

Analysis of Spin Diffusion and Cross Correlation on the Net Nuclear Overhauser Effect in NMR

P. K. Madhu* and Anil Kumar*†

*Department of Physics and †Sophisticated Instruments Facility, Indian Institute of Science, Bangalore 560 012, India

Received September 12, 1996; revised May 1, 1997

The effect of dipole–dipole cross correlations on the net nuclear Overhauser effect (NOE) has been analyzed here for realistic systems by extending the three-spin calculations to four and five spins in order to account for additional cross correlations and spin diffusion. These have been compared with the addition of leakage terms to the three-spin system. The additional spins enhance cross-correlation effects on one hand but on the other act as supplementary relaxation pathways for the magnetization to diffuse. This analysis shows that for a linear array of spins in the long-correlation limit, dipole–dipole cross correlations increase net NOE, while spin diffusion decreases it, and that the cumulative effect is a reduced effect of cross correlations. In other geometries and correlation limits, the effect of cross correlations on net NOE is generally small. © 1997 Academic Press

INTRODUCTION

With the advent of two- and multidimensional NMR, it has become possible to obtain structures of biomolecules in solution. The main information on structure is obtained by monitoring the transfer of polarization from one spin to another in the molecule, also known as the nuclear Overhauser effect (NOE) through the well-known two-dimensional NOESY experiment (1–4). Most interpretations of NOESY cross-peak intensities neglect cross correlations arising from multiple pathways of relaxation of a spin. It has been recently emphasized that cross correlations can give rise to a large multiplet effect as well as a significant net effect (5–9). The multiplet effect, which is a differential relaxation and NOE effect among the various transitions of a spin, the observation of which requires resolution of various transitions of a spin by J coupling, can be suppressed by the use of a nondiscriminatory 90° measuring pulse. On the other hand, it has been shown that the multiplet effect can be put to good use to obtain additional information on the structure of molecules (10–12).

Detailed analyses have also been carried out on the net effect of cross correlations on NOE (8, 13, 14). It has been shown that the net effect is a second-order effect in time and that a significant net effect can build up in a linear arrangement of spins, especially at long mixing times

(13, 14). These conclusions have been based on an analysis carried out for a system of three relaxation-coupled spins (13, 14), which forms the smallest spin system for which dipole–dipole cross correlations can contribute. This three-spin-system analysis, while highlighting the contribution of dipolar cross correlations, is restricted in its applicability, since it assumes an isolated three-spin system, particularly in the long-correlation limit where there are no leakage mechanisms for relaxation. As a result, the nonequilibrium magnetization remains within the spin system for a significant time, building up the effects of cross correlations. If there are additional spins present, they on one hand act as additional sources for cross correlation, while on the other, they act as sources of leakage of magnetization. In order to obtain a realistic estimate of the effects of cross correlations on net NOE, we examine in this paper the effects of addition of fourth and fifth spins to a linear array of three spins and compare the results with nonspecific leakage of magnetization in the three-spin system. It may be mentioned here that there is no simple way that the net effect of cross correlation can be suppressed, and a neglect of the same can lead to systematic errors in the distance estimation.

RESULTS AND DISCUSSIONS

The earlier analysis of the effect of dipole–dipole cross correlations on net NOE in a three-spin system revealed that the effect is largest in a linear geometry of the three spins and in the long-correlation-time regime ($\omega\tau_c > 1$), and that the maximum effect of approximately 25% of the total magnetization appears at long mixing times, for $\omega\tau_c = 10$ (13). For the same three-spin system, Fig. 1 shows the differences in the NOE calculated with and without cross correlation for fixed mixing times (100, 200, and 400 ms) as a function of $\omega\tau_c$ for $\omega = 300$ MHz. This shows that the error arising from the neglect of cross correlations for a given mixing time, although small, is not negligible. The error reaches its maximum value of approximately 1, 2, and 7% of the total magnetization for mixing times of 100, 200, and 400 ms respectively for $\omega\tau_c$ between 1.2 and 1.6. It may be

noted that the net NOE at $\omega\tau_c = 1.6$ with cross correlation, for the above mixing times, is 6, 11, and 16% of the total magnetization, respectively. Thus the error is approximately 16, 18, and 44% of the net NOE at these mixing times.

The error in net NOE for $\tau_m = 400$ ms is a significant fraction of the total NOE for $\omega\tau_c = 1.6$ and decreases significantly for $\omega\tau_c = 10$. Some of our earlier conclusions have been based on the results at $\omega\tau_c = 10$ (13, 14). However, there are many biomolecules which fall in the region of $\omega\tau_c = 2-5$. Figure 2 shows the net NOE calculated with and without cross correlation in the linear three-spin system for $\omega\tau_c = 2, 3$, and 5 as a function of τ_m . These curves show that at intermediate mixing times such as 400 ms, the NOE with cross correlation is 24, 35, and 48% of total magnetization and the error is 7, 5, and 3% of total magnetization at $\omega\tau_c = 2, 3$, and 5 respectively, and therefore the error is a significant fraction (0.28, 0.15, 0.06) of net NOE. For experiments performed near these parameters, cross correlations thus have significant influence on net NOE. The maximum error in net NOE, however, is much larger and appears at very long mixing times, reaching a value of 22, 23, and 24% of the total magnetization at mixing times of 1.8, 2.6, and 3.9 s, respectively, for the three $\omega\tau_c$ values, Fig. 2.

The above analysis is for a three-spin system. However,

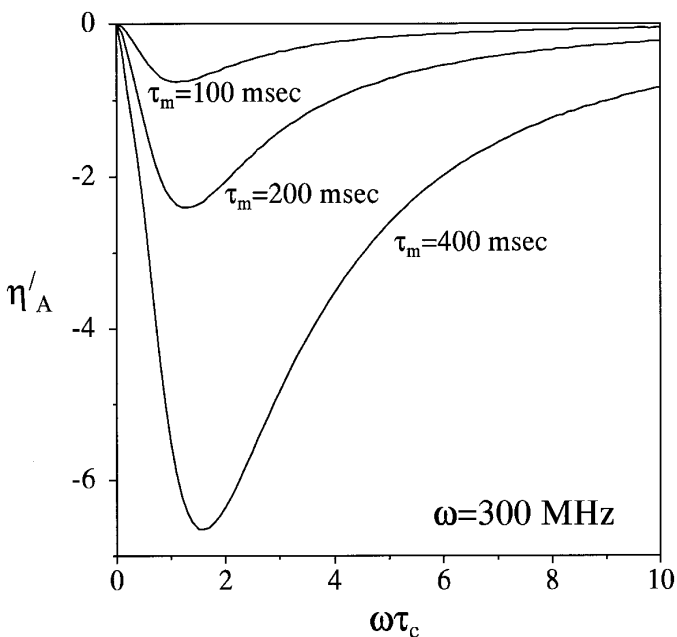


FIG. 1. The difference in the net NOE on spin A (after selective inversion of spin M at $\tau_m = 0$ for a linear arrangement of A, M, and K spins having an interspin distance of 2 Å) calculated with and without cross correlation in percentage is plotted as a function of τ_c for mixing times $\tau_m = 100, 200$, and 400 ms for $\omega = 300$ MHz. η_A is defined as $[A_z(\tau_m)/A_0]\%$ while $\eta'_A = (\eta_A)_w - (\eta_A)_{wo}$, where $(\eta_A)_w$ is the NOE calculated with cross correlation and $(\eta_A)_{wo}$ is the NOE calculated without cross correlation. Identical curves are obtained for spin K in this case.

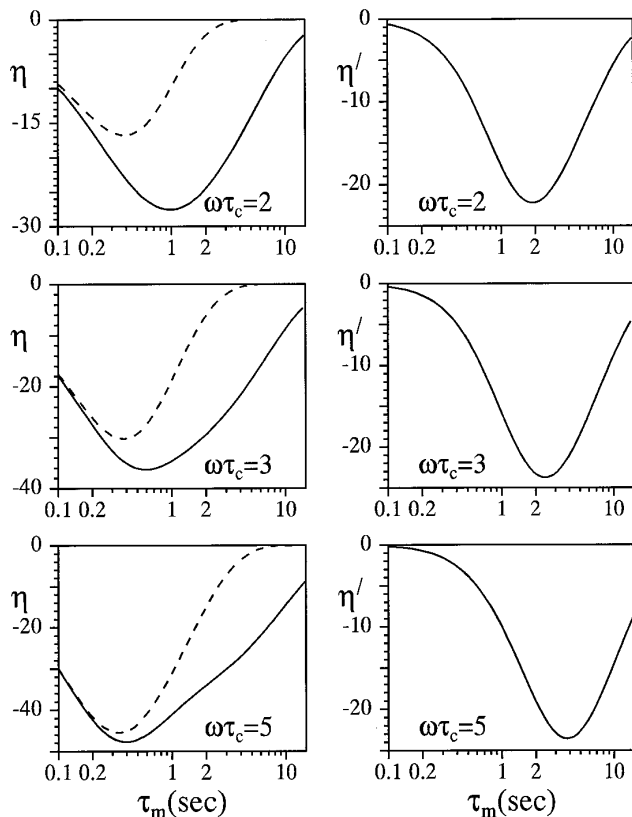


FIG. 2. (Left) Calculated net NOE in percentage on spins A and K after selective inversion of spin M at $\tau_m = 0$ is shown as a function of τ_m for a linear arrangement of A, M, and K spins having an interspin distance of 2 Å. The dashed curves represent the calculated net NOE without cross correlation and the solid curves represent that with cross correlation. (Right) The difference between these two calculated NOE values is shown by solid curves. The top, middle, and bottom traces correspond respectively to $\omega\tau_c = 2, 3$, and 5 for $\omega = 300$ MHz. The NOE values on A and K spins are identical in this case.

in practice unless special experiments involving spin locking of interacting spins are performed (15), there are other relaxation-coupled spins present. These additional spins act as supplementary sources of cross correlations as well as generating more pathways for the magnetization to diffuse, thereby reducing the NOE on the spins of interest. To analyze the effect of spin diffusion and cross correlation on net NOE, the above calculation has been extended to four- (AMKP) and five- (AMKPX) spin systems in linear configuration. Addition of the fourth spin, Fig. 3, for $\omega\tau_c = 2, 3$, and 5, reduces the net NOE, with and without cross correlation on the first spin (A) is relatively unaffected. For example at $\tau_m = 400$ ms, the NOE with cross correlation on the K spin on selective inversion of spin M at $\tau_m = 0$ drops to 18, 26, and 33% of total magnetization, but the errors as a percentage of the total magnetization, given by 6, 5, and 3% for $\omega\tau_c = 2, 3$ and 5 respectively, remain

unchanged. However, the error as a fraction of the total NOE has gone up in this case. On the other hand, at longer mixing times, the net NOE on the K spin as well as the maximum error on both K and A spins is significantly reduced for all $\omega\tau_c$. The maximum error for $\omega\tau_c = 5$ decreases more dramatically than that for $\omega\tau_c = 2$ as well as that for $\omega\tau_c < 1$ (figure not shown), since in the case of $\omega\tau_c = 2$ and for the short-correlation limit ($\omega\tau_c = 0.1$), there is already significant leakage in the relaxation process of the three-spin system, and the addition of the fourth spin only adds a small additional leakage pathway for the magnetization. On the other hand, for the long-correlation limit, $\omega\tau_c \geq 5$, there is little leakage in the three-spin system, resulting in a significant effect of cross correlation (Fig. 2). It may be noted that while both the NOE and the maximum errors are reduced, the errors as a percentage of NOE are still significant.

Addition of a fifth spin linear to the linear array of four spins with interspin distances of 2 Å is analyzed next. Figure 4 shows the net NOE and the error in net NOE on various

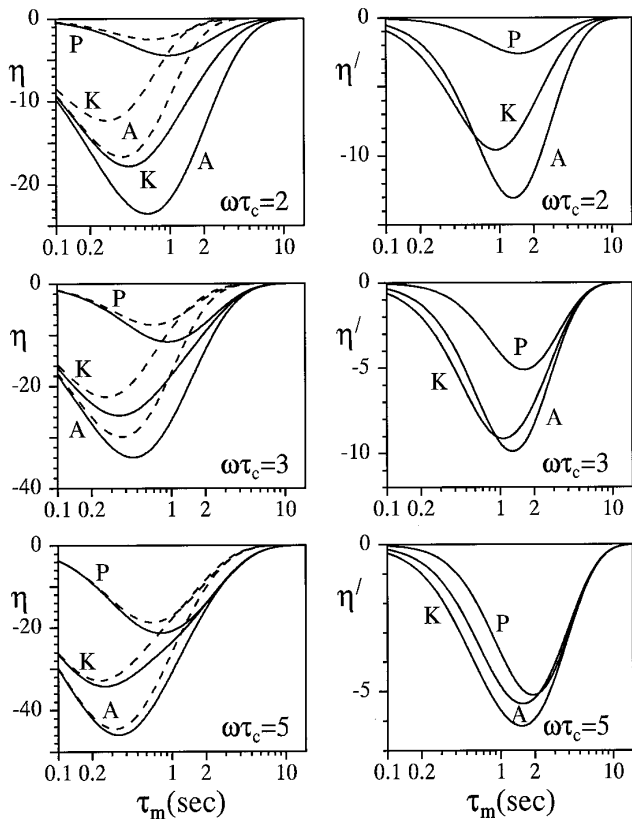


FIG. 3. (Left) Calculated net NOE in percentage on spins A, K, and P after selective inversion of spin M at $\tau_m = 0$ is shown as a function of τ_m for a linear arrangement of A, M, K, and P spins for $\omega\tau_c = 2, 3,$ and 5 with an interspin distance of 2 Å and $\omega = 300$ MHz. The dashed curves represent the calculated net NOE without cross correlation and the solid curves represent that with cross correlation. (Right) The differences between the NOE values calculated with and without cross correlation are shown for each spin.

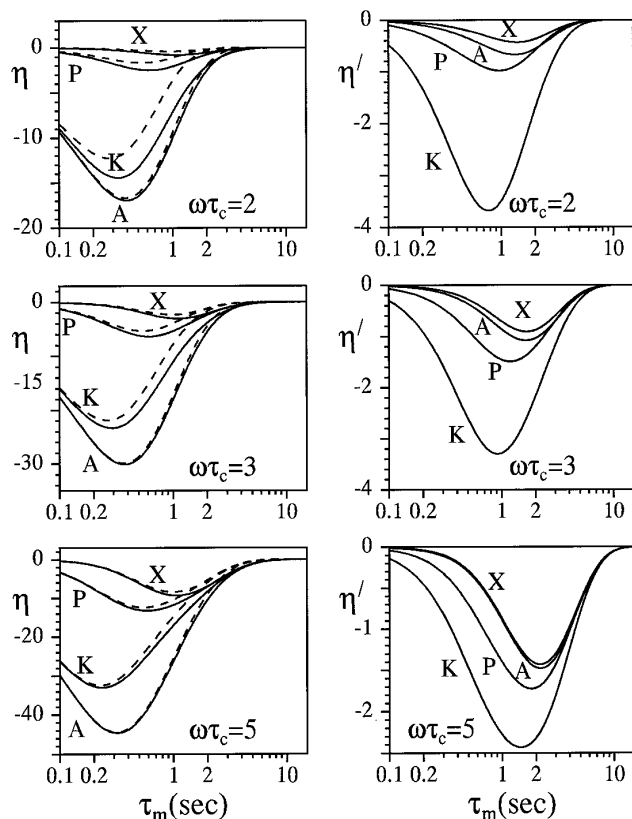


FIG. 4. (Left) Calculated net NOE in percentage on spins A, K, P, and X after selective inversion of spin M at $\tau_m = 0$ is shown as a function of τ_m for a linear arrangement of A, M, K, P, and X spins for $\omega\tau_c = 2, 3,$ and 5 respectively with an interspin distance of 2 Å and $\omega = 300$ MHz. The dashed curves represent the calculated net NOE without cross correlation and the solid curves represent that with cross correlation. (Right) The differences between the NOE values calculated with and without cross correlation are shown.

spins for $\omega\tau_c = 2, 3,$ and 5 when the second spin is inverted at $\tau_m = 0$. It turns out that the maximum errors are small in all cases and are less than those in the four-spin case and that the errors are significantly reduced even at intermediate mixing times such as $\tau_m = 400$ ms. It is noted from these curves that, while the calculated net NOE without cross correlation on A and K spins changes little when the fifth spin is added, the effect of cross correlation decreases significantly. On the other hand, with the addition of the fifth spin, the net NOE calculated with and without cross correlation on the fourth spin (P) (Fig. 4) decreases significantly.

In order to assess whether spin diffusion and cross correlation can be mimicked by a leakage process, we compare the four- and five-spin calculations (including cross correlations) with the three-spin calculation with leakage terms added to the diagonal elements of the relaxation matrix (excluding cross correlations). If this leads to acceptable results, it will establish whether one can use Solomon's equations (containing evolution of only single-spin modes and neglect-

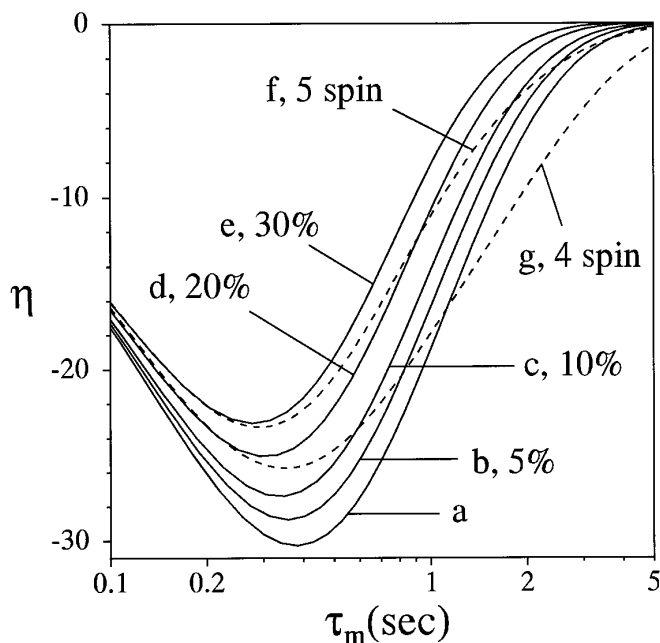


FIG. 5. Net NOE (curve *a*) in percentage on spin K in a linear AMK spin system, calculated without cross correlation after selective inversion of spin M at $\tau_m = 0$. Curves *b*–*e* correspond to the above situation but with the addition of nonselective leakage terms corresponding to 5, 10, 20, and 30% of the average relaxation rates of single-spin orders, $\frac{1}{3}(\rho_A + \rho_M + \rho_K)$, respectively, to each of the diagonal elements of the rate matrix. Dashed curves *f* and *g* correspond to net NOE calculated for the K spin in a linear arrangement of four, AMKP, and five, AMKPX, spin systems, respectively, without any leakage but with cross correlation, after selective inversion of spin M at $\tau_m = 0$. In all the above calculation, the interspin distance is taken as 2 Å, $\omega\tau_c = 3$, and $\omega = 300$ MHz.

ing all cross correlations) with leakage terms added to the diagonal elements, tremendously reducing the dimension of the relaxation matrix to be handled from $2^N \times 2^N$ to a maximum of $N \times N$ (13, 14). Figure 5 shows the calculated NOE on the K spin in the AMK spin system, on inversion of the M spin at $\tau_m = 0$, calculated without cross correlations but with different amounts of leakage added to all the diagonal elements of Solomon's equation, for $\omega\tau_c = 3$. Curve *a* is without any leakage and curves *b*–*f* are, respectively, with 5, 10, 20, and 30% leakages. Figure 5 also shows the calculated transient NOE on K spin in the AMKP and AMKPX spin systems with M being inverted at $\tau_m = 0$, calculated with cross correlations (dashed curves) for $\omega\tau_c = 3$. It is seen from these curves that for short mixing times the four-spin net NOE including cross correlation matches the three-spin net NOE calculation without cross correlations but with 20% leakage. For longer mixing times, the amount of leakage must be reduced, and at very long mixing times beyond 1 s, the three-spin NOE without cross correlation becomes smaller than the four-spin NOE with cross correlation. Leakage then cannot account for the spin diffusion. To match the five-spin case, the leakage term must be 30% for short mix-

ing times and reduced for long mixing times with the three-spin NOE without leakage becoming smaller than the five-spin case after $\tau_m = 3$ s.

The above results indicate that in the presence of significant spin diffusion, the effect of cross correlation on the net NOE becomes small. Both spin diffusion and net effects of cross correlation are second-order processes. In the long-correlation limit for all mixing times for a linear array of spins, cross correlations increase the net NOE as seen by the curves of Figs. 2–4 while spin diffusion tends to decrease it. Thus, the two effects compete against each other and compensate.

CONCLUSIONS

Earlier work on a three-spin system revealed significant effects of cross correlation in net NOE at long mixing times. It predicted an overestimation of distances from NOESY data upon neglect of cross correlation. In this paper, it is shown that, in a linear three-spin system, effects of cross correlation on net NOE are quite significant even for usually employed intermediate mixing times, for the range $\omega\tau_c = 2$ –5. These effects are further analyzed for four- and five-spin systems with linear geometry, as well as compared with a nonspecific leakage in a three-spin system. It is found that, for intermediate mixing times in the above correlation limits, the errors continue to be significant but decrease in magnitude for longer mixing times. Furthermore, it is found that one can mimic the transport of magnetization to outer spins from a finite spin system neglecting cross correlations by using a nonspecific leakage, but only for short mixing times. Thus, we conclude that for intermediate and long mixing times (200–500 ms) for $\omega\tau_c \approx 2$ –5, there is a significant effect of cross correlation on net NOE. It is therefore suggested that unless cross correlations are explicitly taken into account, only semiaccurate distances may be estimated from net NOE. This applies particularly to the so-called full-relaxation-matrix analyses (16–19). It may be emphasized that the best strategy for the analysis of NOE data continues to be based on the principle that a large number of semi-accurate distance estimates (2) are better than a smaller number of accurate distances.

ACKNOWLEDGMENT

We thank Dr. R. C. R. Grace for the various discussions.

REFERENCES

1. Anil Kumar, R. R. Ernst, and K. Wüthrich, *Biochem. Biophys. Res. Commun.* **95**, 1 (1980).
2. K. Wüthrich, "NMR of Proteins and Nucleic Acids," Wiley, New York, 1986.
3. R. R. Ernst, G. Bodenhausen, and A. Wokaun, "Principles of Nuclear Magnetic Resonance in One and Two Dimensions," Oxford, London, 1991.

4. R. R. Ernst, *Angew. Chem. Int. Ed. Engl.* **31**, 805 (1992).
5. L. G. Werbelow and D. M. Grant, *Adv. Magn. Reson.* **9**, 189 (1977).
6. C. Dalvit and G. Bodenhausen, *Adv. Magn. Reson.* **14**, 1 (1990).
7. J. Keeler and F. S. Ferrando, *J. Magn. Reson.* **75**, 96 (1987).
8. T. E. Bull, *J. Magn. Reson.* **72**, 397 (1987).
9. V. V. Krishnan and Anil Kumar, *J. Magn. Reson.* **92**, 293 (1991).
10. R. Brüschweiler, C. Griesinger, and R. R. Ernst, *J. Am. Chem. Soc.* **111**, 8034 (1984).
11. R. Brüschweiler and R. R. Ernst, *J. Chem. Phys.* **96**, 1758 (1992).
12. M. Ernst and R. R. Ernst, *J. Magn. Reson. A* **110**, 202 (1994).
13. P. K. Madhu, R. Christy Rani Grace, and Anil Kumar, *Bull. Magn. Reson.* **16**, 107 (1994).
14. Anil Kumar and P. K. Madhu, *Concepts Magn. Reson.* **8**, 139 (1996).
15. I. Burghardt, R. Konart, and G. Bodenhausen, *Mol. Phys.* **75**, 467 (1992).
16. E. T. Olejniczak, R. T. Gampe, Jr., and S. W. Fesik, *J. Magn. Reson.* **67**, 28 (1980).
17. B. A. Borgias and T. L. James, *J. Magn. Reson.* **79**, 493 (1988).
18. B. A. Borgias, M. Gochin, D. J. Kenwood, and T. L. James, *Prog. NMR Spectrosc.* **22**, 83 (1990).
19. T. L. James, *Curr. Opin. Struct. Biol.* **1**, 1042 (1991).