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Spin Relaxation and Chemical Exchange in NMR Simulations

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Theory for describing the density matrix of a spin system experiencing chemical exchange and relaxation during the steps of an NMR experiment is presented in a form suitable for computation. Features in the theory that arise from exchange are discussed in detail, and comparisons to the exchange-free situation are made. A general computer program to carry out simulations of NMR experiments is described, and several examples of its performance are presented. © 2000 Academic Press

Key Words: **relaxation; chemical exchange; spectral simulation.**

INTRODUCTION

The possibility of investigating molecular dynamics using NMR spectroscopy was recognized very early in the development of NMR. In 1948, Gutowsky and Pake showed a correlation of temperature with NMR lineshape (*1, 2*), which they interpreted in terms of molecular rotations. Important reasons for the early interest and continuing enthusiasm for the application of NMR to studying molecular reorganization include the following:

1. Reactions can be studied without driving the system out of equilibrium (*3, 4*). Disturbing the nuclear spin levels involves minimal energy changes and therefore rarely has an observable effect on chemical reactions.
2. NMR is nondestructive to the sample.
3. NMR is responsive to slower rates of change than optical spectroscopies such as IR and UV.

Thus NMR can reveal either static or time-averaged quantities depending on the rate of the dynamic process and the specific method of detection (*5*). NMR has been useful in studying a variety of dynamical processes with rates from 10^{-2} to 10^{6}/s (*6*).

Chemical exchange during NMR experiments is commonly understood to be any reorganization of the system that causes a change in any parameter of the spin system(s) under examination. Such parameters include chemical shifts, scalar and dipolar coupling interactions, and spin relaxation rates. These topics have been the subject of many books (*3, 4, 6–9*), where elements of theoretical methods for investigating exchange processes are discussed. However, most treatments include relaxation in an approximate way, usually through an empirical (phenomenological) approach (*10*). Authors who treat relaxation in a rigorous way usually employ the development of Redfield (*11*), but in an ad hoc manner, usually by inclusion of only the most important relaxation matrix elements for a specific system under study. To the best of our knowledge, the work we describe here is the first development of a general computer program that is flexible enough to treat arbitrary spin systems in a variety of exchanging situations while retaining all features of the relaxation matrix. In this article, we develop theory for describing chemical exchange with simultaneous treatment of relaxation phenomena in the manner introduced by Redfield. The dipole–dipole, chemical shift anisotropy (with all their associated cross terms), and random fluctuating field relaxation mechanisms are included (*12–14*), but the treatment is general and could be expanded to include other mechanisms. Molecular tumbling is treated either as rotational diffusion (*15, 16*) or by a "model-free" approach (*17*).

THEORY

We adopt the usual set of assumptions about the exchange process (*3*).

1. The molecular reorganizations that interchange nuclei are assumed to happen much faster than spin relaxation. Thus, the spins do not alter their magnetic orientation during the transition from one exchange site to another.
2. The rate of a particular exchange step is determined by the probability (per time) for the occurrence of that molecular motion.
3. Energy differences between the nuclear spin states are assumed to be much smaller than the (chemical) energies associated with the exchange process. That is, changes in the spin levels do not alter the rate of the exchange process.

This standard treatment of chemical exchange treats the exchange step as a stochastic process that exchanges distinguishable nuclei. This is the same approach that is used in the treatment of rotational diffusion which we have also adopted. The atomic motion that brings about the chemical exchange is considered to be governed by classic mechanics. It therefore ignores the Pauli principle and its effect on the (quantum

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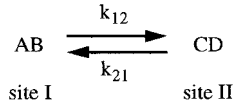

FIG. 1. Exchange of two spins between two sites.

mechanical) spatial wavefunction (*18*), which in turn would predict different exchange rates for the symmetric and antisymmetric nuclear spin wavefunctions.

In this section, we derive equations that describe a spin system that is relaxing while exchange is taking place. The derivation is analogous to the treatment of relaxation alone (*11–14*), but our purpose here is to emphasize the changes that are required when exchange is present. We pattern the treatment of exchange after that of Kaplan (*3, 19*) and Alexander (*20*). Details of our treatment of relaxation have been described previously (*11, 21*).

A two-site exchange process for a two-spin system is used to illustrate the method; we indicate later how this procedure is expanded to account for more exchanging configurations and for more spins. We name two molecular configurations "sites" I and II, which can be interconverted by some process. The interchange of sites I and II is represented in Fig. 1. Here A and B represent the two nuclei in the spin system plus all the parameters that describe that spin system. C and D are the same two nuclei but in a different environment; some or all of the parameters that describe the CD spin system differ from the AB parameters. As a result of the assumptions described above, this means that the exchange event itself does not induce transitions between nuclear spin states so that a nucleus with spin $\alpha(\beta)$ before an exchange will have spin $\alpha(\beta)$ after the exchange.

Alexander (*20*) applies this idea to a quantum mechanical description of the spin system by defining an exchange operator P such that exchange changes wavefunction Ψ^{I}, for exchange site I, into Ψ^{II} as $\Psi^{\mathrm{II}}(t) = P\Psi^{\mathrm{I}}(t)$. In a similar manner, a density matrix can be associated with each exchange site. We designate $\sigma^{\mathrm{I}}(t)$ and $\sigma^{\mathrm{II}}(t)$ to be the time-dependent density matrices in the product bases for sites I and II, respectively. The evolution of the system is described in terms of these density matrices whose time dependence is given by a pair of coupled Liouville (or "master") equations. The equation for $\sigma^{\mathrm{I}}(t)$ is (*3, 19, 20*)

$$\frac{\partial}{\partial t}\,\sigma^{\mathrm{I}}(t) = -\frac{i}{\hbar}\cdot[H^{\mathrm{I}}(t),\,\sigma^{\mathrm{I}}(t)] - k_{12}\cdot\sigma^{\mathrm{I}}(t) + k_{21}\cdot\sigma^{\mathrm{II}}(t) \quad [1]$$

with an equivalent equation for $\sigma^{\mathrm{II}}(t)$. $H^{\mathrm{I}}(t) = H_0^{\mathrm{I}} + H_2^{\mathrm{I}}(t)$ is the complete Hamiltonian of the system at site I, which, in the absence of an RF field, can be separated into a time-independent part and a time-dependent relaxation Hamiltonian. Of course site II will be described by an analogous but different Hamiltonian.

The solution of Eq. [1] is most easily accomplished in the basis of the eigenfunctions of H_0^{I}, and the equation for $H^{\mathrm{II}}(t)$ will be solved in the eigenbasis of H_0^{II}. These equations can be solved in a manner similar to the usual treatment of Redfield equations (*11, 12, 22*). First one transforms into the interaction representation for site I, where each operator (Op) in this representation is designated by a hat (ˆ) as

$$\widehat{\mathrm{Op}} = U^{\mathrm{I}}\mathrm{Op}U^{\mathrm{I}\dagger} \quad \text{with } U^{\mathrm{I}} = e^{iH_0^{\mathrm{I}}t/\hbar}. \quad [2]$$

The Liouville equation in this representation is

$$\frac{\partial\hat{\sigma}^{\mathrm{I}}}{\partial t} = -\frac{i}{\hbar}\cdot[\hat{H}_2^{\mathrm{I}}(t),\,\hat{\sigma}^{\mathrm{I}}] - k_{12}\cdot\hat{\sigma}^{\mathrm{I}} + k_{21}\cdot U^{\mathrm{I}}\sigma^{\mathrm{II}}U^{\mathrm{I}\dagger}. \quad [3]$$

Equation [3] can be simplified by a further transformation of the density matrix. Define

$$\bar{\sigma}^{\mathrm{I}} \equiv e^{k_{12}t}\hat{\sigma}^{\mathrm{I}}. \quad [4]$$

Algebraic manipulation generates the Liouville equation for the evolution of this density matrix:

$$\frac{\partial\bar{\sigma}^{\mathrm{I}}}{\partial t} = -\frac{i}{\hbar}[\hat{H}_2^{\mathrm{I}}(t),\,\bar{\sigma}^{\mathrm{I}}] + e^{k_{12}t}(k_{21}\cdot U^{\mathrm{I}}\sigma^{\mathrm{II}}U^{\mathrm{I}\dagger}). \quad [5]$$

There is no analytical solution for Eq. [5], but it can be expanded and integrated (from initial time t_0 to t) in the usual manner of solving Redfield types of equations. The resulting expression for $\bar{\sigma}^{\mathrm{I}}(t)$ is substituted back into Eq. [5] to generate a modification of the familiar double commutator integral equation found in NMR relaxation theories:

$$\begin{aligned}\frac{\partial}{\partial t}\,\bar{\sigma}^{\mathrm{I}}(t) &= -\frac{i}{\hbar}\cdot\left[\hat{H}_2^{\mathrm{I}}(t),\,\bar{\sigma}^{\mathrm{I}}(t_0) - \frac{i}{\hbar}\int_{t_0}^{t} e^{k_{12}t'}[\hat{H}_2^{\mathrm{I}}(t'),\,\hat{\sigma}^{\mathrm{I}}(t')]dt'\right]\\ &\quad - k_{21}\,\frac{i}{\hbar}\int_{t_0}^{t} e^{k_{12}t'}[\hat{H}_2^{\mathrm{I}}(t),\,U^{\mathrm{I}}(t')\sigma^{\mathrm{II}}(t')U^{\mathrm{I}\dagger}(t')]dt'\\ &\quad + k_{21}\cdot e^{k_{12}t}U^{\mathrm{I}}\sigma^{\mathrm{II}}(t)U^{\mathrm{I}\dagger}. \end{aligned} \quad [6]$$

The effects of the relaxation Hamiltonian must be averaged over the ensemble. $H_2(t)$ is a random function of time with average value zero. The standard arguments (*11*) hold for this case and give the differential equation for $\bar{\sigma}^{\mathrm{I}}(t)$ which can then

be transformed back into the interaction representation to generate the equation

$$\frac{\partial}{\partial t}\hat{\sigma}^{\mathrm{I}}(t) = -\frac{1}{\hbar^2}\int_0^\infty e^{-k_{12}\tau}[\hat{H}_2^{\mathrm{I}}(t),\,[\hat{H}_2^{\mathrm{I}}(t-\tau),\,\hat{\sigma}^{\mathrm{I}}(t)]]d\tau - k_{12}\cdot\hat{\sigma}^{\mathrm{I}}(t) + k_{21}\cdot U^{\mathrm{I}}\sigma^{\mathrm{II}}(t)U^{\mathrm{I}\dagger}. \quad [7]$$

The integral in Eq. [7] differs from the one in the Redfield equation in the absence of exchange only by inclusion of the exponential $e^{-k_{12}\tau}$. It will be seen that this exponential alters the spectral densities. The two "extra" terms in Eq. [7] which evidently describe the gain and loss of elements of the density matrix generated by exchange processes do not affect the subsequent derivation of the Redfield relaxation matrix elements.

The relaxation Hamiltonian can be written as a sum of contributions $H_\mu(t)$ from the individual interactions that generate relaxation. Each of these is conveniently written as a sum of products of spatial factors $F_\mu^{-m}(t)$ (which, because of molecular motion, are time dependent) times spin operators T_μ^m. In the interaction representation, the relaxation Hamiltonian is therefore

$$\hat{H}_2(t) = \sum_\mu \hat{H}_\mu(t) = \sum_\mu \xi_\mu \sum_m (-1)^m F_\mu^{-m}(t)\hat{T}_\mu^m, \quad [8]$$

where the ξ_μ are constants. We rewrite Eq. [7] in terms of this expansion, and expand in the basis of eigenfunctions of H_0^{I}; (Multiply on the left by $\langle\alpha|$ and on the right by $|\alpha'\rangle$, and evaluate the spin operators in terms of specific matrix elements.) This generates

$$\begin{aligned}\left\langle \alpha_{\mathrm{I}}\left|\frac{\partial}{\partial t}\hat{\sigma}^{\mathrm{I}}(t)\right|\alpha'_{\mathrm{I}}\right\rangle &= -\frac{1}{\hbar^2}\sum_\mu\sum_{\mu'}\xi_\mu\xi_{\mu'}\sum_m\sum_{m'}(-1)^{m+m'}\sum_{\beta_{\mathrm{I}},\beta'_{\mathrm{I}}}\int_0^\infty e^{-k_{12}\tau}d\tau \\ &\times\{(e^{i(\omega^{\mathrm{I}}_{\alpha\beta}+\omega^{\mathrm{I}}_{\beta\beta'})t}\langle\alpha|T_\mu^m|\beta\rangle\langle\beta|T_{\mu'}^{m'}|\beta'\rangle\langle\beta'|\hat{\sigma}^{\mathrm{I}}(t)|\alpha'\rangle \\ &\times e^{-i\omega^{\mathrm{I}}_{\beta\beta'}\tau} - e^{i(\omega^{\mathrm{I}}_{\alpha\beta}+\omega^{\mathrm{I}}_{\beta'\alpha'})t}\langle\alpha|T_\mu^m|\beta\rangle\langle\beta|\hat{\sigma}^{\mathrm{I}}(t)|\beta'\rangle \\ &\times\langle\beta'|T_{\mu'}^{m'}|\alpha'\rangle e^{-i\omega^{\mathrm{I}}_{\beta'\alpha'}\tau} - e^{i(\omega^{\mathrm{I}}_{\alpha\beta'}+\omega^{\mathrm{I}}_{\beta'\alpha'})t}\langle\beta'|T_\mu^m|\alpha'\rangle \\ &\times\langle\alpha|T_{\mu'}^{m'}|\beta\rangle\langle\beta|\hat{\sigma}^{\mathrm{I}}(t)|\beta'\rangle e^{-i\omega^{\mathrm{I}}_{\alpha\beta}\tau} + e^{i(\omega^{\mathrm{I}}_{\beta'\alpha'}+\omega^{\mathrm{I}}_{\beta\beta'})t} \\ &\times\langle\beta'|T_\mu^m|\alpha'\rangle\langle\beta|T_{\mu'}^{m'}|\beta'\rangle\langle\alpha|\hat{\sigma}^{\mathrm{I}}(t)|\beta\rangle \\ &\times e^{-i\omega^{\mathrm{I}}_{\beta\beta'}\tau})F_\mu^{-m}(t)F_{\mu'}^{-m'}(t-\tau)\} \\ &- k_{12}\cdot\langle\alpha|\hat{\sigma}^{\mathrm{I}}(t)|\alpha'\rangle + k_{21}\cdot\langle\alpha|U^{\mathrm{I}}\sigma^{\mathrm{II}}(t)U^{\mathrm{I}\dagger}|\alpha'\rangle, \quad [9]\end{aligned}$$

where $\omega^{\mathrm{I}}_{\gamma\gamma'}$ is the frequency of the transition between eigenstates γ and γ' of the Hamiltonian H_0^{I} for exchange site I.

The time integrals in Eq. [9] each have the form

$$\overline{J^{mm'}_{\mu\mu'}}(\omega) = \int_0^\infty e^{-k_{12}\tau}\langle F_\mu^{-m}(t)F_{\mu'}^{-m'}(t-\tau)\rangle e^{i\omega\tau}d\tau. \quad [10]$$

This is a modification of the usual definition of a spectral density where inclusion of exchange has contributed the damping factor $e^{-k_{12}\tau}$. The bar indicates that the spectral density has been altered from the usual form by chemical exchange. Note that only the loss of population affects the correlation function for a site and, hence, the spectral density governing relaxation at that site. That is, the loss of spins from one site caused by chemical exchange leads to a loss of correlation between the spatial functions in Eq. [10]. Any gain in spins at site I, resulting from exchanges from other sites, introduces spins into site I that have no memory of any previous spatial orientation at site I. Thus the overall correlation function is a product of one for exchange $e^{-k_{12}\tau}$ times one for rotational motion $\langle F_\mu^{-m}(t)F_{\mu'}^{-m'}(t-\tau)\rangle$. It is clear from Eq. [10] that spectral densities are different for different exchange sites, but we will not create additional symbols for the formulas; the specific meaning of the spectral densities will be given by the context.

The correlation function in the expression for the spectral density (Eq. [10]) can be evaluated using a model for the molecular motion. We have implemented two commonly used models for molecular motion in liquids: the rotational diffusion model (*13*, *15*, *16*), and the "model-free" method (*17*). The derivations of the expressions for the spectral densities according to each of these approaches follow step-for-step the derivations in the absence of exchange. In the case of rotational diffusion, the penultimate equation in the derivation is (for the specific case of rank 2 tensor interactions)

$$\overline{J^{mm'}_{\mu\mu'}}(\omega) = \delta_{(-m,m')}\frac{(-1)^m}{5}\sum_\kappa c_\kappa(\mu)c_\kappa^*(\mu')\int_{-\infty}^\infty e^{-k_{12}\tau}e^{-\zeta_\kappa\tau}e^{i\omega\tau}d\tau, \quad [11]$$

where inserting $k_{12} = 0$ generates the spectral density in the absence of exchange (*15*). The most important part of Eq. [11] for the present purpose is the identification of ζ_κ as an eigenvalue of the rotation Hamiltonian. The integral in Eq. [11] can be evaluated:

$$\mathrm{Re}\left(\int_{-\infty}^\infty e^{-k_{12}\tau}e^{-\zeta_\kappa\tau}e^{i\omega\tau}d\tau\right) = \frac{k_{12}+\zeta_\kappa}{(k_{12}+\zeta_\kappa)^2+\omega^2}. \quad [12]$$

In the spectral density functions, the exchange process acts as an additional "motion" with a frequency equal to the rate

TABLE 1
Parameters for the Single-Spin System

Site	Population	Chemical shift (Hz)	T_2 (s)	Rate constant (s^{-1})
I	0.2	145.	0.159	$k_{12} = 200.$
II	0.8	−55.	0.159	$k_{21} = 50.$

constant. Because both k_{12} and ζ_κ must be non-negative, chemical exchange decreases the spectral densities, but because typically $k_{12} < 1/\tau_c$, which is proportional to ζ_κ, the decrease in spin relaxation caused by exchange is small.

The model-free approach produces a similar result. The final expression for the spectral density according to that treatment is (*17*)

$$\bar{J}(\omega) = \frac{1}{5} S^2 \frac{(\tau_c^{-1} + k_{12})}{(\tau_c^{-1} + k_{12})^2 + \omega^2} + \frac{1}{5}(1 - S^2) \frac{(\tau_0^{-1} + k_{12})}{(\tau_0^{-1} + k_{12})^2 + \omega^2}. \quad [13]$$

As with the rotational diffusion model, chemical exchange has the effect of an apparent reduction in the orientational correlation time of the system.

The subsequent steps in the derivation of the relaxation equations follow as in standard treatments (*12*) to give

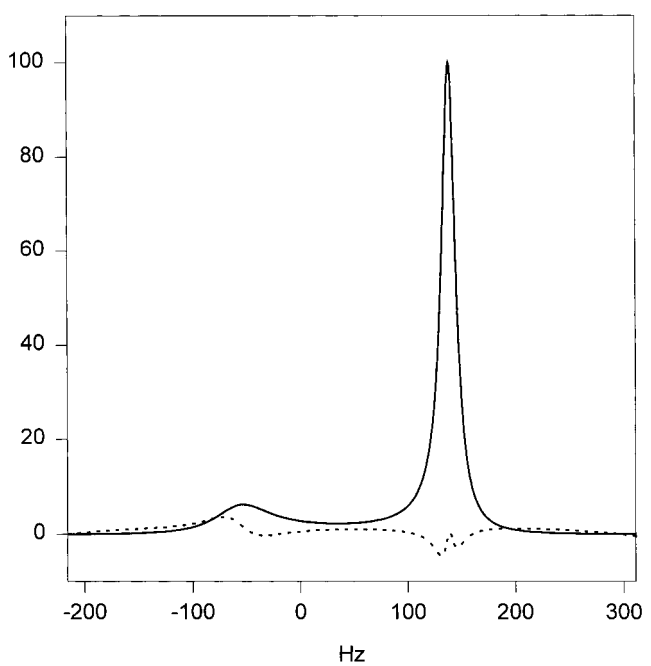

FIG. 2. The *exlax* spectrum for a single spin exchanging between two different sites (solid line), and the difference between spectra computed by *exlax* and DNMR5 multiplied by 100 (dashed line).

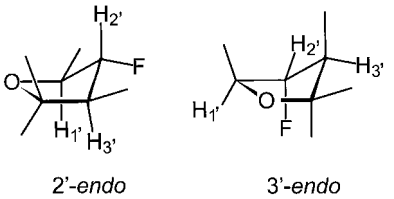

FIG. 3. Conformations of the 2′-fluorodeoxyribose ring of 2′-fluorodeoxyuridine.

$$\frac{\partial}{\partial t}\langle\alpha_1|\hat{\sigma}^{\mathrm{I}}(t)|\alpha_1'\rangle = \sum_{\beta}^{\mathrm{bf}}\sum_{\beta'}^{\mathrm{bf}} e^{i(\omega^{\mathrm{I}}_{\alpha\alpha'} - \omega^{\mathrm{I}}_{\beta\beta'})t}\bar{R}^{\mathrm{I}}_{\alpha\alpha'\beta\beta'}\langle\beta|\hat{\sigma}^{\mathrm{I}}(t)|\beta'\rangle - k_{12}\cdot\langle\alpha|\hat{\sigma}^{\mathrm{I}}(t)|\alpha'\rangle + k_{21}\cdot\langle\alpha|U^{\mathrm{I}}\sigma^{\mathrm{II}}(t)U^{\mathrm{I}\dagger}|\alpha'\rangle, \quad [14]$$

where the elements of the Redfield matrix are

$$\bar{R}^{\mathrm{I}}_{\alpha\alpha'\beta\beta'} = \frac{1}{\hbar^2}\sum_{\mu}\sum_{\mu'}\xi_\mu\xi_{\mu'}\sum_{m}\{\langle\alpha|T^m_\mu|\beta\rangle \times\langle\alpha'|T^m_{\mu'}|\beta'\rangle^*[\overline{\mathcal{J}_{\mu\mu'}}(\omega^{\mathrm{I}}_{\beta'\alpha'}) + \overline{\mathcal{J}_{\mu\mu'}}(\omega^{\mathrm{I}}_{\alpha\beta})] - \delta_{\alpha'\beta'}\sum_{\gamma}^{\mathrm{bf}}\langle\alpha|T^m_\mu|\gamma\rangle\langle\beta|T^m_{\mu'}|\gamma\rangle^*\overline{\mathcal{J}_{\mu\mu'}}(\omega^{\mathrm{I}}_{\gamma\beta}) - \delta_{\alpha\beta}\sum_{\gamma}^{\mathrm{bf}}\langle\gamma|T^m_\mu|\alpha'\rangle\langle\gamma|T^m_{\mu'}|\beta'\rangle^*\overline{\mathcal{J}_{\mu\mu'}}(\omega^{\mathrm{I}}_{\beta'\gamma})\}. \quad [15]$$

The bar over the Redfield matrix elements and over the reduced spectral densities are reminders that exchange is included.

Equation [17] can now be transformed out of the interaction representation to give

$$\frac{\partial}{\partial t}\sigma^{\mathrm{I}}_{\alpha\alpha'} = \sum_{\beta}^{\mathrm{b.f.}}\sum_{\beta'}^{\mathrm{b.f.}}\bar{R}^{\mathrm{I}}_{\alpha\alpha'\beta\beta'}\sigma^{\mathrm{I}}_{\beta\beta'} - i\omega^{\mathrm{I}}_{\alpha\alpha'}\sigma^{\mathrm{I}}_{\alpha\alpha'} - k_{12}\sigma^{\mathrm{I}}_{\alpha\alpha'} + k_{21}\sigma^{\mathrm{II}}_{\alpha\alpha'} \quad [16]$$

Note that the last term consists of the density matrix for site II evaluated over eigenfunctions for the Hamiltonian of site I. Equations such as Eq. [16] are easier to work with if they are reindexed to look like vector equations. For example, we reindex the matrix $\sigma_{\alpha\alpha'}$ into the vector σ_j by defining $j = \alpha' + (\alpha - 1)n$, where n is the maximum value of α'. At the same time, we define $\bar{\mathcal{R}}^{\mathrm{I}}_{\alpha\alpha'\beta\beta'} \equiv \bar{R}^{\mathrm{I}}_{\mathrm{I}^{k\alpha'\beta\beta'}} - i\omega^{\mathrm{I}}_{\alpha\alpha'}\delta_{\alpha\beta}\delta_{\alpha'\beta'}$. In this notation Eq. [16] becomes

TABLE 2
NMR Parameters for 2′-Fluorodeoxyuridine

	2′-*endo* conformation, population 0.1				3′-*endo* conformation, population 0.9			
	Chemical shift (Hz)	Spin–spin coupling (Hz)			Chemical shift (Hz)	Spin–spin coupling (Hz)		
		F	$H_{1'}$	$H_{2'}$		F	$H_{1'}$	$H_{2'}$
F	145	—	—	—	−55	—	—	—
$H_{1'}$	130	19.7	—	—	130	19.7	—	—
$H_{2'}$	−40	52.7	6.0	—	−40	52.7	1.5	—
$H_{3'}$	−130	5.0	0	5.1	−130	21.6	0	5.1

$$\frac{\partial}{\partial t}\,\sigma_j^{\mathrm{I}}(t) = \sum_k \bar{\mathcal{R}}_{jk}^{\mathrm{I}}\sigma_k^{\mathrm{I}}(t) - k_{12}\sigma_j^{\mathrm{I}}(t) + k_{21}\sigma_j^{\mathrm{II}}(t). \quad [17]$$

Because they are interdependent, the differential equations for the density matrices for all the exchange sites must be solved simultaneously. This can be incorporated into our formalism by combining the two (or more) "site-density matrices" into a single column vector. We adopt the convention of evaluating each site-density matrix in the eigenfunctions of its zeroth-order Hamiltonian. Then the combined equations for a system with two exchange sites will be (in vector notation)

$$\frac{d}{dt}\begin{bmatrix} [\sigma^{\mathrm{I}}(t)]_{\mathrm{I}} \\ [\sigma^{\mathrm{II}}(t)]_{\mathrm{II}} \end{bmatrix} = \begin{bmatrix} \bar{\mathcal{R}}^{\mathrm{I}} - k_{12}E & k_{21}T^{\mathrm{I}\leftarrow\mathrm{II}} \\ k_{12}T^{\mathrm{II}\leftarrow\mathrm{I}} & \bar{\mathcal{R}}^{\mathrm{II}} - k_{12}E \end{bmatrix} \cdot \begin{bmatrix} [\sigma^{\mathrm{I}}(t)]_{\mathrm{I}} \\ [\sigma^{\mathrm{II}}(t)]_{\mathrm{II}} \end{bmatrix}, \quad [18]$$

where E is the identity matrix of size $n^2 \times n^2$, and the T matrices are transformation matrices from one basis to another as indicated by their superscripts.

For the general case with N exchanging sites, Eq. [18] becomes

$$\frac{d}{dt}\begin{bmatrix} \sigma^{\mathrm{I}}(t) \\ \sigma^{\mathrm{II}}(t) \\ \cdots \\ \sigma^{N}(t) \end{bmatrix} = \begin{bmatrix} \bar{\mathcal{R}}^{\mathrm{I}} - k_{11}E & k_{21}T^{\mathrm{I}\leftarrow\mathrm{II}} & \cdots & k_{N1}T^{\mathrm{I}\leftarrow N} \\ k_{12}T^{\mathrm{II}\leftarrow\mathrm{I}} & \bar{\mathcal{R}}^{\mathrm{II}} - k_{22}E & \cdots & k_{N2}T^{\mathrm{II}\leftarrow N} \\ \cdots & \cdots & \cdots & \cdots \\ k_{1N}T^{N\leftarrow\mathrm{I}} & k_{2N}T^{N\leftarrow\mathrm{II}} & \cdots & \bar{\mathcal{R}}^{N} - k_{NN}E \end{bmatrix} \cdot \begin{bmatrix} \sigma^{\mathrm{I}}(t) \\ \sigma^{\mathrm{II}}(t) \\ \cdots \\ \sigma^{N}(t) \end{bmatrix}, \quad [19]$$

where $k_{jj} \equiv \sum_{i\neq j}^{N} k_{ij}$.

Equation [19] can be solved by diagonalizing the entire relaxation-exchange matrix that operates on the density matrix on the right-hand side of Eq. [19]. The eigenvectors of that matrix will be the normal modes of the problem, and the real parts of the eigenvalues will be the negatives of the (generalized) relaxation rates.

At this point in derivations such as this (*9*, *11*, *12*), one must point out an inadequacy. Clearly the system must relax to its equilibrium state at infinite time, an outcome not feasible under Eq. [19]. However, this necessary result can be ensured by replacing the density matrix everywhere it occurs in Eq. [19] by the difference-density matrix $\sigma - \sigma_{\mathrm{eq}}$.

Including chemical exchange alters the treatment of relaxation during the application of an RF field. Whereas the equations for describing relaxation during an RF pulse are complicated (*21–26*), the alteration of those equations that is necessary to account for exchange is simple. In the presence of an RF field, the steady state that is attained at infinite time (in the frame rotating at the RF frequency) differs from equilibrium. It is the determination of that steady-state density matrix that is crucial. The important equation is the Liouville equation in the rotating frame (*22*, *24*), which can be written as

$$\frac{\partial}{\partial t}\,\sigma(t) = (R - i\Omega)\sigma(t) - R\sigma_{\mathrm{eq}}, \quad [20]$$

where the matrix Ω is defined as $\Omega_{\alpha\alpha'\beta\beta'} = \omega_{\alpha\alpha'}\delta_{\alpha\beta}\delta_{\alpha'\beta'}$. At infinite time, the time derivative of the density matrix must vanish, and the density matrix becomes the steady-state limit σ_{ss}. Simply inverting $(R - i\Omega)$ to solve $(R - i\Omega)\sigma_{\mathrm{ss}} = R\sigma_{\mathrm{eq}}$ for σ_{ss} is not possible because the operator $(R - i\Omega)$ is linearly dependent. (This is evident because the trace of σ represents a linear dependency in the elements of the density matrix.) Ravikumar *et al.* (*24*) have presented an elegant way out of this difficulty. They reduce the dimensionality of the matrix $(R - i\Omega)$ by one by subtracting the trace relation times the first element of each row of the matrix from that row. Then they discard the first row and the first column from the resulting matrix leaving a nonsingular $n^2 - 1$ dimensional matrix. Inverting this matrix enables one to find all but one of the

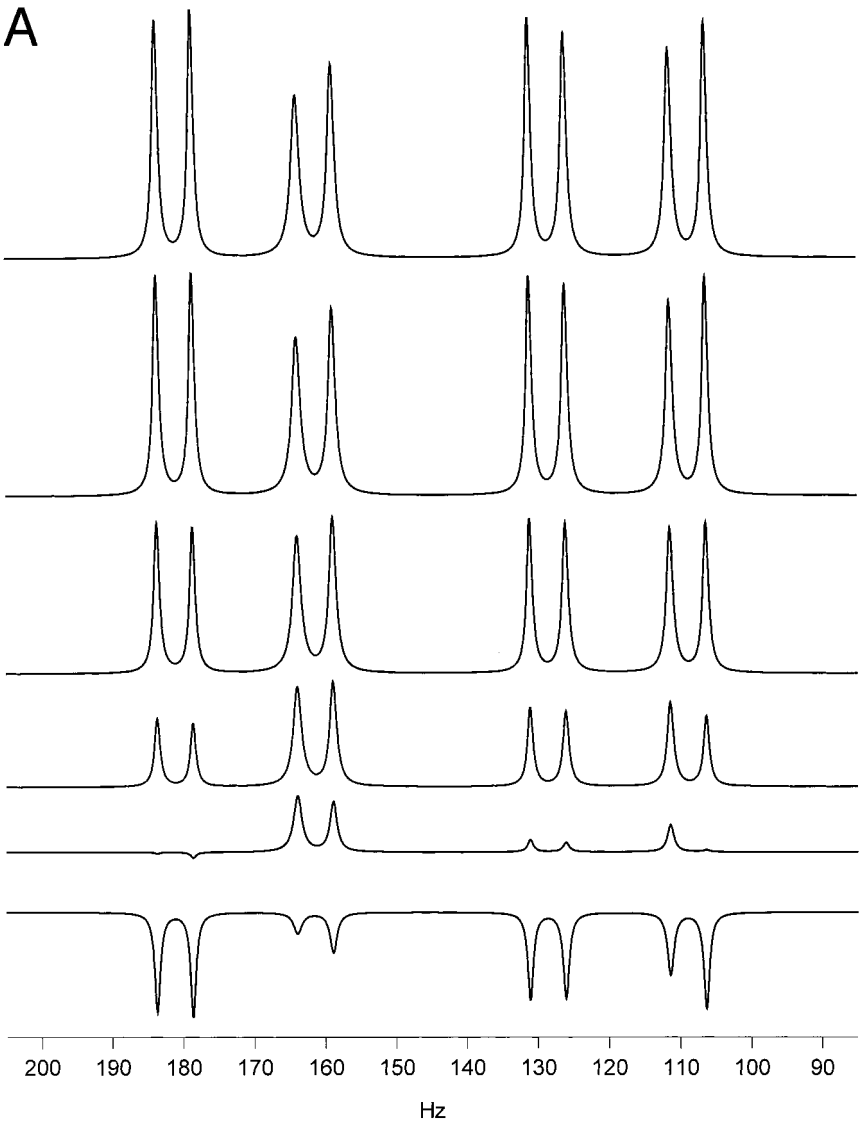

FIG. 4. Computed inversion–recovery ^{19}F NMR spectra of 2′-fluorodeoxyuridine with exchange rate constants 0.1 and 0.9 s^{-1}. Recovery times are 0.2, 0.4, 0.6, 1, 2, and ∞ s. from bottom to top. (A) Low-field 2′-*endo* (10% population) portion of the spectrum. (B) Spectra for the major 3′-*endo* conformation. Spectra within a series are plotted with the same vertical scale.

elements of the steady-state density matrix; the trace relation provides the final element. Their procedure appears to provide a well-balanced solution to this problem. Our situation is only slightly more complicated. In the case of N exchange sites, each σ^J, $I \leq J \leq N$, has a conserved trace equal to the fractional population p^J of that exchange site. Remember that the equilibrium among the chemically exchanging sites is independent of the NMR process. Thus in our Nn^2 dimensional problem, there are N constraints, so we apply the method of Ravikumar *et al.* N times. Specifically, we subtract the trace relation for the density matrix of the Jth exchange site times the first element of each row that corresponds to the Jth exchange site from that row of the matrix. Then we reduce the dimensionality of the problem by eliminating the first row and the first column corresponding to each exchange site. This results in an $Nn^2 - N$ dimensional nonsingular matrix which is inverted, and the rest of the computation proceeds as in the exchange-free situation (*21–25*).

COMPUTER PROGRAM

A FORTRAN program to compute simulations of NMR experiments including exchange and relaxation, *exlax*, has been written based on the theory described above. The program follows the evolution of the density matrix for the system from equilibrium at the start, through an arbitrary sequence of pulses

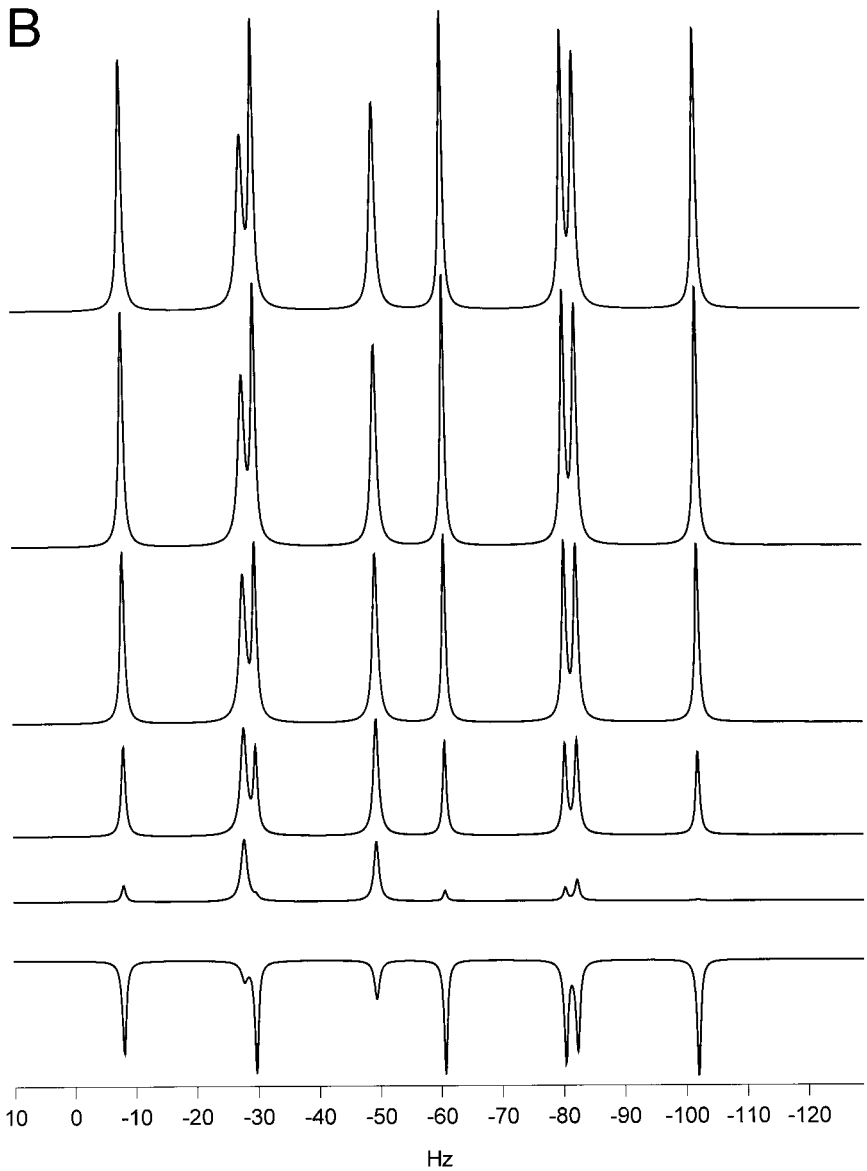

FIG. 4—*Continued*

and delays. An FID is collected by evaluating the magnetization at the appropriate times. The FIDs are written in a format that can be read by a standard NMR processing program. [We use FELIX (*27*) for this purpose.] Copies of *exlax* are available on request; see also references (*28*, *29*).

The general methods described here are open-ended, but the matrices that must be diagonalized grow rapidly as $(N \times 2^{2n}) \times (N \times 2^{2n})$ for n spins and N exchange sites. (The diagonalization of such matrices presents potential numerical difficulties (*21*) because the imaginary frequencies in the diagonal elements can be 10 or more orders of magnitude larger than the Redfield relaxation matrix elements, but we have found no examples that were not solvable by one numerical procedure or another.) While the use of superoperator propagators enables considerable computational efficiency (*29*), the practical limit for our present workstations is a four-spin system, and the maximum number of exchange sites is also 4. This first version of *exlax* was written in a straightforward manner to minimize the chances of errors. Consequently, it does not take advantage of the symmetries of the Redfield procedure; work is underway to improve the program's efficiency. The four-spin two-site heteronuclear examples presented below required an hour on an SGI-Indy for typical 1D simulations. The 2D simulations of three-spin systems in three exchange sites, also presented below, ran in about $2\frac{1}{2}$ h.

To verify the results of our computer program, we made an extensive series of checks to ensure that proper limiting results were obtained for systems in slow and fast exchange limits. We

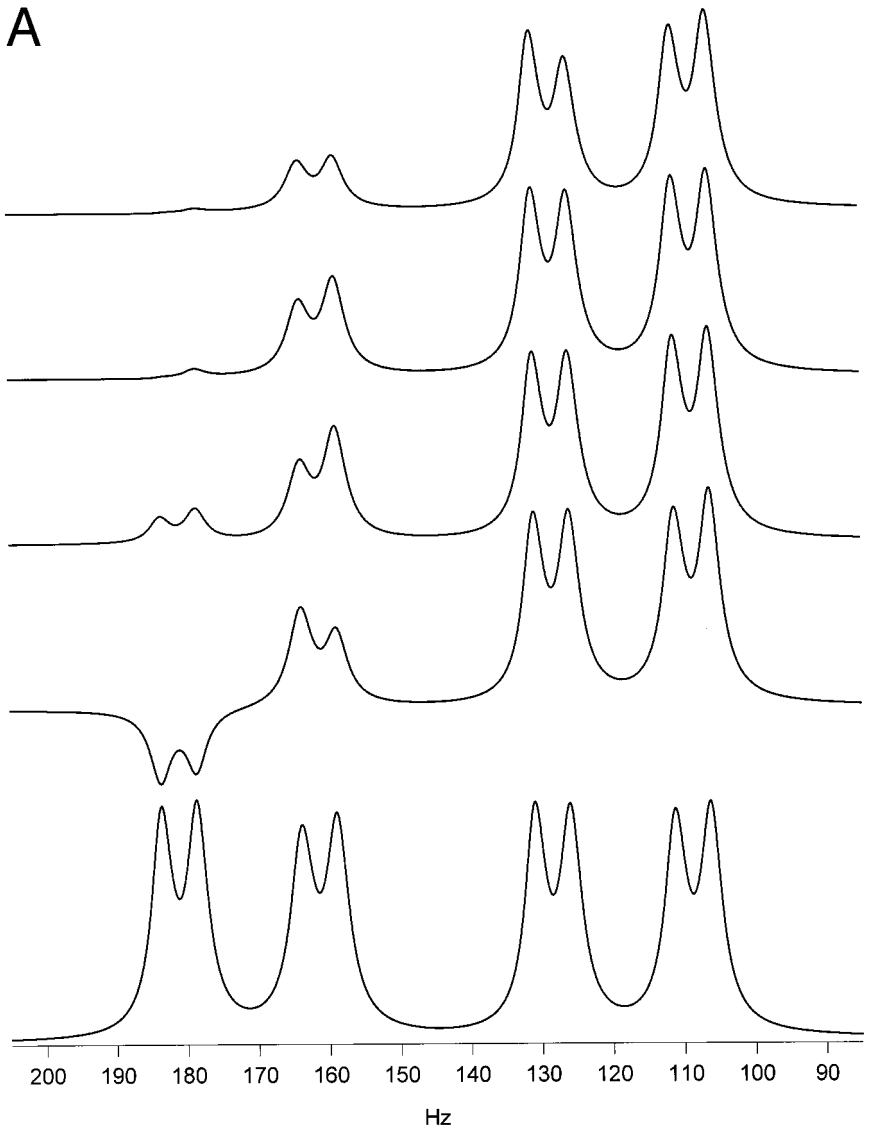

FIG. 5. Computed saturation transfer ^{19}F NMR spectra of 2′-fluorodeoxyuridine with exchange rate constants 1 and 9 s^{-1}. A 25-Hz RF field was applied to the low-field doublet of the minor isomer for 0, 0.1, 0.2, 0.5, and 1 s from bottom to top followed by an analyzing 90° pulse. (A) Low-field 2′-*endo* (10% population) portion of the spectrum. (B) Spectra for the major 3′-*endo* conformation. Spectra within a series are plotted with the same vertical scale. Further details are given in the text.

also made several comparisons to results from the spectral simulation portion of the DNMR5 program developed by Stephenson and Binsch (*30*) in the intermediate exchange region. DNMR5 performs simulations of one-dimensional experiments for systems undergoing exchange, but it includes relaxation phenomenologically by assuming it can be described by an effective relaxation time T_2. In the course of this testing, errors were found in the preliminary version of our program. Several simulations for exchanging systems that were published in a thesis (*31*) are incorrect.

Here we present a detailed comparison of results computed with *exlax* and with DNMR5 for a single spin exchanging between two sites with unequal populations. The parameters of the spin system are listed in Table 1. Transverse relaxation in the DNMR5 program is controlled by a linewidth at half-height that was set to 2 Hz, which corresponds to a T_2 of 0.159 s. This relaxation was included *via* the random field mechanism in *exlax.* The experiment simulated was an ideal 90°_{x} pulse followed by acquisition with relaxation. Figure 2 shows the spectrum predicted by *exlax* as well as the difference between the spectra calculated by DNMR5 and *exlax* for an intermediate value of the exchange rate constants. For comparisons, spectra were "baseline-corrected," the maximum of each spectrum was scaled to 100, and the difference spectrum (*exlax*

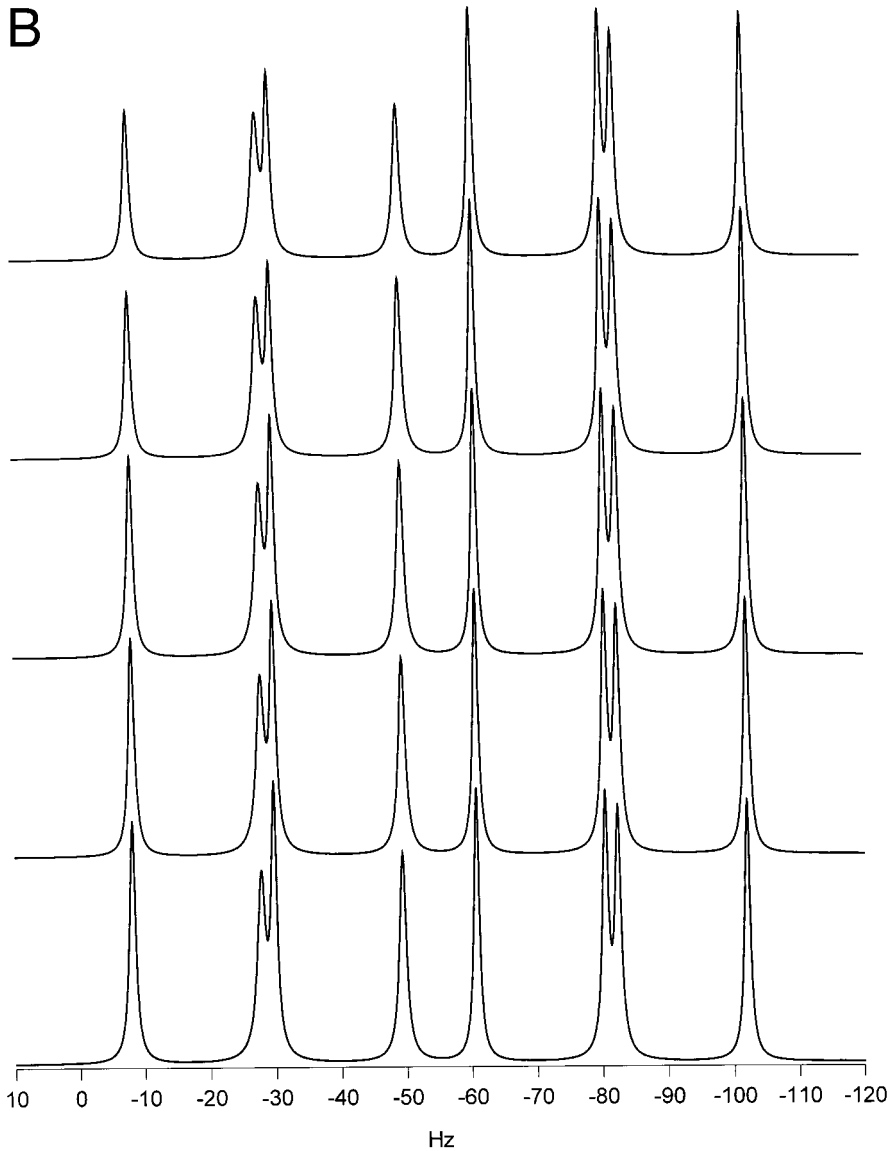

FIG. 5—*Continued*

minus DNMR5) was multiplied by 100. Because this "normalization" procedure does not specifically make the areas under the spectra generated by the two programs equal, the difference will not integrate to zero. The absolute maximum intensity difference between the two spectra was less than 0.1% of the value of the spectra in that region, and the relative difference was always less than 1% in all regions where the spectral intensities are significantly different from zero. We have solved the differential equations generated by this example analytically and verified that each method is indeed solving the proper equations for the behavior of the density matrix and is solving those equations correctly. We consider the agreement between the two types of calculations to be very good; the results shown are typical of the comparisons between *exlax* and DNMR5.

COMPUTATIONAL EXAMPLES

To illustrate the capabilities of the program, we present simulations of several types of experiments on 2′-fluorouridine, a molecule that has been studied extensively by Wheeler and Griffey (*32*). The fluorinated ribose ring of this molecule undergoes a pseudorotation (Fig. 3), and the spins affected by that motion constitute our region of interest. Specifically, we consider the fluorine and protons $H_{1'}$, $H_{2'}$, and $H_{3'}$. The parameters of the spin system in the two configurations used in the

TABLE 3
Parameters of the $H_N{}^{15}NH_\alpha$ System Used in the Simulations

	H_N	H_α	^{15}N
Chemical shift (Hz)			
Site I	550	−650	—
Site II	500	−600	—
Site III	50	−500	—
Coordinates (Å)			
x	−0.469	1.057	0.00
y	−0.883	1.788	0.00
z	0.00	0.00	0.00
Spin coupling constants (Hz)			
H_N	—	—	—
H_α	8	—	—
^{15}N	−90	4	—
CSA parameters			
δ_z	−11.8 ppm	—	108 ppm
η	−0.0085	—	−0.222
α	62°	—	135°
β	90°	—	90°
γ	180°	—	90°

simulation are listed in Table 2. The atomic coordinates were obtained by Wheeler using an optimization based on the MM2 force field (*32*). The spin–spin coupling constants and the proton chemical shifts were the experimental values (*33*). Fluorine's chemical shift was chosen for convenience of display, and its chemical shift anisotropy parameters were chosen to be those of fluoromethane (*34*). The relative populations of the two conformations were estimated by Wheeler to be 90% 3′-*endo* and 10% 2′-*endo*. Dipole–dipole, CSA (on the fluorine only), and all the cross-interactions were included as the relaxation mechanisms. Isotropic rotational diffusion was assumed with a τ_c of 2.5×10^{-10} s. The spectrometer frequency was 188 MHz for fluorine, and the calculated spectra shown are the fluorine part of the spectrum.

Inversion–Recovery

A sequence of inversion recovery simulations (180_F–delay–90_F–acquire F) was carried out for 2′-fluorouridine at several exchange rates. The sequence for slow exchange is shown in Fig. 4 where the recovery behavior of the individual lines is clear. It is evident from Fig. 4 that the various transitions recover at quite different rates. Plots of the logarithms of the recovering magnetization versus the delay time are clearly nonlinear, so any T_1 values are "apparent" ones. These were fairly evenly distributed from 0.39 to 0.63 s. It is striking that the recovery rates for the leftmost pair of intense lines in Fig. 4B that nearly overlap are quite different. The leftmost line of that pair has $T_1 = 0.39$ s, and the rightmost has $T_1 = 0.60$ s. Our simulations predict that the exchange rates themselves have only a small effect on T_1, the range at fast exchange being from 0.48 to 0.65 s. Experimental T_1's at 20°C range from 0.56 to 0.80 s. (*32*), and multiplet structure was not resolved.

Saturation Transfer

To show additional capabilities of the program, we performed simulations of the effects of RF irradiation of one portion of the fluorine multiplet for increasing lengths of time. A weak irradiation, of magnitude $\gamma_F B_1/2\pi = 25$ Hz and at a frequency of 180 Hz relative to the fluorine chemical frequency was used. This field perturbs most strongly the leftmost doublet in the spectrum which is shown in Fig. 4A. Simulations were performed for a range of exchange rates, and results are shown in Fig. 5 for the most illustrative case ($k_{12} = 9\ s^{-1}$ and $k_{21} = 1\ s^{-1}$). Under the conditions simulated, irradiation for 0.1 s results in 2.5 cycles of fluorine rotation, so the net effect is essentially a selective π pulse on the leftmost doublet. Note that the fluorine spectrum of the high population isomer is perturbed as the irradiation time increases due to "saturation" transfer. It is possible that sufficient information is present in the perturbed intensities of the lines of both multiplets to support an analysis that would provide both the exchange rates and details of the relaxation process.

2D Simulation

As an example of a 2D simulation, we consider the ^{15}N, H_N, and H_α spins in an amino acid residue in exchange among three chemical environments. This spin system has been studied previously by us, and for these simulations, we have used many of the parameters of our previous studies of valine (*31, 35*). The geometry is planar with dihedral angle of 180°; the chemical shifts of the protons are varied from one configuration to another with specific values chosen for convenience of display, but the geometry and spin–spin coupling constants are held constant. Details are listed in Table 3. Dipole–dipole and CSA relaxation mechanisms with all cross terms are included; the spectrometer frequency was 600 MHz; isotropic rotational tumbling was assumed with $\tau_c = 15$ ns. The site populations were 0.1, 0.3, and 0.6, respectively, for the three sites, with rate constants for exchange $k_{12} = 9\ s^{-1}$, $k_{21} = 3\ s^{-1}$, $k_{13} = k_{31} = 0$, $k_{23} = 18\ s^{-1}$, $k_{32} = 9\ s^{-1}$. The pulse sequence is (90_ϕ–t_1–90_x–τ–90_x–acquire); TPPI was applied to the first pulse, and the τ delay was 0.2 s. Three 2D spectra computed under different conditions are shown in Figs. 6. The off-diagonal peaks resulting from exchange are clearly evident in Fig. 6C. Also present are the off-diagonal peaks generated by the nuclear Overhauser effect, some of which overlap the peaks caused by exchange. A particularly useful feature of a simulation program is the ability to turn on or off certain interactions. Figure 6B shows the spectrum with exchange set to zero, but all relaxation interactions are included. Sharpening of the spectrum is clearly evident in the 1D section of Fig. 6B. The asymmetry in the H_N lines is caused by CSA, and this asymmetry arises primarily from the cross-interaction of CSA and

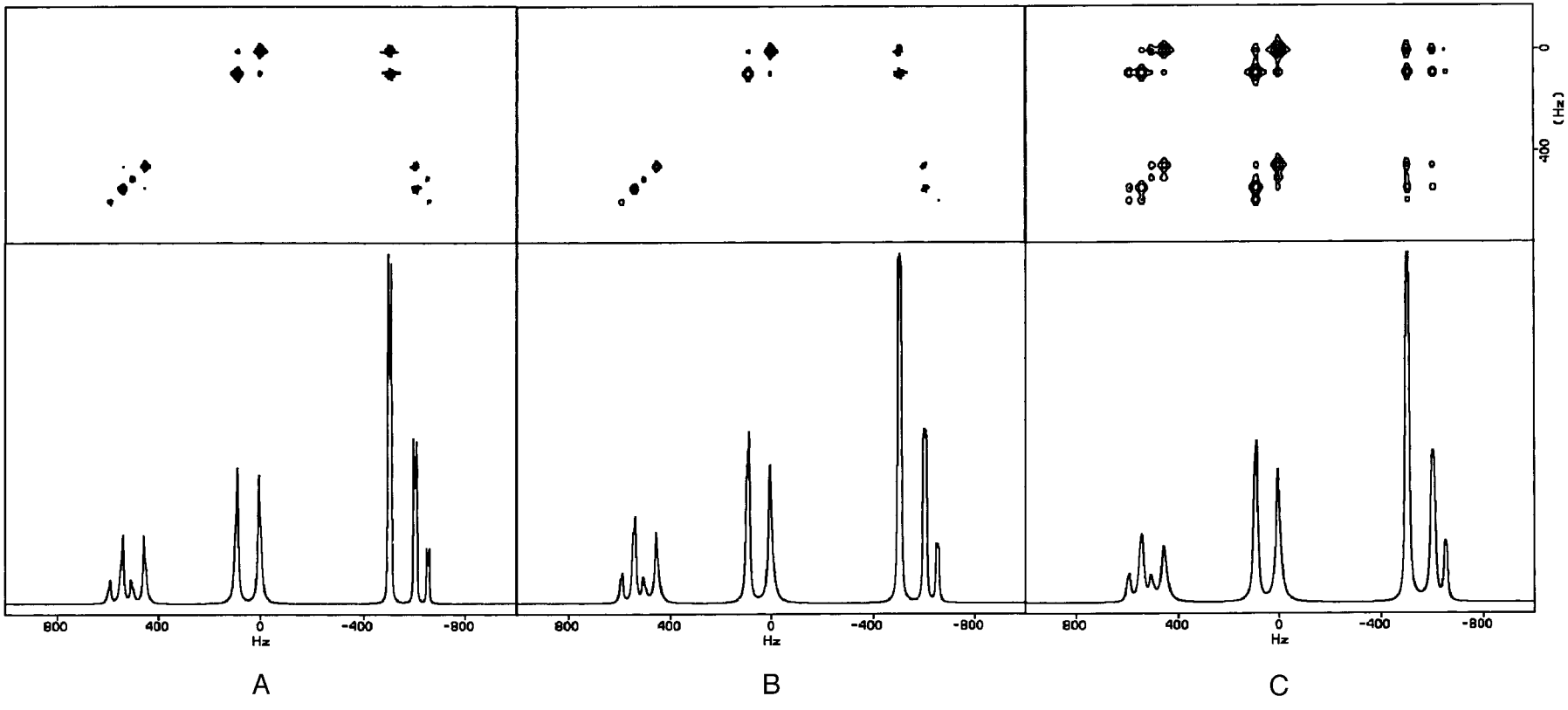

FIG. 6. Computed ^{1}H NMR spectra of an H-^{15}N-C_{α}-H fragment undergoing exchange between three sites with populations 60, 30, and 10%. (A) The 1D spectrum and a portion of the 2D NOESY-exchange spectrum for the system with exchange rate constants equal to zero. CSA and dipole–dipole relaxation is included, but the cross-interactions between the CSA and dipole–dipole mechanisms are excluded. The cross-peaks arise only from relaxation and correspond to the expected NOEs. (B) As in (A), but the cross-interactions between the CSA and dipole–dipole relaxation mechanisms are included; these account for the asymmetry of the H_N doublets. (C) Exchange and complete relaxation (exchange rates are given in the text). The 1D spectra are plotted with the same vertical scale, and the 2D spectra are plotted with the same contour levels.

dipole–dipole relaxation as can be seen in Fig. 6A where that interaction is turned off while all the other relaxation mechanisms are retained. Comparison of these three figures shows clearly that chemical exchange acts as a relaxation mechanism decreasing both the effective T_1's and T_2's of the system.

An additional feature of our program is the ability to select (or to kill) particular quantum coherences that might (not) be of interest. All orders of coherence, including zero, are present at the beginning of the τ delay in this 2D example. Experimentally many of these coherences are killed by instrumental inhomogeneities. We can mimic this effect by "purifying" the density matrix by projecting away the unwanted coherences. We can also study a particular coherence all by itself by selecting it and propagating that density matrix through a pulse sequence.

CONCLUSIONS

We have developed the density matrix treatment of a spin system experiencing the influences of both chemical exchange and relaxation using previously developed separate theories for these effects. Equations for the evolution of the density matrix in the absence and in the presence of an RF field are presented. Based on these theoretical methods, we have written a FORTRAN program, *exlax*, which can be used for the simulation of one- and two-dimensional NMR spectra of up to four nuclear spins undergoing exchange between a maximum of four exchanging sites. Numerous comparisons between the results of our program and previously published experimental results as well as comparisons with the existing simulation programs DNMR5 and ONED2D give us confidence in the reliability of *exlax*.

The useful new features of this program are:

1. It is a general treatment; all the usual relaxation mechanisms operative in liquid samples are included. No extra development or programming is required for the treatment of both T_1 and T_2 in an inversion–recovery experiment such as that presented in Fig. 4.

2. RF fields, in the form of either real pulses, continuous irradiation, or decoupling, are included in a spin-coupled system along with relaxation and exchange. See Fig. 5.

3. Multidimensional experiments can be simulated, again with no additional programming required. See Fig. 6.

exlax can be used to solve problems in a number of different areas. Simulations of the spectra of new NMR pulse sequences for specific systems can help in the development and improvement of those sequences. The ability to simulate the steps in an NMR experiment (different types of pulses, delays, and acquisitions) makes it possible to predict the result of almost any experiment. Simulations can then be used as an aid in the extraction of useful information from experimental results by comparisons of the experimental results with the simulated spectrum for the proposed model of the system. Finally the investigation of the result of the simultaneous treatment of spin relaxation and chemical exchange makes a potentially powerful tool for studies of exchanging systems.

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