OPTIMIZATION OF NOESY AND ROESY THROUGH COMPUTER SIMULATION

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INTRODUCTION AND OBJECTIVE[1,2]

Nuclear Overhauser Effect Spectroscopy in laboratory and rotating frame (NOESY and ROESY) finds significant application in extracting internuclear distances for a range of molecular sizes, taken in conjunction with the nature of extreme narrowing, intermediate and spin diffusion limits interms of the value of the product of the spectrometer frequency and isotropic rotational molecular tumbling correlation time ($\omega_o \tau_c$). Various methods have been proposed to extract internuclear distances of the dipolar network from NOESY and ROESY intensities, which depend upon ω_o, mixing time (τ_m), and τ_c along with the distances. Since the dependence is multidimensional and spin diffusion creates serious problems with respect to bigger molecules, the optimization of τ_m for a particular spectrometer frequency is necessary in order to derive internuclear distances accurately. Pricipally, one can think of two approaches one being computer simulation and the other through trial experiments involving considerable instrumental time and we have used the former approach in this presentation.

METHOD[3-9]

The theory of NOESY and ROESY is well established and the basic expression which describes the behaviour of the intensities with τ_m is given by:

$$a(\tau_m) = \exp(-R\tau_m) \quad \ldots.(1),$$

where $a(\tau_m)$ is the matrix of intensities, the elements of which are normalized to the corresponding equilibrium magnetization when mixing time is set equal to zero. R is the total relaxation matrix and $a(\tau_m)$ can be easily constructed from R, since R is real, symmetric, and positive definite and further Equation (2),

$$\exp(-R\tau_m) = U\exp(-E\tau_m)U^T \quad \ldots.(2),$$

is valid where U and E are the eigen vector and eigen value matrices of R.

The computation of intensity matrix involves the construction of total relaxation matrix, diagonalization of R, exponentiation of the diagonal matrix and the transformation shown in the right hand side of Equation (2) followed by the drawing of the contour plots. Jacobi method is used for the diagonalization and a simple exponentiation of the diagonal elements exponentiate the whole matrix. Last step systematically puts the data of computed intensities with respect to a cross peak in a random file for various combinations of mixing and correlation times and collects different grid of intensities. These are used to draw contours. A simple computer program is written in BASICA for an IBM PC for a two spin system and has been converted in to FORTRAN-77 and modified to work for a maximum of ten spin system (extendable to any number of spin systems). Another option is also provided for the extraction of cross relaxation constants and in turn distances, knowing the correlation time accurately, from the matrix of NOESY or ROESY intensities.

RESULTS AND DISCUSSION

Simulations have been done for two and three spin systems for distances 2.0Å, 2.5Å, and 3.0Å in extreme narrowing and spin diffusion regimes with respect to NOESY and ROESY

(strictly speaking extreme narrowing or spin diffusion has no meaning for ROESY). It is very clear that the contours (Fig. 1) comprise of short and long mixing time regimes on the opposite sides of the saddle point, which corresponds to the mixing time for maximum NOE in connection with the range of $\omega_o\tau_c$'s under consideration. Fig.1 shows the equal intensity contour plot for a distance of 2.5Å in extreme narrowing and spin diffusion limits.

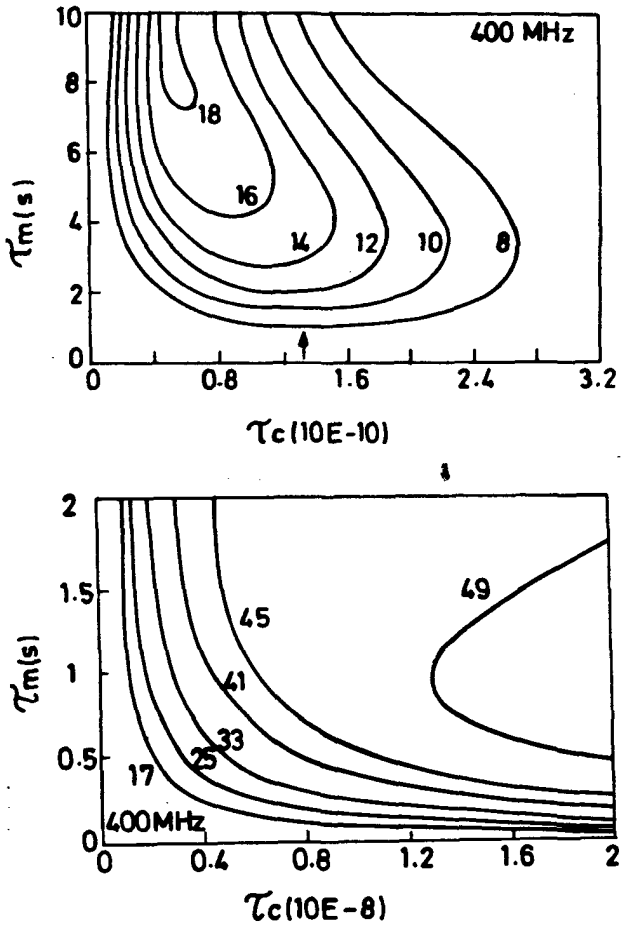


Fig.1 Iso-intensity(%NOE) contour plots for two-spin system(r=2A):400 MHz NOESY top: τ_m vs τ_c -extreme narrowing limit

bottom: same, spin diffusion limit contour numbers indicate % NOE.

It is reasonable to define the optimal τ_m- range as the set of shorter periods leading to between 60% and 80% of the maximum attainable NOE. In this range of τ_m's the time course of NOE is linear. The linearity holds good to a greater extent in the extreme narrowing limit. In the spin diffusion limit, however the NOE build-up is much faster and we take 20% to 80% of the NOE maximum as the optimum intensity range. In a similar fashion we may also relax the so called optimum intensity range for longer distances. Thus one can readily choose the optimal τ_m-range in which the intensity build-up is linear for a given isotropic rotational tumbling correlation time for a two-spin approximated analysis. A τ_m in the above mentioned range with appreciable intensity would also be a good choice for matrix analysis.

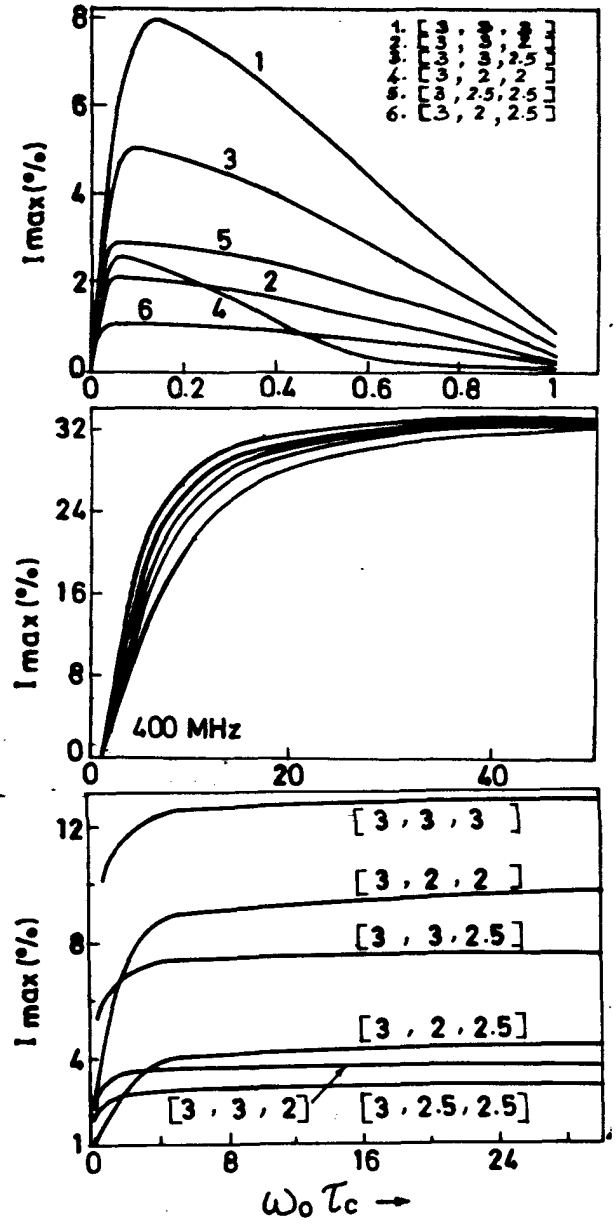
Similarly NOE intensities are computed for various combinations of distances and τ_m's for two typical $\omega_o\tau_c$'s, one in extreme narrowing and the another in the spin diffusion regime. The distances chosen range from 2.0Å to 4.0Å. These intensities may be used to draw strip plots from which one can easily pick out optimal mixing time range for a given distance.

A three spin system is defined by the following 'distance vector':

$$[D_1,D_2,D_3] = \begin{bmatrix} 0.0 & d_{12} & d_{13} \\ d_{21} & 0.0 & d_{23} \\ d_{31} & d_{32} & 0.0 \end{bmatrix} \quad \text{.....(3)}$$

where D_1, D_2, and D_3 are the distances respectively for between the spins 1 and 2, spins 1 and 3, and spins 2 and 3; ie. $D_1=d_{12}=d_{21}$, $D_2=d_{13}=d_{31}$ and $D_3=d_{23}=d_{32}$. This sort of ordered representation of distances may be used to define spatial network of any number of spins. A three spin system consists of six independent combinations for a

particular distance between spins 1 and 2, when we consider 2.0A, 2.5A, and 3.0A as the constituent distances. The results of simulation are presented in the form of $\omega_o \tau_c$ vs I_{max} and $\omega_o \tau_c$ vs τ_{max} plots (Fig. 2) for NOESY and only $\omega_o \tau_c$ vs I_{max} (for simplicity) with respect to ROESY, where I_{max} is maximum NOE% attainable and τ_{max} is the corresponding τ_m. The results are presented only for the distance 3.0Å between spins 1 and 2.



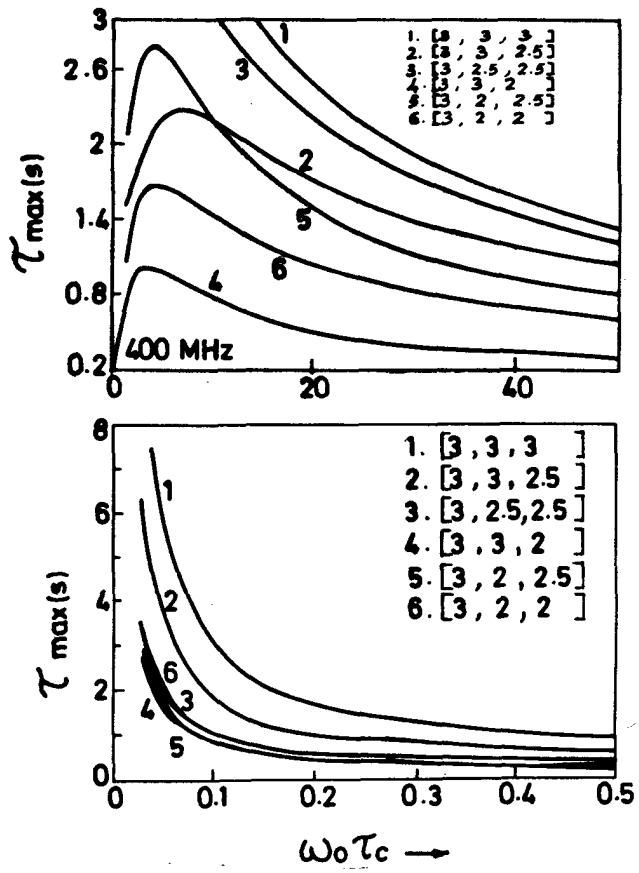


Fig.2.1 Imax vs $\omega_o \tau_c$ for three-spin system for NOESY(extreme narrowing limit) for r=1A between spins 1 & 2.

Fig.2.2 Same as 2.1 for spin diff. limit

Fig.2.3 Same as 2.1 for ROESY

Fig.2.4 τ_{max} vs $\omega_o \tau_c$ for three-spin system for NOESY, extreme narrowing limit

Fig.2.5 Same as 2.4, spin diff. limit

(Figs. 2.1 to 2.3 are in the left top to bottom and Figs. 2.4 and 2.5 are above)

In the extreme narrowing regime it was found that the I_{max} vs $\omega_o \tau_c$ is similar irrespective of the distance

matrix, the exception being when the distance matrix comprises of shorter distances (like [3.0,2.0,2.0]) and is necessarily due to 'three spin effect'[1]. In the spin diffusion limit I_{max} depends exponentially on $\omega_o\tau_c$ and approaches towards the equilibrium I_{max} (I^o_{max}) with longer $\omega_o\tau_c$'s. It is clear that the nature of combination has a little influence on the dependence. $\omega_o\tau_c$ vs I_{max} is exponential for extreme narrowing limit but for long correlation limit it rises sharply, reaches a maximum and descends monotonically to zero with $\omega_o\tau_c$. We have done simulations for hypothetical multispin systems (up to ten spins) keeping all the distances equal to 2.0A and it was found that I_{max} decreases drastically for a particular $\omega_o\tau_c$ when we increase the number of spins;but for spin diffusion limit this does not occur.

In conclusion, the selection of optimal mixing times can be approached in two ways;either through equal intensity contours or through $\omega_o\tau_c$ vs I_{max} in combination with $\omega_o\tau_c$ vs τ_{max} plots. However, sometimes the simulation as a function of distance (instead of $\omega_o\tau_c$) would be very useful. In the case of NOESY with respect to extreme-narrowing regime, and ROESY, It is not necessary to go beyond four or five spins irrespective of the total number of spins involved in the dipolar network. On the other hand, in the spin diffusion limit, we must simulate for the distance matrices involving a maximum number of spins. The advantage of ROESY over NOESY in the spin diffusion limit is that various combinations of a three spin system, with one fixed distance, have very characteristic initial rise and flat portions of I_{max} ($\omega_o\tau_c$ vs I_{max} plot for ROESY in Fig.2). This leads us to derive some qualitative ideas about the spin system, assuming that the particular cross peak is part of a three spin system by looking at I_{max}, knowing approximately $\omega_o\tau_c$. Since we can largely eliminate spin diffusion by choosing a mixing time which is well below τ_{cr} (the mixing time at which the sign change takes place in the NOE build-up), it is advisable to do ROESY experiments for $\omega_o\tau_c$ up to 10, even though NOE intensities are small when compared to NOESY for $\omega_o\tau_c$'s beyond 5. Initial build-up rate approximation is still valid. Thus the observation of 'three spin efeect' is made advantageous for ROESY which is not so in the case of NOESY. Furthermore, along with the advantage of 'three spin effect' we may utilize greater intensity criterion for $\omega_o\tau_c$'s ranging from .1 to 1.

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