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Experimental Control of Spin Diffusion in Liquid State NMR: A Comparison of Methods

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In this paper, we use the formalism of the first directional derivative of the matrix exponential, to analyze and compare several experimental schemes designed to measure cross-relaxation rate constants between two spins, without having to calculate the effects due to spin diffusion. © 1999 Academic Press

Key Words: **first directional derivative; matrix exponential; generator of the evolution; cross-relaxation rate constant; spin diffusion.**

I. INTRODUCTION

In some situations it is interesting to manipulate a spin system or its environment so that the evolution of the spin system becomes independent of one or several parameters that would otherwise affect it. For example, by applying a decoupling sequence to a particular nucleus or group of nuclei the effects of some scalar couplings can be removed. Another example is given by the mechanical rotation of a powder sample along an axis inclined at the magic angle with respect to the static B_0 field, in order to remove or at least to reduce the effects of chemical shift anisotropy and dipolar or first order quadrupolar couplings.

Proving that the response of a spin system to an experimental method does not depend upon a parameter κ that would otherwise affect its evolution requires that we first describe the evolution under this methodology including the parameter κ. In this manner we can determine the extent to which the evolution has become insensitive to the variation of κ, and the domain of values of the other parameters in which the evolution remains independent of κ can be specified.

We shall consider representations in which the density operator describing the state of a spin system at time t is connected to the corresponding operator at time zero by means of a mathematical expression that contains the exponential of some time-independent (super-) operator, the generator of the evolution $\mathscr{L}$, multiplied by the duration of the evolution. In such cases the first directional derivative of the matrix exponential (*1*) can be used to quantify, to first order, the extent to which the evolution is independent of a parameter κ, allowing an evaluation of the adequacy of the experimental method employed. If, in some domain of values of the other experimental parameters, the level of independence of the evolution with respect to κ is satisfactory, we can simplify the description of the evolution of the system, by excluding from it the parameter κ.

If a basis is chosen in operator space, assumed to be of dimension n, the generator of the evolution is represented by an $n \times n$ matrix and the same is true of its exponential. In the following the same symbol will be used to describe a superoperator or the matrix representing it. The space $\mathscr{M}_n$ of $n \times n$ matrices is a vector space of dimension n^2. In this space a particular matrix $\mathscr{B}$ is a vector; therefore it specifies a direction in it. The first derivative of the matrix exponential of $\mathscr{L}$ in the direction of a matrix $\mathscr{B}$ of the same order as the matrix $\mathscr{L}$ characterizes to first order the sensitivity of the evolution with respect to the variation of the parameters associated with $\mathscr{B}$. The matrix $\mathscr{B}$ can be thought of as the $n \times n$ matrix with zero everywhere, except at the positions where the parameter whose effect on the evolution we want to study is located.

In this paper we apply the method of the first derivative of the matrix exponential to analyze and compare experiments that were proposed to avoid the effects of multi-step magnetization transfer, referred to as spin diffusion, in the measurement of the cross-relaxation rate constant between a pair of spins. Four experiments will be considered: MINSY (*2*); a simplified version of BD-NOESY (*3–5*), which we shall denote BD1I-NOESY; QUIET-NOESY (*6*); and the Modified sYnchronouS nutatIoN method, or MYSIN (*7, 8*). We do not pretend that our list of methods is exhaustive. Major experimental methods that are left out of the calculations but not out of the discussions are BD-NOESY itself and the related CBD-NOESY (*9*), as a detailed mathematical analysis was given for them in (*10*). MYSIN was analyzed in some detail in (*11*). For the methods studied, systems containing a maximum of four spins will be considered (α, β, γ, and δ). The dependence of the transfer of magnetization from spin α to spin β will be analyzed as a function of the pattern of cross-relaxation rate constants between the spins α, β, γ, and δ. Isolation of the spin α and the spin β with respect to cross-relaxation with the spin γ and the spin δ, as well as sensitivity issues regarding the

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magnetization transfer between the spin α and the spin β, will be derived from the result of the calculation of the first derivative of the matrix exponential in appropriate directions.

II. THEORY

We assume that the master equation describing the evolution of the density operator representing the state of the nuclear spin system can be written in the laboratory frame as

$$\frac{d}{dt}\sigma(t) = -\iota[H(t), \sigma(t)] + \mathcal{R}[\sigma(t) - \sigma^{\mathrm{eq}}] + \Xi\sigma(t), \quad [1]$$

where $\iota = \sqrt{-1}$, $\sigma(t)$ is the density operator at time t, and σ^{eq} is the density operator at thermal equilibrium. $H(t)$ is the hamiltonian at time t, $\mathcal{R}$ is the (Redfield) relaxation superoperator, and Ξ is the exchange superoperator. We assume that the equation above can be transformed to an interaction frame in which the explicit time dependence of the transformed hamiltonian and relaxation and exchange superoperators are zero or can be neglected. In this frame the master equation reads (*12*)

$$\frac{d}{dt}\sigma^*(t) = -\iota[H_A^*, \sigma^*(t)] + \mathcal{R}_A^*(\sigma^*(t) - \sigma^{\mathrm{eq}*}) + \Xi_A^*\sigma^*(t). \quad [2]$$

The superscript $*$ refers to quantities that have been transformed to the interaction frame, while the subscript A refers to quantities that have been approximated. A formal solution to the equation above is given by (*13*)

$$\sigma^*(t) = \exp\{\mathcal{L}t\}(\sigma^*(0) - \sigma^*(\infty)) + \sigma^*(\infty), \quad [3]$$

where $\mathcal{L} = -\iota[H_A^*, \cdot\,] + \mathcal{R}_A + \Xi_A^*$ and $\sigma^*(\infty)$ is the interaction frame steady-state solution attained asymptotically (i.e., defined as $\lim_{t\to\infty} d/dt\ \sigma(t) = 0$). The superoperator $[H_A^*, \cdot]$ acts on any operator B as

$$[H_A^*, \cdot\,](B) = [H_A^*, B]. \quad [4]$$

$\sigma^*(\infty)$ is related to the equilibrium density operator by the equation

$$\mathcal{L}\sigma^*(\infty) = \mathcal{R}_A\sigma^{\mathrm{eq}*}. \quad [5]$$

If a basis is chosen in operator space, the superoperator $\mathcal{L}$, generator of the evolution, becomes a matrix. The dependence of the evolution upon a particular matrix $\mathcal{B}$ of the same size as $\mathcal{L}$ can be determined to first order by analyzing the first derivative of the matrix exponential $\mathcal{E}_t = \exp(\mathcal{L}t)$ in the direction of the matrix $\mathcal{B}$.

The first directional derivative of the matrix exponential $\mathcal{E}_t(\mathcal{L}) = \exp(\mathcal{L}t)$ evaluated at $\mathcal{L}$ in the direction $\mathcal{B}$ is defined as (*1*)

$$D_{\mathcal{B}}(t, \mathcal{L}) = \lim_{h\to 0}\frac{1}{h}(\mathcal{E}_t(\mathcal{L} + h\mathcal{B}) - \mathcal{E}_t(\mathcal{L})). \quad [6]$$

The number $h \in \mathbb{C}$, $\mathbb{C}$ the space of complex numbers. The matrix $D_{\mathcal{B}}(t, \mathcal{L})$ represents the dominant term in the expansion of the evolution with respect to matrix perturbation $h\mathcal{B}$ evaluated at $\mathcal{L}$. More formally stated,

$$\mathcal{E}_t(\mathcal{L} + h\mathcal{B}) = \mathcal{E}_t(\mathcal{L}) + hD_{\mathcal{B}}(t, \mathcal{L}) + O(h^2\mathcal{B}^2), \quad [7]$$

where $O(h^2\mathcal{B}^2)$ denotes elements of degree two or higher in $h\mathcal{B}$, with $\lim_{h\to 0}(O(h^2\mathcal{B}^2)/h)$ equal to the null matrix. $D_{\mathcal{B}}(t, \mathcal{L})$ can be calculated according to a formula that depends on the computed eigensystem of $\mathcal{L}$ (*1*),

$$D_{\mathcal{B}}(t, \mathcal{E}) = \Psi(\bar{\mathcal{B}} \odot \Phi(t))\Psi^{-1}, \quad [8]$$

where $\bar{\mathcal{B}} = \Psi^{-1}\mathcal{B}\Psi$, Ψ the matrix whose columns are the eigenvectors of $\mathcal{E}$. The expression $\bar{\mathcal{B}} \odot \Phi(t)$ denotes the Hadamard product (entrywise product) of the matrix $\bar{\mathcal{B}}$ and the matrix $\Phi(t)$, whose entries are given by

$$\Phi_{ij}(t) = \Phi_{ji}(t) = \begin{cases} \dfrac{\exp(t\lambda_i) - \exp(t\lambda_j)}{\lambda_i - \lambda_j} & \text{if } \lambda_i \neq \lambda_j \\ t\exp(t\lambda_i) & \text{if } \lambda_i = \lambda_j, \end{cases} \quad [9]$$

where λ_i, $i = 1, 2, \ldots, 5$, are the eigenvalues of $\mathcal{E}$. If $\mathcal{F}$ is the product of two exponentials, $\mathcal{F} = \mathcal{E}_t(\mathcal{L}_1)\mathcal{E}_t(\mathcal{L}_2)$, it can be easily proven using Eq. [6] that the first directional derivative of $\mathcal{F}$ in the direction $\mathcal{B}$ satisfies the "Leibniz rule" for derivations

$$D_{\mathcal{B}}(t, \mathcal{L}_1, \mathcal{L}_2) = D_{\mathcal{B}}(t, \mathcal{L}_1)\mathcal{E}_t(\mathcal{L}_2) + \mathcal{E}_t(\mathcal{L}_1)D_{\mathcal{B}}(t, \mathcal{L}_2). \quad [10]$$

Generalization to superoperator $\mathcal{F}$, consisting of the products of more than two exponentials, $\mathcal{E}_t(\mathcal{L}_1)$, $\mathcal{E}_t(\mathcal{L}_2)$, . . . , and possibly containing other time-independent superoperators as product elements, is straightforward and will be exemplified below.

III. APPLICATION

We apply below the formalism presented in Section II to analyze a class of experiments that were designed to avoid the calculation of the effects of spin diffusion in the measurement of the cross-relaxation rate constant between a pair of spins.

The direct measurement of cross-relaxation or exchange rate constants via conventional multi-dimensional methods is made difficult in large molecules, due to multi-step magnetization transfer processes, also known as spin diffusion. In structure calculations spin diffusion is usually taken into account by performing refinement procedures using a full relaxation matrix analysis (*14*). This method, computational by nature, is partially based on theoretical assumptions. As a consequence pulse sequences are sought to provide a direct experimental measurement of cross-relaxation rate constants, at least in particular situations, so that the assumptions used for structure calculations can be supported experimentally. Using the first directional derivative of the matrix exponential four experiments are analyzed: MINSY (*2*); BD1I-NOESY, a simplified version of BD-NOESY (*3–5*); QUIET-NOESY (*6*); and the synchronous nutation method (*15*) in its modified form, MYSIN (*7, 8*). We want to stress that even if BD1I-NOESY is derived from the BD-NOESY experiment, our results apply only to the simplified version and not to the original experiment itself. As mentioned above BD-NOESY and CBD-NOESY were carefully analyzed in earlier works. In this paper we wish to be able to compare QUIET-NOESY with a simplified version of BD-NOESY. We shall discuss the respective advantages of BD-NOESY and CBD-NOESY later in the paper. For each method studied we derive expressions for the first directional derivative in appropriate directions in systems containing a maximum of four spins (α, β, γ, and δ), but often it will be sufficient to consider three-spin systems only (α, β, and γ), especially in the cases of MINSY and MYSIN. In the four methods, NOE transfer can take place during the mixing time τ_m. During that time each method proposes a scheme to isolate spin α and spin β with respect to cross-relaxation with spin γ and spin δ.

MINSY attempts to isolate the spins α and β with respect to cross-relaxation with the spins γ and δ by continuously irradiating, during the mixing time, selected bands of frequencies into which the spins γ and δ resonate. BD1I-NOESY performs a single band-selective inversion at specific instants during the mixing time, in frequency bands containing the spins γ and δ. QUIET-NOESY inverts selectively spins α and β at specified instants during the mixing time. During MYSIN the spins α and β are forced to nutate synchronously for the entire mixing time; at half the duration of the mixing time the phase of the RF field is switched by 180°, so that the nutation is performed in the reverse sense as compared to the first half of it. Of the four methods, MYSIN is the only one that does not necessitate a "read" pulse to record the results of the experiment. At the same time that cross-relaxation is active between the spins α and β, the same process is used to overcome spectral overlap (*8*).

Our analysis is performed in the idealized situation characterized by the following assumptions:

(1) Relaxation behavior is based only on dipole–dipole interactions, neglecting dipole–dipole cross-correlations.

(2) Irradiation in MINSY and MYSIN is assumed to be continuous and at a constant amplitude ω. Off-resonance effects due to RF fields and interference of RF sidebands are not taken into account. For the effects of the interference of RF sidebands, the reader should consult Ref. (*15*).

(3) The inversion pulses in BD1I-NOESY and QUIET-NOESY are assumed to be instantaneous. For the effects of real soft inversion pulses the reader should consult Ref. (*16*).

Symbols of the forms ρ_μ and ρ^t_μ, $\mu = \alpha, \beta, \gamma, \delta$, stand respectively for longitudinal or transverse self-relaxation rate constant. Symbols of the form $\sigma_{\mu\nu}$, $\mu, \nu = \alpha, \beta, \gamma, \delta$, stand for cross-relaxation rate constants between spins μ and ν. The symbol ω is reserved for the amplitude of a radiofrequency field. The calculations of the matrix exponential and its first directional derivative, in suitable directions, are made using a program written by the author that utilizes routines of the C version of the linear algebraic package LAPACK (*17*) for finding eigenvalues and eigenvectors of matrices.

Within the assumptions expressed above, the generator of the evolution $\mathcal{L}_{\text{MINSY}}$ active during the mixing time of a MINSY experiment is written in a three-spin system and in the basis set $\{I_{z\alpha}, I_{z\beta}, I_{z\gamma}, I_{y\gamma}\}$ as

$$\mathcal{L}_{\text{MINSY}} = -\begin{pmatrix} \rho_\alpha & \sigma_{\alpha\beta} & \sigma_{\alpha\gamma} & 0 \\ \sigma_{\alpha\beta} & \rho_\beta & \sigma_{\beta\gamma} & 0 \\ \sigma_{\alpha\gamma} & \sigma_{\beta\gamma} & \rho_\gamma & \omega \\ 0 & 0 & -\omega & \rho^t_\gamma \end{pmatrix}. \qquad [11]$$

At the end of the mixing time in the MINSY experiment the density operator $\sigma(\tau_m)$ is written according to Eq. [3] as

$$\sigma(\tau_m) = \exp\{\mathcal{L}_{\text{MINSY}}\tau_m\}(\sigma(0) - \sigma_{\text{MINSY}}(\infty)) + \sigma_{\text{MINSY}}(\infty). \qquad [12]$$

We calculate directional derivatives of the expression

$$\mathcal{F}_{\text{MINSY}} = \exp\{\mathcal{L}_{\text{MINSY}}\tau_m\} \qquad [13]$$

in directions $\mathcal{B}$, that is, according to Eq. [6], quantities of the form $D_{\mathcal{B}}(\tau_m, \mathcal{L}_{\text{MINSY}})$ with

$$\mathcal{L}_{\text{MINSY}} = \begin{pmatrix} -5 & 1 & 3 & 0 \\ 1 & -7 & 3 & 0 \\ 3 & 3 & -7 & 75 \\ 0 & 0 & -75 & -9 \end{pmatrix}. \qquad [14]$$

The values chosen for the various self- and cross-relaxation rate constants are quite arbitrary but may correspond to the relaxation rate constants of spins of proton in a particular residue of a small protein (*18*). In Fig. 1 the dotted–dashed curve represents, as a function of the duration of the mixing time, the quantity

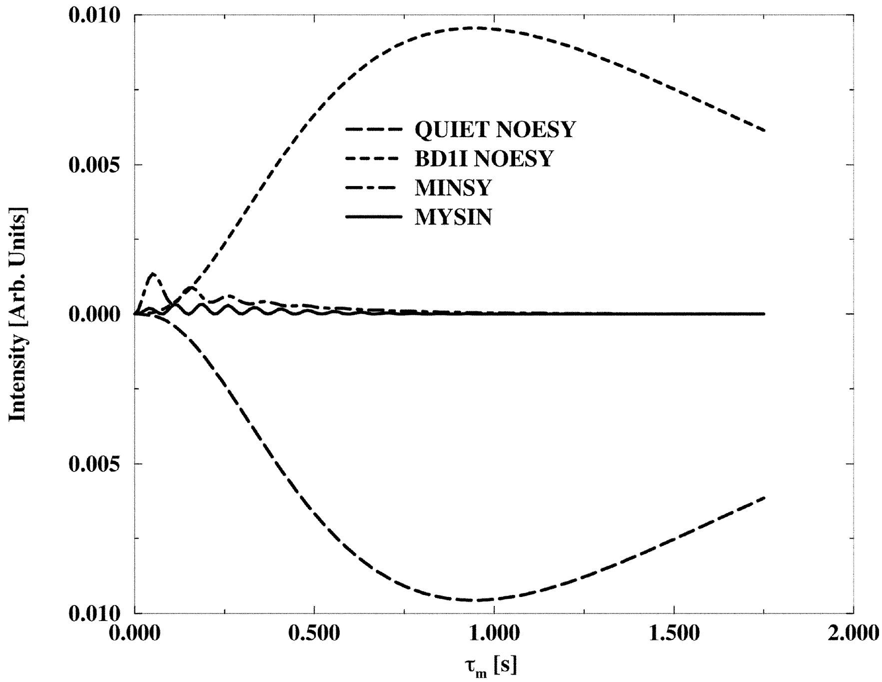

FIG. 1. Variation as a function of the mixing time τ_{m} of the quantities $Tr(D_{\mathscr{B}^1}(\tau_{\mathrm{m}},\mathscr{L}_{\mathrm{MINSY}})I_{z\alpha},I_{z\beta})$ (dotted–dashed curve), $\mathrm{Tr}(D_{\mathscr{B}^1}(\tau_{\mathrm{m}},\ \mathrm{BD1I})I_{z\alpha},\ I_{z\beta})$ (dashed curve), $\mathrm{Tr}(D_{\mathscr{B}^1}(\tau_{\mathrm{m}},\ \mathrm{QUIET})I_{z\alpha},\ I_{z\beta})$ (long-dashed curve), $\mathrm{Tr}(D_{\mathscr{B}^1}(\tau_{\mathrm{m}},\ \mathrm{MYSIN})I_{y\alpha},\ I_{y\beta})$ (solid curve). In each case $\mathscr{B}^1$ is the matrix of the same dimensions as the generator of the evolution for the particular experiment, with zero everywhere except at the positions which in the generator of the evolution contain the elements $\sigma_{\alpha\gamma}$ which are equal to one. See the text for explicit details about the values of the matrix element of the particular generators of the evolution.

$$\mathrm{Tr}(D_{\mathscr{B}^1}(\tau_{\mathrm{m}},\ \mathscr{L}_{\mathrm{MINSY}})I_{z\alpha},\ I_{z\beta}), \qquad [15]$$

where Tr is the symbol for the trace and $\mathscr{B}^1$ is the 4×4 matrix with zero everywhere except at the positions $\mathscr{B}^1_{13}$ and $\mathscr{B}^1_{31}$ which are equal to one. The quantity in Eq. [15] thus represents the sensitivity of the transfer of longitudinal magnetization from spin α to spin β with respect to a variation of the cross-relaxation rate constant $\sigma_{\alpha\gamma}$. In Fig. 2 the dotted–dashed curve represents, as a function of the duration of the mixing time, the quantity

$$\mathrm{Tr}(D_{\mathscr{B}^2}(\tau_{\mathrm{m}},\ \mathscr{L}_{\mathrm{MINSY}})I_{z\alpha},\ I_{z\beta}), \qquad [16]$$

where $\mathscr{B}^2$ is the 4×4 matrix with zero everywhere except at the positions $\mathscr{B}^2_{12}$ and $\mathscr{B}^2_{21}$ which are equal to one. The quantity in Eq. [16] thus represents the sensitivity of the transfer of longitudinal magnetization from spin α to spin β with respect to a variation of the cross-relaxation rate constant $\sigma_{\alpha\beta}$.

The difference between the two curves is striking. We observe that the transfer of longitudinal magnetization from spin α to spin β is much more sensitive to a variation of the cross-relaxation rate constant $\sigma_{\alpha\beta}$ than to a variation of the cross-relaxation rate constant $\sigma_{\alpha\gamma}$. To a good extent it can be said that during a MINSY experiment the spin α and the spin β are well isolated from cross-relaxation with the spin γ. Isolation becomes almost perfect for long mixing time.

BD1I-NOESY and QUIET-NOESY share exactly the same generator of the evolution $\mathscr{L}_{\mathrm{BD1I/QUIET}}$, namely in the basis set $\{I_{z\alpha},\ I_{z\beta},\ I_{z\gamma},\ I_{z\delta}\}$,

$$\mathscr{L}_{\mathrm{BD1I/QUIET}} = -\begin{pmatrix} \rho_\alpha & \sigma_{\alpha\beta} & \sigma_{\alpha\gamma} & \sigma_{\alpha\delta} \\ \sigma_{\alpha\beta} & \rho_\beta & \sigma_{\beta\gamma} & \sigma_{\beta\delta} \\ \sigma_{\alpha\gamma} & \sigma_{\beta\gamma} & \rho_\gamma & \sigma_{\gamma\delta} \\ \sigma_{\alpha\delta} & \sigma_{\beta\delta} & \sigma_{\gamma\delta} & \rho_\delta \end{pmatrix}. \qquad [17]$$

In this paper we shall assume that both BD1I- and QUIET-NOESY perform two experiments, whose outputs are subtracted. In the first experiment, the spin α is inverted. The operator performing this inversion will be denoted by J_α. Following a duration $\tau_{\mathrm{m}}/2$ after the inversion of the spin α, BD1I-NOESY inverts selectively spins in the frequency band in which spin γ (operator J_γ) resonates or in the frequency band in which spin δ (operator J_δ) resonates. Alternatively the spins in both frequency bands containing respectively spins γ and δ can be inverted (operator $J_{\gamma\delta}$). Then the system is again left to evolve by itself for another period of time $\tau_{\mathrm{m}}/2$. QUIET-NOESY follows the same time development except that instead of inverting the spins γ and δ, the sequence inverts the spins α and β at time $t = \tau_{\mathrm{m}}/2$ (inversion operator $J_{\alpha\beta}$). The

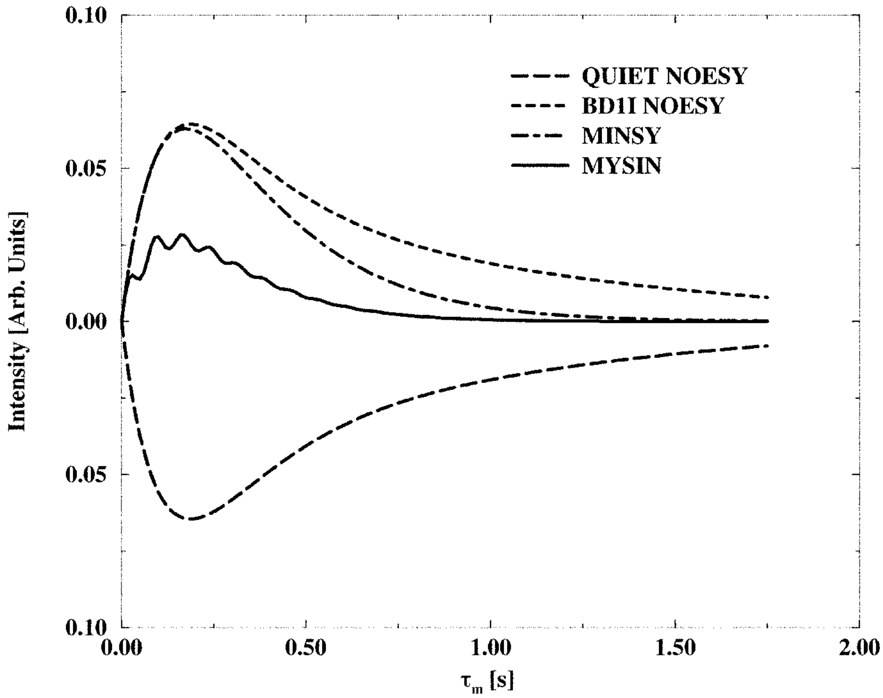

FIG. 2. Variation as a function of the mixing time τ_m of the quantities $\mathrm{Tr}(D_{\mathscr{B}^2}(\tau_m, \mathscr{L}_{\mathrm{MINSY}})I_{y\alpha}, I_{y\beta})$ (dotted–dashed curve), $\mathrm{Tr}(D_{\mathscr{B}^2}(\tau_m, \mathrm{BD1I})I_{z\alpha}, I_{z\beta})$ (dashed curve), $\mathrm{Tr}(D_{\mathscr{B}^2}(\tau_m, \mathrm{QUIET})I_{z\alpha}, I_{z\beta})$ (long-dashed curve), $\mathrm{Tr}(D_{\mathscr{B}^2}(\tau_m, \mathrm{MYSIN})I_{y\alpha}, I_{y\beta})$ (solid curve). In each case $\mathscr{B}^2$ is the matrix of the same dimensions as the generator of the evolution for the particular experiment, with zero everywhere except at the positions which in the generator of the evolution contain the elements $\sigma_{\alpha\beta}$ which are equal to one. See the text for explicit details about the values of the matrix element of the particular generators of the evolution.

control experiment that is to be subtracted from the first experiment follows the same time development, with the difference that the initial inversion of the spin α is not performed. It thus consists of an inversion at time $\tau_m/2$, the same one that is performed after this duration in the first experiment, followed by a period of free evolution of duration $\tau_m/2$. After subtraction, and with the operator utilized for the inversion at time $\tau_m/2$ denoted as J, the density operator reads

$$\sigma_{\mathrm{BD1I/QUIET}}(\tau_m) = \left\{\exp\left\{\mathscr{L}_{\mathrm{BD1I/QUIET}}\,\frac{\tau_m}{2}\right\}J \times \exp\left\{\mathscr{L}_{\mathrm{BD1I/QUIET}}\,\frac{\tau_m}{2}\right\}\right\}(J_\alpha\sigma^{\mathrm{eq}} - \sigma^{\mathrm{eq}}). \quad [18]$$

As $(J_\alpha\sigma^{\mathrm{eq}} - \sigma^{\mathrm{eq}}) = 2I_{z\alpha}$ we get

$$\sigma_{\mathrm{BD1I/QUIET}}(\tau_m) = \left\{\exp\left\{\mathscr{L}_{\mathrm{BD1I/QUIET}}\,\frac{\tau_m}{2}\right\}J \times \exp\left\{\mathscr{L}_{\mathrm{BD1I/QUIET}}\,\frac{\tau_m}{2}\right\}\right\}2I_{z\alpha}. \quad [19]$$

Depending on the nature of the inversion J chosen at time $\tau_m/2$, $\sigma_{\mathrm{BD1I}}(\tau_m)$ or $\sigma_{\mathrm{QUIET}}(\tau_m)$ is obtained.

In the following we calculate directional derivatives in the direction $\mathscr{B}$ of expressions of the form

$$\mathscr{F}_{\mathrm{BD1I/QUIET}} = \left\{\exp\left\{\mathscr{L}_{\mathrm{BD1I/QUIET}}\,\frac{\tau_m}{2}\right\}J \times \exp\left\{\mathscr{L}_{\mathrm{BD1I/QUIET}}\,\frac{\tau_m}{2}\right\}\right\}. \quad [20]$$

Denoting by $D_{\mathscr{B}}(\tau_m, \mathrm{BD1I/QUIET})$ the directional derivative of $\mathscr{F}_{\mathrm{BD1I/QUIET}}$ in the direction $\mathscr{B}$ and using the “Leibniz rule,” Eq. [10], we can show that

$$D_{\mathscr{B}}(\tau_m, \mathrm{BD1I/QUIET}) = D_{\mathscr{B}}\left(\frac{\tau_m}{2}, \mathscr{L}_{\mathrm{BD1I/QUIET}}\right)J\mathscr{E}_{\tau_m/2}(\mathscr{L}_{\mathrm{BD1I/QUIET}}) + \mathscr{E}_{\tau_m/2}(\mathscr{L}_{\mathrm{BD1I/QUIET}})JD_{\mathscr{B}}\left(\frac{\tau_m}{2}, \mathscr{L}_{\mathrm{BD1I/QUIET}}\right). \quad [21]$$

In the following we shall, when necessary, particularize $D_{\mathscr{B}}(\tau_m, \mathrm{BD1I/QUIET})$ to $D_{\mathscr{B}}(\tau_m, \mathrm{BD1I})$ or $D_{\mathscr{B}}(\tau_m, \mathrm{QUIET})$. It

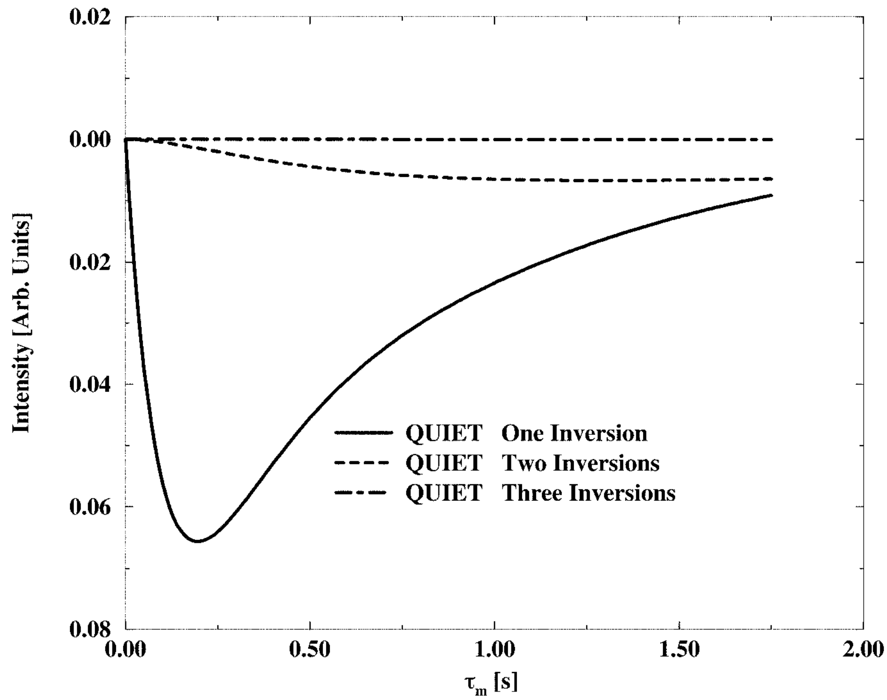

FIG. 3. Variation as a function of the mixing time τ_m of the quantities $\mathrm{Tr}(D_{\mathscr{B}}(\tau_m, \mathrm{QUIET})I_{z\alpha}, I_{z\beta})$, where $\mathscr{B}$ is a matrix of the same dimensions as the generator of the evolution $\mathscr{L}_{\mathrm{QUIET}}$, with zero everywhere except at the positions which in the generator of the evolution contain the element $\sigma_{\alpha\beta}$ which are equal to one. The solid curve represents a single inversion in the middle of the mixing time. The dashed curve represents two inversions during the mixing time, one taking place at $\tau_m/4$, the other at $3\tau_m/4$. The dotted–dashed curve represents three inversions during the mixing time, one taking place at $\tau_m/6$, the second at $\tau_m/2$, and the third at $5\tau_m/6$. See the text for explicit details about $\mathscr{L}_{\mathrm{QUIET}}$.

should be noted that multiple inversions during the mixing time were proposed for both methods, but to the author's knowledge they were never implemented (see discussion below).

In Fig. 1 the dashed curve represents, as a function of the mixing time, the quantity

$$\mathrm{Tr}(D_{\mathscr{B}^1}(\tau_m, \mathrm{BD1I})I_{z\alpha}, I_{z\beta}) \quad [22]$$

with

$$\mathscr{L}_{\mathrm{BD1I}} = \begin{pmatrix} -5 & 1 & 3 & 0 \\ 1 & -7 & 3 & 0 \\ 3 & 3 & -7 & 0 \\ 0 & 0 & 0 & -5 \end{pmatrix} \quad [23]$$

and $\mathscr{B}^1$ defined as the 4×4 matrix with zero everywhere except at the positions $\mathscr{B}^1_{13}$ and $\mathscr{B}^1_{31}$ which are equal to one. In Fig. 2 the dashed curve represents, as a function of the mixing time, the quantity

$$\mathrm{Tr}(D_{\mathscr{B}^2}(\tau_m, \mathrm{BD1I})I_{z\alpha}, I_{z\beta}) \quad [24]$$

for the same $\mathscr{L}_{\mathrm{BD1I}}$ as above and $\mathscr{B}^2$ the 4×4 matrix with zero everywhere except at the positions $\mathscr{B}^2_{12}$ and $\mathscr{B}^2_{21}$ which are equal to one.

With $\mathscr{L}_{\mathrm{QUIET}} = \mathscr{L}_{\mathrm{BD1I}}$ the long-dashed curves in Figs. 1 and 2 represent, for QUIET-NOESY, quantities corresponding to those given in Eqs. [22] and [24]. These curves demonstrate that in a three-spin system BD1I-NOESY and QUIET-NOESY are up to a sign totally equivalent. With these two methods isolation of spin α and spin β with respect to cross-relaxation with spin γ is very good for short mixing times but tends to degrade rapidly for longer times. The sensitivity of the transfer of longitudinal magnetization between spin α and spin β to a variation of $\sigma_{\alpha\beta}$ is the best of the four experiments analyzed. One should keep in mind however that the inversion in the middle of the mixing time was assumed to be instantaneous. In a real experiment the sensitivity will be reduced.

Isolation is improved by using multiple inversions (curves not shown). However, as presented in Fig. 3, the transfer of longitudinal magnetization between spin α and spin β becomes insensitive to a variation of the cross-relaxation rate constant $\sigma_{\alpha\beta}$ if multiple inversions are used. In this figure the quantity

$$\mathrm{Tr}(D_{\mathscr{B}}(\tau_m, \mathrm{QUIET})I_{z\alpha}, I_{z\beta}) \quad [25]$$

is plotted as a function of the mixing time, for one inversion at $\tau_m/2$ (solid curve); two inversions, one at $\tau_m/4$, the other at

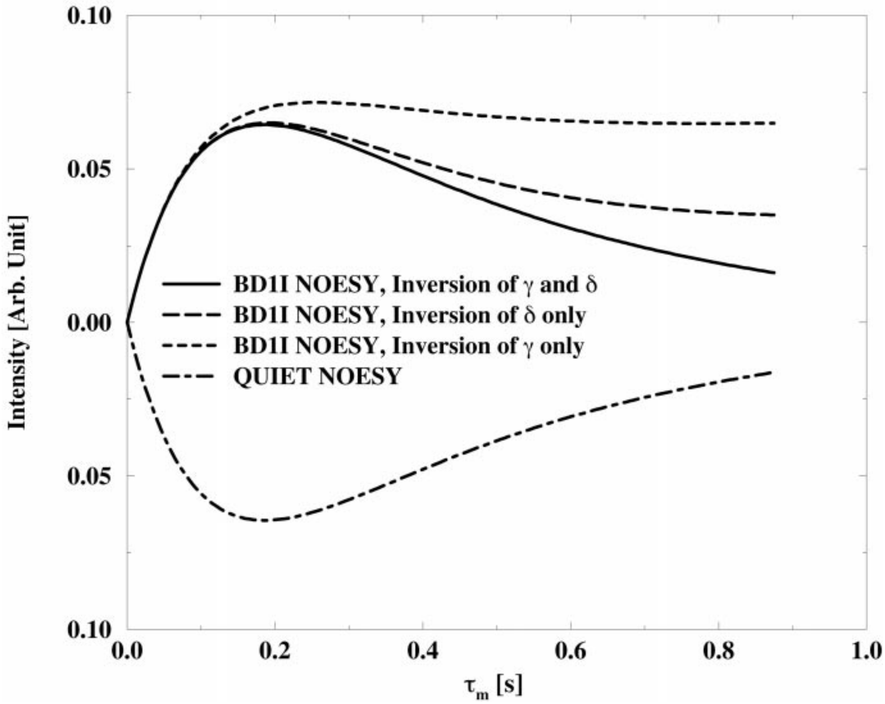

FIG. 4. Variation as a function of the mixing time τ_m of the quantities $\mathrm{Tr}(D_{\mathcal{B}}(\tau_m, \mathrm{BD1I})I_{z\alpha}, I_{z\beta})$ when the spins γ and δ are both inverted in the middle of the mixing time (solid curve), $\mathrm{Tr}(D_{\mathcal{B}}(\tau_m, \mathrm{BD1I})I_{z\alpha}, I_{z\beta})$ when only the spin γ is inverted in the middle of the mixing time (long-dashed curve), $\mathrm{Tr}(D_{\mathcal{B}}(\tau_m, \mathrm{BD1I})I_{z\alpha}, I_{z\beta})$ when only the spin δ is inverted in the middle of the mixing time (dashed curve), and $\mathrm{Tr}(D_{\mathcal{B}}(\tau_m, \mathrm{QUIET})I_{z\alpha}, I_{z\beta})$ (dotted–dashed curve). The matrix $\mathcal{B}$ is of the same dimensions as the generator of the evolution $\mathcal{L}_{\mathrm{BD1I/QUIET}}$, with zero everywhere except at the positions which in the generator of the evolution contain the elements $\sigma_{\alpha\beta}$ which are equal to one. See the text for explicit details about $\mathcal{L}_{\mathrm{BD1I}}$ and $\mathcal{L}_{\mathrm{QUIET}}$.

$3\tau_m/4$ (dashed curve); and three inversions, one at $\tau_m/6$, the second at $\tau_m/2$, and the third at $5\tau_m/6$ (dotted–dashed curve). All the inversions are assumed to be instantaneous. The same $\mathcal{L}_{\mathrm{QUIET}}$ as that above is used and $\mathcal{B}$ is the 4×4 matrix with zero everywhere except at the positions $\mathcal{B}_{12}$ and $\mathcal{B}_{21}$ which are equal to one. Of course the control experiment is also modified; the inversions during the mixing time are made in correspondence with those made in the experiment from which it will be subtracted. With our choices for the parameters and three inversions during the mixing time, the sensitivity of the transfer of magnetization from the spin α to the spin β relative to a variation of $\sigma_{\alpha\beta}$ becomes very low. It is not identically zero as the corresponding curve in Fig. 3 seems to indicate. This result may be explained by noting that the transfer of magnetization from spin α to spin β is proportional to the deviation of the magnetization of spin α from its equilibrium value. We consider the experiment that utilized three doubly selective inversions during the mixing time. The spin α is initially inverted and we choose a duration for the mixing time such that the maximum transfer from the spin α to the spin β by cross-relaxation will take place during the periods 0 to $\tau_m/6$ and $\tau_m/2$ to $5\tau_m/6$. During the period $\tau_m/6$ to $\tau_m/2$ cross-relaxation is reduced, as now the magnetization on the spin α is assumed to be closer to its equilibrium value. Overall, during the period 0 to $\tau_m/2$ the cross-relaxation from the spin α to the spin β will be less than that in the experiment with only a single inversion during the mixing time. As the mixing time passes, the deviation away from equilibrium of spin α magnetization and spin β magnetization tends to equalize. Thus the loss in magnetization transfer experienced during the first part of the mixing time cannot be recovered during the second part. Therefore, a longer mixing time would be necessary to compensate for this loss in magnetization transfer. This explains why an experiment with a greater number of doubly selective inversions during the same mixing time becomes less sensitive to a variation of the cross-relaxation rate constant between the spins that are selectively inverted. This points to the fact that a QUIET-NOESY experiment in which the doubly selective inversions are considered to be instantaneous is not equivalent in the limit of continuous inversions to the MYSIN experiment. But, in such situations, we must be careful in the implementation of the formalism of the first directional derivative of the matrix exponential. We will explain this later while discussing the BD-NOESY experiment.

The difference between BD1I-NOESY and QUIET-NOESY is exemplified in Fig. 4. In this figure the quantity

$$\mathrm{Tr}(D_{\mathcal{B}}(\tau_m, \mathrm{BD1I/QUIET})I_{z\alpha}, I_{z\beta}) \qquad [26]$$

is plotted as a function of the mixing time, with $\mathcal{L}_{\mathrm{BD1I/QUIET}}$ equal to

$$\mathcal{L}_{\mathrm{BD1I}} = \begin{pmatrix} -5 & 1 & 3 & 1 \\ 1 & -7 & 3 & 0 \\ 3 & 3 & -9 & 2 \\ 1 & 0 & 2 & -5 \end{pmatrix}. \quad [27]$$

This quantity follows (up to a sign) exactly the same time development in BD1I-NOESY and QUIET-NOESY if and only if the spins γ and δ are inverted in the middle of the mixing time. If however only γ (resp. δ) is inverted we can observe that in BD1I-NOESY the quantity becomes more sensitive to spin diffusion with the non-inverted spin. BD1I-NOESY requires, before the start of the experiment, knowledge of the spin diffusion network of the pair of spins α and β, if one wishes to obtain a spin-diffusion-free measurement of the cross-relaxation rate constant $\sigma_{\alpha\beta}$. In such situations it is advantageous to use QUIET-NOESY, which does not necessitate this prior knowledge. However if the spin diffusion network is known, BD1I-NOESY enables one to make a detailed analysis of spin diffusion within the network, by allowing one to quench selectively specific relaxation pathways.

In MYSIN the spins α and β are continuously irradiated during the mixing time τ_{m} by means of an amplitude modulated RF field with a modulation frequency equal to half the frequency difference between the spins α and β. At time $t = \tau_{\mathrm{m}}/2$ the phase of the RF field is switched by 180° and keeps this value for the remaining half of the mixing time. During the first half of the mixing time the superoperator, generator of the evolution for MYSIN, $\mathcal{L}_{\mathrm{MYSIN}}$, is written in the basis set $\{I_{z\alpha}, I_{z\beta}, I_{y\alpha}, I_{y\beta}, I_{z\gamma}\}$ as

$$\mathcal{L}_{\mathrm{MYSIN}} = -\begin{pmatrix} \rho_\alpha & \sigma_{\alpha\beta} & \omega & 0 & \sigma_{\alpha\gamma} \\ \sigma_{\alpha\beta} & \rho_\beta & 0 & \omega & \sigma_{\beta\gamma} \\ -\omega & 0 & \rho^{\mathrm{t}}_\alpha & 0 & 0 \\ 0 & -\omega & 0 & \rho^{\mathrm{t}}_\beta & 0 \\ \sigma_{\alpha\gamma} & \sigma_{\beta\gamma} & 0 & 0 & \rho_\gamma \end{pmatrix}. \quad [28]$$

During the second half of the mixing time the generator of the evolution for MYSIN is the transposed $\mathcal{L}^{\mathrm{T}}_{\mathrm{MYSIN}}$ of $\mathcal{L}_{\mathrm{MYSIN}}$. It is assumed that the resonance frequencies of the spins α and β are well separated so that transverse cross-relaxation can be neglected.

At the end of the mixing time the density operator is written as

$$\sigma_{\mathrm{MSIN}}(\tau_{\mathrm{m}}) = \left\{\exp\left\{\frac{\mathcal{L}^{\mathrm{T}}_{\mathrm{MYSIN}}\tau_{\mathrm{m}}}{2}\right\}\exp\left\{\frac{\mathcal{L}_{\mathrm{MYSIN}}\tau_{\mathrm{m}}}{2}\right\}\right\} \times (\sigma^*(0) - \sigma^*(\infty)) + \sigma^*(\infty). \quad [29]$$

We calculate directional derivatives in the direction $\mathcal{B}$ of an expression of the form

$$\mathcal{F}_{\mathrm{MYSIN}} = \left\{\exp\left\{\frac{\mathcal{L}^{\mathrm{T}}_{\mathrm{MYSN}}\tau_{\mathrm{m}}}{2}\right\}\exp\left\{\frac{\mathcal{L}_{\mathrm{MYSN}}\tau_{\mathrm{m}}}{2}\right\}\right\}. \quad [30]$$

Denoting by $D_{\mathcal{B}}(\tau_{\mathrm{m}}, \mathrm{MYSIN})$ the directional derivative of $\mathcal{F}_{\mathrm{MYSIN}}$ in the direction $\mathcal{B}$ and using the Leibniz rule enunciated above, we obtain an expression of the form

$$D_{\mathcal{B}}(\tau_{\mathrm{m}}, \mathrm{MYSIN}) = D_{\mathcal{B}}\left(\frac{\tau_{\mathrm{m}}}{2}, \mathcal{L}^{\mathrm{T}}_{\mathrm{MYSIN}}\right)\mathcal{E}_{\tau_{\mathrm{m}}/2}(\mathcal{L}_{\mathrm{MYSIN}}) + \mathcal{E}_{\tau_{\mathrm{m}}/2}(\mathcal{L}_{\mathrm{MYSIN}})\, D_{\mathcal{B}}\left(\frac{\tau_{\mathrm{m}}}{2}, \mathcal{L}_{\mathrm{MYSIN}}\right). \quad [31]$$

In Fig. 1 the solid curve represents the time development during the mixing time of the quantity

$$\mathrm{Tr}(D_{\mathcal{B}^1}(\tau_{\mathrm{m}}, \mathrm{MYSIN})I_{y\alpha}, I_{y\beta}) \quad [32]$$

with

$$\mathcal{L}_{\mathrm{MYSIN}} = \begin{pmatrix} -5 & 1 & 75 & 0 & 3 \\ 1 & -7 & 0 & 75 & 3 \\ -75 & 0 & -7 & 0 & 0 \\ 0 & -75 & 0 & -9 & 0 \\ 3 & 3 & 0 & 0 & -7.1 \end{pmatrix} \quad [33]$$

and $\mathcal{B}^1$ defined as the 5×5 matrix with zero everywhere except at the positions $\mathcal{B}^1_{15}$ and $\mathcal{B}^1_{51}$. In Fig. 2 the solid curve represents the time development during the mixing time of the quantity

$$\mathrm{Tr}(D_{\mathcal{B}^2}(\tau_{\mathrm{m}}, \mathrm{MYSIN})I_{y\alpha}, I_{y\beta}) \quad [34]$$

for $\mathcal{B}^2$ defined as the 5×5 matrix with zero everywhere except at the positions $\mathcal{B}^2_{12}$ and $\mathcal{B}^2_{21}$. From these figures we can conclude that during a MYSIN experiment the transfer of magnetization from spin α to spin β is extremely well isolated from any cross-relaxation transfer emanating from spin γ. However the transfer of magnetization from spin α to spin β is the least sensitive of the four methods studied to a variation of $\sigma_{\alpha\beta}$.

In Fig. 5, we present for MYSIN and QUIET-NOESY the dependence of the first directional derivative in an appropriate direction upon variation of the value for some matrix element in $\mathcal{L}$. For MYSIN the quantity

$$\mathrm{Tr}(D_{\mathcal{B}}(\tau_{\mathrm{m}}, \mathrm{MYSIN})I_{y\alpha}, I_{y\beta}) \quad [35]$$

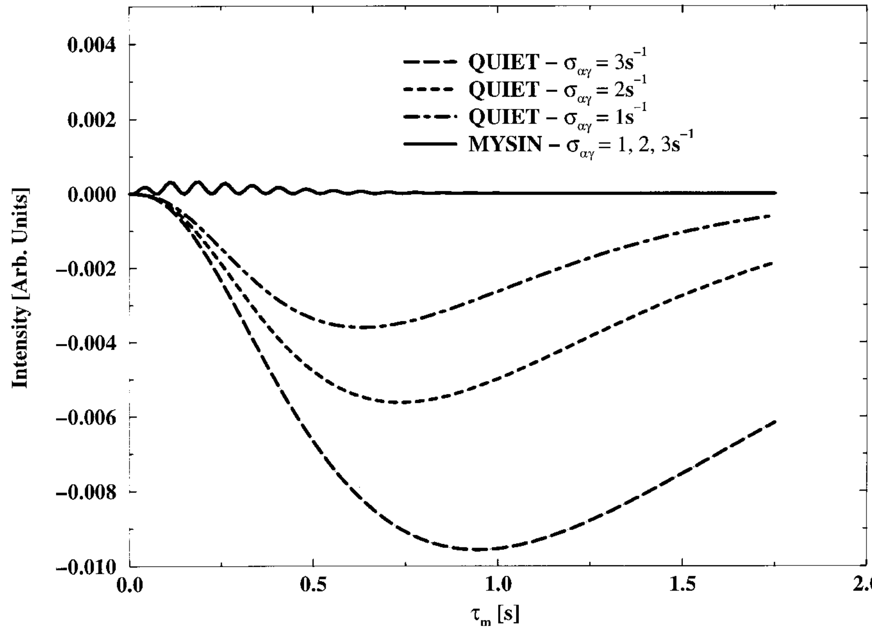

FIG. 5. Variation as a function of the mixing time τ_m of the quantities $\mathrm{Tr}(D_{\mathcal{B}}(\tau_m, \mathrm{QUIET})I_{z\alpha}, I_{z\beta})$, long-dashed curve ($\sigma_{\alpha\gamma} = 3\ s^{-1}$), dashed curve ($\sigma_{\alpha\gamma} = 2\ s^{-1}$), dotted–dashed curve ($\sigma_{\alpha\gamma} = 1\ s^{-1}$); and $\mathrm{Tr}(D_{\mathcal{B}}(\tau_m, \mathrm{MYSIN})I_{y\alpha}, I_{y\beta})$, solid curve (superimposition of the three cases $\sigma_{\alpha\gamma} = 1, 2, 3\ s^{-1}$). For all the curves $\mathcal{B}$ is the matrix of the same dimensions as the respective generator of the evolution with zero everywhere except at the positions which in the generator of the evolution contain the elements $\sigma_{\alpha\gamma}$ which are equal to one. See the text for explicit details about $\mathcal{L}_{\mathrm{MYSIN}}$ and $\mathcal{L}_{\mathrm{QUIET}}$.

is plotted as a function of the mixing time with $\mathcal{B}$ defined as the 5×5 matrix with zero everywhere except at the positions $\mathcal{B}_{15}$ and $\mathcal{B}_{51}$ which are equal to one. For QUIET-NOESY the quantity

$$\mathrm{Tr}(D_{\mathcal{B}}(\tau_m, \mathrm{QUIET})I_{z\alpha}, I_{z\beta}) \qquad [36]$$

is plotted as a function of the mixing time with $\mathcal{B}$ the 4×4 matrix with zero everywhere except at the positions $\mathcal{B}_{13}$ and $\mathcal{B}_{31}$ which are equal to one. $\mathcal{L}_{\mathrm{MYSIN}}$ and $\mathcal{L}_{\mathrm{QUIET}}$ are as given in Eqs. [33] and [23]. The set of curves indicates, in particular cases, the times at which higher order terms in the development (see Eq. [7]) of the matrix exponential become effective. In the case of MYSIN we can observe that the quantity defined in Eq. [35] is not sensitive at all to variation of the value of $\sigma_{\alpha\gamma}$. On the other hand in QUIET-NOESY the quantity defined in Eq. [36] increases in absolute value with increasing values of $\sigma_{\alpha\gamma}$.

We remark that in Fig. 2 all the curves intersect the *y* axis for a mixing time $\tau_m = 0$. The curves in the figure represent a variation in the transfer of magnetization from spin α to spin β upon variation of the cross-relaxation rate constant $\sigma_{\alpha\beta}$. When the duration of the mixing time is zero there cannot be any transfer of magnetization no matter how different the cross-relaxation rate constant is. This result indicates that even though it would be preferable to use a short mixing time to avoid the effects of spin diffusion, we must bear in mind the fact that the mixing time must be long enough to allow for good precision in the measurement of the cross-relaxation rate constant.

As mentioned at the beginning of the paper CBD-NOESY (*9*) and BD-NOESY (*3–5*) were carefully analyzed in (*10*). The particular relationship that exists between the cross-relaxation rate constant in the laboratory frame σ_{NOE} and the cross-relaxation rate constant in the rotating frame σ_{ROE} for a rigid molecule in the spin diffusion limit (*19*) is the cornerstone on which CBD-NOESY is based, to provide cross-peak intensities connecting two different spectral regions, which are free of multistep magnetization transfer involving one or more steps contained within a single region. For spins belonging to parts of the molecule in which the spectral densities of motion at ω_0 and/or $2\omega_0$ are not negligible, the relation which we referred to above between σ_{NOE} and σ_{ROE} is no longer valid, and the results obtained from the experiment cannot be interpreted as easily. In BD-NOESY the experimenter is advised to utilize multiple selective inversion pulses to invert one or several spectral bands with a repetition rate for the pulses that is fast compared to the cross-relaxation rate constant. In the limit where the pulses are square the irradiation becomes almost continuous and the experiment is equivalent to MINSY. If the pulses are amplitude modulated, an analysis using the first directional derivative becomes problematic as the steady-state $\sigma^*(\infty)$, knowledge of which is necessary in order to solve Eq. [3], cannot be approximated by the null operator. In such a case, Eq. [3] could be resolved step by step, with a different steady state for each piecewise constant value of the amplitude modulated pulse, and the final value of the solution pertaining to one particular step must be used as the initial value of the adjacent step. Of course the same is true for the inversion(s) in QUIET-NOESY, or for the single inversion in BD1I-NOESY. However, as long as the durations of the inversions are kept short relative to the entire mixing time, we can approximate the steady-state operator $\sigma^*(\infty)$ to be the null operator at all times. We have used this approximation in our analysis by considering instantaneous inversions.

IV. CONCLUSION

We have demonstrated that the first derivative of the matrix exponential is a very useful tool for analyzing and comparing with precision a class of experiments aimed at achieving the same goals. In our case, using this methodology we have studied a class of experiments designed to measure directly the value of the cross-relaxation rate constant between a chosen pair of spins α and β. MINSY, BD1I-NOESY, QUIET-NOESY, and MYSIN were analyzed and compared. If to this list of experiments we add the BD-NOESY and CBD-NOESY experiments that were analyzed previously by different means, we are in a better position to choose the right experiment to face particular situations (type of molecule, cross-relaxation patterns, and so on). If it can be known in advance that the molecule or region of the molecule studied is rigid and has an overall tumbling correlation time which places it in the spin diffusion limit then CBD-NOESY is the experiment of choice. When these two conditions are not met another method must be employed. For short mixing times it was shown that the four methods analyzed in this paper provide good isolation of the spins α and β with respect to cross-relaxation with spin outside the pair. For long mixing times MINSY and MYSIN are proven to be better in this respect. MINSY, QUIET-NOESY, and BD1I-NOESY offer the best sensitivity with respect to a variation of the cross-relaxation rate constant $\sigma_{\alpha\beta}$. As they avoid the artifacts associated with continuous irradiation, BD1I-NOESY and QUIET-NOESY should be chosen when the mixing time can be kept short. If this condition can be satisfied and prior knowledge of the spin diffusion network of the spins α and β cannot be acquired, QUIET-NOESY must be employed. MINSY or BD-NOESY should be utilized when longer mixing times are needed. MYSIN necessitates a careful calibration of the double irradiation pulse. However, besides offering an almost perfect isolation for any duration of the mixing time, MYSIN enables us to overcome spectral overlap. In some situations this method can thus be employed with profit.

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