Computer Simulations of High Resolution NMR Spectra

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1 Introduction

The evolution of a spin system during the application of an RF pulse is a central aspect of high resolution NMR spectroscopy. An appropriately applied field can lead to saturation or decoupling effects. When a field is used to maintain a spin-locked condition, both scalar and dipolar interactions come into play, leading to coherence transfers and nuclear Overhauser effects that form the basis of TOCSY, ROESY and related experiments. These experiments have important roles in structural studies of biological macromolecules in solution[1-5]. The accompanying bad news is that the interpretation of these experiments is tricky; computer simulations can be very helpful in assisting the analysis of these spectra.

The theoretical formalism of relaxation in the presence of an RF field can be written elegantly in superoperator notation[6], or in a more conventional notation laden with superscripts and subscripts.[7] The latter approach displays more details and shows how the presence of the RF field introduces new combinations of frequencies into the relaxation expressions. Of course, it can be shown that both methods generate identical results. Details of the theoretical developments formulated in this lab are given in reference 7 and in a manuscript in preparation.

We have used our theoretical results to extend the program GAMMA[8] to include the effects of relaxation in the presence of an RF field and present here the results of several calculations of double resonance or rotating frame experiments that illustrate the capabilities thereby developed. For the examples presented, only dipole-dipole relaxation was included, and isotropic diffusional tumbling was assumed with a correlation time of 1.0 nsec. In all cases, the spectrometer proton frequency was 500 MHz.

2 Applications

Presaturation - The first example is a presaturation experiment on a three spin system whose parameters were chosen to mimic three protons in the *trans* conformation of a glycine residue. Details are given in Table 1.

Table 1: Spin System 1 Parameters

$R_{12} = 1.75Å$	$J_{12} = -16.3$ Hz	$v = -700$ Hz
$R_{13} = 3.00Å$	$J_{13} = 4.0$ Hz	$v = -950$ Hz
$R_{23} = 2.53Å$	$J_{23} = 12.0$ Hz	$v = 950$ Hz

The pulse sequence consisted of a pre-irradiation period followed by an analyzing 90° pulse, as shown in Figure 1.

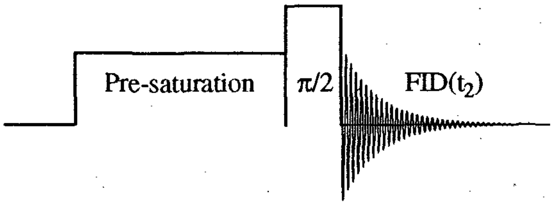

Figure 1. Pre-saturation Pulse Sequence

The initial pulse length was chosen to be long enough to establish a steady state, and the RF frequency chosen to match the chemical shift of proton 2. The resulting spectra for several RF field strengths are shown in Figure 2. Notice the

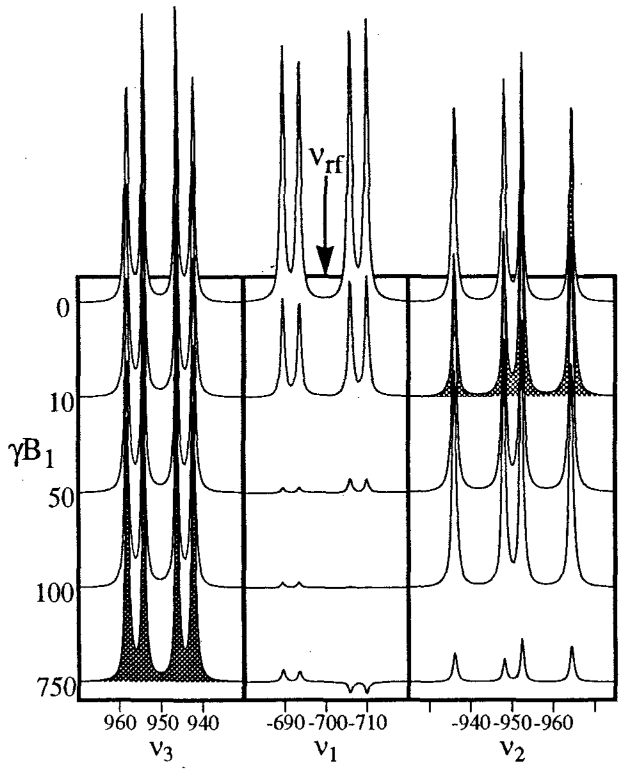

Figure 2. Simulation of Presaturation. Shading is used to highlight NOE enhanced intensities. Units on both axes are Hz.

non-symmetrical effects on spins 1 and 3.

Decoupling - The second example illustrates the decoupling of a two spin proton system with a chemical shift difference of 1000 Hz and a spin coupling constant of 10 Hz. The protons were 2Å apart. After an initial 90° pulse, an RF field of a specified magnitude was applied during the collection of the FID. The pulse sequence is shown in Figure 3. Some spectra are shown in Figure 4. Notice the slight oscillation in the peak height as a function of the strength of the decoupling field.

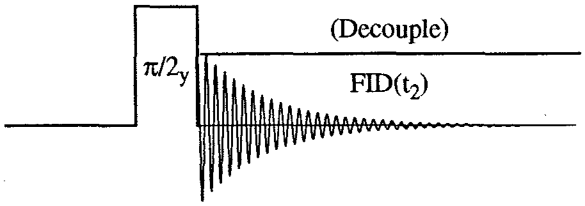

Figure 3. Decoupling Pulse Sequence

ROESY/TOCSY Simulations - Two-dimensional rotating frame experiments may produce coherence transfer (TOCSY) and spin-spin cross relaxation (ROESY) effects simultaneously, and ambiguities of interpretation arise when both kinds of effects are present. Griesinger *et al.* have described a strategy for suppressing ROESY effects when doing TOCSY experiments[9]. The interpretation of ROESY experiments is complicated because coherence transfer (COSY) and indirect interactions (HOHAHA) can lead to spectral effects that interfere with or imitate rotating frame Overhauser features[10]. Methods for suppressing these unwanted effects have been proposed[3], and it has been suggested that use of a train of short RF pulses to generate an effective spin-locking field leads to diminution of COSY-type cross peaks[11].

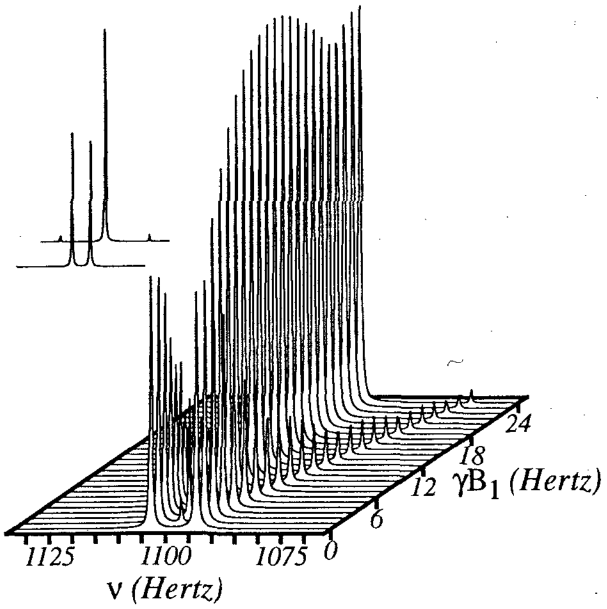

Figure 4. Simulation of Decoupling. Spectra at the first and last field strengths are shown to the upper left.

Here we simulate several variations of ROESY experiments. First, consider the pulse sequence shown in Figure 5 in which a continuous spin-locking pulse is applied.

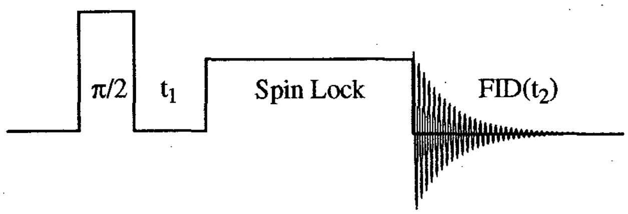

Figure 5. CW ROESY Pulse Sequence

We apply this to an equilateral triangle of protons as described in Table 2. The 2D spectra that

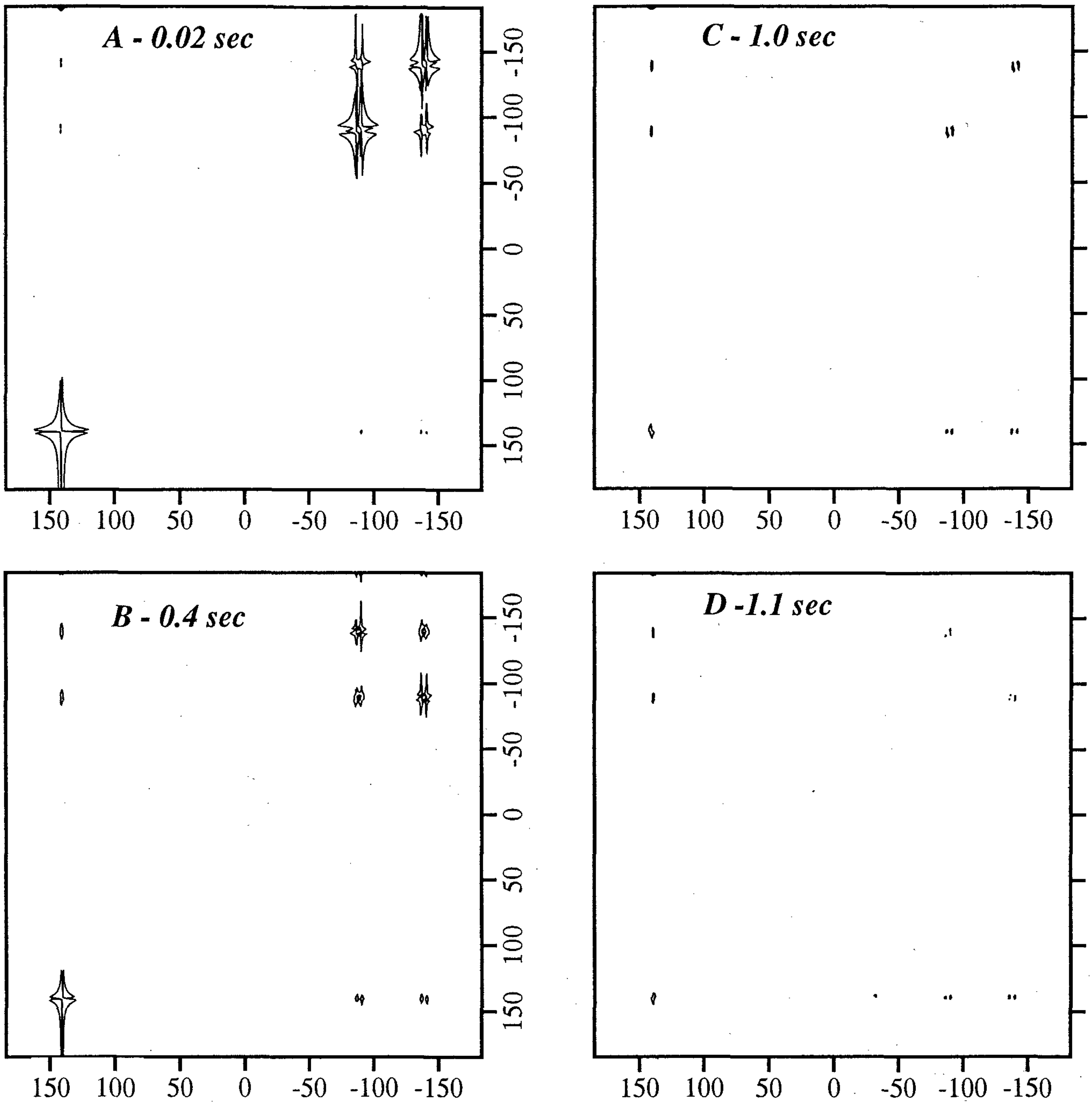

Figure 6. 2D ROESY spectra for differing spin-lock times.

result from several different spin-lock times are shown in Figure 6. A point to notice here is that the ROESY peaks grow more slowly than the COSY peaks, reaching their maximum intensity well after the latter have begun to subside.The-ROESY peaks also persist much longer and decay more smoothly than both the COSY and diagonal peaks for protons 2 and 3. The plots for 1.0 and 1.1 sec. spin-lock times show oscillation in both the latter features while the ROESY peaks decay smoothly.

Table 2: Spin System 2 Parameters

$R_{12}=\sqrt{3}$Å	$J_{12}=0$	v = 140 Hz
$R_{13}=\sqrt{3}$Å	$J_{13}=0$	v = -90 Hz
$R_{23}=\sqrt{3}$Å	J_{23} = 4.0 Hz	v = -140 Hz

Interesting contrasts to these results arise in changing to an isosceles triangle configuration. The spin system is given in Table 3. In this geometry, the ratio of the 1-3 distance to the 1-2 distance is $\sqrt{2}$. Thus, the direct 1-2 dipole-dipole interaction is 8 times stronger than the corresponding 1-3 interaction. Keeping a fixed geometry and a spin-lock time of 0.1 sec., the 2-3 spin-spin coupling is varied from 0 to 10 Hz as we repeat the pulse sequence of Figure 5. Slices through the resulting 2D spectra at 140 Hz are shown in Figure 7.

Table 3: Spin System 3 Parameters

$R_{12}=\sqrt{3}$Å	$J_{12}=0$	$v = 140$ Hz
$R_{13}=\sqrt{6}$Å	$J_{13}=0$	$v = -90$ Hz
$R_{23}=\sqrt{3}$Å	$J_{23}=0$ - 10 Hz	$v = -140$ Hz

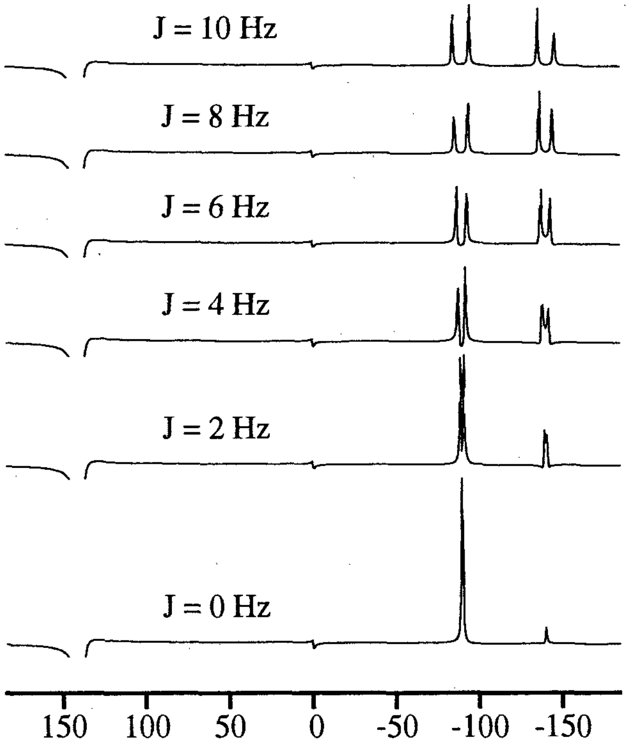

Figure 7. The effect of J_{23} variation on ROESY/ TOCSY experiment.

Indeed, the simulation with $J_{23} = 0$ shows an 8:1 ratio of NOE peak intensities. However, variation of J_{23} generates a ROESY-like peak in the location of the 1-3 interaction that becomes even larger than the true 1-2 ROESY peak for some values of J_{23}. This situation provides an excellent example of a spectrum that cannot directly yield reliable information about internuclear distances.

In the next example, the frequency of the spin-locking RF field is varied. In this simulation spin system of Table 3 is used with $J_{23} = 4$ Hz. It is subjected to a 1.0 sec. spin-locking pulse as depicted in the ROESY pulse sequence in Figure 5. The frequency of the RF field is indicated by the arrow on each of the stacked plots in Figure 8. While the 1-2 ROESY peak is relatively unaffected by the frequency of the RF field, the 1-3 peaks which are generated by indirect HOHAHA effects show extreme sensitivity both in magnitude and sign. As has been suggested in the literature, several experiments should be run with different RF frequencies to discriminate between these interactions.

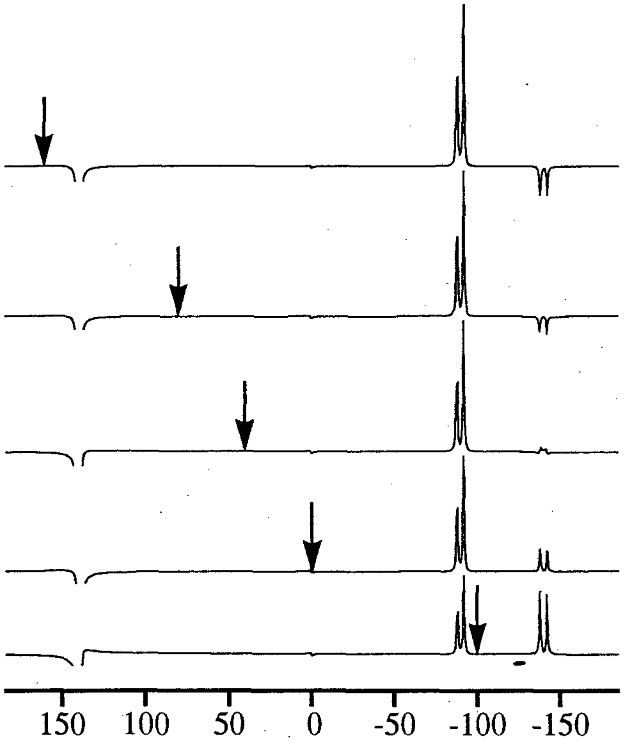

Figure 8. The effect of varying the applied RF frequency on ROESY/TOCSY experiment.

As a final demonstration, we investigate the suggestion that dividing the spin-locking period into a sequence of numerous pulse-delay steps can emphasize the ROESY interactions. Again, the three spin proton system in Table 3 is used with $J_{23} = 4$ Hz. It was subjected to the ROESY pulse sequence utilizing a pulse train as shown in Figure 9. The length of the pulse and delay steps were varied keeping the average γB_1 constant at 2000 Hz by adjusting the number of pulses per second. This simulation has been performed with pulse lengths that correspond to individual rotations of 180°, 30°, and 10°, and also with CW irradiation. The contour plot using 30° pulses is presented in Figure 10. For comparison, the plot which was simulated using continuous irradiation during the spin lock is shown in Figure 11. Cross sections of these contour plots taken at -90 Hz are shown in Figure 12.

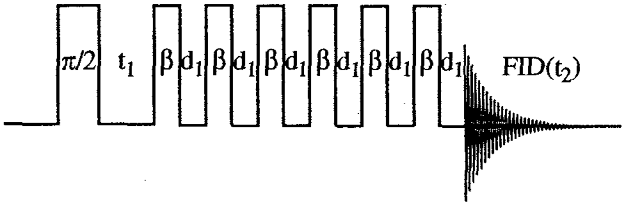

Figure 9. ROESY Pulse Sequence with pulse train

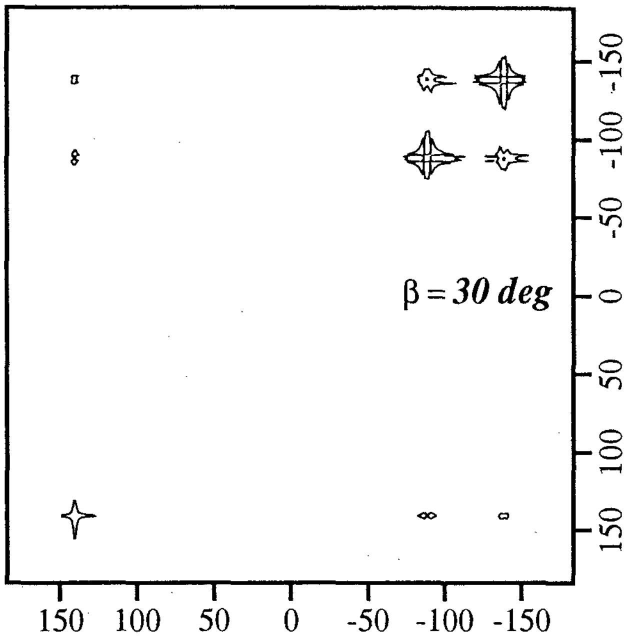

Figure 10. Simulation of ROESY/TOCSY experiment using a spin-locking pulse train.

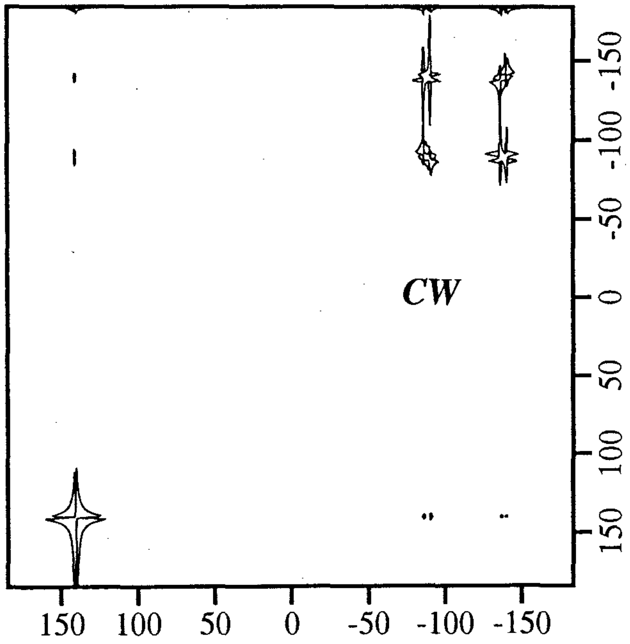

Figure 11. Simulation of ROESY/TOCSY using continuous irradiation for spin-locking.

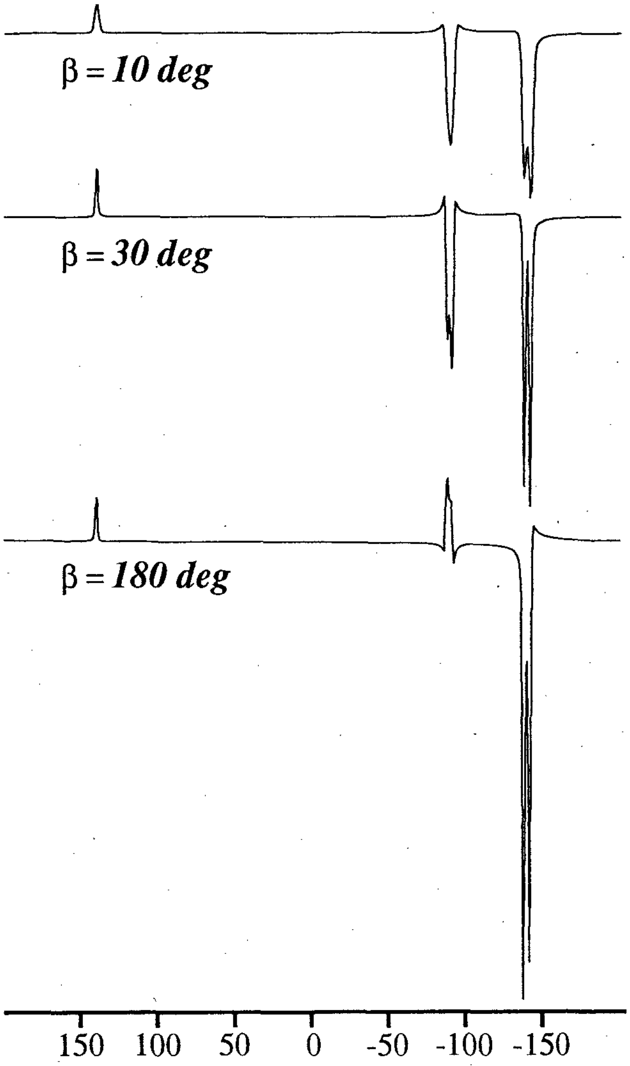

Figure 12. Cross sections of ROESY/TOCSY simulations using a spin-locking pulse train.

Shortening the pulse length indeed suppress the COSY peaks, but they remain even for the shortest pulses. Also, the limit of the sequence of shorter and more frequent pulses gives the same result as continuous irradiation. These two processes appear to become the same once the delay between pulses is sufficiently short that a negligible precession occurs before the next pulse.

3 Conclusions

It is clear that full computer simulation may be essential for the correct interpretation of ROESY experiments. Our initial results indicate that there may be additional information about structure and dynamics that can be extracted from classical one-dimensional multiple irradiation experiments with the aid of simulations. An appreciable expansion of this program in terms of the size of spin systems that can be handled is probably needed to make such applications practical; this along with other expansions of the program's capabilities is planned.

4 References

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