+ S^2(u^2-1) & -C^2- S^2(u^2-1) & -v^2S^2 \\ C^2 & S^2 & C^2 & S^2 \end{bmatrix} \begin{bmatrix} u & 0 & 0 & 0 \\ 0 & v & 0 & 0 \\ 0 & 0 & v & 0 \\ 0 & 0 & 0 & u \end{bmatrix} \quad (6)$$

where S = Sin(γ / 2) ; C = Cos(γ / 2); u = Cos θ + Sin θ ;v = Cos θ - Sin θ and $tan(2\theta) = J_{AB}/(\delta_A - \delta_B)$ defines the strength ofthe coupling.

Table.1.

Frequencies ω_1	Frequencies ω_2	Intensities $\star(-S_{2\alpha}S_{2\beta})$
(1) Diagonal peaks		
1-3	1-3	$v^4[4C_\alpha^2C_\beta^2 + 4S_\alpha^2S_\beta^2\{u^2 + (u^2-1)^2\} - (1 - C_{2\alpha}C_{2\beta})(1-u^2)]$
3-4	3-4	
1-2	1-2	$u^4[4C_\alpha^2C_\beta^2 + 4S_\alpha^2S_\beta^2\{v^2 + (1-v^2)^2\} - (1 - C_{2\alpha}C_{2\beta})(1-v^2)]$
2-4	2-4	
(2) Auto-peaks		
1-3	2-4	$u^2v^2[-4S_\alpha^2S_\beta^2(1-u^2v^2) + 2(1 - C_{2\alpha}C_{2\beta}) + (1-v^2)(C_{2\alpha} - C_{2\beta})]$
3-4	1-2	
2-4	1-3	$u^2v^2[-4S_\alpha^2S_\beta^2(1-u^2v^2) + 2(1 - C_{2\alpha}C_{2\beta}) - (1-v^2)(C_{2\alpha} - C_{2\beta})]$
1-2	3-4	
(3) Cross-peaks		
1-3	3-4	$v^4[-2C_\alpha^2C_\beta^2 - 2S_\alpha^2S_\beta^2\{u^4 + (u^2-1)^2\} + (1 - C_{2\alpha}C_{2\beta})v^2]$
3-4	1-3	
2-4	1-2	$u^4[-2C_\alpha^2C_\beta^2 - 2S_\alpha^2S_\beta^2\{v^4 + (1-v^2)^2\} + (1 - C_{2\alpha}C_{2\beta})u^2]$
1-2	2-4	
1-3	1-2	$(u^2v^2/2)[4C_\alpha^2C_\beta^2 + 4S_\alpha^2S_\beta^2\{3 - 2u^2v^2\} - 2(1 - C_{2\alpha}C_{2\beta})$ $-(u^2-v^2)(C_{2\alpha} - C_{2\beta})]$
2-4	3-4	
1-2	1-3	
3-4	2-4	

C_{2i} = Cos(i) ; S_{2i} = Sin(i) ; $C_i = Cos(\frac{i}{2})$; $S_i = Sin(\frac{i}{2})$ where i = α, β and u = Cos θ + Sin θ ; v = Cos θ - Sin θ.

The origin of these cross-peaks lies in the creation of a initial state in which the initial perturbation is distributed over all the transitions of a strongly coupled spin system as well as due to the non-linear measurement of the strongly coupled spin system by the third 90^o pulse. The initial state in this experiment can also be described using magnetization modes(12,13). For an AB system the initial state in terms of the magnetization modes at various cross-sections parallel to ω_2 is given in Table.2. From these it is seen that the single spin modes of both the spins are created in each cross-section. This is the origin of the cross-peaks in strongly coupled spins. In the limit of weak coupling (u = v = 1) each cross-section contains only one single spin mode belonging to the inverted spin and the cross-peaks are absent.

ABX spin system

If there are two groups of spins which are strongly coupled among themselves but weakly coupled to others then it is not a priory clear that there will be cross-peaks between the two groups. This is investigated here with the help of an ABX spin system. Fig.2 shows the standard NOESY ($\alpha = \beta = 90^o$) spectrum calculated using eqn.[5] for an ABX spin system with zero mixing time. Actual intensities of the peaks in the 2D spectrum is obtained by multiplying the expressions given in Table.3 with the corresponding 1D intensities in both ω_1 and ω_2 dimensions of that particular peak. From this it is seen that every peak has a cross-peak to every other peak. The cross-peaks in this spectrum including those between A and B spins and between AB spins and X spin arise due to the strong coupling among the A and B spins, and disappear under weak coupling approximation. The appearance of these cross-peaks needs further investigation in terms of whether they are due to the non-linearity of the second or the third pulse. To investigate this, calculations have been carried out for the cases when the excitation pulse is small(in the linear regime) or the detection pulse is small(in the linear regime). The following results were noted from these experiments:

(i) $90^o - \alpha - 90^o$ Experiment

The ABX spin system has eight AB-transitions and six X-transitions two of which are between states which are unperturbed by strong coupling (the so called pure states 1,2,7 and 8)(14). In this system in the $90^o - \alpha - 90^o$ experiment there are no cross-peaks from the

Table.2.

At ω_1	ω_{12}	ω_{13}	ω_{24}	ω_{34}
$< \Delta A_z >_{\tau_m=0}$	$-u^2(1-v^2)$	$-v^2(1+u^2)$	$-u^2(1+v^2)$	$v^2(1-v^2)$
$< \Delta B_z >_{\tau_m=0}$	$-u^2(1+v^2)$	$v^2(1-v^2)$	$-u^2(1-v^2)$	$-v^2(1+u^2)$
$< \Delta A_z B_z >_{\tau_m=0}$	0	0	0	0

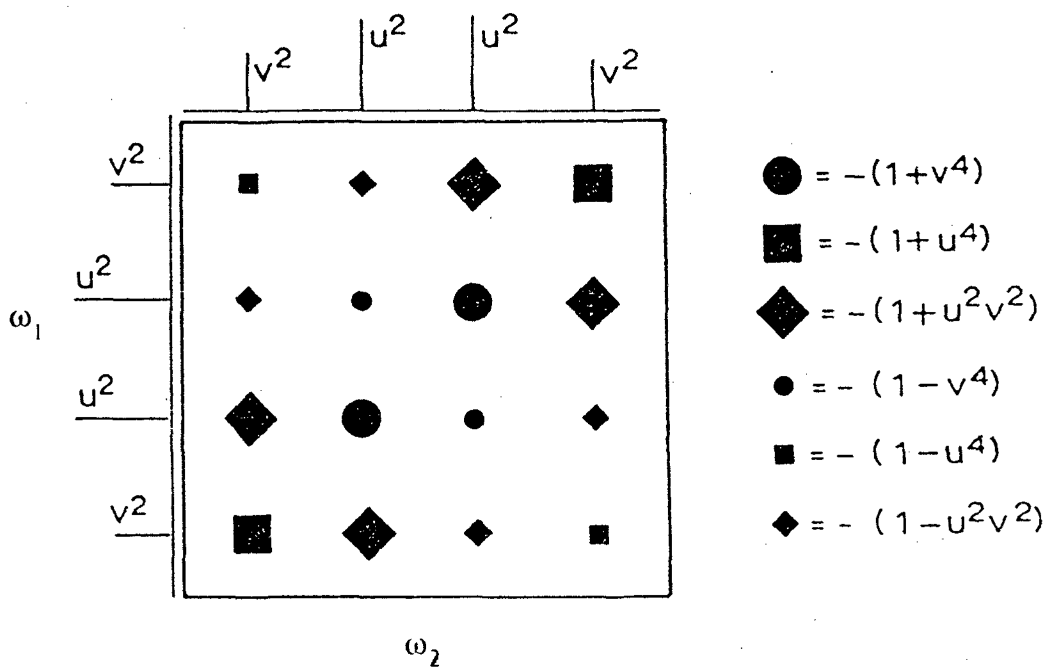
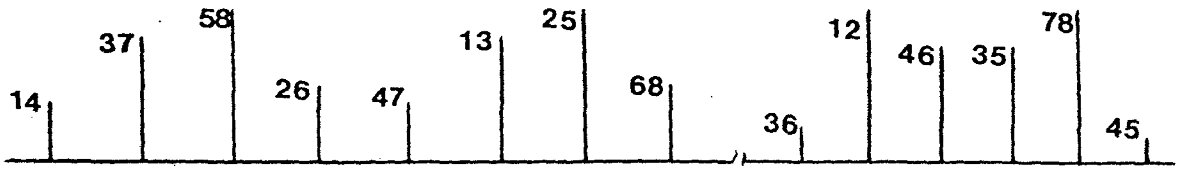


Figure 1. Schematic spectrum of an AB spin system calculated for the $90^o-90^o-90^o$ 2D NOESY experiment with zero mixing time. The symbols represent $P_{M\times N}(90^o) \times P_{N\times M}(90^o)$, the $|F_x|^2$ are given along the 1D spectra and the final intensities are obtained using eq [5].



ω_1	v_+^2	u_+^2	u_-^2	v_-^2	v_+^2	u_+^2	u_-^2	v_-^2	S_-^2	1	C_-^2	C_-^2	1	S_-^2
X S_-^2	A_1X_6	A_2X_6	A_3X_6	A_4X_6	B_1X_6	B_2X_6	B_3X_6	B_4X_6	X_{16}	X_{26}	X_{36}	X_{46}	X_{56}	X_6
X 1	A_1X_5	A_2X_5	A_3X_5	A_4X_5	B_1X_5	B_2X_5	B_3X_5	B_4X_5	X_{15}	X_{25}	X_{35}	X_{45}	X_5	
X C_-^2	A_1X_4	A_2X_4	A_3X_4	A_4X_4	B_1X_4	B_2X_4	B_3X_4	B_4X_4	X_{14}	X_{24}	X_{34}	X_4		
X C_-^2	A_1X_3	A_2X_3	A_3X_3	A_4X_3	B_1X_3	B_2X_3	B_3X_3	B_4X_3	X_{13}	X_{23}	X_3			
X 1	A_1X_2	A_2X_2	A_3X_2	A_4X_2	B_1X_2	B_2X_2	B_3X_2	B_4X_2	X_{12}	X_2				
X S_-^2	A_1X_1	A_2X_1	A_3X_1	A_4X_1	B_1X_1	B_2X_1	B_3X_1	B_4X_1	X_1					
B v_-^2	A_1B_4	A_2B_4	A_3B_4	A_4B_4	B_{14}	B_{24}	B_{34}	B_4						
B u_-^2	A_1B_3	A_2B_3	A_3B_3	A_4B_3	B_{13}	B_{23}	B_3							
B u_+^2	A_1B_2	A_2B_2	A_3B_2	A_4B_2	B_{12}	B_2								
B v_+^2	A_1B_1	A_2B_1	A_3B_1	A_4B_1	B_1									
A v_-^2	A_{14}	A_{24}	A_{34}	A_4										
A u_-^2	A_{13}	A_{23}	A_3											
A u_+^2	A_{12}	A_2												
A v_+^2	A_1													

ω_2

$$
\begin{aligned}
A_i &= B_i && \text{for } i = 1 \text{ to } 4 \\
A_{ij} &= B_{ij} \\
A_iB_j &= A_jB_i && \text{for } i \neq j \text{ and } j > i \\
A_iX_j &= -B_iX_j && \text{for } i = 1 \text{ to } 4 \text{ and} \\
A_iX_5 &= A_iX_2 && \text{for } j = 1 \text{ to } 6 \\
B_iX_5 &= B_iX_2 \\
A_1B_2 &= A_3B_4 \\
A_{12} &= A_{34}
\end{aligned}
$$

$$
\begin{aligned}
X_2 &= X_5 = X_{25} \\
X_{12} &= X_{15} \\
X_{23} &= X_{35} \\
X_{24} &= X_{45} \\
X_{26} &= X_{56} \\
A_1X_1 &= A_4X_6 \\
A_1X_3 &= A_3X_4 \\
A_2X_4 &= A_4X_3 \\
A_3X_1 &= A_2X_6
\end{aligned}
$$

Figure 2.

Table 3. Intensities of the peaks

Peaks	Strong Coupling	Weak Coupling	Peaks	Strong Coupling	Weak Coupling
A_1	$-(1+s/b_2^2)$	-2	A_1B_1	s/b_2^2-1	0
A_2	$-(1+sb_2^2)$	-2	A_2B_2	sb_2^2-1	0
A_3	$-(1+sb_1^2)$	-2	A_3B_3	sb_1^2-1	0
A_4	$-(1+s/b_1^2)$	-2	A_4B_4	s/b_1^2-1	0
A_{12}	$-(1+s)$	-2	A_1B_2	$s-1$	0
A_{13}	$-(1+sb_4/b_3)$	-2	A_1B_3	sb_4/b_3-1	0
A_{14}	$-(1+s/b_1b_2)$	-2	A_1B_4	sb_3/b_4-1	0
A_{23}	$-(1+sb_1b_2)$	-2	A_2B_3	sb_1b_2-1	0
A_{24}	$-(1+sb_3/b_4)$	-2	A_2B_4	s/b_1b_2-1	0
X_1	$-(a_4^2+ka_3^2)$	0	A_1X_1	$a_4m_1u_+^2-a_3m_2u_-^2$	0
X_2	$-(1+k)$	-2	A_1X_2	$[n_3m_1-m_2u_-^2]$	0
X_3	$-(a_1^2+ka_2^2)$	-2	A_1X_3	$a_2m_2u_-^2-a_1m_1v_+^2$	0
X_4	$-(a_2^2+ka_1^2)$	-2	A_1X_4	$a_1[n_3m_1-m_2u_-^2]$	0
X_6	$-(a_3^2+ka_4^2)$	0	A_1X_6	$-a_4[n_3m_1-m_2u_-^2]$	0
X_{12}	sa_3-a_4	0	A_2X_1	$-a_3[n_1m_1-m_2v_-^2]$	0
X_{13}	$ka_2a_4-a_1a_3$	0	A_2X_2	$-[n_1m_1-m_2v_-^2]$	0
X_{14}	$ka_1a_4-a_2a_3$	0	A_2X_3	$-a_2[n_1m_1-m_2v_-^2]$	0
X_{16}	$-a_3a_4(1+k)$	0	A_2X_4	$a_1m_2v_-^2-a_2m_1u_+^2$	0
X_{23}	$-(a_1+sa_2)$	-2	A_2X_6	$a_3m_1v_+^2-a_4m_2v_-^2$	0
X_{24}	$-(a_2+sa_1)$	-2	A_3X_2	$-[n_2m_2-m_1v_+^2]$	0
X_{26}	sa_4-a_3	0	A_3X_3	$a_2[n_2m_2-m_1v_+^2]$	0
X_{34}	$-a_1a_2(1+k)$	0	A_3X_6	$-a_4[n_2m_2-m_1v_+^2]$	0
X_{36}	$ka_2a_3-a_1a_4$	0	A_4X_1	$-a_3[n_4m_2-m_1u_+^2]$	0
X_{46}	$ka_1a_3-a_2a_4$	0	A_4X_2	$-[n_4m_2-m_1u_+^2]$	0
			A_4X_4	$a_1[n_4m_2-m_1u_+^2]$	0

$$
\begin{array}{llllll}
a_1 = u_+u_-/C_-; & b_1 = v_+/u_+ & & \\
a_2 = v_+v_-/C_-; & b_2 = v_-/u_- & & \\
a_3 = u_+v_-/S_-; & b_3 = u_+/u_- & & \\
a_4 = u_-v_+/S_-; & b_4 = v_+/v_- & & \\
n_1 = u_+v_+v_-/u_-; & m_1 = u_+^2 - v_+^2 & & \\
n_2 = u_-v_+v_-/u_+; & m_2 = u_-^2 - v_-^2 & & \\
n_3 = u_+u_-v_+/v_-; & k = (u_+^4 + v_+^4 + u_-^4 + v_-^4)/4 & & \\
n_4 = u_+u_-v_-/v_+; & s = (u_+^2v_+^2 + u_-^2v_-^2)/2 & & \\
C_- = Cos(\theta_+ - \theta_-); & u_+ = Cos(\theta_+) + Sin(\theta_+); & u_- = Cos(\theta_-) + Sin(\theta_-) \\
S_- = Sin(\theta_+ - \theta_-); & v_+ = Cos(\theta_+) - Sin(\theta_+); & v_- = Cos(\theta_-) - Sin(\theta_-)
\end{array}
$$

X transitions between pure states to all AB transitions, while there are cross-peaks between the other X transitions to all AB transitions and also cross-peaks between all AB transitions to all X transitions. The selective inversion of $X^{(12)}$ or $X^{(78)}$ by a small angle α pulse does not cause any perturbation of the strongly coupled states and hence there are no cross-peaks from these transitions to all AB transitions. The 90^o third pulse mixes the X-magnetisation unequally between all the X transitions giving rise to the auto-peaks. On the other hand, the selective inversion of an AB transition perturbs the strongly coupled states leading to cross-peaks to X transitions between mixed states. The non-linear detection pulse in turn mixes the intensities of all the X transitions giving rise to cross-peaks to even the X transitions between pure states. The spectrum is not symmetrical(15).

(ii) $90^o - 90^o - \alpha$ Experiment

In this experiment there are no cross-peaks between all AB transitions to X transitions between pure states, while there are cross-peaks to all X transitions between the mixed states and also cross-peaks between all X transitions to all AB transitions. This is due to the fact that the second 90^o pulse perturbs unequally all the transitions of AB as well as the X spin. The mixed states of the AB spins do not give directly any cross-peak to X-pure transitions. Since the detection pulse is a small angle pulse it does not mix the X transitions between pure and mixed states and therefore there are no cross-peaks from AB to X-pure transitions. The appearance of cross-peaks between X transitions between pure states and the AB transitions is due to the mixing produced between the various transitions of the X spin by the second 90^o pulse. This state of the system is faithfully measured by the detection pulse. Here also the spectrum is not symmetrical(15).

The results of $90^o - \alpha - 90^o$ and $90^o - 90^o - \alpha$ experiments are transpose of each other. This is due to the fact that $P_{N\times M}(\alpha_x) = -P^T_{M\times N}(\alpha_x)$. The conversion of populations into coherences and vice versa are described by mirror operations(15).

Experimental

Experimental observation of these cross-peaks was carried out in acetone oriented in liquid crystal ZLI 1167. Acetone oriented in liquid crystal is a strongly coupled spin system of the type $(A_3A'_3)$ with $C_{3v} \otimes C_{3v}$ symmetry. The spectra is shown in Fig.3. From this spectrum it is clear that there are cross-peaks from every peak to all others within the same irreducible representation. Theoretical simulations of these cross-peaks show a very good match with the experimental results(6), confirming the existence of strong coupling induced cross-peaks in the 2D NOE experiments even in the absence of relaxation.

B. CROSS-CORRELATIONS IN 2D NOE

If a spin has more than one pathway for relaxation, then there can be cross-terms between these pathways that may contribute to the relaxation of the spin. For example, if there is another spin nearby, and the mutual dipolar interaction contributes to the relaxation of the spin and if in addition the first spin has a partial relaxation by CSA, there can be cross-terms between the dipolar relaxation and CSA(16). If on the other hand there is a third spin contributing to the relaxation of the first two through dipolar relaxation then there can be cross-terms between various dipolar interactions and between the dipolar and CSA interactions contributing to the relaxation of the various spins. These cross-terms known as cross-correlations are often neglected in the relaxation analysis such as those using generalized Solomons equations(17). It turns out that while the cross-terms may be significant in magnitude their manifestation in a particular experiment may be small. For example the dominant effect of the cross-terms is to make the relaxation of various transitions of a spin unequal. In a given spin system or in an experiment if these transitions are not resolved then this dominant effect of cross-terms is absent. This can happen for example when the spins are not J-coupled or if one uses a 90^o pulse for measuring the intensities of the multiplet. In the later case the non-linearity of the pulse yields an average intensity over all the transitions of a spin obliterating the multiplet effect and largely the cross-correlation effects. The use of a small flip angle for the measuring pulse is a necessary requirement for the observation of the multiplet effect and in turn the cross-correlation effects in the 1D and the standard NOESY experiments(7).

In two-dimensional NOE experiment the most significant attempts to observe the effect of cross-correlations have been made by Bodenhausen and his group(7,16,18-20). One of the experiments they have used is a small flip angle NOESY experiment namely $90^o - t_1 - \alpha - \tau_m - \alpha - t_2$, where α is small. Each cross-section of the small flip angle NOESY (NOESY $90^o - \alpha - \alpha$) is then equivalent to a 1D difference transient NOE experiment in which the peak corresponding to the diagonal peak is selectively inverted. This experiment has both the direct pumping effects and the cross-correlation induced multiplet effects present which are measured by the small angle third pulse. For example the intensities of the X diagonal and the AX cross-peak multiplet in a weakly coupled three spin (AMX) system, in the initial rate approximation are given by(7),

$$\omega_1 \downarrow \; \overset{\rightarrow \omega_2}{\begin{matrix} & X_1 & X_2 & X_3 & X_4 \end{matrix}}$$

$$\begin{matrix} X_1 \\ X_2 \\ X_3 \\ X_4 \end{matrix} \begin{bmatrix} d_{\downarrow\downarrow} & l^{\downarrow}_{1A} & l^{\downarrow}_{1M} & l_{2AM} \\ l^{\downarrow}_{1A} & d_{\downarrow\uparrow} & l_{0AM} & l^{\uparrow}_{1M} \\ l^{\downarrow}_{1M} & l_{0AM} & d_{\uparrow\downarrow} & l^{\uparrow}_{1A} \\ l_{2AM} & l^{\uparrow}_{1M} & l^{\uparrow}_{1A} & d_{\uparrow\uparrow} \end{bmatrix}$$

$$\begin{matrix} A_1 \\ A_2 \\ A_3 \\ A_4 \end{matrix} \begin{bmatrix} r_1^{(1)} & p_1^{(1)} & r_1^{(0)} & p_1^{(0)} \\ p_2^{(1)} & r_2^{(1)} & p_2^{(0)} & r_2^{(0)} \\ r_3^{(0)} & p_3^{(0)} & r_3^{(1)} & p_3^{(1)} \\ p_4^{(0)} & r_4^{(0)} & p_4^{(1)} & r_4^{(1)} \end{bmatrix} \quad (7)$$

where X_1, X_2, X_3, X_4 are the four X transitions and A_1, A_2, A_3, A_4 are the four A transitions. The expressions for the various intensities of the peaks are given in ref (7) except that when the cross-correlations due to CSA and dipole-dipole interaction are included $W^{\uparrow\uparrow} \neq W^{\downarrow\downarrow}$, $W^{\uparrow\downarrow} \neq W^{\downarrow\uparrow}$ and $l^{\uparrow}_{1i} \neq l^{\downarrow}_{1i}$ where i = A, M or X. The r and p terms signify regressive and progressive peaks respectively. From eqn[7] it is seen that while the cross-correlation information is contained in the small flip angle NOESY experiment, it is coupled with the direct pumping effects.

We propose here simple modifications to the small flip angle NOESY (NOESY $90^o - \alpha - \alpha$). If the second or the third pulse is made 90^o then the intensities in the initial rate approximation are obtained as averages of the multiplets in either ω_1 or ω_2 direction respectively. This removes the direct pumping effects from the 2D spectra. The following results are obtained.

$90^o - \alpha - 90^o$ NOESY

The intensities of the various peaks in the initial rate approximation are given by

$$\omega_1 \downarrow \; \overset{\rightarrow \omega_2}{\begin{matrix} & X_1 & X_2 & X_3 & X_4 \end{matrix}}$$

$$\begin{matrix} X_1 \\ X_2 \\ X_3 \\ X_4 \end{matrix} \begin{bmatrix} R_1 & R_1 & R_1 & R_1 \\ R_2 & R_2 & R_2 & R_2 \\ R_3 & R_3 & R_3 & R_3 \\ R_4 & R_4 & R_4 & R_4 \end{bmatrix} \quad (8)$$

$$\begin{matrix} A_1 \\ A_2 \\ A_3 \\ A_4 \end{matrix} \begin{bmatrix} C_1 & C_1 & C_1 & C_1 \\ C_2 & C_2 & C_2 & C_2 \\ C_3 & C_3 & C_3 & C_3 \\ C_4 & C_4 & C_4 & C_4 \end{bmatrix}$$

where

$$\begin{aligned} R_1 &= d_{\downarrow\downarrow} + l_{2AM} + l^{\downarrow}_{1M} + l^{\downarrow}_{1A} \\ &= -2[1 - (\rho_x + \Delta^X_{AX} + \Delta^X_{MX} \\ &\qquad +\delta_X)]\tau_m \\ R_2 &= d_{\downarrow\uparrow} + l_{0AM} + l^{\uparrow}_{1M} + l^{\downarrow}_{1A} \\ &= -2[1 - (\rho_x + \Delta^X_{AX} - \Delta^X_{MX} \\ &\qquad -\delta_X)]\tau_m \\ R_3 &= d_{\uparrow\downarrow} + l_{0AM} + l^{\downarrow}_{1M} + l^{\uparrow}_{1A} \\ &= -2[1 - (\rho_x - \Delta^X_{AX} + \Delta^X_{MX} \\ &\qquad -\delta_X)]\tau_m \\ R_4 &= d_{\uparrow\uparrow} + l_{2AM} + l^{\uparrow}_{1M} + l^{\uparrow}_{1A} \\ &= -2[1 - (\rho_x - \Delta^X_{AX} - \Delta^X_{MX} \\ &\qquad +\delta_X)]\tau_m \end{aligned} \quad (9)$$

$$\begin{aligned} C_1 &= r_1^{(1)} + p_1^{(1)} + r_1^{(0)} + p_1^{(0)} \\ &= 2(\sigma_{AX} + \Delta^X_{AX} + \delta_X)\tau_m \\ C_2 &= r_2^{(1)} + p_2^{(1)} + r_2^{(0)} + p_2^{(0)} \\ &= 2(\sigma_{AX} - \Delta^X_{AX} - \delta_X)\tau_m \\ C_3 &= r_3^{(1)} + p_3^{(1)} + r_3^{(0)} + p_3^{(0)} \\ &= 2(\sigma_{AX} + \Delta^X_{AX} - \delta_X)\tau_m \\ C_4 &= r_4^{(1)} + p_4^{(1)} + r_4^{(0)} + p_4^{(0)} \\ &= 2(\sigma_{AX} - \Delta^X_{AX} + \delta_X)\tau_m \end{aligned} \quad (10)$$

Here ρ_x is the rate of self relaxation of spin X, σ_{AX} is the cross-relaxation rate between spins A and X, $\delta_X = \delta_{AXMX}$, which gives the cross-correlation rate between the dipolar vectors AX and MX and Δ^X_{AX} gives the cross-correlation raten between the dipolar vector AX and the CSA of spin X. The expressions for the spectral density functions for the various relaxation rates (ρ, σ, Δ, δ) are given in ref(11). The intensities of the various peaks in each multiplet are identical in ω_2 dimension and differ in ω_1 dimension, the differences directly yielding the cross-correlations. If the multiplet is resolved in the ω_1 dimension the difference in the intensities of the inner or the outer lines gives the dipole-CSA cross-correlations(Δ's) and the difference between the inner and outer lines gives the dipole-dipole cross-correlations(δ's). The diagonal multiplet result is identical to the differences in the initial rates of recovery of the outer and inner multiplets in inversion-recovery T_1 measurements (21). However many analyses of inversion recovery measurements including (21) ignore CSA-dipole cross-correlations, while retaining dipole-dipole cross-correlations.

$90^o - 90^o - \alpha$ NOESY

The intensities of the diagonal and the cross-peak multiplets in the initial rate approximation in this case are given by

$\rightarrow \omega_2$, $\downarrow \omega_1$

$$\begin{array}{c} \\ X_1 \\ X_2 \\ X_3 \\ X_4 \end{array} \begin{array}{c} \begin{array}{cccc} X_1 & X_2 & X_3 & X_4 \end{array} \\ \begin{bmatrix} R_1 & R_2 & R_3 & R_4 \\ R_1 & R_2 & R_3 & R_4 \\ R_1 & R_2 & R_3 & R_4 \\ R_1 & R_2 & R_3 & R_4 \end{bmatrix} \end{array} \quad (11)$$

$$\begin{array}{c} A_1 \\ A_2 \\ A_3 \\ A_4 \end{array} \begin{bmatrix} C_1' & C_2' & C_3' & C_4' \\ C_1' & C_2' & C_3' & C_4' \\ C_1' & C_2' & C_3' & C_4' \\ C_1' & C_2' & C_3' & C_4' \end{bmatrix}$$

where

$$\begin{aligned} C_1' &= 2(\sigma_{AX} + \Delta^A_{AX} + \delta_A)\tau_m \\ C_2' &= 2(\sigma_{AX} - \Delta^A_{AX} - \delta_A)\tau_m \\ C_3' &= 2(\sigma_{AX} + \Delta^A_{AX} - \delta_A)\tau_m \\ C_2' &= 2(\sigma_{AX} - \Delta^A_{AX} + \delta_A)\tau_m \end{aligned} \quad (12)$$

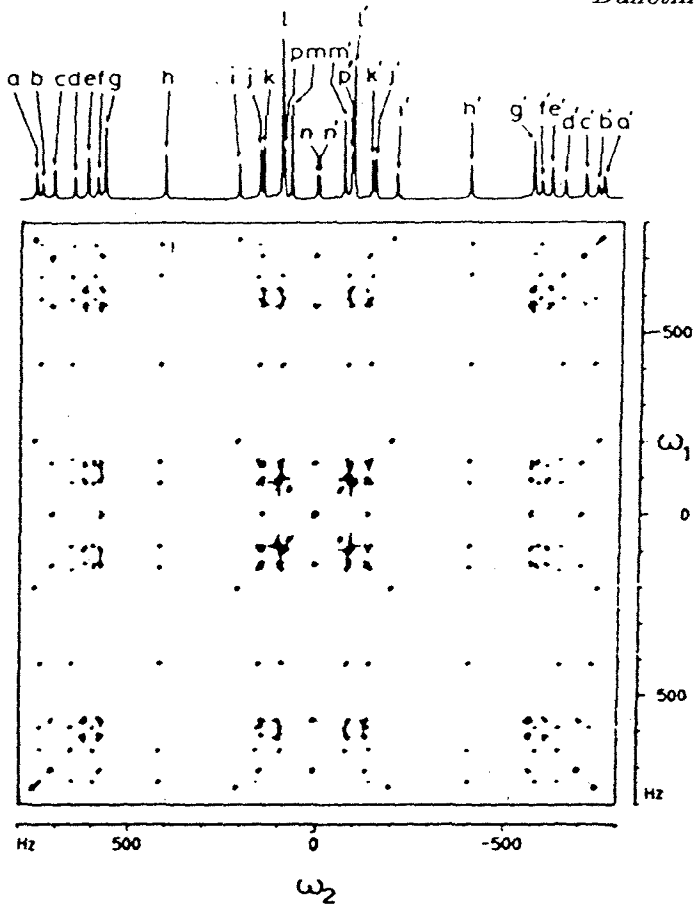


Figure 3. 2D NOESY spectrum of oriented acetone recorded at 400 MHz with $\tau_m = 20$ μsec. The cross-peaks are mainly due to strong coupling. Zero- quantum interference during τ_m was shifted out in another experiment and the residual strong coupling peaks showed satisfactory correlation with the calculated intensities(6).

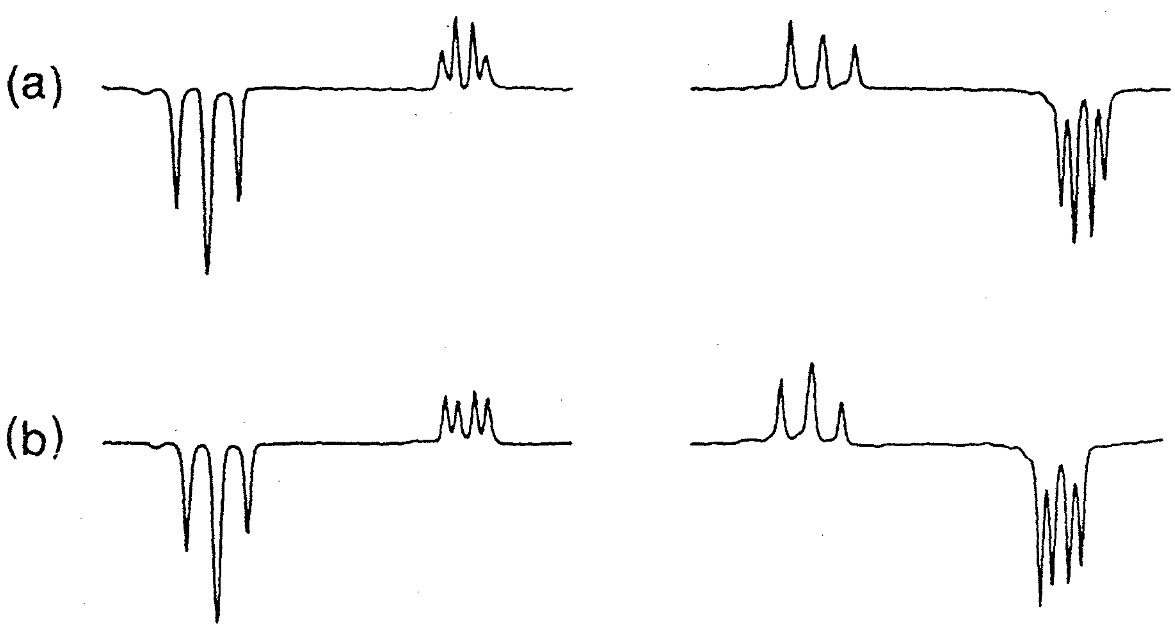


Figure 4. Cross-sections taken from (a) $90^o - 90^o - 15^o$ (b) $90^o - 90^o - 90^o$ 2D NOESY experiment with $\tau_m = \tau_0 + k$ using a 400 MHz spectrometer. τ_0 was 400 msec and k was randomly varied between 10 and 1000 msec.

From these expressions it is seen that the intensities differ in ω_2 and an averaging takes place along ω_1 . The second 90^o pulse excites the multiplet as a whole, the correct state being monitored by the small angle α pulse. The differences in the intensities again yield the cross-correlations except that in this case the AX multiplet yields δ_X and Δ^X_{AX}. The diagonal multiplet has intensities identical to $90^o - \alpha - 90^o$ experiment. Since in the $90^o - 90^o - \alpha$ experiment the intensities differ in ω_2 domain which is easier to resolve, this experiment may be preferred over the $90^o - \alpha - 90^o$ experiment. In addition, since all the lines of a multiplet along ω_1 have equal intensities, a ω_1 -decoupled $(90^o - (\Delta + t_1)/2 - 180^o - (\Delta - t_1)/2 - 90^o - \tau_m - \alpha - t_2)$ NOESY experiment can replace the undecoupled $(90^o - t_1 - 90^o - \tau_m - \alpha - t_2)$ NOESY experiment without loss of information.

Experimental

Two-dimensional NOESY experiment was carried out in 2,3-dibromo propionic acid using the $90^o - 90^o - \alpha$ sequence with small $\alpha(\simeq 15^o)$ and a mixing time of 400msec plus a random variation from 10 to 1000msec. Some of the cross-sections are shown in Fig.4. The differences between the intensities of various transitions of a multiplet indicate the presence of cross-correlations. Lack of any particular symmetry in these multiplets indicates the presence of both dipole-dipole and CSA-dipole cross-correlations.

CONCLUSIONS

The use of 90^o angle for the excitation or detection pulses allows an easier method for studying the cross-correlations in 2D NOE experiment. One can use either the second or the third pulse as small angle pulse to highlight the cross-correlation effects. In strongly coupled spins the non-linearity of the pulses can give rise to cross-peaks even in the absence of relaxation. The origin of these cross-peaks arising due to the non-linearity of the second or the third pulse are discussed with the help of an ABX spin system.

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