MULTIPLE-QUANTUM ARTIFACTS IN SINGLE-QUANTUM TWO-DIMENSIONAL CORRELATED NMR SPECTRA OF STRONGLY COUPLED SPINS

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Artifacts in the form of cross peaks have been observed along two- and three-quantum diagonals in single-quantum two-dimensional correlated (COSY) spectra of several peptides and oligonucleotides. These have been identified as due to the presence of a non-equilibrium state of kind I (a state describable by populations which differ from equilibrium) of strongly coupled spins carried over from one experiment to the next in the COSY algorithm.

1. Introduction

The two-dimensional correlated (COSY) experiment has become popular in the resonance assignments of biological systems; the cross peaks reveal the coupling networks of the spin systems in the molecule. However this experiment suffers from artifacts such as t_1 noise, tailing from strong peaks and data accumulation and processing artifacts arising from a limited data set and use of filter functions. It is highly desirable that these artifacts are kept to a minimum in number and intensity, and are identified.

Artifacts in the form of peaks along two-quantum and three-quantum diagonals have recently been observed in the COSY spectra of several peptides and oligonucleotides (fig. 1). In this communication it is demonstrated that many of these artifacts arise due to a non-equilibrium state of kind I [1] of strongly coupled spins being carried over from one experiment to the next in the COSY algorithm. Such a non-equilibrium gets converted into multiple-quantum coherences in strongly coupled spins which appear at two-quantum and three-quantum frequencies in the COSY spectra.

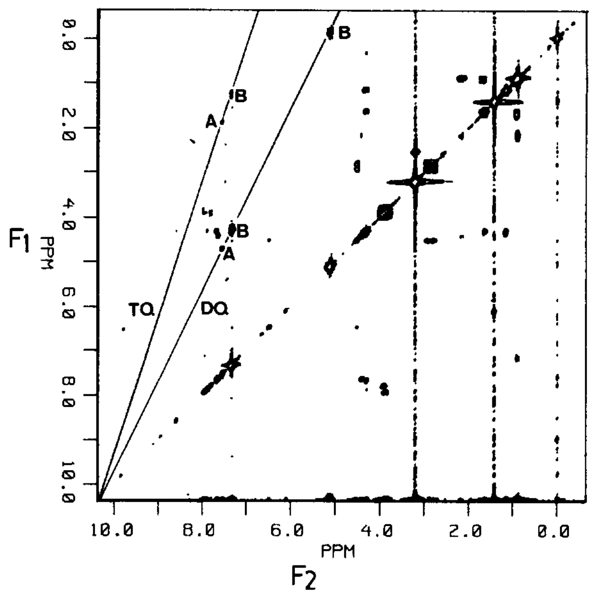

Fig. 1. COSY spectrum of the peptide Boc–Asp (OBzl)–Leu–Thr–$(Gly)_3$–Val (OBzl) dissolved in a mixture of $CDCl_3$ and $(CD_3)_2SO$, recorded at 270 MHz. The observed artifacts lie on the double-quantum ($\omega_1 = 2\omega_2$) and triple-quantum ($\omega_1 = 3\omega_2$) diagonals. The class A artifacts appear on the chloroform resonance (F_2 = 7.56 ppm). The class B artifacts appear on the phenyl group (7.34 ppm) forming an AA'BB'C spin system and on the CH_2 group (5.11 ppm) forming an AB spin system of the *o*-benzyl group in the peptide. The resonances of this.peptide have been assigned earlier [14]. The relaxation delay (RD) in the experiment was 0.5 s.

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2. Experiment and discussion

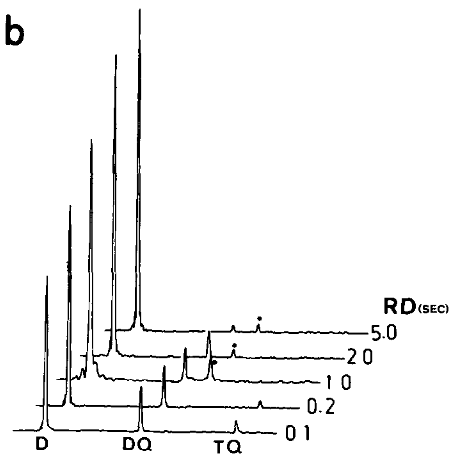
A series of COSY spectra were recorded for various values of relaxation delay (RD) (fig. 2). Based on these measurements the observed artifacts are classified into two classes.

Class A: Those which increase in intensity along with the diagonal as RD is increased.

Class B: Those which decrease in intensity as RD is increased.

Artifacts of class A are not due to carry-over effects, and are probably due to problems associated with data accumulation or data processing of strong sharp peaks [2–4] and are of no interest to the present analysis.

Artifacts of class B have been analysed as due to carry-over of a non-equilibrium state of kind I of strongly coupled spins, which give rise to multiple-quantum coherences during t_1 and are detected as multiple-quantum transitions (MQT) in COSY spectra. Since the MQT artifacts are seen only at $\omega_1 \approx n\omega_2$ (fig. 1) the observed MQT are neither of type I, type II or type III of weakly coupled spins [5]. However the type I MQTs among strongly coupled spins would appear at $\omega_1 \approx n\omega_2$. The observed MQTs therefore arise from very strongly coupled spins all the resonances of which lie within a small range of a few Hertz. In the COSY algorithm, at the start of the second experiment just before the pulse (point 2 in fig. 2a), the non-equilibrium state of kind II [1] can be ruled out, since all off-diagonal elements of the density matrix are expected to decay during pe-



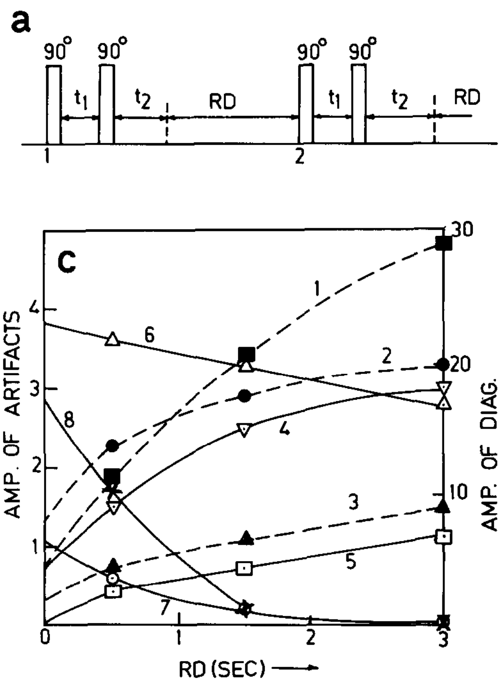


Fig. 2. (a) 2D COSY algorithm, having relaxation delay RD between each scan. (b) Cross sections parallel to F_1 axis, taken from fig. 1, at phenyl resonance (7.34 ppm), for various relaxation delays RD. The diagonal peak (D) grows in intensity while the double-quantum (DQ) and triple-quantum (TQ) artifacts decrease in intensity as RD is increased. The peaks indicated by asterisks are from the tails of the HDO resonances. (c) The experimental amplitudes of the diagonal and their class A or class B artifacts for various RD. The *Y* scale on the right-hand side corresponds to the diagonal peak amplitudes (dashed curves 1, 2 and 3) and that on the left corresponds to the amplitudes of the artifacts (full curves 4–8). Phenyl ring resonances at 7.34 ppm (diag, 1; class B artifacts DQ, 6; TQ, 7); CH_2 resonances (diag, 2; class B artifact DQ, 8); $CHCl_3$ (diag, 3; class A artifacts, 4, 5).

riod t_2 + RD (RD was varied from 0.1 to 5.0 s and t_2 = 0.171 s). Any significant failure of this decay would have resulted in a large number of various types of MQTs rather than just the specific ones observed. Therefore during RD only the non-equilibrium state of kind I need be considered.

The evolution of the diagonal elements of the density matrix during RD is through coupled rate equations:

$$\frac{d\chi_\alpha}{dt} = \sum_\beta W_{\alpha\beta}\chi_\beta \,, \quad (1)$$

where χ_α are the deviations of the diagonal elements of the density matrix from equilibrium and W are transition probabilities between states α and β [1,6–9].

For two strongly coupled spins (AB) eq. (1) has been solved in closed form for (i) mutual dipole–dipole relaxation and (ii) an isotropic random field with equal fields at sites A and B having arbitrary correlation between these fields [10]. For (iii), isotropic random fields having unequal fields at the two sites, a closed-form solution has not been found and numerical solutions have been performed.

For a single 90° pulse the amplitudes of the double- and zero-quantum coherences of an AB spin system, prepared in the non-equilibrium state of kind I, are given by [1]

$$\mathrm{DQ(AB)} = \tfrac{1}{4}[(P_1 - P_2) - (P_3 - P_4)] - \tfrac{1}{4}\sin 2\theta\,(P_2 - P_3)\,,$$

$$\mathrm{ZQ(AB)} = \tfrac{1}{4}\cos 2\theta\,[(P_1 - P_2) - (P_3 - P_4)] + \tfrac{1}{8}\sin 4\theta\,(P_2 - P_3)\,. \quad (2)$$

The first term, $[(P_1 - P_2) - (P_3 - P_4)]$, in the above equations expresses the lack of spin-temperature in the spin system, while the second term $(P_2 - P_3)$ expresses difference in spin temperature of sub-systems formed by energy levels [(1,2), (3,4)] and [(1,3), (2,4)]. Eq. (2) can be re-expressed as

$$\mathrm{DQ(AB)} = \tfrac{1}{4}\Delta_S - \tfrac{1}{4}\sin 2\theta\,\Delta_T\,,$$

$$\mathrm{ZQ(AB)} = \tfrac{1}{4}\cos 2\theta\,\Delta_S + \tfrac{1}{8}\sin 4\theta\,\Delta_T\,. \quad (3)$$

These equations show that even when the spin systems obey a Boltzmann distribution ($\Delta_S = 0$) [12], but have different spin temperatures for the sub-systems ($\Delta_T \neq 0$), double- and zero-quantum coherences will be created by a single pulse whenever the spins are strongly coupled. (The zero-quantum coherences of strongly coupled spins have nearly zero frequency and overlap with axial peaks.)

A detailed analysis of the evolution of Δ_S and Δ_T has been carried out for various relaxation mechanisms and for various initial conditions (the details will be published elsewhere [10]) and the following features are observed.

(i) *For weakly coupled spins:* If at RD = 0, Δ_S = 0, it remains so for all values of time and the system remains in an internal equilibrium, yielding no MQT. If $\Delta_S \neq 0$ at RD = 0 then it remains so for significant values of RD ($\approx T_1$) and would give rise to several MQTs for all types of spins, which have not been observed. In the present calculations the solutions have therefore been restricted to Δ_S = 0 at RD = 0.

(ii) *For strongly coupled spins:* For Δ_S = 0 and $\Delta_T \neq 0$ at t = 0, Δ_T decays with time, while Δ_S goes through a maximum and falls, indicating passage through a non-equilibrium in which no spin temper-

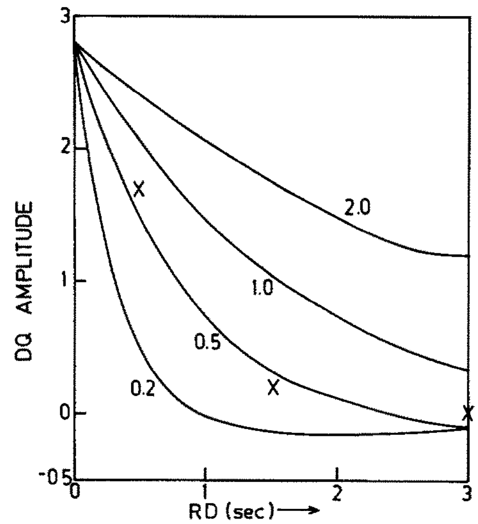


Fig. 3. Calculated amplitudes of double-quantum artifact of CH_2 protons (forming an AB spin system with δ = 6.2 Hz, J = 12.4 Hz), as a function of RD, for dipolar relaxation having T_D = 0.2, 0.5, 1.0 and 2.0 s. The experimental amplitudes are also shown with crosses (curve 8 of fig. 2c). The calculated amplitudes with the initial condition Δ_S = 0, $\Delta_T \neq 0$ are normalized to the extrapolated experimental value for RD = 0. Definition of T_D as in ref. [6].

ature can be defined during the intermediate periods [1]. There are significant MQT amplitudes for this situation, for various relaxation mechanisms.

Fig. 3 contains plots of calculated double-quantum amplitudes for the strongly coupled two-spin system (AB) of CH_2 protons of the *o*-benzyl group, as a function of RD for dipolar relaxation along with the experimental amplitudes. The general behaviour of the time dependence of the double-quantum amplitudes calculated for several parameters of the random field mechanism is also similar to the curves in fig. 3. The experimental time dependence is well explained by these calculations, confirming the origin of these artifacts as due to carry-over of the non-equilibrium state of kind I of strongly coupled spins.

The above analysis has been carried out for a two-spin system. A numerical solution of eq. (1) for higher-order spin systems is expected to show similar behaviour [10].

Recently Müller et al. [13] have observed forbidden peaks in multiple-quantum filtered COSY spectra and multiple-quantum spectra, arising due to unequal spin–spin relaxation of degenerate coherences. The multiple-quantum artifacts discussed in the present communication, on the other hand, are due to the presence of non-equilibrium states of kind I during spin–lattice relaxation of strongly coupled spins.

3. Conclusions

The observed MQT artifacts in COSY spectra are of a rather general nature and can occur whenever strongly coupled spins are present in the sample. Furthermore, these artifacts cannot be cancelled by the usual phase cycling procedures [2], since the carry-over effects will exist between experiments having different t_1 values in the COSY algorithm.

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