

A Method for Simulation of NOESY, ROESY, and Off-Resonance ROESY Spectra

Peter Allard,¹ Magnus Helgstrand, and Torleif Hård

Center for Structural Biochemistry, The Royal Institute of Technology, Novum, S-141 57 Huddinge, Sweden

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A formalism is proposed for simulation of NOESY, ROESY, and, more specifically, off-resonance ROESY nuclear magnetic resonance spectra. The off-resonance ROESY experiment has several advantages compared to standard NOESY and ROESY experiments. A simplified formalism which allows rapid computer simulation of the development of magnetization, including relaxation, in the presence of an RF field is of general use, in particular in the implementation and interpretation of off-resonance ROESY experiments. The relevant matrix equations can be derived either from the classical Bloch and Solomon equations or from the quantum mechanical homogeneous master equation in the basis of the Cartesian product operators. Examples of simulated spectra and behavior of magnetization during pulse sequences are shown. In addition, we present the full quantum mechanical theory for a two-spin system derived from the homogeneous master equation. The proposed formalism, here applied to off-resonance ROESY, has many potential applications, e.g., in the development and analysis of ROESY and TOCSY mixing sequences, selective pulses, and decoupling in which the complete spin dynamics, including relaxation, is taken into account. © 1997 Academic Press

effective field at a well-defined angle from the longitudinal axis during the cross-relaxation mixing time. This angle can be manipulated by changing the magnitude and the frequency of the applied RF field. The experiment can be considered a combination of the NOESY and ROESY experiments because the observed auto- and cross-relaxation rates are linear combinations of longitudinal and transverse relaxation rates (7, 8). The off-resonance ROESY experiment provides the possibility of determining both longitudinal and transverse cross-relaxation rates with high accuracy. Thus it will, in principle, provide increased precision in distances used in structure determinations as well as information on dynamics (9). It has previously been shown for NOESY data that a complete analysis of experimental data using the Solomon equations (10) will increase the precision of the measured distances compared to the common isolated spin pair approximation (11, 12). Here, we present a similar procedure useful for the analysis of off-resonance ROESY spectra and experiments. Simplified algorithms for the description of the off-resonance ROESY experiments have been proposed earlier (13). However, this new approach is, in our opinion, more direct and can produce both spectra and time-dependent magnetization trajectories. A second potential use of the novel formalism is in the simulation of large spin systems during selective pulses without neglecting relaxation.

In addition, we present the corresponding form of the full theory for a scalar-coupled two-spin system derived using the homogeneous master equation in the basis of the Cartesian product operators. The complete theory should be useful for the development and analysis of different types of mixing sequences used in, for example, ROESY and TOCSY spectroscopy, when both relaxation and homonuclear Hartmann–Hahn effects need to be considered.

INTRODUCTION

Distances between protons in biological molecules are usually measured based on cross-relaxation in the laboratory (NOESY) or rotating (ROESY) reference frames. Problems associated with NOESY (1, 2) include spin diffusion in macromolecules (3) and the absence of longitudinal cross-relaxation for molecules with an intermediate correlation time ($\approx 1/\omega_0$). The ROESY (4, 5) experiment is less sensitive to these effects but a quantitative interpretation might be prohibited by potential homonuclear Hartmann–Hahn transfer of magnetization (5, 6). The transfer of magnetization by Hartmann–Hahn matching can, on the other hand, be avoided in off-resonance ROESY (7, 8) experiments. In off-resonance ROESY the nuclear spins are aligned along an

THEORY

The system of equations we propose to use for the simulation of NOESY, ROESY, and off-resonance ROESY spectra

¹ To whom correspondence should be addressed. Fax: 46-8-608-92-90. E-mail: peter@csb.ki.se.

can be thought of either as an extension of the Solomon equations (10) using the Bloch equations (14) in the frame rotating with the frequency of the RF field, or as a special case of the quantum mechanical homogeneous master equation (15–18) in the basis of Cartesian product operators (15, 16).

In our derivations below, we first use arguments based on classical mechanics to set up a matrix representation of the evolution of magnetization in a dipole–dipole-coupled (but not scalar-coupled) two-spin system. We then go on to verify this representation by deriving the corresponding matrix using quantum mechanics. The result is a matrix representation of the spin dynamics occurring in a scalar-coupled and dipole–dipole-coupled two-spin system in the presence of an external RF field, i.e., a description of the complete theory for such a system. From our quantum mechanical treatment we identify the result arrived at using classical mechanics, if we remove the effect of scalar coupling.

The only approximation made in the quantum mechanical derivation is that we assume that $\omega_e \tau_c \ll 1$, where ω_e is the angular nuclear spin precession rate about the effective field and τ_c is the correlation time of the dipole–dipole interactions. With this approximation the relaxation behavior is the same as in the absence of the RF field, but in a frame tilted relative to the static magnetic field (7, 19). In the final step of the quantum mechanical derivation we also set the scalar coupling constant to zero; i.e., we ignore scalar couplings. This means that Hartmann–Hahn magnetization transfer is not taken into account. This is not necessarily a serious limitation because it can be avoided to a large extent in off-resonance ROESY by limiting the angle of the effective field. The treatment is in other aspects general and does for instance take the relaxation of like and unlike spins properly into account (10, 20). The matrix representation of spin systems where scalar coupling is neglected can easily be extended to three or more spins. The application of this formalism in the simulation of NOESY, ROESY, and off-resonance ROESY spectra is straightforward and is the major result that we want to communicate here.

Classical Mechanics

We first describe a simple derivation based on classical mechanics. The Bloch equations in the rotating frame can be written as (19)

$$\begin{aligned}\frac{dM_x(t)}{dt} &= -M_x(t)\lambda - M_y(t)\Omega + M_z(t)\omega_y \\ \frac{dM_y(t)}{dt} &= M_x(t)\Omega - M_y(t)\lambda - M_z(t)\omega_x \\ \frac{dM_z(t)}{dt} &= -M_x(t)\omega_y + M_y(t)\omega_x - (M_z(t) - M_0)\rho\end{aligned}\quad [1]$$

with

$$\begin{aligned}\omega_x &= -\gamma B_1^r \cos(\phi) \\ \omega_y &= -\gamma B_1^r \sin(\phi) \\ \Omega &= \omega_0 - \omega_{\text{RF}},\end{aligned}\quad [2]$$

where Ω is the chemical shift offset frequency in rad s^{-1} and ω_0 is the Larmor frequency; B_1^r , ω_{RF} , and ϕ are the strength, frequency, and phase of the applied RF field, respectively; γ is the magnetogyric ratio; ω_x and ω_y are the magnetic field components along the x and y axes in rad s^{-1} , respectively; λ is the relaxation rate of transverse magnetization; ρ is the relaxation rate of longitudinal magnetization; and M_0 is the equilibrium magnetization. In matrix form this equation is

$$\frac{d}{dt} \begin{bmatrix} M_x \\ M_y \\ M_z \end{bmatrix} = - \begin{bmatrix} \lambda & \Omega & -\omega_y \\ -\Omega & \lambda & \omega_x \\ \omega_y & -\omega_x & \rho \end{bmatrix} \begin{bmatrix} M_x \\ M_y \\ M_z \end{bmatrix} + \begin{bmatrix} 0 \\ 0 \\ M_0\rho \end{bmatrix}.\quad [3]$$

The matrix can be extended to include several spins. If we add one spin and label the spins I and S , and at the same time add one additional row and column, the result is

$$\frac{d}{dt} \begin{bmatrix} E/2 \\ I_x \\ I_y \\ I_z \\ S_x \\ S_y \\ S_z \end{bmatrix} = - \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & \lambda_I & \Omega_I & -\omega_y & 0 & 0 & 0 \\ 0 & -\Omega_I & \lambda_I & \omega_x & 0 & 0 & 0 \\ 0 & \omega_y & -\omega_x & \rho_I & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \lambda_S & \Omega_S & -\omega_y \\ 0 & 0 & 0 & 0 & -\Omega_S & \lambda_S & \omega_x \\ 0 & 0 & 0 & 0 & \omega_y & -\omega_x & \rho_S \end{bmatrix} \begin{bmatrix} E/2 \\ I_x \\ I_y \\ I_z \\ S_x \\ S_y \\ S_z \end{bmatrix} + \begin{bmatrix} 0 \\ 0 \\ 0 \\ M_{I0}\rho_I \\ 0 \\ 0 \\ M_{S0}\rho_S \end{bmatrix}.\quad [4]$$

Matrix elements σ and μ corresponding to longitudinal and transverse cross-relaxation, respectively, can be included in analogy with the Solomon equations (4, 7, 10). The E in the magnetization vector stands for unity. The vector of steady-state magnetization can be included in the first column in order to convert the inhomogeneous differential equation into a homogeneous differential equation (15, 16). We obtain

$$\frac{d}{dt} \begin{bmatrix} E/2 \\ I_x \\ I_y \\ I_z \\ S_x \\ S_y \\ S_z \end{bmatrix} = - \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & \lambda_I & \Omega_I & -\omega_y & \mu & 0 & 0 \\ 0 & -\Omega_I & \lambda_I & \omega_x & 0 & \mu & 0 \\ -2\Theta_I & \omega_y & -\omega_x & \rho_I & 0 & 0 & \sigma \\ 0 & \mu & 0 & 0 & \lambda_S & \Omega_S & -\omega_y \\ 0 & 0 & \mu & 0 & 0 & -\Omega_S & \lambda_S & \omega_x \\ -2\Theta_S & 0 & 0 & \sigma & \omega_y & -\omega_x & \rho_S \end{bmatrix} \begin{bmatrix} E/2 \\ I_x \\ I_y \\ I_z \\ S_x \\ S_y \\ S_z \end{bmatrix} \quad [5]$$

with

$$\begin{aligned} \Theta_I &= \rho_I M_{I0} + \sigma M_{S0} \\ \Theta_S &= \sigma M_{I0} + \rho_S M_{S0}. \end{aligned} \quad [6]$$

The factor 2 that is multiplied with Θ in the first column is due to the multiplication with $E/2$ when forming the constant term in the differential equation. The matrix thus formed is our final result and it can easily be extended to large spin systems since the size of the matrix increases linearly with the number of spins and is equal to $(3N + 1) \times (3N + 1)$ for N spins. The correction factor for equilibrium magnetization is then

$$\Theta_I = \sum_{X \neq I} (\rho_{IX} M_{I0} + \sigma_{IX} M_{X0}) \quad [7]$$

for each longitudinal I_z magnetization, where the sum is taken over all dipole–dipole-coupled spins.

Quantum Mechanics

In order to more formally derive the set of equations to be used in this paper we need to calculate the homogeneous form of the quantum mechanical master equation (17) in the basis of Cartesian product operators (15, 16). The common inhomogeneous form of the quantum mechanical master equation (20, 21), the Liouville–von Neumann equation for the density operator σ , can be written as

$$\frac{d}{dt} \sigma = -i[H, \sigma] - \hat{\Gamma}(\sigma - \sigma_0) \quad [8]$$

or

$$\frac{d}{dt} \sigma = -(i\hat{H} + \hat{\Gamma})\sigma + \hat{\Gamma}\sigma_0 \quad [9]$$

with

$$\hat{H}\sigma = [H, \sigma]. \quad [10]$$

The master equation, Eq. [8], consists of two terms. The first term contains the spin Hamiltonian H , and it includes all “coherent” influences on the ensemble of spins. The second term accounts for the relaxation of the density operator to equilibrium (σ_0) during and after a perturbation. It contains the relaxation superoperator $\hat{\Gamma}$, which usually is calculated according to Redfield theory (20, 22).

We start with the coherent part of the master equation and consider a scalar-coupled two-spin system, in which both spins have spin quantum numbers of $\frac{1}{2}$. First, we need a basis in Hilbert space for our calculations. The angular momentum operators for a single spin $\frac{1}{2}$ in the basis of $|\alpha\rangle$ and $|\beta\rangle$, the Pauli spin matrices, are (19)

$$\begin{aligned} \frac{1}{2} E &= \frac{1}{2} \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix}, \quad I_x = \frac{1}{2} \begin{bmatrix} 0 & 1 \\ 1 & 0 \end{bmatrix}, \\ I_y &= \frac{1}{2} \begin{bmatrix} 0 & -i \\ i & 0 \end{bmatrix}, \quad I_z = \frac{1}{2} \begin{bmatrix} 1 & 0 \\ 0 & -1 \end{bmatrix}. \end{aligned} \quad [11]$$

The matrix forms of the 16 Cartesian product operators (23) in the direct product basis are calculated from the four single-spin Pauli spin matrices for the two spins (21) according to

$$B_{rs}^{2\text{spin}} = 2 \cdot B_r^{1\text{spin}} \otimes B_s^{1\text{spin}} \quad [12]$$

$$\Gamma_{rs} = 15 \frac{R^2}{\hbar^2} \sum_{q=-2}^{q=2} J[q\omega] \times \text{Tr}\{[A_q, B_r^{2\text{spin}}]^\dagger \cdot [A_q, B_s^{2\text{spin}}]\}, \quad [17]$$

with

$$B^{1\text{spin}} = \frac{1}{2}E, I_x, I_y, I_z. \quad [13]$$

The result is 16 square matrices with the dimension 4×4 for a spin system consisting of two coupled spins $\frac{1}{2}$ (19). The time-independent rotating frame Hamiltonian for a coupled homonuclear two-spin system in the frame rotating with the frequency of the RF field can be written using these matrices as

$$H = \Omega_I I_z + \Omega_S S_z + \pi J(2I_x S_x + 2I_y S_y + 2I_z S_z) + \omega_x(I_x + S_x) + \omega_y(I_y + S_y), \quad [14]$$

where Ω_I and Ω_S are the chemical shift offset frequencies in rad s^{-1} , J is the scalar coupling constant for the two spins in Hz, and ω_x and ω_y are the magnetic field components along the x and y axes, respectively, in rad s^{-1} .

In order to solve the Liouville–von Neumann equation in the superspace (17) the spin Hamiltonian must be expressed in this space. This can be done by calculating the commutator superoperator for the spin Hamiltonian according to (21)

$$\hat{H} = H \otimes \tilde{E} - E \otimes \tilde{H}, \quad [15]$$

where \sim denotes the transpose. The superoperator form of the spin Hamiltonian is a 16×16 matrix in the level-shift basis of the form $|\alpha\alpha\rangle \langle\alpha\alpha|$, etc. The basis of this matrix can be changed to the basis of Cartesian product operators for two spins $\frac{1}{2}$ using (21)

$$H_{rs} = \langle B_r^{2\text{spin}} | \hat{H} | B_s^{2\text{spin}} \rangle \quad [16]$$

in which $B^{2\text{spin}}$ have been transformed into 16-unit-long column vectors from the 4×4 matrices by appending rows one through four after each other (21).

The relaxation term in the master equation is calculated using Redfield theory (20, 22). The elements of the Redfield relaxation matrix for dipole–dipole relaxation in the basis of Cartesian product operators can most easily be calculated using Wigner 3- J symbols (24, 25) according to

where \dagger denotes the adjoint, with

$$A_q = \sum_{\mu=-1}^{\mu=1} \begin{bmatrix} 1 & 1 & 2 \\ \mu & q-\mu & -q \end{bmatrix} I_\mu S_{q-\mu} \quad [18]$$

$$R = \left(\frac{\mu_0}{4\pi} \right) \left(\frac{\hbar^2 \gamma_I \gamma_S}{r_{IS}^3} \right) \quad [19]$$

and

$$I_1 = 2 \left[\frac{-1}{\sqrt{2}} (I_x + iI_y) \otimes \frac{1}{2} E \right] \quad [20]$$

$$I_0 = 2 \left[I_z \otimes \frac{1}{2} E \right] \quad [21]$$

$$I_{-1} = 2 \left[\frac{1}{\sqrt{2}} (I_x - iI_y) \otimes \frac{1}{2} E \right] \quad [22]$$

$$S_1 = 2 \left[\frac{1}{2} E \otimes \frac{-1}{\sqrt{2}} (S_x + iS_y) \right] \quad [23]$$

$$S_0 = 2 \left[\frac{1}{2} E \otimes S_z \right] \quad [24]$$

$$S_{-1} = 2 \left[\frac{1}{2} E \otimes \frac{1}{\sqrt{2}} (S_x - iS_y) \right] \quad [25]$$

in which I and S are the spherical spin operators for the two interacting spins in the product basis calculated according to Eq. [12] using the Pauli spin matrices defined in Eq. [11], μ_0 is the permeability of vacuum, \hbar is Plank's constant divided by 2π , and r_{IS} is the distance between spins I and S . The angular Larmor frequencies of spins I and S are labeled ω_I and ω_S , respectively. It should be noted that the relaxation matrix elements are calculated without the usual secular approximations that are made when unlike spins are assumed, i.e., that resonances do not overlap.

The explicit matrix representation of the inhomogeneous master equation in the basis of Cartesian product operators is thus

$$\frac{d}{dt} \begin{bmatrix} E/2 \\ I_x \\ I_y \\ I_z \\ S_x \\ S_y \\ S_z \\ 2I_x S_z \\ 2I_y S_z \\ 2I_z S_x \\ 2I_z S_y \\ 2I_x S_x \\ 2I_x S_y \\ 2I_y S_x \\ 2I_y S_y \\ 2I_z S_z \end{bmatrix} = - \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & \lambda_I & \Omega_I & -\omega_y & \mu & 0 & 0 & 0 & \pi J & 0 & -\pi J & 0 & 0 & 0 & 0 \\ 0 & -\Omega_I & \lambda_I & \omega_x & 0 & \mu & 0 & -\pi J & 0 & \pi J & 0 & 0 & 0 & 0 & 0 \\ 0 & \omega_y & -\omega_x & \rho_I & 0 & 0 & \sigma & 0 & 0 & 0 & 0 & 0 & \pi J & -\pi J & 0 \\ 0 & \mu & 0 & 0 & \lambda_S & \Omega_S & -\omega_y & 0 & -\pi J & 0 & \pi J & 0 & 0 & 0 & 0 \\ 0 & 0 & \mu & 0 & -\Omega_S & \lambda_S & \omega_x & \pi J & 0 & -\pi J & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & \sigma & \omega_y & -\omega_x & \rho_S & 0 & 0 & 0 & 0 & 0 & -\pi J & \pi J & 0 \\ 0 & 0 & \pi J & 0 & 0 & -\pi J & 0 & \rho_I^a & \Omega_I & \mu^a & 0 & \omega_y & -\omega_x & 0 & -\omega_y \\ 0 & -\pi J & 0 & 0 & \pi J & 0 & 0 & -\Omega_I & \rho_I^a & 0 & \mu^a & 0 & \omega_y & -\omega_x & \omega_x \\ 0 & 0 & -\pi J & 0 & 0 & \pi J & 0 & \mu^a & 0 & \rho_S^a & \Omega_S & \omega_y & 0 & -\omega_x & 0 \\ 0 & \pi J & 0 & 0 & -\pi J & 0 & 0 & 0 & \mu^a & -\Omega_S & \rho_S^a & 0 & \omega_y & 0 & -\omega_x \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & -\omega_y & 0 & -\omega_y & 0 & \lambda^{xx} & \Omega_S & \Omega_I & \mu^{xyxy} & \mu^{xzcz} \\ 0 & 0 & 0 & -\pi J & 0 & 0 & \pi J & \omega_x & 0 & 0 & -\omega_y & -\Omega_S & \lambda^{xy} & \mu^{xyyx} & \Omega_I & 0 \\ 0 & 0 & 0 & \pi J & 0 & 0 & -\pi J & 0 & -\omega_y & \omega_x & 0 & -\Omega_I & \mu^{xyyx} & \lambda^{yx} & \Omega_S & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \omega_x & 0 & \omega_x & \mu^{xyyy} & -\Omega_I & -\Omega_S & \lambda^{yy} & \mu^{yyzz} \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & \omega_y & -\omega_x & \omega_y & -\omega_x & \mu^{xzzz} & 0 & 0 & \mu^{yyzz} & \rho_{IS}^{2sp} \end{bmatrix} \begin{bmatrix} E/2 \\ I_x \\ I_y \\ I_z \\ S_x \\ S_y \\ S_z \\ 2I_x S_z \\ 2I_y S_z \\ 2I_z S_x \\ 2I_z S_y \\ 2I_x S_x \\ 2I_x S_y \\ 2I_y S_x \\ 2I_y S_y \\ 2I_z S_z \end{bmatrix} + \begin{bmatrix} 0 \\ 0 \\ 0 \\ \Theta_I \\ 0 \\ 0 \\ \Theta_S \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix} \quad [26]$$

with

$$\Theta_I = \rho_I M_{I0} + \sigma M_{S0}$$

$$\Theta_S = \sigma M_{I0} + \rho_S M_{S0}$$

and

$$\lambda^{xy} = \lambda^{yx} = \frac{1}{4} \left(\frac{\mu_0}{4\pi} \right)^2 \left(\frac{\hbar^2 \gamma_I^2 \gamma_S^2}{r_{IS}^6} \right)$$

$$\times [J(0) + 3J(\omega) + 3J(2\omega)] \quad [33]$$

$$\lambda^{xx} = \lambda^{yy} = \frac{1}{4} \left(\frac{\mu_0}{4\pi} \right)^2 \left(\frac{\hbar^2 \gamma_I^2 \gamma_S^2}{r_{IS}^6} \right)$$

$$\times [3J(\omega) + 3J(2\omega)] \quad [34]$$

$$\lambda_I = \lambda_S = \frac{1}{4} \left(\frac{\mu_0}{4\pi} \right)^2 \left(\frac{\hbar^2 \gamma_I^2 \gamma_S^2}{r_{IS}^6} \right) \times \left[\frac{5}{2} J(0) + \frac{9}{2} J(\omega) + 3J(2\omega) \right] \quad [27]$$

$$\rho_I = \rho_S = \frac{1}{4} \left(\frac{\mu_0}{4\pi} \right)^2 \left(\frac{\hbar^2 \gamma_I^2 \gamma_S^2}{r_{IS}^6} \right) \times [J(0) + 3J(\omega) + 6J(2\omega)] \quad [28]$$

$$\mu = \frac{1}{4} \left(\frac{\mu_0}{4\pi} \right)^2 \left(\frac{\hbar^2 \gamma_I^2 \gamma_S^2}{r_{IS}^6} \right) [2J(0) + 3J(2\omega)] \quad [29]$$

$$\sigma = \frac{1}{4} \left(\frac{\mu_0}{4\pi} \right)^2 \left(\frac{\hbar^2 \gamma_I^2 \gamma_S^2}{r_{IS}^6} \right) [-J(0) + 6J(2\omega)] \quad [30]$$

$$\rho_I^a = \rho_S^a = \frac{1}{4} \left(\frac{\mu_0}{4\pi} \right)^2 \left(\frac{\hbar^2 \gamma_I^2 \gamma_S^2}{r_{IS}^6} \right) \times \left[\frac{5}{2} J(0) + \frac{3}{2} J(\omega) + 3J(2\omega) \right] \quad [31]$$

$$\mu^a = \frac{1}{4} \left(\frac{\mu_0}{4\pi} \right)^2 \left(\frac{\hbar^2 \gamma_I^2 \gamma_S^2}{r_{IS}^6} \right) [2J(0)] \quad [32]$$

$$\rho_{IS}^{2sp} = \frac{1}{4} \left(\frac{\mu_0}{4\pi} \right)^2 \left(\frac{\hbar^2 \gamma_I^2 \gamma_S^2}{r_{IS}^6} \right) [6J(\omega)] \quad [35]$$

$$\mu^{xyyx} = \frac{1}{4} \left(\frac{\mu_0}{4\pi} \right)^2 \left(\frac{\hbar^2 \gamma_I^2 \gamma_S^2}{r_{IS}^6} \right) [-J(0) + 3J(2\omega)] \quad [36]$$

$$\mu^{xyyy} = \frac{1}{4} \left(\frac{\mu_0}{4\pi} \right)^2 \left(\frac{\hbar^2 \gamma_I^2 \gamma_S^2}{r_{IS}^6} \right) [-3J(2\omega)] \quad [37]$$

$$\mu^{xzzz} = \mu^{yyzz} = \frac{1}{4} \left(\frac{\mu_0}{4\pi} \right)^2 \left(\frac{\hbar^2 \gamma_I^2 \gamma_S^2}{r_{IS}^6} \right) [-3J(\omega)] \quad [38]$$

$$J(\omega) = \frac{2}{5} \frac{\tau_c}{(1 + \omega^2 \tau_c^2)}, \quad [39]$$

where τ_c is the correlation time; λ is the relaxation rate of transverse in-phase magnetization; ρ is the relaxation rate of longitudinal magnetization; μ is the transverse cross-relaxation rate; σ is the longitudinal cross-relaxation rate; ρ_I^a is the relaxation rate of transverse I magnetization that is antiphase with respect to S ; μ^a is the cross-relaxation rate between antiphase coherences; λ^{xx} , λ^{xy} , λ^{yx} , and λ^{yy} represent the relaxation rates of the multiple-quantum coherences; ρ_{IS}^{2sp} is the relaxation rate

of longitudinal two-spin order; μ^{xyx} and μ^{xyy} are cross-relaxation rates between multiple-quantum coherence components; and finally, μ^{xzz} and μ^{yzz} are cross-relaxation rates not observed between unlike spins. The (*ad hoc*) correction vector for equilibrium magnetization with the elements Θ_I and Θ_S is calculated by multiplying the relaxation matrix $\hat{\Gamma}$ with the equilibrium density operator vector σ_0 .

The inhomogeneous differential equation corresponding to the inhomogeneous quantum mechanical master equation can be rewritten into a homogeneous differential equation with help of the previously unused first column (15, 16):

$$\frac{d}{dt} \begin{bmatrix} E/2 \\ I_x \\ I_y \\ I_z \\ S_x \\ S_y \\ S_z \\ 2I_x S_z \\ 2I_y S_z \\ 2I_z S_x \\ 2I_x S_y \\ 2I_z S_y \\ 2I_x S_x \\ 2I_x S_y \\ 2I_y S_x \\ 2I_y S_y \\ 2I_z S_z \end{bmatrix} = - \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & \lambda_I & \Omega_I & -\omega_y & \mu & 0 & 0 & 0 & \pi J & 0 & -\pi J & 0 & 0 & 0 & 0 & 0 \\ 0 & -\Omega_I & \lambda_I & \omega_x & 0 & \mu & 0 & -\pi J & 0 & \pi J & 0 & 0 & 0 & 0 & 0 & 0 \\ -2\Theta_I & \omega_y & -\omega_x & \rho_I & 0 & 0 & \sigma & 0 & 0 & 0 & 0 & 0 & \pi J & -\pi J & 0 & 0 \\ 0 & \mu & 0 & 0 & \lambda_S & \Omega_S & -\omega_y & 0 & -\pi J & 0 & \pi J & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \mu & 0 & -\Omega_S & \lambda_S & \omega_x & \pi J & 0 & -\pi J & 0 & 0 & 0 & 0 & 0 & 0 \\ -2\Theta_S & 0 & 0 & \sigma & \omega_y & -\omega_x & \rho_S & 0 & 0 & 0 & 0 & 0 & -\pi J & \pi J & 0 & 0 \\ 0 & 0 & \pi J & 0 & 0 & -\pi J & 0 & \rho_I^a & \Omega_I & \mu^a & 0 & \omega_y & -\omega_x & 0 & 0 & -\omega_y \\ 0 & -\pi J & 0 & 0 & \pi J & 0 & 0 & -\Omega_I & \rho_I^a & 0 & \mu^a & 0 & 0 & \omega_y & -\omega_x & \omega_x \\ 0 & 0 & -\pi J & 0 & 0 & \pi J & 0 & \mu^a & 0 & \rho_S^a & \Omega_S & \omega_y & 0 & -\omega_x & 0 & -\omega_y \\ 0 & \pi J & 0 & 0 & -\pi J & 0 & 0 & 0 & \mu^a & -\Omega_S & \rho_S^a & 0 & \omega_y & 0 & -\omega_x & \omega_x \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & -\omega_y & 0 & -\omega_y & 0 & \lambda^{xx} & \Omega_S & \Omega_I & \mu^{xyx} & \mu^{xzz} \\ 0 & 0 & 0 & -\pi J & 0 & 0 & \pi J & \omega_x & 0 & 0 & -\omega_y & -\Omega_S & \lambda^{xy} & \mu^{xyx} & \Omega_I & 0 \\ 0 & 0 & 0 & \pi J & 0 & 0 & -\pi J & 0 & -\omega_y & \omega_x & 0 & -\Omega_I & \mu^{xyx} & \lambda^{yx} & \Omega_S & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \omega_x & 0 & \omega_x & \mu^{xyy} & -\Omega_I & -\Omega_S & \lambda^{yy} & \mu^{yzz} \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & \omega_y & -\omega_x & \omega_y & -\omega_x & \mu^{xzz} & 0 & 0 & \mu^{yzz} & \rho_{IS}^{2sp} \end{bmatrix} \begin{bmatrix} E/2 \\ I_x \\ I_y \\ I_z \\ S_x \\ S_y \\ S_z \\ 2I_x S_z \\ 2I_y S_z \\ 2I_z S_x \\ 2I_x S_y \\ 2I_z S_y \\ 2I_x S_x \\ 2I_x S_y \\ 2I_y S_x \\ 2I_y S_y \\ 2I_z S_z \end{bmatrix}. \quad [40]$$

The extra elements in the first column will not affect the eigenvalues, or rates, but only the eigenvectors, and thus the position of the eventual equilibrium (15, 16).

Equation [40] represents the complete theory for two spins $\frac{1}{2}$ in the presence of an RF field, if $\omega_e \tau_c \ll 1$ is assumed as discussed earlier. It is possible to extend the complete theory to involve three (or more) spins. Such a derivation would follow the same general lines as used here for the two-spin system, i.e., deriving the appropriate three-spin operator basis and inserting the operators to generate the matrix elements of a homogeneous differential equation (equivalent to Eq. [40]) describing the evolution of the three-spin system.

In the absence of scalar coupling the upper left 7×7 part of the matrix in Eq. [40] is block diagonal with respect to the rest of the matrix and can therefore be treated separately. We now have a matrix equation that is identical to that arrived at using classical mechanics, Eq. [5].

It should be noted that the chemical shifts and the scalar coupling which motivate the secular approximation when calculating the relaxation matrix for unlike spins are included in the coherent part of the homogeneous master equation.

These terms will keep the magnetization oscillating between x and y magnetization and thus make the nonsecular cross-relaxation pathways ineffective unless the spins are either spin-locked in the RF field or have the same chemical shift (10, 26).

Off-Resonance ROESY

The coherent part of the matrix equation will keep the magnetization in precession about an effective field with an effective frequency at a specified angle from the z axis, θ_i (19). By introducing a rotating frame, tilted with the

angle θ_i , it has previously been shown (7, 19) that the effective cross- and auto-relaxation rates in the tilted rotating frame are linear combinations of transverse and longitudinal relaxation rates in the rotating frame as defined by Solomon (10). The effective relaxation rates in the tilted frame are thus

$$\begin{aligned} \sigma'_{IS} &= \cos(\theta_I)\cos(\theta_S)\sigma_{IS} + \sin(\theta_I)\sin(\theta_S)\mu_{IS} \\ \rho'_I &= \cos^2(\theta_I)\rho_I + \sin^2(\theta_I)\lambda_I \end{aligned} \quad [41]$$

with

$$\tan(\theta) = -\frac{\gamma B_1}{\Omega} = \frac{\omega_I}{\Omega}, \quad [42]$$

where Ω is the difference between the RF field frequency and the resonance frequency of the spin under consideration. These are the equations usually used in the evaluation of off-resonance ROESY experiments.

Simulating Spectra

The solution to the homogeneous master equation, which is a homogeneous differential equation of the first order, is

$$\sigma(t) = \exp[-\hat{P}t] \cdot \sigma(t=0), \quad [43]$$

where $\exp[-\hat{P}t]$ is a superoperator relaxation propagator (18) and P is the matrix either in Eq. [5] or in Eq. [40]. The expectation value of an arbitrary operator Obs, corresponding to an observable, can be calculated according to

$$\langle \text{Obs} \rangle = \langle \text{Obs}^\dagger | \sigma(t) \rangle. \quad [44]$$

The operators corresponding to observable x and y magnetization are $I_x + S_x$ and $I_y + S_y$, respectively. The detectable magnetization can thus be calculated as

$$\begin{aligned} \langle I_x + S_x \rangle &= \langle (I_x + S_x)^\dagger | \sigma(t) \rangle \\ \langle I_y + S_y \rangle &= \langle (I_y + S_y)^\dagger | \sigma(t) \rangle. \end{aligned} \quad [45]$$

A complete FID can be calculated recursively with (18)

$$\begin{aligned} \text{FID}_x(k) &= \langle (I_x + S_x)^\dagger | \exp[-\hat{P}t_{\text{dwell}}] | \sigma_{k-1} \rangle \\ \text{FID}_y(k) &= \langle (I_y + S_y)^\dagger | \exp[-\hat{P}t_{\text{dwell}}] | \sigma_{k-1} \rangle, \end{aligned} \quad [46]$$

where

$$| \sigma_k \rangle = \exp[-\hat{P}t_{\text{dwell}}] | \sigma_{k-1} \rangle. \quad [47]$$

The computational method is the same whether or not the homogeneous master equation is solved with or without scalar couplings.

RESULTS

The simulations described below were carried out assuming that the spins were not scalar-coupled using Eqs. [5], [7], [43], [46], and [47], except for one of the simulations presented in Fig. 1, for which the complete theory, Eqs. [27]–[40], [43], [46], and [47], was applied. The simulations were performed using Matlab software running on a Silicon Graphics Indy workstation with a 150-MHz MIPS R5000 processor.

Test of Formalism

A number of tests were performed in order to evaluate and verify the simplified formalism.

The longitudinal part of Eq. [5] corresponds to the Solomon equations and it behaves accordingly. Both selective and nonselective transient experiments as well as steady-state NOE effects are correctly reproduced.

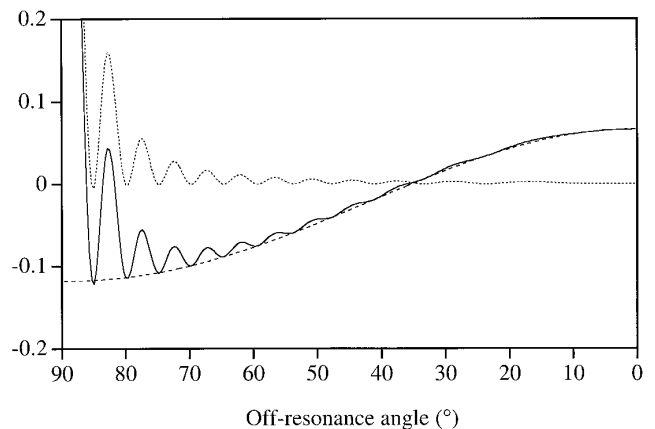


FIG. 1. The transfer of magnetization from spin 1 to spin 2 of a scalar-coupled two-spin system calculated using the simplified theory (dashed line) and the complete theory (solid line) for all off-resonance angles between ROESY ($\theta_1 = 90^\circ$) and NOESY ($\theta_1 = 0^\circ$). The difference between the simplified and the exact theory is illustrated with a dotted line. The application of the simplified theory involves use of Eqs. [5], [7], [27]–[30], [43], [46], and [47] and the complete theory of Eqs. [27]–[40], [43], [46], and [47] as described in the text. A unity-sized magnetization vector was placed along the effective field for spin 1 and the buildup of the corresponding magnetization vector for spin 2 after a mixing time of 60 ms is shown. The chemical shifts were 0 and -200 Hz for spin 1 and 2, respectively. The strength of the RF field was 5111 Hz. The distance between the spins and the correlation time was 2.5 \AA and 2.5 ns, respectively. The scalar coupling constant was 10 Hz and the spectrometer frequency was 500 MHz.

No ROESY transfer of magnetization is observed in the transverse plane if the two spins do not have the same chemical shift, unless both spins are spin-locked by the RF field. If the spins are spin-locked using an on-resonance RF field the observed transfer of magnetization corresponds to equations given by Bothner-By *et al.* (4).

If two spins have the same chemical shift the observed transverse relaxation rate becomes the sum of the diagonal auto-relaxation rate λ and the off-diagonal cross-relaxation rate μ . This is the correct relaxation rate for like spins (10, 20). With different chemical shifts and without an RF field the two spins relax independently with their respective transverse relaxation rates for unlike spins, λ .

Effect of Homonuclear Hartmann–Hahn Transfer

In Fig. 1 we show the transfer of magnetization from spin 1 to spin 2 calculated using both the complete and the simplified theory for all off-resonance angles between ROESY ($\theta_1 = 90^\circ$) and NOESY ($\theta_1 = 0^\circ$). The full theory is described by a solid line, the simplified theory by a dashed line, and the difference between the two cases by a dotted line.

A unity-sized magnetization vector was placed along the effective field for spin 1 and the buildup of the corresponding

magnetization vector for spin 2 after a constant mixing time was followed in Fig. 1 for different off-resonance angles. The off-resonance angle is defined for spin 1.

The scalar coupling was 10 Hz and the chemical shifts of spin 1 and 2 were 0 and -200 Hz, respectively. The distance between the two spins was 2.5 \AA . A single correlation time of 2.5 ns and a spectrometer frequency of 500 MHz were assumed in order to calculate the relaxation rates. The strength of the RF field was 5111 Hz and a mixing time of 60 ms was used.

The Hartmann–Hahn matching shows an oscillatory behavior as a function of the off-resonance angle as can be observed in Fig. 1. Similar effects can also be simulated using the equations presented by Bax (27). In this particular case it can also be observed that the Hartmann–Hahn transfer is much less effective than the ROESY transfer for off-resonance angles less than 60° . The relative contribution due to Hartmann–Hahn transfer is, of course, dependent on the exact input parameters, such as coupling constant, distance, and correlation time.

Simulation of an Off-Resonance ROESY Pulse Sequence

In Fig. 2 we show the off-resonance ROESY pulse sequence (A) by Berthault *et al.* (9) and the x , y , and z magnetization of spin 1 (B) and 2 (C) of a two-spin system during the pulse sequence. A single transient with all pulse and receiver phases set to X was used in the simulation and the idealized effect of pulsed field gradients was added to the original pulse sequence before and after the spin lock in order to purge transverse magnetization (not shown). The simulated x , y , and z magnetizations are presented as dotted, dashed, and solid lines, respectively.

The pulse sequence consists of a selective 270° Gaussian pulse directly followed by a hard 90° pulse. Only spin 1 is effectively excited by the Gaussian pulse. The magnetizations of spins 1 and 2 are now aligned along the z axis and the $-y$ axis, respectively. The mixing time starts with a pulsed field gradient which purges the magnetization of spin 2. The carrier frequency is then changed to a suitable offset and an adiabatic rotation to the effective field is performed using a slow buildup of the RF field (20). Only spin 1 has large magnetization components aligned along the effective field after the adiabatic rotation since the magnetization of spin 2 was purged. A continuous wave off-resonance RF field is now turned on during the mixing time, followed by an adiabatic rotation of the magnetization of both spins back to the z axis. A second pulsed field gradient is used in order to purge residual transverse magnetization and the frequency is changed back to the original value. The pulse sequence ends with a hard 90° read pulse before acquiring data.

The shift of carrier frequency was accomplished by simply adding an offset frequency to the offset chemical shift terms

Ω during the spin lock in the simulation. Dephasing of transverse magnetization due to the pulsed field gradients was simulated by multiplication with a unity matrix in which the diagonal elements corresponding to x and y magnetization were set to zero.

The chemical shifts were 0 and 1000 Hz for spin 1 and 2, respectively. The longitudinal auto-relaxation rates were 2 s^{-1} and the transverse auto-relaxation rates were 7 s^{-1} . The longitudinal and transverse cross-relaxation rates were -1 and 4 s^{-1} , respectively. A Gaussian 270° pulse consisting of 512 steps with a total length of 10 ms , a truncation level of 1%, and a strength of 181 Hz was used as a selective pulse. The mixing time was 100 ms including two adiabatic rotations of 3 ms length and 1024 steps, respectively. The adiabatic rotations work well if a sufficient number of steps are used to construct the linear amplitude gradients. The amount of magnetization that becomes aligned along the effective field at an angle θ after an adiabatic rotation consisting of n steps is $\cos(\theta/n)^n$; thus 64 steps are enough for transfer of 98% of the magnetization from the z axis to the

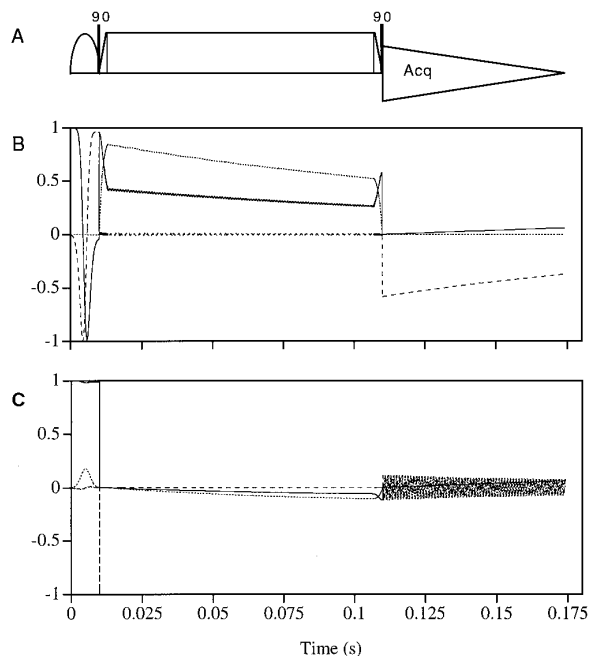


FIG. 2. The off-resonance ROESY pulse sequence (A) by Berthault *et al.* (9) and the x , y , and z magnetization of spins 1 (B) and 2 (C) during the pulse sequence. The x , y , and z magnetizations are presented as dotted, dashed, and solid lines, respectively. Transient magnetization vectors were calculated using Eqs. [5], [7], [43], [46], and [47]. The input data used in the simulation are described in the text. A single transient with all phases set to X was used in the simulation and the effect of pulsed field gradients was added to the original pulse sequence before and after the spin lock in order to purge transverse magnetization (not shown). A Gaussian 270° pulse was used as a selective pulse. Only spin 1 was effectively excited by the Gaussian pulse. The mixing time was 100 ms including two adiabatic rotations.

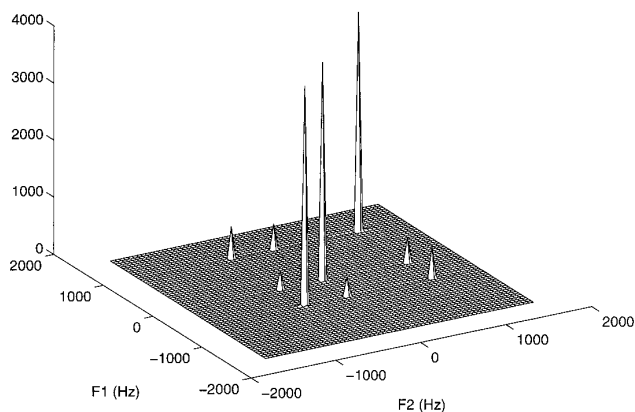


FIG. 3. The simulated off-resonance ROESY 2D spectrum of a three-spin system calculated using Eqs. [5], [7], [43], [46], and [47] presented in the text. The pulse sequence used is the 2D analogue of the pulse sequence presented in Fig. 2, but in which the shaped pulse has been replaced by a hard 90° pulse directly followed by a t_1 period (28). A two-step phase cycle of X , $-X$ was applied on the first pulse and the receiver. The input data used in the simulation are given in the text. The mixing time was 100 ms. The number of complex points calculated in both dimensions was 64, using the method of States *et al.* (29) to obtain absorption mode in the indirect dimension. The acquisition time was 0.02 s in both dimensions.

xy plane. The strength of the spin-lock field applied during the mixing time was 20 kHz and it was applied with an offset of 10 kHz. The hard pulses were applied with a field strength of 25 kHz, corresponding to a 90° pulse length of $10 \mu\text{s}$.

The off-resonance angle θ for spin 1 was 63.4° during the mixing time and the x and z components of the magnetization correspond to this angle; see Fig. 2B. The net magnetization of spin 1 decays as a result of relaxation and magnetization is appearing on spin 2 due to cross-relaxation. The simulation shows that the transverse cross-relaxation dominates over the longitudinal cross-relaxation since the magnetization of spin 2 is appearing along the negative x and z axes during the mixing time (Fig. 2C).

Simulation of a 2D Off-Resonance ROESY Spectrum

In Fig. 3 we show the simulated off-resonance ROESY 2D spectrum of a three-spin system. The pulse sequence used is the 2D analogue of the pulse sequence used in Fig. 2, but in which the shaped pulse has been replaced by a hard 90° pulse directly followed by a t_1 period (28). A two-step phase cycle of X , $-X$ was applied on the first pulse and the receiver.

The chemical shifts for spins 1, 2, and 3 were 1000, 0, and -500 Hz, respectively. All longitudinal and transverse auto-relaxation rates were 3 and 20 s^{-1} , respectively. The transverse cross-relaxation rates were 3 s^{-1} . The longitudinal cross-relaxation rates between spin 1–2, 1–3, and 2–3 were

-0.4 , -0.5 , and -0.3 s^{-1} , respectively. The mixing time including adiabatic rotations was 100 ms. The length of the adiabatic rotations was 3 ms with 1024-step linear amplitude ramps. The spin-lock power was 10 kHz and the offset frequency was 50 kHz. The 90° pulse length was $10 \mu\text{s}$, corresponding to a transmitter power of 25 kHz. The number of complex points calculated in both dimensions was 64, using the method of States *et al.* (29) to obtain absorption mode in the indirectly detected dimension. The acquisition time was 0.02 s in both dimensions. Pulsed field gradients and the shift of frequency were simulated as described previously.

The total time required for the simulation was about 25 s. Several matrices corresponding to superoperator propagators could be multiplied together in advance of the main program loop in order to minimize the time required for the simulation.

The result is an ordinary 2D spectrum with three diagonal peaks and three cross peaks, presented as a stack plot (Fig. 3). The different cross-relaxation rates can be observed as different intensities of the cross peaks. All cross peaks are positive since the longitudinal cross-relaxation dominates over the transverse cross-relaxation at this particular off-resonance angle. The sharp shapes of the peaks are due to the limited digital resolution. No window functions were used during the processing of the theoretical free induction decays.

DISCUSSION

It is of course possible to simulate off-resonance ROESY spectra using the complete quantum mechanical theory (20, 21, 30) as described in homogeneous form by Eq. [40]. The problem is that the size of the matrix to be diagonalized increases rapidly with the number of spins involved. For N spins $\frac{1}{2}$ the matrix is $4^N \times 4^N$, and this size prohibits simulations of large spin systems. With the simplified matrix representation presented here, the size of the matrix to be diagonalized is only $(3N + 1) \times (3N + 1)$, and it increases linearly with the number of spins involved. Simulations of large spin systems are therefore feasible.

For simulations of NOESY experiments, the matrix equation to be solved is approximately three times larger for the new method compared to the common relaxation matrix approach, used in the determination of distances without the isolated spin pair approximation (11, 12). The time required for solving the eigensystem for a matrix is proportional to N^3 . The method presented in this paper is therefore 27 times more computer intensive. The present simulation method also needs the chemical shifts for all dipole–dipole-coupled spins as input. This is not needed when using the longitudinal Solomon equations (10).

Since the solution to the equation system is in the form of a superoperator propagator (18), several parts of a pulse

sequence, i.e., pulses and delays, with their respective superoperator propagators, can be combined into a single superoperator propagator. Multidimensional spectra with many pulses can therefore be efficiently calculated. A spin lock using a DANTE (31) sequence should not require much more computational time than a spin lock using a continuous wave RF field (18). It should be noted that this is true both for the complete theory, Eq. [40], and for the simplified theory, Eq. [5].

We find the matrix representation of the complete homogeneous master equation, Eq. [40], interesting in itself. For instance, all transformation rules for Cartesian product operators (23) can easily be extracted by inspection of anti-symmetric off-diagonal elements connecting the product operators for the two-spin system. The diagonal elements correspond to the relaxation rates of the product operators and the symmetric off-diagonal elements correspond to the cross-relaxation rates. It should be noted that since the secular approximations performed when assuming that the spins are unlike are not made in this case, several cross-relaxation rates appear that are normally not considered. These pathways are only effective if the spins are spin-locked or have the same chemical shift.

One effect that neither the complete theory nor the simplified version takes into account is the experimental inhomogeneity of the RF field. Such inhomogeneities will usually destroy many of the coherences that oscillate around the effective field while the spin-locked coherences survive (32).

The method presented here can be applied to any system of dipole–dipole-coupled spins for any combination of frequency offsets, spin-lock field strengths, mixing times, adiabatic or selective pulses, etc. The drawback of the formulation (for many-spin systems) is that scalar coupling effects have been neglected. However, in practice it is known that scalar coupling has only minor effects on off-resonance ROESY spectra when the magnetization is not spin-locked in the transverse plane, i.e., for $\theta < \text{ca. } 60^\circ$ (7, 9). An illustration of this effect is also provided for a special case (Fig. 1).

In addition, we have obtained the corresponding matrix representation of the complete theory for a two-spin system. Thus, in cases where scalar coupling cannot be neglected the full theory as represented by Eq. [40] should be used instead.

CONCLUSIONS

We have presented a simple formalism that allows for efficient simulation of NOESY, ROESY, and off-resonance ROESY spectra. The method is easy to use as well as computationally efficient and therefore possible to apply on large spin systems. Both NMR spectra and time-dependent magnetization trajectories are easily calculated. The derivation is

general with the exception that strong scalar coupling effects and therefore homonuclear Hartmann–Hahn effects are neglected.

In addition, we have presented the complete quantum mechanical theory for a two-spin system using the homogeneous master equation in the basis of Cartesian product operators. This theory will accurately include homonuclear Hartmann–Hahn effects as well as relaxation which should be useful for the analysis and optimization of mixing sequences as well as shaped pulses. Research in this direction is currently in progress in our laboratory.

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