
CHAPTER

5

RELAXATION AND DYNAMIC PROCESSES

Previous chapters have utilized the density matrix and product operator formalisms to describe the evolution of the density operator under the chemical shift, scalar coupling, dipolar coupling, and rf Hamiltonians, which are responsible for the chemical shifts, multiplet structures, and coherence transfer phenomena observed by NMR spectroscopy. In principle, NMR experiments begin from the equilibrium state, in which all coherences (off-diagonal elements of the density operator) are zero and the populations of the energy levels of the system (diagonal elements of the density operator) are described by the Boltzmann distribution. Although multiple pulse and multidimensional NMR techniques permit generation of off-diagonal density matrix elements and observation of complex coherence transfer processes, eventually the equilibrium state is restored. As with similar phenomena in other areas of spectroscopy, the process by which an arbitrary density operator returns to the equilibrium operator is called nuclear magnetic, or spin, *relaxation*. The present chapter describes the general theoretical framework of spin relaxation. Consequences of spin relaxation processes for particular multidimensional NMR experiments are described in Chapters 6 and 7, and experimental methods for studying spin relaxation and protein dynamics are described in Chapter 8.

As relaxation is one of the fundamental aspects of magnetic resonance, an extensive literature on theoretical and experimental aspects of relaxation has developed since the earliest days of NMR spectroscopy [see McConnell (1) and references therein]. Relaxation has important consequences for the NMR experiment: (i) relaxation rate constants for single-quantum transverse operators determine the natural linewidths of the resonances detected during the acquisition period, (ii) relaxation rate constants for operators of interest during multidimensional experiments determine the linewidths of resonances in indirectly detected dimensions, unless constant-time or very short evolution periods are utilized, (iii) relaxation rate constants for longitudinal magnetization and coherences generated by the pulse sequence determine the length of the recycle delay needed between acquisitions, and (iv) relaxation rate constants for spin operators created during coherence transfer sequences affect overall sensitivity. Conversely, unique information about the physical processes governing relaxation, including molecular motions and intramolecular distances, is available from NMR experiments. In particular, dipolar cross-relaxation gives rise to the nuclear Overhauser effect (NOE) and makes possible the determination of three-dimensional molecular structures by NMR spectroscopy. Additionally, a variety of chemical kinetic processes can be studied through effects manifested in the NMR spectrum; in many cases, such phenomena are studied while the molecular system remains in chemical equilibrium.

Because the theoretical formalism describing relaxation is more complicated mathematically than is the product operator formalism emphasized in this text, the present treatment emphasizes applications of semiclassical relaxation theory. More detailed descriptions of the derivation of the relaxation equations are presented elsewhere (1–4), and numerous review articles are available (5–10).

5.1 Introduction and Survey of Theoretical Approaches

Introductory theoretical treatments of optical spectroscopy emphasize the role of spontaneous and stimulated emission in relaxation from excited states back to the ground state of a molecule. The probability per unit time, W , for transition from the upper to lower energy state of an isolated magnetic dipole by spontaneous emission of a photon of energy $\Delta E = \hbar\omega_0$ is given by (2, 11)

$$W = \frac{\mu_0 \gamma^2 \hbar \omega_0^3}{6\pi c^3}, \quad [5.1]$$

in which c is the speed of light. For an ^1H spin with a Larmor frequency of 500 MHz, $W \approx 10^{-21} \text{ s}^{-1}$; thus, spontaneous emission is a completely ineffective relaxation mechanism for nuclear magnetic resonance. Stimulated emission also can be shown to have a negligible influence on nuclear spin relaxation, although calculation of transition probabilities is complicated by consideration of the coil in the probe (11). Spontaneous and stimulated emissions are important in optical spectroscopy because the relevant photon frequencies are orders of magnitude larger than the rf frequencies relevant in NMR spectroscopy.

Instead, nuclear spin relaxation is a consequence of coupling of the spin system to the *surroundings*. The surroundings have historically been termed the *lattice* following the early studies of NMR relaxation in solids where the surroundings genuinely were a solid lattice. The lattice includes other degrees of freedom of the molecule containing the spins of interest (such as rotational degrees of freedom) as well as other molecules comprising the system. The energy levels of the lattice are assumed to be quasi-continuous with populations that are described by a Boltzmann distribution. Furthermore, the lattice is assumed to have an infinite heat capacity and consequently to be in thermal equilibrium at all times. The lattice modifies the local magnetic fields at the locations of the nuclei. As a consequence, the local fields weakly couple the lattice and the spin system. Stochastic Brownian rotational motions of molecules in liquids render local magnetic fields time dependent. More precisely, the local fields are composed of a rotationally invariant, and consequently time-independent, component and a rotationally variant, time-dependent component. The time-dependent local magnetic fields can be resolved into components perpendicular and parallel to the main static field. In addition, the fields can be decomposed by Fourier analysis into a superposition of harmonically varying magnetic fields with different frequencies. Thus, the Hamiltonian acting on the spins is given by

$$\begin{aligned}\mathcal{H} &= \mathcal{H}_z + \mathcal{H}_{\text{local}}(t) \\ &= \mathcal{H}_z + \mathcal{H}_{\text{local}}^{\text{isotropic}} + \mathcal{H}_{\text{local}}^{\text{anisotropic}}(t) \\ &= \mathcal{H}_z + \mathcal{H}_{\text{local}}^{\text{isotropic}} + \mathcal{H}_{\text{longitudinal}}^{\text{anisotropic}}(t) + \mathcal{H}_{\text{transverse}}^{\text{anisotropic}}(t),\end{aligned}\quad [5.2]$$

in which \mathcal{H}_z is the Zeeman Hamiltonian, $\mathcal{H}_{\text{local}}^{\text{isotropic}}$ contains the isotropic chemical shift and scalar coupling interactions, and $\mathcal{H}_{\text{local}}^{\text{anisotropic}}(t)$, $\mathcal{H}_{\text{longitudinal}}^{\text{anisotropic}}(t)$, $\mathcal{H}_{\text{transverse}}^{\text{anisotropic}}(t)$ are the Hamiltonians for stochastic anisotropic interactions. The anisotropic Hamiltonians have an ensemble average of zero by construction owing to the rotational invariance

of isotropic solution. Additionally, the correlations between stochastic fluctuations tend to zero for $t \gg \tau_c$, in which τ_c is defined as the correlation time of the stochastic process (*vide infra*). In isotropic solution, τ_c is approximately the rotational correlation time of the molecular species.

Relaxation processes that require exchange of energy with the lattice are termed *nonadiabatic*. Transverse components of the stochastic local field are responsible for nonadiabatic contributions to relaxation. If the Fourier spectrum of the fluctuating transverse magnetic fields at the location of a nucleus contains components with frequencies corresponding to the energy differences between eigenstates of the spin system, then transitions between eigenstates can occur. In this case, transition of the spin system from a higher (lower) energy state to a lower (higher) energy state is accompanied by an energy-conserving transition of the lattice from a lower (higher) to higher (lower) energy state. A transition of the spin system from higher energy to lower energy is more probable because the lattice is always in thermal equilibrium and has a larger population in the lower energy state. Thus, exchange of energy between the spin system and the lattice brings the spin system into a state of thermal equilibrium in which the populations of the stationary states have the Boltzmann distribution. Furthermore, transitions between stationary states caused by nonadiabatic processes decrease the lifetimes of these states and thereby broaden the energies of the stationary states through a Heisenberg uncertainty relationship. As a result, resonance frequencies vary randomly and phase coherence between spins is reduced over time. Consequently, nonadiabatic fluctuations that cause transitions between states result in both thermal equilibration of the spin state populations and decay of off-diagonal coherences.

Relaxation processes that do not require exchange of energy with the lattice are termed *adiabatic*. Fluctuating fields parallel to the main static field are responsible for adiabatic contributions to relaxation. These fluctuating fields generate variations in the total magnetic field in the z -direction and, consequently, in the energies in the nuclear spin energy levels. Thus, adiabatic processes cause resonance frequencies of affected spins to vary randomly. Over time, the spins lose phase coherence and off-diagonal elements of the density matrix decay to zero. The populations of the states are not altered and no energy is exchanged between the spin system and the lattice, because transitions between stationary states do not occur. For macromolecules with $\omega\tau_c \gg 1$, the adiabatic contributions to the relaxation of off-diagonal elements of the density matrix are much more important compared to nonadiabatic lifetime effects.

5.1.1 RELAXATION IN THE BLOCH EQUATIONS

In the simplest theoretical approach to spin relaxation, the relaxation of isolated spins is characterized in the Bloch equations [1.28] by two phenomenological first-order rate constants: the *spin-lattice*, or *longitudinal*, relaxation rate constant, R_1 , and the *spin-spin*, or *transverse*, relaxation rate constant, R_2 (12). In the following discussion, rate constants rather than time constants are utilized; the two quantities are reciprocals of each other ($T_1 = 1/R_1$ and $T_2 = 1/R_2$). The spin-lattice relaxation rate constant describes the recovery of the longitudinal magnetization to thermal equilibrium, or, equivalently, return of the populations of the energy levels of the spin system (diagonal elements of the density operator) to the equilibrium Boltzmann distribution. The spin-spin relaxation rate constant describes the decay of the transverse magnetization to zero, or, equivalently, the decay of transverse single-quantum coherences (off-diagonal elements of the density matrix). Nonadiabatic processes contribute to *both* spin-lattice and spin-spin relaxation. Adiabatic processes contribute *only* to spin-spin relaxation; spin-lattice relaxation is not affected because adiabatic processes do not change the populations of stationary states.

The Bloch formulation provides qualitative insights into the effects of relaxation on the NMR experiment, and the phenomenological rate constants can be measured experimentally. For example, the Bloch equations predict that the FID is the sum of exponentially damped sinusoidal functions and that, following perturbation of a spin system from equilibrium, R_2 governs the length of time that the FID can be observed and R_1 governs the minimum time required for equilibrium to be restored. The Bloch formulation does not provide a microscopic explanation of the origin or magnitude of the relaxation rate constants, nor is it extendible to more complex, coupled spin systems. For example, in dipolar-coupled two-spin systems, multiple spin operators, such as zero-quantum coherence, have relaxation rate constants that differ from both R_1 and R_2 .

In the spirit of the Bloch equations, the results for a product operator analysis of the evolution of a spin system under a particular pulse sequence can be corrected approximately for relaxation effects simply by adding an exponential damping factor for each temporal period *post hoc*. If product operator analysis of a two-dimensional pulse sequence yields a propagator $\mathbf{U} = \mathbf{U}_a(t_2)\mathbf{U}_m\mathbf{U}_e(t_1)\mathbf{U}_p$, in which \mathbf{U}_p is the propagator for the preparation period, etc., then relaxation effects

approximately are included by writing

$$\sigma(t_1, t_2) = U\sigma(0)U^{-1} \exp[-R_p t_p - R_e t_1 - R_m t_m - R_a t_2], \quad [5.3]$$

in which R_p is the relaxation rate constant for the operators of interest during the preparation time, t_p , etc. Cross-correlation and cross-relaxation effects are assumed to be negligible.

For example, the signal recorded in an ^1H - ^{15}N HSQC spectrum is found by product operator analysis to be proportional to $\cos(\omega_N t_1) \cos(\omega_H t_2) \cos(\pi J_{\text{H}^{\text{N}}\text{H}^\alpha} t_2)$, in which ω_N and ω_H are the Larmor frequencies of the ^{15}N and $^1\text{H}^{\text{N}}$ spins, respectively and $J_{\text{H}^{\text{N}}\text{H}^\alpha}$ is the scalar coupling constant between the amide and α ^1H spins. The phenomenological approach modifies this expression to $\cos(\omega_N t_1) \cos(\omega_H t_2) \cos(\pi J_{\text{H}^{\text{N}}\text{H}^\alpha} t_2) \exp[-R_{2\text{N}} t_1 - R_{2\text{H}} t_2]$, in which $R_{2\text{N}}$ and $R_{2\text{H}}$ are the transverse relaxation rate constants for the ^{15}N and ^1H operators present during t_1 and t_2 , respectively, and relaxation during the INEPT sequences has been ignored. Relaxation effects on HSQC spectra are discussed in additional detail in Section 7.1.2.4. As a second example, product operator analysis of the INEPT pulse sequence [2.285], in the absence of relaxation, yields a density operator term proportional to $-2I_z S_y \sin(2\pi J_{IS} t)$. Coherence transfer is maximized for $2t = 1/(2J_{IS})$ [2.287]. If relaxation is considered, the result is modified to $-2I_z S_y \sin(2\pi J_{IS} t) \exp(-2R_{2I} t)$, in which R_{2I} is the relaxation rate of the I spin operators present during the period $2t$. Maximum coherence transfer is obtained for

$$2t = (\pi J_{IS})^{-1} \tan^{-1}(\pi J_{IS}/R_{2I}) \leq 1/(2J_{IS}). \quad [5.4]$$

5.1.2 THE SOLOMON EQUATIONS

Spin-lattice relaxation for interacting spins can be treated theoretically by considering the rates of transitions of the spins between energy levels, as was demonstrated first by Bloembergen, Pound, and Purcell (13). Figure 5.1 shows the energy levels for a two-spin system with transition frequencies labeled. The four energy levels are labeled in the normal way as $|m_I m_S\rangle$. The rate constants for transitions between the energy levels are denoted by W_0 , W_I , W_S , and W_2 , and are distinguished according to which spins change spin state during the transition. Thus, W_I denotes a relaxation process involving an I spin flip, W_S denotes a relaxation process involving an S spin flip, W_0 is a relaxation process in

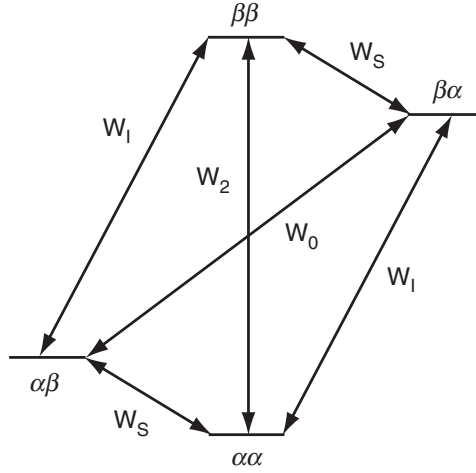


FIGURE 5.1 Transitions and associated rate constants for a two-spin system.

which both spins are flipped in opposite senses (flip-flop transition), and W_2 is a relaxation process in which both spins are flipped in the same sense (flip-flip transition). A differential equation governing the population of the state $|\alpha\alpha\rangle$ is written by inspection:

$$\frac{dP_{\alpha\alpha}}{dt} = -(W_I + W_S + W_2)P_{\alpha\alpha} + W_I P_{\beta\alpha} + W_S P_{\alpha\beta} + W_2 P_{\beta\beta} + K, \quad [5.5]$$

in which $P_{\gamma\delta}$ is the population of the state $|\gamma\delta\rangle$ and K is a constant chosen to ensure that the population $P_{\gamma\delta}$ returns to the equilibrium value $P_{\gamma\delta}^0$. At equilibrium, $dP_{\alpha\alpha}/dt = 0$; therefore, the value of K is found by setting the left-hand side of [5.5] equal to zero:

$$K = (W_I + W_S + W_2)P_{\alpha\alpha}^0 - W_I P_{\beta\alpha}^0 - W_S P_{\alpha\beta}^0 - W_2 P_{\beta\beta}^0. \quad [5.6]$$

Writing $\Delta P_{\gamma\delta} = P_{\gamma\delta} - P_{\gamma\delta}^0$ yields an equation for the deviation of the population of the $|\alpha\alpha\rangle$ state from the equilibrium population,

$$\frac{d\Delta P_{\alpha\alpha}}{dt} = -(W_I + W_S + W_2)\Delta P_{\alpha\alpha} + W_I \Delta P_{\beta\alpha} + W_S \Delta P_{\alpha\beta} + W_2 \Delta P_{\beta\beta}. \quad [5.7]$$

Similar equations are written for the other three states:

$$\begin{aligned}\frac{d\Delta P_{\alpha\beta}}{dt} &= -(W_0 + W_I + W_S)\Delta P_{\alpha\beta} + W_0\Delta P_{\beta\alpha} + W_I\Delta P_{\beta\beta} + W_S\Delta P_{\alpha\alpha}, \\ \frac{d\Delta P_{\beta\alpha}}{dt} &= -(W_0 + W_I + W_S)\Delta P_{\beta\alpha} + W_0\Delta P_{\alpha\beta} + W_I\Delta P_{\alpha\alpha} + W_S\Delta P_{\beta\beta}, \\ \frac{d\Delta P_{\beta\beta}}{dt} &= -(W_I + W_S + W_2)\Delta P_{\beta\beta} + W_I\Delta P_{\alpha\beta} + W_S\Delta P_{\beta\alpha} + W_2\Delta P_{\alpha\alpha}.\end{aligned}\tag{5.8}$$

Now, recalling that $\langle I_z \rangle(t) = \text{Tr}\{\sigma(t)I_z\} = \sigma_{11} + \sigma_{22} - \sigma_{33} - \sigma_{44} = P_{\alpha\alpha} + P_{\alpha\beta} - P_{\beta\alpha} - P_{\beta\beta}$ and $\langle S_z \rangle(t) = \text{Tr}\{\sigma(t)S_z\} = \sigma_{11} - \sigma_{22} + \sigma_{33} - \sigma_{44} = P_{\alpha\alpha} - P_{\alpha\beta} + P_{\beta\alpha} - P_{\beta\beta}$ leads to

$$\begin{aligned}\frac{d\Delta I_z(t)}{dt} &= -(W_0 + 2W_I + W_2)\Delta I_z(t) - (W_2 - W_0)\Delta S_z(t), \\ \frac{d\Delta S_z(t)}{dt} &= -(W_0 + 2W_S + W_2)\Delta S_z(t) - (W_2 - W_0)\Delta I_z(t),\end{aligned}\tag{5.9}$$

in which $\Delta I_z(t) = \langle I_z \rangle(t) - \langle I_z^0 \rangle$ and $\langle I_z^0 \rangle$ is the equilibrium magnitude of the I_z operator. Corresponding relationships hold for S_z . Making the identifications

$$\begin{aligned}\rho_I &= W_0 + 2W_I + W_2, \\ \rho_S &= W_0 + 2W_S + W_2, \\ \sigma_{IS} &= W_2 - W_0\end{aligned}\tag{5.10}$$

leads to the Solomon equations for a two-spin system (14):

$$\begin{aligned}\frac{d\Delta I_z(t)}{dt} &= -\rho_I\Delta I_z(t) - \sigma_{IS}\Delta S_z(t), \\ \frac{d\Delta S_z(t)}{dt} &= -\rho_S\Delta S_z(t) - \sigma_{IS}\Delta I_z(t).\end{aligned}\tag{5.11}$$

The Solomon equations reveal that the temporal evolution of the I spin longitudinal magnetization depends not only on its own departure from equilibrium (*autorelaxation*), but also on the state of the S spin longitudinal magnetization and *vice versa*. The time dependence of the two magnetizations are linked: this connection is called *cross-relaxation*. The rate constants ρ_I and ρ_S are the *autorelaxation rate constants* (or the spin–lattice relaxation rate constants, R_{1I} and R_{1S} , in the Bloch terminology) for the I and S spins, respectively, and σ_{IS} is the *cross-relaxation rate constant* for exchange of magnetization between the two spins.

The Solomon equations are extended to N interacting spins as

$$\frac{d\Delta I_{kz}(t)}{dt} = -\rho_k \Delta I_{kz}(t) - \sum_{j \neq k} \sigma_{kj} \Delta I_{jz}(t), \quad [5.12]$$

in which

$$\rho_k = \sum_{k \neq j} \rho_{kj} \quad [5.13]$$

reflects the relaxation of the k th spin by all other spins (in the absence of interference effects; see Section 5.2.1). Equation [5.12] is written in matrix nomenclature as

$$\frac{d\Delta \mathbf{M}_z(t)}{dt} = -\mathbf{R} \Delta \mathbf{M}_z(t), \quad [5.14]$$

in which \mathbf{R} is an $N \times N$ matrix with elements $R_{kk} = \rho_k$ and $R_{kj} = \sigma_{kj}$, and $\Delta \mathbf{M}_z(t)$ is an $N \times 1$ column vector with entries $\Delta M_k(t) = \Delta I_{kz}(t)$. The Solomon equations in matrix form have the formal solution

$$\Delta \mathbf{M}_z(t) = e^{-\mathbf{R}t} \Delta \mathbf{M}_z(0) = \mathbf{U}^{-1} e^{-\mathbf{D}t} \mathbf{U} \Delta \mathbf{M}_z(0), \quad [5.15]$$

in which \mathbf{D} is a diagonal matrix of the eigenvalues of \mathbf{R} , \mathbf{U} is a unitary matrix, and

$$\mathbf{D} = \mathbf{U} \mathbf{R} \mathbf{U}^{-1} \quad [5.16]$$

is the similarity transformation that diagonalizes \mathbf{R} . These differential equations show that if the populations of the energy levels of the spin system are perturbed from equilibrium, then relaxation of a particular spin is in general a multiexponential process.

For a two-spin system,

$$\begin{aligned} \mathbf{R} &= \begin{bmatrix} \rho_I & \sigma_{IS} \\ \sigma_{IS} & \rho_S \end{bmatrix}, \\ \mathbf{D} &= \begin{bmatrix} \lambda_+ & 0 \\ 0 & \lambda_- \end{bmatrix}, \\ \lambda_{\pm} &= \frac{1}{2} \left\{ (\rho_I + \rho_S) \pm [(\rho_I - \rho_S)^2 + 4\sigma_{IS}^2]^{1/2} \right\}, \\ \mathbf{U} &= \begin{bmatrix} \frac{-\sigma_{IS}}{[(\rho_I - \lambda_+)^2 + \sigma_{IS}^2]^{1/2}} & \frac{-\sigma_{IS}}{[(\rho_I - \lambda_-)^2 + \sigma_{IS}^2]^{1/2}} \\ \frac{\rho_I - \lambda_+}{[(\rho_I - \lambda_+)^2 + \sigma_{IS}^2]^{1/2}} & \frac{\rho_I - \lambda_-}{[(\rho_I - \lambda_-)^2 + \sigma_{IS}^2]^{1/2}} \end{bmatrix}, \end{aligned} \quad [5.17]$$

and upon substituting into [5.15], the result obtained is

$$\begin{bmatrix} \Delta M_I(t) \\ \Delta M_S(t) \end{bmatrix} = \begin{bmatrix} a_{II}(t) & a_{IS}(t) \\ a_{SI}(t) & a_{SS}(t) \end{bmatrix} \begin{bmatrix} \Delta M_I(0) \\ \Delta M_S(0) \end{bmatrix}, \quad [5.18]$$

in which the matrix elements, $a_{ij}(t)$, are given by

$$\begin{aligned} a_{II}(t) &= \frac{1}{2} \left[\left(1 - \frac{\rho_I - \rho_S}{(\lambda_+ - \lambda_-)} \right) \exp(-\lambda_- t) + \left(1 + \frac{\rho_I - \rho_S}{(\lambda_+ - \lambda_-)} \right) \exp(-\lambda_+ t) \right], \\ a_{SS}(t) &= \frac{1}{2} \left[\left(1 + \frac{\rho_I - \rho_S}{(\lambda_+ - \lambda_-)} \right) \exp(-\lambda_- t) + \left(1 - \frac{\rho_I - \rho_S}{(\lambda_+ - \lambda_-)} \right) \exp(-\lambda_+ t) \right], \\ a_{IS}(t) &= a_{SI}(t) = \frac{-\sigma_{IS}}{(\lambda_+ - \lambda_-)} [\exp(-\lambda_- t) - \exp(-\lambda_+ t)]. \end{aligned} \quad [5.19]$$

These equations frequently are written in the form

$$\begin{aligned} a_{II}(t) &= \frac{1}{2} \left[\left(1 - \frac{\rho_I - \rho_S}{R_C} \right) + \left(1 + \frac{\rho_I - \rho_S}{R_C} \right) \exp(-R_C t) \right] \exp(-R_L t), \\ a_{SS}(t) &= \frac{1}{2} \left[\left(1 + \frac{\rho_I - \rho_S}{R_C} \right) + \left(1 - \frac{\rho_I - \rho_S}{R_C} \right) \exp(-R_C t) \right] \exp(-R_L t), \\ a_{IS}(t) &= a_{SI}(t) = \frac{-\sigma_{IS}}{R_C} [1 - \exp(-R_C t)] \exp(-R_L t) \end{aligned} \quad [5.20]$$

by defining the cross-rate constant, R_C and a leakage rate constant, R_L :

$$\begin{aligned} R_C &= \lambda_+ - \lambda_- = [(\rho_I - \rho_S)^2 + 4\sigma_{IS}^2]^{1/2}, \\ R_L &= \lambda_-. \end{aligned} \quad [5.21]$$

The leakage rate constant results in irreversible relaxation toward Boltzmann equilibrium for both spins, while the cross-rate constant determines the rate of magnetization transfer between spins. If $\rho_I = \rho_S = \rho$, and $\sigma_{IS} = \sigma$, [5.19] simplifies to

$$\begin{aligned} a_{II}(t) &= a_{SS}(t) = \frac{1}{2} [1 + \exp(-2\sigma t)] \exp\{-(\rho - \sigma)t\}, \\ a_{IS}(t) &= a_{SI}(t) = -\frac{1}{2} [1 - \exp(-2\sigma t)] \exp\{-(\rho - \sigma)t\}. \end{aligned} \quad [5.22]$$

The time dependence of the matrix elements $a_{II}(t)$ and $a_{IS}(t)$ are illustrated in Fig. 5.2.

To illustrate aspects of longitudinal relaxation as exemplified by the Solomon equations, four different experiments are analyzed. For

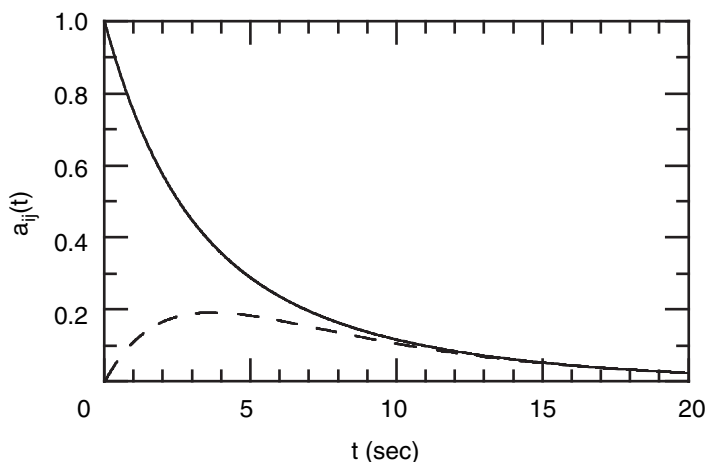


FIGURE 5.2 Time dependence of (—) $a_{II}(t)$ and (- - -) $a_{IS}(t)$ calculated using [5.22] with $\rho = 0.30 \text{ s}^{-1}$ and $\sigma = -0.15 \text{ s}^{-1}$.

simplicity, a homonuclear spin system with $\gamma_I = \gamma_S$, $\rho_I = \rho_S = \rho$, and $\sigma_{IS} = \sigma$ are assumed. The experiments use the generalized pulse sequence:

$$180^\circ - t - 90^\circ - \text{acquire}. \quad [5.23]$$

The initial state of the longitudinal magnetization is prepared by application of the 180° pulse to equilibrium magnetization. The longitudinal magnetization relaxes according to the Solomon equations during the delay t . The final state of the longitudinal magnetization is converted into transverse magnetization by the 90° pulse and recorded during the acquisition period.

In the *selective inversion recovery* experiment, the 180° pulse is applied selectively to the I spin. The initial conditions are $\Delta I_z(0) = \langle I_z \rangle(0) - \langle I_z^0 \rangle = -2\langle I_z^0 \rangle$, and $\Delta S_z(0) = \langle S_z \rangle(0) - \langle S_z^0 \rangle = 0$. The time decay of the I spin magnetization is given by

$$\langle I_z \rangle(t) / \langle I_z^0 \rangle = 1 - \exp\{-(\rho - \sigma)t\} [1 + \exp(-2\sigma t)] \quad [5.24]$$

and is generally biexponential. In the *initial rate regime*, the slope of the recovery curve is given by

$$\left. \frac{d(\langle I_z \rangle(t) / \langle I_z^0 \rangle)}{dt} \right|_{t \rightarrow 0} = 2\rho. \quad [5.25]$$

In the *nonselective inversion recovery* experiment, the 180° pulse affects both the I and S spins equally. The initial conditions are $\Delta I_z(0) = \langle I_z \rangle(0) - \langle I_z^0 \rangle = -2\langle I_z^0 \rangle$ and $\Delta S_z(0) = \langle S_z \rangle(0) - \langle S_z^0 \rangle = -2\langle S_z^0 \rangle$. The time course of the I spin magnetization is given by

$$\begin{aligned} \langle I_z \rangle(t) / \langle I_z^0 \rangle &= 1 - \exp\{-(\rho + \sigma)t\} [1 + \exp(-2\sigma t)] \\ &\quad + (\langle S_z^0 \rangle / \langle I_z^0 \rangle) \exp\{-(\rho + \sigma)t\} [1 - \exp(-2\sigma t)] \\ &= 1 - 2 \exp\{-(\rho + \sigma)t\}, \end{aligned} \quad [5.26]$$

in which the second line is obtained by using $\langle I_z^0 \rangle / \langle S_z^0 \rangle = \gamma_S / \gamma_I = 1$. The recovery curve is monoexponential with rate constant $\rho + \sigma$. In the initial rate regime,

$$\left. \frac{d(\langle I_z \rangle(t) / \langle I_z^0 \rangle)}{dt} \right|_{t \rightarrow 0} = 2(\rho + \sigma), \quad [5.27]$$

in which $\rho + \sigma = 2(W_I + W_2)$ does not depend on W_0 . For macromolecules (Section 5.3), $W_0 \gg W_I \gg W_2$; consequently, recovery of equilibrium magnetization following a nonselective 180° pulse is inefficient.

In the *transient NOE experiment*, the S spin longitudinal magnetization is inverted with a selective 180° pulse to produce initial conditions $\Delta I_z(0) = \langle I_z \rangle(0) - \langle I_z^0 \rangle = 0$ and $\Delta S_z(0) = \langle S_z \rangle(0) - \langle S_z^0 \rangle = -2\langle S_z^0 \rangle$. The time course of the I spin magnetization is given by

$$\begin{aligned} \langle I_z \rangle(t) / \langle I_z^0 \rangle &= 1 + (\langle S_z^0 \rangle / \langle I_z^0 \rangle) \exp\{-(\rho + \sigma)t\} [1 - \exp(-2\sigma t)] \\ &= 1 + \exp\{-(\rho + \sigma)t\} [1 - \exp(-2\sigma t)], \end{aligned} \quad [5.28]$$

and is biexponential. In the initial rate regime,

$$\left. \frac{d(\langle I_z \rangle(t) / \langle I_z^0 \rangle)}{dt} \right|_{t \rightarrow 0} = 2\sigma. \quad [5.29]$$

Thus, the initial rate of change of the I spin intensity in the transient NOE experiment is proportional to the cross-relaxation rate, σ . In this *initial rate approximation*, in which $\langle I_z \rangle(0) = \langle I_z^0 \rangle$, solving [5.28] to first order in the mixing time, τ_m , the time during which cross-relaxation occurs, gives

$$\langle I_z \rangle(\tau_m) = \langle I_z^0 \rangle + 2\sigma\tau_m \langle S_z^0 \rangle = \langle I_z^0 \rangle (1 + 2\sigma\tau_m). \quad [5.30]$$

Therefore, for $\tau_m = 0$, the I spin magnetization is equal to its equilibrium value, but as τ_m increases, the I spin magnetization has an

additional contribution that is proportional to the mixing time and the cross-relaxation rate constant. This change in the magnitude of the I spin magnetization due to perturbation of the S spin is the *NOE enhancement*.

In the *decoupled inversion recovery experiment*, the S spin is irradiated by a weak selective rf field (so as not to perturb the I spin) throughout the experiment in order to equalize the populations across the S spin transitions. In this situation, $\langle S_z \rangle(t) = 0$ for all t , and the S spins are said to be saturated. Equation [5.11] reduces to

$$\begin{aligned} \frac{d\langle I_z \rangle(t)}{dt} &= -\rho[\langle I_z \rangle(t) - \langle I_z^0 \rangle] + \sigma\langle S_z^0 \rangle \\ &= -\rho[\langle I_z \rangle(t) - \langle I_z^0 \rangle(1 + \sigma/\rho)]. \end{aligned} \quad [5.31]$$

Following the 180° pulse, $\Delta I_z(0) = \langle I_z \rangle(0) - \langle I_z^0 \rangle = -2\langle I_z^0 \rangle$ and the time course of the I spin magnetization is given by

$$\langle I_z \rangle(t)/\langle I_z^0 \rangle = 1 + \sigma/\rho - (2 + \sigma/\rho) \exp(-\rho t). \quad [5.32]$$

In the initial rate regime,

$$\left. \frac{d(\langle I_z \rangle(t)/\langle I_z^0 \rangle)}{dt} \right|_{t \rightarrow 0} = 2\rho + \sigma. \quad [5.33]$$

In this case, the recovery curve is monoexponential with rate constant ρ . These analyses indicate that, even for an isolated two-spin system, the time dependence of the longitudinal magnetization usually is biexponential. The actual time course observed depends upon the initial condition of the spin system prepared by the NMR pulse sequence. Examples of the time courses of the I spin magnetization for these experiments are given in Fig. 5.3.

The preceding derivation does not provide theoretical expressions that relate the transition rate constants, W_0 , W_I , W_S , and W_2 to particular stochastic Hamiltonians. The semiclassical relaxation theory as described in Section 5.2 will be used to obtain such expressions, rather than pursuing further the original approach of Bloembergen et al. (13). As will be shown, the transition rate constants depend upon the different frequency components of the stochastic magnetic fields [5.118]. Thus, the transition characterized by W_I is induced by molecular motions that produce fields oscillating at the Larmor frequency of the I spin, and the transition characterized by W_S is induced by molecular motions that produce fields oscillating at the Larmor frequency of the S spin. The W_0

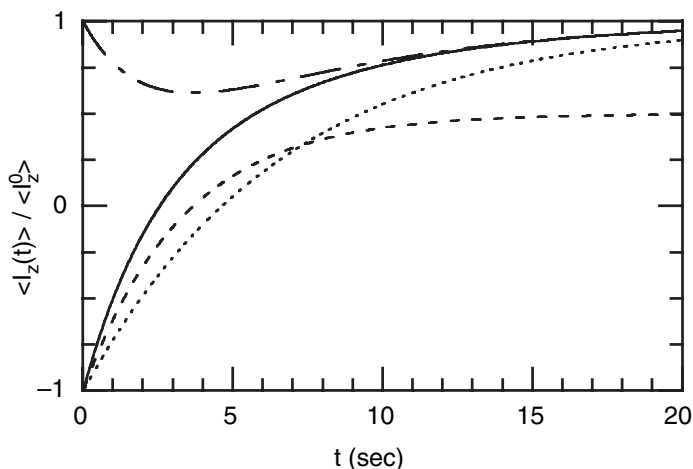


FIGURE 5.3 Magnetization decays for inversion recovery experiments. (—) Selective inversion recovery calculated using [5.24]; (···) nonselective inversion recovery calculated using [5.26]; (---) transient NOE recovery calculated using [5.28]; and (- - -) decoupled inversion recovery calculated using [5.32]. Calculations were performed for a homonuclear IS spin system with $\gamma_I = \gamma_S$, $\rho = 0.30 \text{ s}^{-1}$, and $\sigma = -0.15 \text{ s}^{-1}$.

pathway is induced by fields oscillating at the *difference* of the Larmor frequencies of the I and S spins, and the W_2 pathway is induced by fields oscillating at the *sum* of the Larmor frequencies of the two spins. Most importantly, the cross-relaxation rate constant is nonzero only if $W_2 - W_0 \neq 0$; therefore, the relaxation mechanism must generate nonzero rate constants for the flip-flip (double-quantum) and/or flip-flop (zero-quantum) transitions. For biological macromolecules, dipolar coupling between nuclear spins is the main interaction for which W_2 and W_0 are nonzero. The Solomon equations are central to the study of the NOE and will be discussed in additional detail in Section 5.5.

5.1.3 RANDOM-PHASE MODEL FOR TRANSVERSE RELAXATION

A very simple model for the effect of longitudinal stochastic fluctuations on the transverse relaxation of nuclear spins will now be derived. The instantaneous longitudinal component of the Hamiltonian experienced by a spin is

$$\mathcal{H}_{\text{longitudinal}}(t) = \{\omega_0 + \omega(t)\} I_z. \quad [5.34]$$

The local, stochastic component to the precession frequency of the spin, $\omega(t)$, varies due to molecular motion. The average value of $\omega(t) = 0$ by construction, because any nonzero average value is incorporated into ω_0 . The time dependence of the complex magnetization is given by

$$\frac{dM^+(t)}{dt} = i\{\omega_0 + \omega(t)\}M^+(t). \quad [5.35]$$

This equation will be solved in a slightly unusual manner, in order to provide insights into later derivations. First, the effect of ω_0 is removed by changing to the variable $M^{T+}(t) = \exp(-i\omega_0 t)M^+(t)$; this is identical to a transformation to a rotating frame resonant with ω_0 . The new variable satisfies the differential equation:

$$\begin{aligned} \frac{dM^{T+}(t)}{dt} &= -i\omega_0 \exp(-i\omega_0 t)M^+(t) + \exp(-i\omega_0 t) \frac{dM^+(t)}{dt} \\ &= -i\omega_0 \exp(-i\omega_0 t)M^+(t) + i \exp(i\omega_0 t)\{\omega_0 + \omega(t)\}M^+(t) \\ &= i\omega(t)M^{T+}(t), \end{aligned} \quad [5.36]$$

which is integrated to give the solution for a particular $\omega(t)$:

$$M^{T+}(t) = \exp\left[i \int_0^t \omega(t') dt'\right] M^+(0), \quad [5.37]$$

where $M^{T+}(0) = M^+(0)$. The observed signal is obtained by averaging over all possible instances of $\omega(t)$, which will be indicated by overbars:

$$\begin{aligned} \overline{M^{T+}(t)} &= \overline{\exp\left[i \int_0^t \omega(t') dt'\right] \overline{M^+(0)}} \\ &= \left\{ 1 + i \overline{\int_0^t \omega(t') dt'} - \frac{1}{2} \overline{\int_0^t \omega(t') dt' \int_0^t \omega(t'') dt''} + \dots \right\} \overline{M^+(0)} \\ &= \left\{ 1 - \frac{1}{2} \int_0^t \int_0^t \overline{\omega(t')\omega(t'')} dt' dt'' + \dots \right\} \overline{M^+(0)}. \end{aligned} \quad [5.38]$$

Between lines two and three, the order of averaging and integration has been reversed and the condition $\overline{\omega(t)} = 0$ has been used. This equation provides an expression for the ensemble average magnetization. The assumption has been made that the variation in $\omega(t)$ is uncorrelated with the variation in $M^+(0)$ so that the ensemble averaging can be performed

independently. A differential form of this equation is obtained by differentiating [5.38]:

$$\frac{d\overline{M^{T+}}(t)}{dt} = - \int_0^t \overline{\omega(t)\omega(t')} dt' \overline{M^+}(0). \quad [5.39]$$

Equation [5.39] is converted into a differential equation for $\overline{M^{T+}}(t)$ by making the following two assumptions: (i) $\overline{M^+}(0)$ can be replaced with $\overline{M^{T+}}(t)$ on the right-hand side of [5.39] and (ii) the limit of the integral can be extended from t to infinity. The result is

$$\frac{d\overline{M^{T+}}(t)}{dt} = - \int_0^\infty \overline{\omega(t)\omega(t')} dt' \overline{M^{T+}}(t). \quad [5.40]$$

The function $\overline{\omega(t)\omega(t')}$ is called the autocorrelation function of the stochastic process. Autocorrelation functions play a central role in the analysis of stochastic processes. If $t=t'$, the value of the autocorrelation function is simply the mean square amplitude of the process (i.e., the variance of the fluctuations). For stationary random processes, the autocorrelation function depends only on the time difference, $\tau = t - t'$. The autocorrelation function will be denoted $C(\tau)$. The relaxation rate constant, R_2 , is then identified as

$$R_2 = \int_0^\infty \overline{\omega(t)\omega(t-\tau)} d\tau = \int_0^\infty C(\tau) d\tau. \quad [5.41]$$

With this expression, [5.40] is transformed back to an equation for $\overline{M^+}(t)$:

$$\frac{d\overline{M^+}(t)}{dt} = \{i\omega_0 - R_2\} \overline{M^+}(t), \quad [5.42]$$

which is simply the Bloch equation for evolution of $\overline{M^+}(t)$. To proceed, the assumption will be made that $C(\tau) = C(0) \exp(-\tau/\tau_c)$, in which $C(0) = \overline{\omega^2(t)}$ is the variance of the fluctuating fields and τ_c is the characteristic time over which the fields vary. The final result after performing the integral is

$$R_2 = \overline{\omega^2(t)} \tau_c. \quad [5.43]$$

This expression shows that the relaxation rate constant depends on the mean square magnitude of the fluctuating fields and the time scale of the fluctuations. This result, although derived for a very simple model,

contains much of the qualitative physics of the more complete treatment to be presented in Section 5.2.

Now, the two assumptions made in deriving [5.40] are justified *post facto*. To first order, the fractional change in $\overline{M^{T+}}(t)$ is given by $[\overline{M^{T+}}(t) - \overline{M^{T+}}(0)]/\overline{M^{T+}}(0) = -R_2 t$. For a time $t \ll 1/R_2$, $\overline{M^{T+}}(t)$ and $\overline{M^{T+}}(0)$ differ negligibly and $\overline{M^{T+}}(t)$ can be substituted for $\overline{M^{T+}}(0)$ in [5.39]. The correlation time, τ_c , typically is related to the time scale for molecular rotational diffusion in solution; consequently, the time scales of interest for spin relaxation satisfy $t \gg \tau_c$. In this case, the value of the integrand in [5.39] is zero for times greater than t and the upper limits of the integral can be extended to infinity. As a result of these two assumptions, the present theory is valid on a so-called coarse-grained time scale $\tau_c \ll t \ll 1/R_2$. The restrictions on $t \ll 1/R_2$ would appear to constitute a fatal weakness because relaxation in NMR experiments frequently must be considered for times $T \geq 1/R_2$. To rectify this, T conceptually is defined as $T = nt$, in which n is an integer and t satisfies the coarse-grained temporal restrictions. Relaxation over the period T is calculated by piecewise evaluation of [5.42] for each of the n intervals in succession. In the limit of $n \rightarrow \infty$ while $t \rightarrow 0$, the expected exponential relaxation behavior is obtained even for $T > 1/R_2$.

To finish the analysis, the variance in $\omega(t)$ must be determined. The variance will depend, of course, on the particular relaxation mechanism of interest. As an illustration, relaxation due to the CSA interaction will be considered. For the CSA Hamiltonian with an axially symmetric CSA tensor (Section 5.4.4),

$$\mathcal{H}_{\text{local}}^{\text{anisotropic}}(t) = \sqrt{\frac{2}{3}}\gamma\Delta\sigma B_0 \left\{ \sqrt{\frac{2}{3}}Y_2^0[\Omega(t)]I_z + \frac{1}{2}Y_2^{-1}[\Omega(t)]I^+ - \frac{1}{2}Y_2^1[\Omega(t)]I^- \right\}, \quad [5.44]$$

in which $\Delta\sigma$ is the CSA, $Y_2^q[\Omega]$ are modified spherical harmonic functions given in Table 5.1, and $\Omega(t) = \{\theta(t), \phi(t)\}$ are the time-dependent angles defining the orientation of the symmetry axis of the CSA principal axis system in the laboratory frame. The term proportional to I_z represents the fluctuating longitudinal interactions (giving rise to adiabatic relaxation) and the terms proportional to I^+ and I^- represent the fluctuating transverse interactions (giving rise to nonadiabatic relaxation). An expression for $\omega(t)$ is obtained from the longitudinal component of the CSA Hamiltonian in [5.44]:

$$\mathcal{H}_{\text{longitudinal}}^{\text{anisotropic}}(t) = \omega(t)I_z = \frac{2}{3}\gamma\Delta\sigma B_0 Y_2^0[\Omega(t)]I_z. \quad [5.45]$$

TABLE 5.1
Modified Second-Order Spherical Harmonics^a

q	Y_2^q	$Y_2^{-q} = (-1)^q Y_2^{q*}$
0	$(3 \cos^2 \theta - 1)/2$	$(3 \cos^2 \theta - 1)/2$
1	$-\sqrt{3/2} \sin \theta \cos \theta e^{i\phi}$	$\sqrt{3/2} \sin \theta \cos \theta e^{-i\phi}$
2	$\sqrt{3/8} \sin^2 \theta e^{i2\phi}$	$\sqrt{3/8} \sin^2 \theta e^{-i2\phi}$

^aThe modified spherical harmonic functions are normalized (to give the conventional spherical harmonic functions) by multiplying by $\sqrt{5/(4\pi)}$.

For the present, the assumption will be made that the molecule reorients isotropically; therefore the probability distribution for the orientation of the principal axis system is $p(\theta, \phi) = 1/(4\pi)$. The form of $\overline{\omega^2(t)}$ is:

$$\overline{\omega^2(t)} = \left(\frac{2}{3} \gamma \Delta \sigma B_0 \right)^2 \frac{1}{4\pi} \int_0^{2\pi} \int_0^\pi Y_2^0[\theta]^2 \sin \theta \, d\theta \, d\phi = \frac{4}{45} (\gamma \Delta \sigma B_0)^2. \quad [5.46]$$

For $B_0 = 11.7$ T, the predicted transverse relaxation rate constant for a ^{13}C spin with $\Delta\sigma = 200$ ppm in a molecule with $\tau_c = 10$ ns is 22 s^{-1} . An exact calculation (using expressions presented in Table 5.8; see Section 5.4.4) yields $R_2 = 22.5 \text{ s}^{-1}$. The difference between the two results arises through neglect in the present derivation of the lifetime broadening (nonadiabatic) effects of fluctuating transverse fields.

5.1.4 BLOCH, WANGSNES, AND REDFIELD THEORY

A microscopic semiclassical theory of spin relaxation was formulated by Bloch, Wangsness, and Redfield (BWR) and has proved to be the most useful approach for practical applications (15, 16). In the semiclassical approach, the spin system is treated quantum mechanically and the surroundings (the heat bath or lattice) are treated classically. This treatment suffers primarily from the defect that the spin system evolves toward a final state in which energy levels of the spin system are populated equally. Equivalently, the semiclassical theory is formally correct only for an infinite Boltzmann spin temperature; at finite temperatures, an *ad hoc* correction to the theory is required to ensure that the spin system relaxes toward an equilibrium state in which the populations are properly described by a Boltzmann distribution. A fully quantum mechanical treatment of spin relaxation overcomes this defect

and predicts the proper approach to equilibrium; however, the computational details of the quantum mechanical relaxation theory are outside the scope of this text (2, 16).

5.2 The Master Equation

In the semiclassical theory of spin relaxation, the Hamiltonian for the system is written as the sum of a deterministic quantum mechanical Hamiltonian that acts only on the spin system, \mathcal{H}_0 , and a stochastic Hamiltonian, $\mathcal{H}_1(t)$, that couples the spin system to the lattice:

$$\mathcal{H}(t) = \mathcal{H}_0 + \mathcal{H}_1(t). \quad [5.47]$$

The Hamiltonian $\mathcal{H}_1(t)$ is regarded as a time-dependent perturbation acting on the main time-independent Hamiltonian, \mathcal{H}_0 . This Hamiltonian is written in the absence of an applied rf field (see Section 5.2.3 for the effects of rf fields). The Liouville equation of motion of the density operator is (Section 2.2.3)

$$\frac{d\sigma(t)}{dt} = -i[\mathcal{H}_0 + \mathcal{H}_1(t), \sigma(t)] = -i\{\hat{L}_0 + \hat{L}_1(t)\}\sigma(t), \quad [5.48]$$

in which $\hat{L}(t) = [\mathcal{H}(t), \cdot]$ is the commutation superoperator or Liouvillian. By formally identifying \hat{L}_0 and $\hat{L}_1(t)$ with ω_0 and $\omega(t)$, respectively, the ideas used in the random-phase model are used to solve [5.48]. First, the explicit influence of \mathcal{H}_0 is removed by transforming to the new variable,

$$\sigma^T(t) = \exp(i\hat{L}_0 t)\sigma(t) = \exp(i\mathcal{H}_0 t)\sigma(t) \exp(-i\mathcal{H}_0 t), \quad [5.49]$$

in which the second equality is established by expanding the exponential factors in Taylor series. The change of variables transforms the Liouville equation into a new reference frame, which is called the *interaction frame*:

$$\begin{aligned} \frac{d\sigma^T(t)}{dt} &= i\hat{L}_0 \exp(i\hat{L}_0 t)\sigma(t) + \exp(i\hat{L}_0 t) \frac{d\sigma(t)}{dt} \\ &= i \exp(i\hat{L}_0 t)\hat{L}_0\sigma(t) - i \exp(i\hat{L}_0 t)\{\hat{L}_0 + \hat{L}_1(t)\}\sigma(t) \\ &= -i \exp(i\hat{L}_0 t)\{\hat{L}_1(t)\sigma(t)\} \\ &= -i\hat{L}_1^T(t)\sigma^T(t) \\ &= -i[\mathcal{H}_1^T(t), \sigma^T(t)], \end{aligned} \quad [5.50]$$

in which $\hat{L}_1^T(t) = [\mathcal{H}_1^T(t),]$ and

$$\mathcal{H}_1^T(t) = \hat{L}_0 \mathcal{H}_1(t) = \exp \{i\mathcal{H}_0 t\} \mathcal{H}_1(t) \exp \{-i\mathcal{H}_0 t\}. \quad [5.51]$$

The transformation into the interaction frame is isomorphous to the rotating-frame transformation; however, important differences exist between the two. The rotating-frame transformation removes the explicit time dependence of the rf Hamiltonian and renders the Hamiltonian time independent in the rotating frame. The Hamiltonian \mathcal{H}_0 is active in the rotating frame. The interaction-frame transformation removes the explicit dependence on \mathcal{H}_0 ; however, $\mathcal{H}_1^T(t)$ remains time dependent. As discussed in Section 5.2.3, the rotating-frame and interaction-frame transformations are performed sequentially in some circumstances.

Equation [5.50] is solved by the same formal approach used in the random-phase model for relaxation to yield an analog to [5.40],

$$\frac{d\sigma^T(t)}{dt} = - \int_0^\infty d\tau [\overline{\mathcal{H}_1^T(t), [\mathcal{H}_1^T(t-\tau), \sigma^T(t) - \sigma_0]}], \quad [5.52]$$

in which the overbar indicates ensemble averaging over the stochastic Hamiltonians and $\sigma^T(t)$ now designates the ensemble average of the density matrix (the overbar is omitted for convenience). This equation is subject to the following assumptions:

1. The ensemble average of $\mathcal{H}_1^T(t)$ is zero. Any components of $\mathcal{H}_1^T(t)$ that do not vanish upon ensemble averaging are incorporated into \mathcal{H}_0 .
2. $\mathcal{H}_1^T(t)$ and $\sigma^T(t)$ are uncorrelated so that the ensemble average is taken independently for each quantity.
3. The times of interest satisfy $\tau_c \ll t \ll 1/R$, in which τ_c is the characteristic correlation time for $\mathcal{H}_1^T(t)$ and R is the relevant relaxation rate constant. In liquids, τ_c is on the order of the rotational diffusion correlation time for the molecule, 10^{-12} – 10^{-7} s.
4. $\sigma^T(t)$ can be replaced by $\sigma^T(t) - \sigma_0$, in which σ_0 is the equilibrium density operator. By construction, $\sigma_0^T = \sigma_0$.

Assumptions 1, 2, and 3 are similar to assumptions made for the random-phase model derived in Section 5.1.3. Assumption 4 ensures that the spin system relaxes toward thermal equilibrium, a concern that did not arise in the random-phase model for transverse relaxation. The term σ_0 naturally enters the differential equation in a full quantum

mechanical derivation. More detailed discussions of the range of validity of these assumptions are found elsewhere (2, 3).

In order to transform [5.52] back into the laboratory frame, the transformation properties of $\mathcal{H}_1(t)$ must be established. The approach to be utilized follows the derivation of the nuclear spin Hamiltonian in Section 2.8. The stochastic Hamiltonian is decomposed as

$$\mathcal{H}_1(t) = \sum_{q=-k}^k (-1)^q F_k^{-q}(t) \mathbf{A}_k^q, \quad [5.53]$$

in which $F_k^q(t)$ is a random function of spatial variables, \mathbf{A}_k^q is a tensor spin operator, and k is the rank of the tensor (2, 17, 18). Additionally, $\mathbf{A}_k^{-q} \equiv (-1)^q \mathbf{A}_k^{q\dagger}$ and $F_k^{-q}(t) \equiv (-1)^q F_k^{q*}(t)$. For the Hamiltonians of interest in NMR spectroscopy, k is one or two, and the decomposition is always possible. The \mathbf{A}_k^q are chosen to be spherical tensor operators because these operators have simple transformation properties under rotations. To proceed, the operators \mathbf{A}_k^q are expanded in terms of basis operators

$$\mathbf{A}_k^q = \sum_p \mathbf{A}_{kp}^q \quad [5.54]$$

that satisfy the relationship

$$\hat{L}_0 \{ \mathbf{A}_{kp}^q \} \equiv [\mathcal{H}_0, \mathbf{A}_{kp}^q] = \omega_p^q \mathbf{A}_{kp}^q. \quad [5.55]$$

Here \mathbf{A}_{kp}^q and ω_p^q are called the eigenfunctions and eigenfrequencies of the Hamiltonian commutation superoperator. The index p serves to distinguish spin operators with the same order q but distinct eigenfrequencies. This additional label is important particularly for the dipolar Hamiltonian, because the interacting spins will have different eigenfrequencies in the absence of magnetic equivalence. Equation [5.55] implies the additional property

$$\exp(i\hat{L}_0 t) \mathbf{A}_{kp}^q = \exp(i\mathcal{H}_0 t) \mathbf{A}_{kp}^q \exp(-i\mathcal{H}_0 t) = \exp(i\omega_p^q t) \mathbf{A}_{kp}^q, \quad [5.56]$$

which is proved as usual by expanding the exponential factors in the Taylor series.

For example, if $\mathcal{H}_0 = \omega_I I_z + \omega_S S_z$, then the single-element operator $2I_z S^+ = I^\alpha S^+ - I^\beta S^+ = |\alpha\alpha\rangle\langle\alpha\beta| - |\beta\alpha\rangle\langle\beta\beta|$ (see [2.215]) is an

eigenoperator with eigenfrequency ω_S :

$$\begin{aligned}
 & [\mathcal{H}_0, I^\alpha S^+ - I^\beta S^+] \\
 &= (\omega_I I_z + \omega_S S_z)(|\alpha\alpha\rangle\langle\alpha\beta| - |\beta\alpha\rangle\langle\beta\beta|) \\
 &\quad - (|\alpha\alpha\rangle\langle\alpha\beta| - |\beta\alpha\rangle\langle\beta\beta|)(\omega_I I_z + \omega_S S_z) \\
 &= \omega_I(I_z|\alpha\alpha\rangle\langle\alpha\beta| - I_z|\beta\alpha\rangle\langle\beta\beta| - |\alpha\alpha\rangle\langle\alpha\beta|I_z + |\beta\alpha\rangle\langle\beta\beta|I_z) \\
 &\quad + \omega_S(S_z|\alpha\alpha\rangle\langle\alpha\beta| - S_z|\beta\alpha\rangle\langle\beta\beta| - |\alpha\alpha\rangle\langle\alpha\beta|S_z + |\beta\alpha\rangle\langle\beta\beta|S_z) \\
 &= \frac{1}{2}\omega_I(|\alpha\alpha\rangle\langle\alpha\beta| + |\beta\alpha\rangle\langle\beta\beta| - |\alpha\alpha\rangle\langle\alpha\beta| - |\beta\alpha\rangle\langle\beta\beta|) \\
 &\quad + \frac{1}{2}\omega_S(|\alpha\alpha\rangle\langle\alpha\beta| - |\beta\alpha\rangle\langle\beta\beta| + |\alpha\alpha\rangle\langle\alpha\beta| - |\beta\alpha\rangle\langle\beta\beta|) \\
 &= \omega_S(|\alpha\alpha\rangle\langle\alpha\beta| - |\beta\alpha\rangle\langle\beta\beta|) \\
 &= \omega_S(I^\alpha S^+ - I^\beta S^+). \tag{5.57}
 \end{aligned}$$

Applying [5.56], in the interaction frame,

$$\begin{aligned}
 \mathbf{A}_k^{qT} &= \exp\{i\mathcal{H}_0 t\} \mathbf{A}_k^q \exp\{-i\mathcal{H}_0 t\} = \sum_p \exp\{i\mathcal{H}_0 t\} \mathbf{A}_{kp}^q \exp\{-i\mathcal{H}_0 t\} \\
 &= \sum_p \mathbf{A}_{kp}^q \exp\{i\omega_p^q t\}, \tag{5.58}
 \end{aligned}$$

$$\mathbf{A}_k^{-qT} = \exp\{i\mathcal{H}_0 t\} \mathbf{A}_k^{-q} \exp\{-i\mathcal{H}_0 t\} = \sum_p \mathbf{A}_{kp}^{-q} \exp\{-i\omega_p^q t\}, \tag{5.59}$$

in which $\omega_p^{-q} = -\omega_p^q$. Substituting [5.53] and [5.58] into [5.52] yields

$$\begin{aligned}
 \frac{d\sigma^T(t)}{dt} &= - \sum_{q,q'} \sum_{p,p'} (-1)^{q+q'} \exp\{i(\omega_p^q + \omega_{p'}^{q'})t\} [\mathbf{A}_{kp'}^{q'}, [\mathbf{A}_{kp}^q, \sigma^T(t) - \sigma_0]] \\
 &\quad \times \int_0^\infty \overline{F_k^{-q'}(t) F_k^{-q}(t - \tau)} \exp\{-i\omega_p^q \tau\} d\tau. \tag{5.60}
 \end{aligned}$$

The random processes $F_k^q(t)$ and $F_k^{q'}(t)$ are assumed to be statistically independent unless $q' = -q$; therefore, the ensemble average in [5.60]

vanishes if $q' \neq -q$. Thus,

$$\begin{aligned} \frac{d\sigma^T(t)}{dt} = & - \sum_{q=-k}^k \sum_{p,p'} \exp\left\{i(\omega_p^q - \omega_{p'}^q)t\right\} [\mathbf{A}_{kp}^{-q}, [\mathbf{A}_{kp}^q, \sigma^T(t) - \sigma_0]] \\ & \times \int_0^\infty \overline{F_k^q(t) F_k^{-q}(t - \tau)} \exp\{-i\omega_p^q \tau\} d\tau. \end{aligned} \quad [5.61]$$

A second simplification of this equation is commonly utilized. Terms in [5.61] in which $|\omega_p^q + \omega_{p'}^{-q}| = |\omega_p^q - \omega_{p'}^q| \gg 0$ are *nonsecular* in the sense of perturbation theory (Section 5.2.2), and do not affect the long-time behavior of $\sigma^T(t)$ because the rapidly oscillating factors $\exp\{i(\omega_p^q - \omega_{p'}^q)t\}$ average to zero much more rapidly than relaxation occurs. Furthermore, if none of the eigenfrequencies are degenerate, terms in [5.61] are *secular* and nonzero only if $p = p'$. Thus,

$$\frac{d\sigma^T(t)}{dt} = - \sum_{q=-k}^k \sum_p [\mathbf{A}_{kp}^{-q}, [\mathbf{A}_{kp}^q, \sigma^T(t) - \sigma_0]] \int_0^\infty \overline{F_k^q(t) F_k^{-q}(t - \tau)} \exp\{-i\omega_p^q \tau\} d\tau. \quad [5.62]$$

The correlation functions $\overline{F_k^q(t) F_k^{-q}(t - \tau)}$ are real, even-valued, functions of τ for diffusive stochastic processes of interest in the theory of spin relaxation in macromolecules. The real part of the integral in [5.62] is called the *power spectral density function*, $j^q(\omega)$:

$$\begin{aligned} j^q(\omega) &= 2 \operatorname{Re} \left\{ \int_0^\infty \overline{F_k^q(t) F_k^{-q}(t - \tau)} \exp\{-i\omega\tau\} d\tau \right\} \\ &= \operatorname{Re} \left\{ \int_{-\infty}^\infty \overline{F_k^q(t) F_k^{-q}(t - \tau)} \exp\{-i\omega\tau\} d\tau \right\} \\ &= \operatorname{Re} \left\{ \int_{-\infty}^\infty \overline{F_k^q(t) F_k^{-q}(t + \tau)} \exp\{-i\omega\tau\} d\tau \right\}. \end{aligned} \quad [5.63]$$

The factor of two is introduced in the first line of [5.63] for convenience in extending the lower limit of the integral. Thus, the power spectral density is an even function of ω and $j^{-q}(\omega_p^{-q}) = j^{-q}(-\omega_p^q) = j^{-q}(\omega_p^q) = j^q(\omega_p^q)$. The imaginary part of the integral,

$$\begin{aligned} k^q(\omega) &= \operatorname{Im} \left\{ \int_0^\infty \overline{F_k^q(t) F_k^{-q}(t - \tau)} \exp\{-i\omega\tau\} d\tau \right\} \\ &= \operatorname{Im} \left\{ \int_0^\infty \overline{F_k^q(t) F_k^{-q}(t + \tau)} \exp\{-i\omega\tau\} d\tau \right\} \end{aligned} \quad [5.64]$$

is an odd function of ω . Consequently, $k^{-q}(\omega_p^{-q}) = k^{-q}(-\omega_p^q) = -k^{-q}(\omega_p^q) = -k^q(\omega_p^q)$, $k^q(0) = 0$, and

$$\begin{aligned}
 & \sum_{q=-k}^k \sum_p [\mathbf{A}_{kp}^{-q}, [\mathbf{A}_{kp}^q, \sigma^T(t) - \sigma_0]] k^q(\omega_p^q) \\
 &= \sum_{q=0}^k \sum_p \left\{ [\mathbf{A}_{kp}^{-q}, [\mathbf{A}_{kp}^q, \sigma^T(t) - \sigma_0]] - [\mathbf{A}_{kp}^q, [\mathbf{A}_{kp}^{-q}, \sigma^T(t) - \sigma_0]] \right\} k^q(\omega_p^q) \\
 &= \sum_{q=0}^k \sum_p [[\mathbf{A}_{kp}^{-q}, \mathbf{A}_{kp}^q], \sigma^T(t) - \sigma_0] k^q(\omega_p^q). \tag{5.65}
 \end{aligned}$$

Further, in the high-temperature limit, $\sigma_0 \propto \mathcal{H}_0$; consequently, using [5.55] yields $[[\mathbf{A}_{kp}^{-q}, \mathbf{A}_{kp}^q], \sigma_0] = 0$. With this notation (19),

$$\begin{aligned}
 \frac{d\sigma^T(t)}{dt} &= -\frac{1}{2} \sum_{q=-k}^k \sum_p [\mathbf{A}_{kp}^{-q}, [\mathbf{A}_{kp}^q, \sigma^T(t) - \sigma_0]] j^q(\omega_p^q) \\
 &\quad + i \sum_{q=0}^k \sum_p [[\mathbf{A}_{kp}^{-q}, \mathbf{A}_{kp}^q], \sigma^T(t)] k^q(\omega_p^q). \tag{5.66}
 \end{aligned}$$

Equation [5.66] is transformed to the laboratory frame to yield the Liouville–von Neumann differential equation for the density operator:

$$\frac{d\sigma(t)}{dt} = -i[\mathcal{H}_0, \sigma(t)] - i[\Delta, \sigma(t)] - \hat{\Gamma}(\sigma(t) - \sigma_0), \tag{5.67}$$

in which the relaxation superoperator is

$$\hat{\Gamma} = \frac{1}{2} \sum_{q=-k}^k \sum_p j^q(\omega_p^q) [\mathbf{A}_{kp}^{-q}, [\mathbf{A}_{kp}^q, \cdot]] \tag{5.68}$$

and

$$\Delta = - \sum_{q=0}^k \sum_p k^q(\omega_p^q) [\mathbf{A}_{kp}^{-q}, \mathbf{A}_{kp}^q]. \tag{5.69}$$

The second term in [5.67] represents second-order frequency shifts of the resonance lines, which are called dynamic frequency shifts, and Δ is called the dynamic frequency shift operator. This term has the same

form as the first term and thus Δ can be incorporated into \mathcal{H}_0 , by redefining $\mathcal{H}_0 + \Delta \rightarrow \mathcal{H}_0$, to give the final result:

$$\frac{d\sigma(t)}{dt} = -i[\mathcal{H}_0, \sigma(t)] - \hat{\Gamma}(\sigma(t) - \sigma_0). \quad [5.70]$$

The functions $j^q(\omega)$ and $k^q(\omega)$ obey the Kramers–Krönig relation and form a Hilbert transform pair. Dynamic frequency shifts are not considered further herein, but are discussed extensively elsewhere (2, 20).

Equation [5.70] is converted into an equation for product, or other basis operators, by expanding the density operator in terms of the basis operators to yield the matrix form of the master equation,

$$db_r(t)/dt = \sum_s \{-i\Omega_{rs}b_s(t) - \Gamma_{rs}[b_s(t) - b_{s0}]\}, \quad [5.71]$$

in which

$$\Omega_{rs} = \langle \mathbf{B}_r | [\mathcal{H}_0, \mathbf{B}_s] \rangle / \langle \mathbf{B}_r | \mathbf{B}_r \rangle \quad [5.72]$$

is a characteristic frequency,

$$\begin{aligned} \Gamma_{rs} &= \langle \mathbf{B}_r | \hat{\Gamma} \mathbf{B}_s \rangle / \langle \mathbf{B}_r | \mathbf{B}_r \rangle \\ &= \frac{1}{2} \sum_{q=-k}^k \sum_p \left\{ \langle \mathbf{B}_r | [\mathbf{A}_{kp}^{-q}, [\mathbf{A}_{kp}^q, \mathbf{B}_s]] \rangle / \langle \mathbf{B}_r | \mathbf{B}_r \rangle \right\} j^q(\omega_p^q) \end{aligned} \quad [5.73]$$

is the rate constant for relaxation between the operators \mathbf{B}_r and \mathbf{B}_s , and

$$b_j(t) = \langle \mathbf{B}_j | \sigma(t) \rangle / \langle \mathbf{B}_j | \mathbf{B}_j \rangle. \quad [5.74]$$

For normalized basis operators with $\text{Tr}\{\mathbf{B}_r^2\} = \text{Tr}\{\mathbf{B}_s^2\}$, $\Gamma_{rs} = \Gamma_{sr}$.

Equations [5.71]–[5.74] are the main results of this section for relaxation in the laboratory reference frame. As shown by [5.71], the evolution of the base operators for a spin system is described by a set of coupled differential equations. Diagonal elements Γ_{rr} are the rate constants for auto- or self-relaxation of \mathbf{B}_r ; off-diagonal elements Γ_{rs} are the rate constants for cross-relaxation between \mathbf{B}_r and \mathbf{B}_s . Cross-relaxation between operators with different coherence orders is precluded as a consequence of restricting [5.60] (and hence [5.71]) to terms satisfying $q' = -q$. For example, cross-relaxation does not occur between zero- and single-quantum coherence. Furthermore, if none of the transitions in the spin system is degenerate (to within approximately a linewidth), then cross-relaxation rate constants between off-diagonal elements of the density operator in the laboratory reference frame are

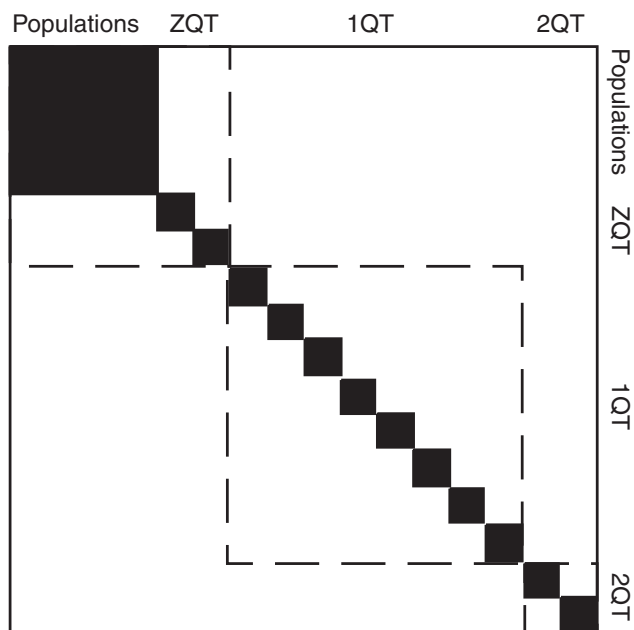


FIGURE 5.4 Redfield kite. Solid blocks indicate nonzero relaxation rate constants between operators in the absence of degenerate transitions. Populations have nonzero cross-relaxation rate constants, but all other coherences relax independently. If transitions are degenerate, the dashed-outline blocks indicate the additional nonzero cross-relaxation rate constants observed between coherences with the same coherence level.

also zero through the secular approximation. Consequently, the matrix of relaxation rate constants between operators has a characteristic block diagonal form, known as the Redfield kite, illustrated in Fig. 5.4.

Two critical requirements for a stochastic Hamiltonian to be effective in causing relaxation are encapsulated in [5.70] and [5.71]: (i) the double commutator $[\mathbf{A}_{kp}^{-q}, [\mathbf{A}_{kp}^q, \sigma(t) - \sigma_0]]$ must not vanish and (ii) the spectral density function for the random process that modulates the spin interactions must have significant components at the characteristic frequencies of the spin system, ω_p^q . The former requirement can be regarded as a kind of selection rule for whether the term in the stochastic Hamiltonian that depends upon the operator \mathbf{A}_{kp}^q is effective in causing relaxation of the density operator. In most cases, the stochastic random process is a consequence of molecular reorientational motions. This observation is central to the dramatic differences in spin relaxation and, thus, in NMR spectroscopy, of rapidly rotating small

molecules and slowly rotating macromolecules. Calculation of relaxation rate constants involves two steps: (i) calculation of the double commutator and trace formation over the spin variables and (ii) calculation of the spectral density function. These two calculations are pursued in the following sections.

5.2.1 INTERFERENCE EFFECTS

In many instances, more than one stochastic Hamiltonian capable of causing relaxation of a given spin may be operative. In this circumstance, [5.53] is generalized to

$$\mathcal{H}_1(t) = \sum_m \sum_{q=-k}^k (-1)^q F_{mk}^{-q}(t) \mathbf{A}_{mk}^q, \quad [5.75]$$

in which the summation over the index m refers to the different relaxation interactions or stochastic Hamiltonians. Using [5.75] rather than [5.53] in this derivation leads once more to [5.71], with Γ_{rs} given by a generalization of [5.73]

$$\begin{aligned} \Gamma_{rs} &= \frac{1}{2} \sum_m \sum_q \sum_p \left\{ \left\langle \mathbf{B}_r | [\mathbf{A}_{mkp}^{-q}, [\mathbf{A}_{mkp}^q, \mathbf{B}_s]] \right\rangle / \langle \mathbf{B}_r | \mathbf{B}_r \rangle \right\} j^q(\omega_p^q) \\ &\quad + \frac{1}{2} \sum_{\substack{m,n \\ m \neq n}} \sum_q \sum_p \left\{ \left\langle \mathbf{B}_r | [\mathbf{A}_{mkp}^{-q}, [\mathbf{A}_{nkp}^q, \mathbf{B}_s]] \right\rangle / \langle \mathbf{B}_r | \mathbf{B}_r \rangle \right\} j_{mn}^q(\omega_p^q) \\ &= \sum_m \Gamma_{rs}^m + \sum_{\substack{m,n \\ m \neq n}} \Gamma_{rs}^{mn}, \end{aligned} \quad [5.76]$$

in which the cross-spectral density is

$$j_{mn}^q(\omega) = \text{Re} \left\{ \int_{-\infty}^{\infty} \overline{F_{mk}^q(t) F_{nk}^{-q}(t + \tau)} \exp\{-i\omega\tau\} d\tau \right\}. \quad [5.77]$$

Here Γ_{rs}^m is the autorelaxation rate constant due to the m th relaxation mechanism and Γ_{rs}^{mn} is the relaxation rate constant arising from interference or cross-correlation between the m th and n th relaxation mechanisms.

Clearly, $j_{mn}^q(\omega) = 0$ unless the random processes $F_{mk}^q(t)$ and $F_{nk}^q(t)$ are correlated. In the absence of correlation between the different relaxation mechanisms, $\Gamma_{rs}^{mn} = 0$ for all m and n and each mechanism contributes additively to relaxation of the spin system.

The two most frequently encountered interference or cross-correlation effects in biological macromolecules arise from interference between dipolar and anisotropic chemical shift interactions and interference between the dipolar interactions of different pairs of spins. The prototypical example of the former is the interference between the dipolar and CSA interactions for ^{15}N (21). The prototypical example of the latter is the interference between the dipolar interactions in an I_2S or I_3S spin system such as a methylene (I_2 represents the two methylene ^1H spins; S represents either a remote ^1H or the methylene ^{13}C) or methyl group (I_3 represents the three methyl ^1H spins; S represents either a remote ^1H or the methyl ^{13}C) (10). Interference effects can result in cross-relaxation between pairs of operators for which cross-relaxation would not be observed otherwise. The observation of otherwise “forbidden” cross-relaxation pathways is one of the hallmarks of interference effects (22). Relaxation interference also forms the basis for Transverse Relaxation Optimized Spectroscopy (TROSY), in which interference between relaxation mechanisms is used to obtain narrower resonance linewidths (Chapter 7).

5.2.2 LIKE SPINS, UNLIKE SPINS, AND THE SECULAR APPROXIMATION

A distinction frequently is made between like and unlike spins and relaxation rate constants are derived independently for each case (2). Like spins are defined as spins with identical Larmor frequencies and unlike spins are defined as spins with widely different Larmor frequencies. Such distinctions can obscure the generality of the theory embodied in [5.71]. In actuality, the presence of spins with degenerate Larmor frequencies has straightforward consequences for relaxation. First, particular operators \mathbf{A}_{kp}^q in [5.53] may become degenerate (i.e., have the same eigenfrequency, ω_p^q) and are therefore secular with respect to each other. Thus, prior to applying the secular condition, the set of \mathbf{A}_{kp}^q must be redefined as

$$\mathbf{A}_{kp}^q = \sum_m \mathbf{A}_{km}^q, \quad [5.78]$$

in which the summation extends over the operators for \mathbf{A}_{km}^q for which $\omega_p^q = \omega_m^q$. For example, operators with eigenfrequencies of 0 and $\omega_I - \omega_S$ belong to different orders p for unlike spins; the eigenfrequencies are degenerate for like spins and the corresponding operators would be summed to yield a single operator with eigenfrequency of zero. Second,

for spins that are magnetically equivalent, such as the three ^1H spins in a methyl group, basis operators that exhibit the maximum symmetry of the chemical moiety are derived using group theory (18, 23). Although such basis operators simplify the resulting calculations, the group theoretical treatment of relaxation of magnetically equivalent spins is beyond the scope of the present text; the interested reader is referred to the original literature (18, 23). The following discussions focus on spin systems without degenerate transitions. Results of practical interest that arise as a consequence of degeneracy are presented as necessary.

The concepts of like and unlike spins arise as limiting cases because the secular approximation has been imposed in deriving [5.62] from [5.61]. This assumption, although widely applied, is not necessary to relaxation theory (6). If the secular approximation is not applied, then [5.61] is transformed directly to the laboratory frame to yield

$$\begin{aligned} \frac{d\sigma(t)}{dt} = & -i[\mathcal{H}_0, \sigma(t)] \\ & - \sum_{q=-k}^k \sum_{p,p'} [\mathbf{A}_{kp'}^{-q}, [\mathbf{A}_{kp}^q, \sigma(t) - \sigma_0]] \int_0^\infty \overline{F_k^q(t) F_k^{-q}(t-\tau)} \exp\{-i\omega_p^q \tau\} d\tau. \end{aligned} \quad [5.79]$$

Ignoring dynamic frequency shifts for simplicity, this equation is identical to [5.70] except that the relaxation superoperator is redefined as

$$\hat{\Gamma} = \frac{1}{2} \sum_{q=-k}^k \sum_{p,p'} j^q(\omega_p^q) [\mathbf{A}_{kp'}^{-q}, [\mathbf{A}_{kp}^q, \cdot]]. \quad [5.80]$$

Thus, if the secular approximation is not invoked, then the relaxation superoperator contains additional terms.

To illustrate the main consequences of not utilizing the secular approximation, [5.61] in the interaction frame will be analyzed again. The density operator is expanded in the set of eigenoperators of the Hamiltonian commutation superoperator,

$$\sigma(t) = \sum_s b_s(t) \mathbf{B}_s, \quad [5.81]$$

in which, for simplicity, \mathbf{B}_s is used to represent the eigenoperators, rather than \mathbf{A}_{kp}^q , and the index s runs over all combinations of q and p . Using [5.56],

$$\sigma^T(t) = \sum_s b_s(t) e^{i\omega_s t} \mathbf{B}_s. \quad [5.82]$$

Equation [5.61] is written as

$$\begin{aligned} \frac{db_r(t)}{dt} = & -i\omega_r b_r(t) - \sum_s (b_s(t) - b_{s0} e^{-i\omega_s t}) \sum_q \sum_{p,p'} \exp\left\{i(\omega_s - \omega_r + \omega_p^q - \omega_{p'}^q)t\right\} \\ & \times \left\{ \langle \mathbf{B}_r | [\mathbf{A}_{kp'}^{-q}, [\mathbf{A}_{kp}^q, \mathbf{B}_s]] \rangle / \langle \mathbf{B}_r | \mathbf{B}_r \rangle \right\} \int_0^\infty \overline{F_k^q(t) F_k^{-q}(t-\tau)} \exp\{-i\omega_p^q \tau\} d\tau. \end{aligned} \quad [5.83]$$

The rate constant for relaxation between the operators \mathbf{B}_r and \mathbf{B}_s is given by:

$$\Gamma_{rs} = \frac{1}{2} \sum_q \sum_{p,p'} \left\{ \langle \mathbf{B}_r | [\mathbf{A}_{kp'}^{-q}, [\mathbf{A}_{kp}^q, \mathbf{B}_s]] \rangle / \langle \mathbf{B}_r | \mathbf{B}_r \rangle \right\} j^q(\omega_p^q), \quad [5.84]$$

provided that $\omega_s - \omega_r + \omega_p^q - \omega_{p'}^q = 0$. This condition can be regarded as a generalization of the secular requirement that $\omega_p^q - \omega_{p'}^q = 0$. For autorelaxation, $r=s$ and the usual secular condition is obtained; thus, the autorelaxation rate constants are not affected by whether or not the secular condition is applied. If $r \neq s$, then [5.84] predicts that additional cross-relaxation rate constants will be nonzero compared to results obtained from [5.73] when the secular approximation is utilized. If only two operators are considered, then

$$\frac{d}{dt} \begin{bmatrix} b_r(t) \\ b_s(t) \end{bmatrix} = \begin{bmatrix} -i\omega_r - \rho_r & -\eta \\ -\eta & -i\omega_s - \rho_s \end{bmatrix} \begin{bmatrix} b_r(t) - b_{r0} \\ b_s(t) - b_{s0} \end{bmatrix}, \quad [5.85]$$

in which $\rho_k = \Gamma_{kk}$ and $\eta = \Gamma_{rs}$. The eigenvalues of this equation are

$$\lambda_{\pm} = i\bar{\omega} - \bar{\rho} \pm \frac{1}{2} (-\Delta\omega^2 + \Delta\rho^2 + 4\eta^2 - 2i\Delta\omega\Delta\rho)^{1/2}, \quad [5.86]$$

in which $\bar{\omega} = (\omega_r + \omega_s)/2$, $\bar{\rho} = (\rho_r + \rho_s)/2$, $\Delta\omega = (\omega_r - \omega_s)$, and $\Delta\rho = (\rho_r - \rho_s)$. Whenever $|\Delta\omega^2 - \Delta\rho^2| \gg 4\eta^2$, η can be neglected in [5.85] and each operator evolves independently. Essentially, cross-relaxation between two basis operators is negligible if the difference between eigenfrequencies is greater than the linewidth. The additional cross-relaxation pathway predicted by neglect of the secular approximation is suppressed because the two operators evolve relative to each other. Over a time period $t = 2\pi/\Delta\omega$, the instantaneous effect of cross-relaxation is averaged to zero. As $\omega_s \rightarrow \omega_r$, $\Delta\omega \rightarrow 0$, and cross-relaxation gradually becomes effective. When the secular approximation is not imposed, distinctions between like and unlike spins do not arise because the relative precession frequencies of pairs of operators naturally control

which cross-relaxation terms will be effective. The drawback to the neglect of the secular approximation is that many more terms must be evaluated in the summations in [5.84] compared with [5.73]. Dipolar relaxation in a scalar-coupled spin system, discussed in Section 5.4.2, is a practical example of the concepts discussed herein.

5.2.3 RELAXATION IN THE ROTATING FRAME

In the presence of an applied rf field [for example, in a Rotating-Frame Overhauser Effect Spectroscopy (ROESY) or TOCSY experiment], the transformation into the interaction frame involves, first, a transformation into a rotating frame to remove the time dependence of $\mathcal{H}_{\text{rf}}(t)$, followed by transformation into the interaction frame of the resulting time-independent Hamiltonian. If $\mathcal{H}_0 \approx \mathcal{H}_z$ — that is if the Zeeman Hamiltonian is dominant (i.e., ignoring the scalar coupling Hamiltonian) — then the interaction frame is equivalent to a doubly rotating tilted frame. As a consequence of the initial rotating-frame transformation, the eigenfrequencies ω_p^q used as arguments of the spectral density function $j^q(\omega_p^q)$ are modified to $\omega_p^q + \omega_p^{q(\text{rf})}$, in which $\omega_p^{q(\text{rf})}$ is defined by:

$$\sum_{i=1}^K \omega_{\text{rf},i} [I_{zi}, \mathbf{A}_{kp}^q] = \omega_p^{q(\text{rf})} \mathbf{A}_{kp}^q, \quad [5.87]$$

in which $\omega_{\text{rf},i}$ is the frequency of the rotating frame for the i th spin and K is the number of irradiated spins in the spin system. Homonuclear spin operators transform identically under the rotating-frame transformation; therefore, the relaxation superoperator in the rotating frame is calculated as for like spins (Section 5.2.2). For macromolecules with $\omega_1 \tau_c \ll 1$, in which ω_1 is the strength of the applied rf field and τ_c is the rotational correlation time of the molecule, $j^q(\omega_p^q + \omega_p^{q(\text{rf})}) \approx j^q(\omega_p^q)$ (Section 5.3). In this case, approximate values for the relaxation rate constants in the rotating frame are calculated using [5.73] by first transforming the operators in the tilted frame, \mathbf{B}'_r and \mathbf{B}'_s to the laboratory frame. Thus (24),

$$\Gamma'_{rs} = \langle \mathbf{U}^{-1} \mathbf{B}'_r \mathbf{U} | \hat{\Gamma} \{ \mathbf{U}^{-1} \mathbf{B}'_s \mathbf{U} \} \rangle / (\mathbf{B}'_r | \mathbf{B}'_r). \quad [5.88]$$

For an rf field applied with x -phase, the transformation \mathbf{U} is defined as a y -rotation,

$$\mathbf{U} = \exp \left\{ i \sum_{i=1}^K \theta_i I_{yi} \right\}, \quad [5.89]$$

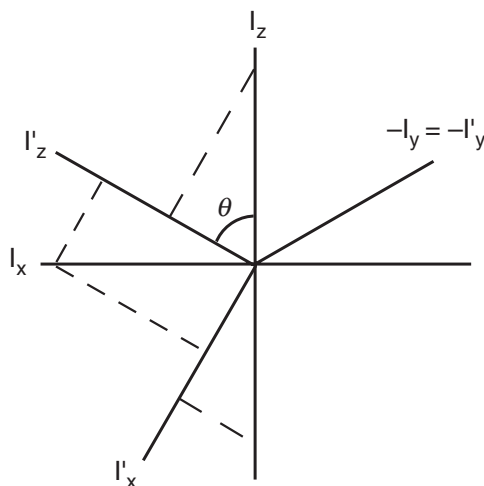


FIGURE 5.5 Relative orientations of the laboratory and tilted reference frames used to determine the transformation [5.89].

in which θ_i is defined by [1.21] (if \mathbf{B}'_r and \mathbf{B}'_s refer to different spins, then θ_i may differ for each spin). The relative orientations of the tilted and untilted reference frames are illustrated in Fig. 5.5 for a single spin. If $\theta = 0$, either because $\omega_1 = 0$ or because $\omega_1 \ll |\omega_0 - \omega_{rf}|$, then [5.88] reduces to [5.73].

In general, operators that do not commute with the Hamiltonian in the rotating frame decay rapidly as a consequence of rf inhomogeneity (Section 3.5.3). Thus, if a cw rf field is applied, as in a basic ROESY experiment, only operators with effective frequencies in the rotating frame equal to zero must be considered: such operators are usually limited to longitudinal operators and homonuclear zero-quantum operators. If the rf field is phase modulated to compensate for resonance offset and rf inhomogeneity, e.g., by applying a suitably constructed coherent decoupling scheme, such as DIPSI-2 (decoupling in the presence of scalar interaction), single- and multiple-quantum operators also must be considered (25). For operators containing transverse components in the rotating frame, the relaxation rate constant given by [5.88] is an instantaneous rate constant; the effective average rate constant is obtained by averaging the rate constant over the trajectory followed by the operator under the influence of the Hamiltonian in the rotating frame (26).

5.3 Spectral Density Functions

A general expression for the spectral density function is given by [5.63]. As discussed elsewhere, for relaxation in isotropic liquids in the high-temperature limit (27),

$$j^q(\omega) = (-1)^q j^0(\omega) \equiv (-1)^q j(\omega), \quad [5.90]$$

therefore, only one spectral density function need be calculated. The relaxation mechanisms of interest in the present context arise from tensorial operators of rank $k=2$. The random functions $F_2^0(t)$ are factored to give

$$F_2^0(t) = c_0(t) Y_2^0[\Omega(t)] \quad [5.91]$$

and, consequently,

$$\begin{aligned} j(\omega) &= \text{Re} \left\{ \int_{-\infty}^{\infty} \overline{c_0(t)c_0(t+\tau) Y_2^0[\Omega(t)] Y_2^0[\Omega(t+\tau)]} \exp(-i\omega\tau) d\tau \right\} \\ &= \text{Re} \left\{ \int_{-\infty}^{\infty} C(\tau) \exp(-i\omega\tau) d\tau \right\}, \end{aligned} \quad [5.92]$$

in which the *stochastic correlation function* is given by

$$C(\tau) = \overline{c_0(t)c_0(t+\tau) Y_2^0[\Omega(t)] Y_2^0[\Omega(t+\tau)]}, \quad [5.93]$$

where $c_0(t)$ is a function of physical constants and spatial variables, $Y_2^0[\Omega(t)]$ is a modified second-order spherical harmonic function, and $\Omega(t) = \{\theta(t), \phi(t)\}$ are polar angles in the laboratory reference frame. The polar angles define the orientation of a unit vector that points in the principal direction for the interaction. For the dipolar interaction, the unit vector points along the line between the two nuclei (or between the nucleus and the electron for paramagnetic relaxation). For CSA interaction with an axially symmetric chemical shift tensor, the unit vector is collinear with the symmetry axis of the tensor. For the quadrupolar interaction, the unit vector is collinear with the symmetry axis of the electric field gradient tensor. The modified spherical harmonics are given in Table 5.1 (28). The functions $c_0(t)$ for dipolar, CSA, and quadrupolar interactions are given in Table 5.2. As a molecule rotates stochastically in solution due to Brownian motion, the oscillating magnetic fields produced are not distributed uniformly over all frequencies. A small organic molecule tumbles at a greater rate as compared to a biological macromolecule in the same solvent, and the

TABLE 5.2
Spatial Functions for Relaxation Mechanisms

Interaction	$c(t)$
Dipolar	$-\sqrt{6}(\mu_0/4\pi)\hbar\gamma_I\gamma_S r_{IS}(t)^{-3}$
CSA ^a	$\Delta\sigma\gamma_I B_0/\sqrt{3}$
Quadrupolar ^b	$e^2qQ/[4\hbar I(2I-1)]$

^aThe chemical shift tensor is assumed to be axially symmetric with principal values $\sigma_{zz} = \sigma_{\parallel}$, $\sigma_{xx} = \sigma_{yy} = \sigma_{\perp}$, and $\Delta\sigma = \sigma_{\parallel} - \sigma_{\perp}$.

^b Q is the nuclear quadrupole moment and e is the charge of the electron. The electric field gradient tensor is assumed to be axially symmetric with the principal value of the field gradient defined by $V_{zz} = eq$, and $V_{xx} = V_{yy}$.

distribution of oscillating magnetic fields resulting from rotational diffusion of the two molecules will be different. The power spectral density function measures the contribution to orientational (rotational) dynamics of the molecule from motions with frequency components in the range ω to $\omega + d\omega$.

For a rigid spherical molecule undergoing rotational Brownian motion, $c_0(t) = c_0$ is a constant and the autospectral density function is

$$j(\omega) = d_{00}J(\omega), \quad [5.94]$$

in which the orientational spectral density function is

$$J(\omega) = \text{Re} \left\{ \int_{-\infty}^{\infty} C_{00}^2(\tau) \exp\{-i\omega\tau\} d\tau \right\}, \quad [5.95]$$

the orientational correlation function is

$$C_{00}^2(\tau) = \overline{Y_2^0[\Omega(t)]Y_2^0[\Omega(t+\tau)]}, \quad [5.96]$$

and $d_{00} = c_0^2$. For *isotropic* rotational diffusion of a rigid rotor or spherical top, the correlation function is given by (10)

$$C_{00}^2(\tau) = \frac{1}{5} \exp[-\tau/\tau_c], \quad [5.97]$$

in which the *correlation time*, τ_c , is approximately the average time for the molecule to rotate by 1 radian. The correlation time varies due to molecular size, solvent viscosity, and temperature, but generally τ_c is of the order of picoseconds for small molecules and of the order of

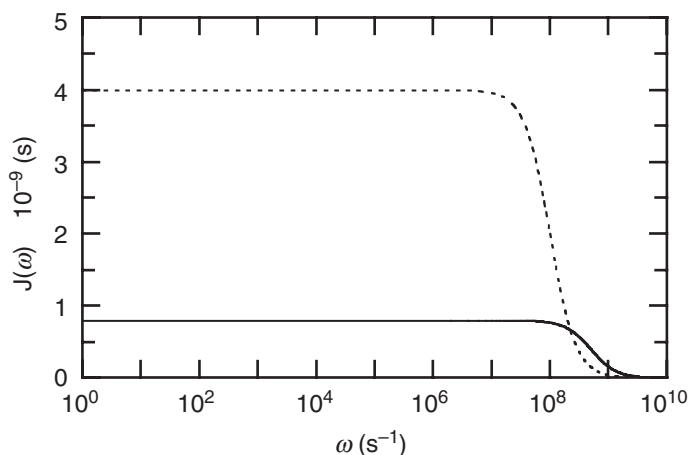


FIGURE 5.6 Spectral density functions for an isotropic rotor. Calculations were performed using [5.98] with (—) $\tau_c = 2$ ns and (···) $\tau_c = 10$ ns.

nanoseconds for biological macromolecules in aqueous solution (Section 1.4). The corresponding spectral density function is

$$J(\omega) = \frac{2}{5} \frac{\tau_c}{(1 + \omega^2 \tau_c^2)}. \quad [5.98]$$

The functional form of the spectral density function for a rigid rotor is Lorentzian; a graph of $J(\omega)$ versus ω is shown in Fig. 5.6. The logarithmic plot of $J(\omega)$ is relatively constant for $\omega^2 \tau_c^2 \ll 1$ and then begins to decrease rapidly at $\omega^2 \tau_c^2 \approx 1$. If molecular motion is sufficiently rapid to satisfy $\omega_p^2 \tau_c^2 \ll 1$ for $\omega_p^q \neq 0$, then $J(\omega_p^q) \approx J(0)$. This limit is called the *extreme narrowing* regime. For sufficiently slow molecular motion, $\omega_p^2 \tau_c^2 \gg 1$, then $J(\omega_p^q) \propto \omega_p^{q-2}$. This limit is called the *slow tumbling*, or *spin diffusion* regime.

Local fields are modulated stochastically by relative motions of nuclei in a molecular reference frame as well as by overall rotational Brownian motion. Rigorously for isotropic rotational diffusion and approximately for anisotropic rotational diffusion, the total correlation function is factored as (29)

$$C(\tau) = C_O(\tau) C_I(\tau). \quad [5.99]$$

The correlation function for overall motion, $C_O(\tau)$, is given by [5.96] or [5.97]. The correlation function for internal motions, $C_I(\tau)$, is given

by [5.93], in which the orientational variables are defined in a fixed molecular reference frame, rather than the laboratory reference frame. Calculations of $C_l(\tau)$ have been performed for a number of diffusion and lattice jump models for internal motions.

Rather than describing in detail calculations of spectral density functions for diffusion and jump models of intramolecular motions, two useful limiting cases of N -site models are given without proof [see Brüschweiler (17) for a more extensive review]. The N -site lattice jump models assume that the nuclei of the relevant spins jump instantaneously between N allowed conformations. Therefore, the transition rates reflect the lifetimes of each conformation. The spectral density function depends upon the time scale of the variation in the spatial variables, $c_0(t)$. If the transition rates between sites approaches zero, then

$$j(\omega) = J(\omega) \sum_{k=1}^N p_k c_{0k}^2 = J(\omega) \overline{c_0^2}, \quad [5.100]$$

in which p_k is the population and c_{0k} is the value of the spatial function for site k . If the transition rates between sites approaches infinity, then

$$j(\omega) = J(\omega) \sum_{q=-2}^q \left| \sum_{k=1}^N p_k c_{0k} Y_2^q(\Omega_k) \right|^2 = J(\omega) \sum_{q=-2}^2 \left| \overline{c_0 Y_2^q(\Omega)} \right|^2, \quad [5.101]$$

in which Ω_k are the polar angles for site k .

An extremely useful treatment that incorporates intramolecular motions in addition to overall rotational motion is provided by the Lipari-Szabo model free formalism (29, 30). In this treatment, the spectral density function is given by

$$j(\omega) = \frac{2}{5} \overline{c_0^2} \left[\frac{S^2 \tau_c}{1 + (\omega \tau_c)^2} + \frac{(1 - S^2) \tau}{1 + (\omega \tau)^2} \right], \quad [5.102]$$

in which $\tau^{-1} = \tau_c^{-1} + \tau_e^{-1}$, S^2 is the square of the generalized order parameter that characterizes the amplitude of intramolecular motions in a molecular reference frame, and τ_e is the effective correlation time for internal motions. The order parameter is defined by

$$S^2 = \left[\overline{c_0^2} \right]^{-1} \sum_{q=-2}^2 \left| \overline{c_0 Y_2^q(\Omega)} \right|^2, \quad [5.103]$$

in which the overbar indicates an ensemble average performed over the equilibrium distribution of orientations Ω in the molecular reference

frame. The order parameter satisfies the inequality, $0 \leq S^2 \leq 1$, in which lower values indicate larger amplitudes of internal motions. A significant advantage of the Lipari-Szabo formalism is that specification of the microscopic motional model is not required. If τ_c approaches infinity, [5.102] reduces to the same form as [5.100]; if τ_c approaches zero, [5.102] reduces to the same form as [5.101]. Equation [5.102] has been used extensively to analyze spin relaxation in proteins (31, 32).

The expressions given in [5.100], [5.101], and [5.102] are commonly encountered in discussions of dipolar relaxation between two spins, I and S . Using $c_0(t)$ from Table 5.2 gives

$$j(\omega) = \zeta J(\omega) \overline{r_{IS}^{-6}}, \quad [5.104]$$

$$j(\omega) = \zeta J(\omega) \sum_{q=-2}^2 \left| \frac{Y_2^q(\Omega_k)}{r_{IS}^3} \right|^2, \quad [5.105]$$

$$j(\omega) = \frac{2}{5} \zeta \overline{r_{IS}^{-6}} \left[\frac{S^2 \tau_c}{1 + (\omega \tau_c)^2} + \frac{(1 - S^2) \tau}{1 + (\omega \tau)^2} \right], \quad [5.106]$$

$$S^2 = \left[\overline{r_{IS}^{-6}} \right]^{-1} \sum_{q=-2}^2 \left| \frac{\overline{Y_2^q(\Omega)}}{r_{IS}^3} \right|^2, \quad [5.107]$$

in which $\zeta = 6[(\mu_0/4\pi)\hbar\gamma_I\gamma_S]^2$. Equation [5.104] (slow internal motion) is called “ r^{-6} averaging” and [5.105] (fast internal motion) is called “ r^{-3} averaging” with respect to the conformations of the molecule. The former equation is appropriate for treating the effects of aromatic ring flips and the latter equation is appropriate for treating methyl group rotations (33, 34).

The spectral density function [5.100] can be modified to include cross-correlation between relaxation interactions with fixed relative orientations (35). The cross-spectral density function is given by

$$j_{mn}(\omega) = \overline{c_0^m c_0^n} P_2(\cos \theta_{mn}) J(\omega), \quad [5.108]$$

in which $P_2(x) = (3x^2 - 1)/2$, and θ_{mn} is the angle between the principal axes of the two interactions. The cross-spectral density function for the Lipari-Szabo model free formalism is given by

$$j_{mn}(\omega) = \frac{2}{5} \overline{c_0^m c_0^n} \left[\frac{S_{mn}^2 \tau_c}{1 + (\omega \tau_c)^2} + \frac{\{P_2(\cos \theta_{mn}) - S_{mn}^2\} \tau}{1 + (\omega \tau)^2} \right], \quad [5.109]$$

in which

$$S_{mm}^2 = [c_0^m c_0^n]^{-1} \sum_{q=-2}^2 \left| \overline{c_0^m Y_2^q(\Omega_m)} \right| \left| \overline{c_0^n Y_2^q(\Omega_n)} \right|. \quad [5.110]$$

Other expressions for $j(\omega)$ have been derived for molecules that exhibit anisotropic rotational diffusion or specific internal motional models (10). Spin relaxation measurements have proved to be a powerful approach for experimental investigation of the rotational diffusion anisotropy of macromolecules (36–38).

5.4 Relaxation Mechanisms

A very large number of physical interactions give rise to stochastic Hamiltonians capable of mediating spin relaxation. In the present context, only the intramolecular magnetic dipolar, anisotropic chemical shift, quadrupolar, and scalar coupling interactions will be discussed. Intramolecular paramagnetic relaxation has the same Hamiltonian as for nuclear dipolar relaxation, except that the interaction occurs between a nucleus and an unpaired electron. Other relaxation mechanisms are of minor importance for macromolecules or are only of interest in very specialized cases. For spin-1/2 nuclei in diamagnetic biological macromolecules, the dominant relaxation mechanisms are the magnetic dipolar and anisotropic chemical shift mechanisms. For nuclei with spin $> 1/2$, notably ^{14}N and ^2H in proteins, the dominant relaxation mechanism is the quadrupolar interaction.

Relaxation rate constants for nuclei in proteins depend upon a large number of factors, including overall rotational correlation times, internal motions, the geometrical arrangement of nuclei, and the relative strengths of the applicable relaxation mechanisms. If the overall correlation time and the three-dimensional structural coordinates of the protein are known, relaxation rate constants are calculated in a relatively straightforward manner using expressions derived in the following sections. In general, ^1H relaxation in proteins is dominated by dipolar interactions with other ^1H spins (within approximately 5 Å) and by interactions with directly bonded heteronuclei. The latter arise from dipolar interactions with ^{13}C and ^{15}N in labeled proteins or from scalar relaxation of the second kind between the quadrupolar ^{14}N nuclei and amide ^1H nuclei. Relaxation of protonated ^{13}C and ^{15}N heteronuclei is dominated by dipolar interactions with the directly bonded ^1H spins, and secondarily by CSA (for ^{15}N spins and aromatic ^{13}C spins). Relaxation

of unprotonated heteronuclei is dominated by CSA interactions and dipolar interactions with remote ^1H spins.

5.4.1 INTRAMOLECULAR DIPOLAR RELAXATION FOR IS SPIN SYSTEM

Any nucleus with a nonzero spin angular momentum generates an instantaneous magnetic dipolar field that is proportional to the magnetic moment of the nucleus. As the molecule tumbles in solution, this field fluctuates and constitutes a mechanism for relaxation of nearby spins. Most importantly for structure elucidation, the efficacy of dipolar relaxation depends on the nuclear moments and on the *inverse sixth power of the distance between the interacting nuclei*. As a result, nuclear spin relaxation can be used to determine distances between nuclei. Hydrogen nuclei have a large magnetogyric ratio; therefore, dipole–dipole interactions cause the most efficient relaxation of ^1H spins and constitute a sensitive probe for internuclear distances.

Initially, a two-spin system, IS , will be considered with $\omega_I \gg \omega_S$ and scalar coupling constant $J_{IS} = 0$. The energy levels of the spin system and the associated transition frequencies are shown in Fig. 5.7. The terms A_{2p}^q are given in Table 5.3. The spatial functions for the different interactions are given in Tables 5.1 and 5.2.

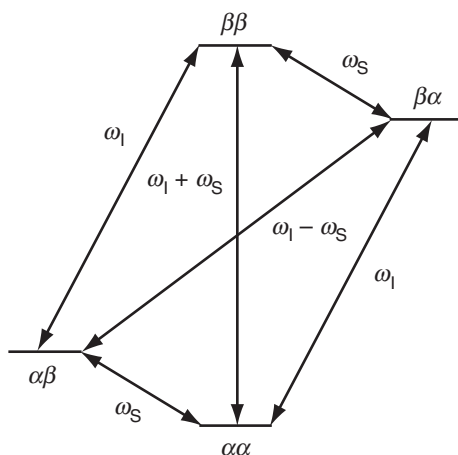


FIGURE 5.7 Transitions and associated eigenfrequencies for a two-spin system.

TABLE 5.3
Tensor Operators for the Dipolar Interaction

q	p	A_{2p}^q	$A_{2p}^{-q} = (-1)^q A_{2p}^{q\dagger}$	ω_p^q
0	0	$(2/\sqrt{6})I_z S_z$	$(2/\sqrt{6})I_z S_z$	0
0	-1	$-1/(2\sqrt{6})I^- S^+$	$-1/(2\sqrt{6})I^+ S^-$	$\omega_S - \omega_I$
0	1	$-1/(2\sqrt{6})I^+ S^-$	$-1/(2\sqrt{6})I^- S^+$	$\omega_I - \omega_S$
1	0	$-(1/2)I_z S^+$	$(1/2)I_z S^-$	ω_S
1	1	$-(1/2)I^+ S_z$	$(1/2)I^- S_z$	ω_I
2	0	$(1/2)I^+ S^+$	$(1/2)I^- S^-$	$\omega_I + \omega_S$

TABLE 5.4
Commutator Relationships^a

$$\begin{aligned}
 [I_x, I_y] &= iI_z \\
 [I_\alpha, 2I_\beta S_\gamma] &= 2[I_\alpha, I_\beta]S_\gamma \\
 [2I_\alpha S_\gamma, 2I_\beta S_\epsilon] &= [I_\alpha, I_\beta]\delta_{\gamma\epsilon}
 \end{aligned}$$

^a $I_\alpha = I_x, I_y$, or I_z ; $S_\gamma = S_x, S_y$, or S_z .
Equivalent expressions for S operators
are obtained by exchanging I
and S labels; $\delta_{\gamma\epsilon}$ is the Kronecker
delta.

The relaxation rate constants are calculated using [5.73]. To aid in the calculation of the double commutators, the commutation relations given in Table 5.4 are useful. To begin, the identity operator is disregarded because it has no effect on the relaxation equations. Next, the zero-order block consists of the operators with total coherence order equal to zero, I_z , S_z , $2I_z S_z$, $I^+ S^-$, and $I^- S^+$, and has dimension 5×5 . The operators with coherence order equal to ± 1 form a series of blocks of dimension 2×2 : $\{I^+, 2I^+ S_z\}$, $\{I^-, 2I^- S_z\}$, $\{S^+, 2I_z S^+\}$, and $\{S^-, 2I_z S^-\}$. The operators with coherence order equal to ± 2 form a series of blocks of dimension 1×1 : $\{I^- S^-\}$ and $\{I^+ S^+\}$. Because of the secular approximation, the longitudinal operators I_z , S_z , and $2I_z S_z$ do not cross-relax with the zero-quantum operators $I^+ S^-$ and $I^- S^+$. Dipolar cross-relaxation between the operators $2I_z S_z$ and I_z or between $2I_z S_z$ and S_z does not occur; therefore, the $2I_z S_z$ operator relaxes independently of the I_z and S_z operators. Similarly, dipolar cross-relaxation between

in-phase and antiphase operators, such as I^+ , $2I^+S_z$, does not occur. These results are obtained by direct calculation of the cross-relaxation rate constants or are anticipated using the symmetry of the dipole Hamiltonian and group theory arguments beyond the scope of this text (10, 18, 23). Cross-relaxation between these operators does arise due to interference between dipolar and CSA relaxation mechanisms (Section 5.5.4) (21).

The relaxation matrix for the zero-order block for longitudinal magnetization has dimensions 2×2 , with individual elements, Γ_{rs} , giving the rate constant for relaxation between operators \mathbf{B}_r and \mathbf{B}_s for $r, s = \{1, 2\}$, $\mathbf{B}_1 = I_z$, and $\mathbf{B}_2 = S_z$. The double commutators $[\mathbf{A}_{2p}^{-q}, [\mathbf{A}_{2p}^q, I_z]]$ are calculated as follows for each combination of p and q in Table 5.3:

$$\begin{aligned}
 [A_{20}^0, [A_{20}^0, I_z]] &= (2/3)[I_z S_z, [I_z S_z, I_z]] = 0, \\
 [A_{21}^0, [A_{21}^0, I_z]] &= (1/24)[I^- S^+, [I^+ S^-, I_z]] \\
 &= -(1/24)[I^- S^+, I^+ S^-] = (1/24)\{I_z - S_z\}, \\
 [A_{2-1}^0, [A_{2-1}^0, I_z]] &= (1/24)[I^+ S^-, [I^- S^+, I_z]] \\
 &= (1/24)[I^+ S^-, I^- S^+] = (1/24)\{I_z - S_z\}, \\
 [A_{20}^{-1}, [A_{20}^{-1}, I_z]] &= -(1/4)[I_z S^-, [I_z S^+, I_z]] = 0, \\
 [A_{20}^1, [A_{20}^1, I_z]] &= -(1/4)[I_z S^+, [I_z S^-, I_z]] = 0, \quad [5.111] \\
 [A_{21}^{-1}, [A_{21}^{-1}, I_z]] &= -(1/4)[I^- S_z, [I^+ S_z, I_z]] \\
 &= (1/4)S_z^2[I^-, I^+] = -(1/8)I_z, \\
 [A_{21}^1, [A_{21}^1, I_z]] &= -(1/4)[I^+ S_z, [I^- S_z, I_z]] = -(1/24)S_z^2[I^-, I^+] \\
 &= -(1/8)I_z, \\
 [A_{20}^{-2}, [A_{20}^{-2}, I_z]] &= (1/4)[I^- S^-, [I^+ S^+, I_z]] = -(1/4)[I^- S^-, I^+ S^+] \\
 &= (1/4)\{S_z + I_z\}, \\
 [A_{20}^2, [A_{20}^2, I_z]] &= (1/4)[I^+ S^+, [I^- S^-, I_z]] = (1/4)[I^+ S^+, I^- S^-] \\
 &= (1/4)\{S_z + I_z\}.
 \end{aligned}$$

For autorelaxation of the I_z operator, the preceding operators are premultiplied by I_z and the trace operation is performed:

$$\begin{aligned}
 (1/24)\langle I_z | \{I_z - S_z\} \rangle &= (1/24)\langle I_z^2 - I_z S_z \rangle \\
 &= (1/24)\{ \langle \alpha\alpha | I_z^2 - I_z S_z | \alpha\alpha \rangle + \langle \alpha\beta | I_z^2 - I_z S_z | \alpha\beta \rangle \\
 &\quad + \langle \beta\alpha | I_z^2 - I_z S_z | \beta\alpha \rangle + \langle \beta\beta | I_z^2 - I_z S_z | \beta\beta \rangle \} \\
 &= 1/24, \\
 -(1/8)\langle I_z | I_z \rangle &= -(1/8)\langle I_z^2 \rangle \\
 &= -(1/8)\{ \langle \alpha\alpha | I_z^2 | \alpha\alpha \rangle + \langle \alpha\beta | I_z^2 | \alpha\beta \rangle \\
 &\quad + \langle \beta\alpha | I_z^2 | \beta\alpha \rangle + \langle \beta\beta | I_z^2 | \beta\beta \rangle \} \quad [5.112] \\
 &= -1/8, \\
 (1/4)\langle I_z | \{S_z + I_z\} \rangle &= (1/4)\langle I_z^2 + I_z S_z \rangle \\
 &= (1/4)\{ \langle \alpha\alpha | I_z^2 + I_z S_z | \alpha\alpha \rangle + \langle \alpha\beta | I_z^2 + I_z S_z | \alpha\beta \rangle \\
 &\quad + \langle \beta\alpha | I_z^2 + I_z S_z | \beta\alpha \rangle + \langle \beta\beta | I_z^2 + I_z S_z | \beta\beta \rangle \} \\
 &= 1/4.
 \end{aligned}$$

For cross-relaxation between the S_z and the I_z operator, the operators of [5.112] are premultiplied by S_z and the trace operation is performed:

$$\begin{aligned}
 (1/24)\langle S_z | \{I_z - S_z\} \rangle &= (1/24)\langle I_z S_z - S_z^2 \rangle \\
 &= (1/24)\{ \langle \alpha\alpha | I_z S_z - S_z^2 | \alpha\alpha \rangle + \langle \alpha\beta | I_z S_z - S_z^2 | \alpha\beta \rangle \\
 &\quad + \langle \beta\alpha | I_z S_z - S_z^2 | \beta\alpha \rangle + \langle \beta\beta | I_z S_z - S_z^2 | \beta\beta \rangle \} \\
 &= -1/24, \\
 (1/8)\langle S_z | I_z \rangle &= -(1/8)\langle I_z S_z \rangle \\
 &= -(1/8)\{ \langle \alpha\alpha | I_z S_z | \alpha\alpha \rangle + \langle \alpha\beta | I_z S_z | \alpha\beta \rangle \quad [5.113] \\
 &\quad + \langle \beta\alpha | I_z S_z | \beta\alpha \rangle + \langle \beta\beta | I_z S_z | \beta\beta \rangle \} \\
 &= 0, \\
 (1/4)\langle S_z | \{S_z + I_z\} \rangle &= (1/4)\langle S_z^2 + I_z S_z \rangle \\
 &= (1/4)\{ \langle \alpha\alpha | S_z^2 + I_z S_z | \alpha\alpha \rangle + \langle \alpha\beta | S_z^2 + I_z S_z | \alpha\beta \rangle \\
 &\quad + \langle \beta\alpha | S_z^2 + I_z S_z | \beta\alpha \rangle + \langle \beta\beta | S_z^2 + I_z S_z | \beta\beta \rangle \} \\
 &= 1/4.
 \end{aligned}$$

Auto- and cross-relaxation rate constants of the S_z operator are obtained by exchanging I and S operators in the preceding expressions. Substituting the values of these trace operations into [5.73] (and using $\langle I_z | I_z \rangle = 1$) yields

$$\begin{aligned}\Gamma_{11} &= (1/24)\{j(\omega_I - \omega_S) + 3j(\omega_I) + 6j(\omega_I + \omega_S)\}, \\ \Gamma_{22} &= (1/24)\{j(\omega_I - \omega_S) + 3j(\omega_S) + 6j(\omega_I + \omega_S)\}, \\ \Gamma_{12} &= (1/24)\{-j(\omega_I - \omega_S) + 6j(\omega_I + \omega_S)\}.\end{aligned}\quad [5.114]$$

If the I and S spins are separated by a constant distance, r_{IS} , then,

$$\begin{aligned}\Gamma_{11} &= (d_{00}/4)\{J(\omega_I - \omega_S) + 3J(\omega_I) + 6J(\omega_I + \omega_S)\}, \\ \Gamma_{22} &= (d_{00}/4)\{J(\omega_I - \omega_S) + 3J(\omega_S) + 6J(\omega_I + \omega_S)\}, \\ \Gamma_{12} &= (d_{00}/4)\{-J(\omega_I - \omega_S) + 6J(\omega_I + \omega_S)\},\end{aligned}\quad [5.115]$$

in which

$$d_{00} = (\mu_0/4\pi)^2 \hbar^2 \gamma_I^2 \gamma_S^2 r_{IS}^{-6}. \quad [5.116]$$

Thus, the evolution of the longitudinal operators, I_z and S_z , is governed by

$$\begin{aligned}d(\langle I_z \rangle(t) - \langle I_z^0 \rangle)/dt &= -\Gamma_{11}(\langle I_z \rangle(t) - \langle I_z^0 \rangle) - \Gamma_{12}(\langle S_z \rangle(t) - \langle S_z^0 \rangle), \\ d(\langle S_z \rangle(t) - \langle S_z^0 \rangle)/dt &= -\Gamma_{22}(\langle S_z \rangle(t) - \langle S_z^0 \rangle) - \Gamma_{12}(\langle I_z \rangle(t) - \langle I_z^0 \rangle).\end{aligned}\quad [5.117]$$

Making the identification $\Gamma_{11} = \rho_I (= R_{1I})$, $\Gamma_{22} = \rho_S (= R_{1S})$, and $\Gamma_{12} = \sigma_{IS}$ puts [5.117] into the form of the Solomon equations [5.11], in which ρ_I and ρ_S are the autorelaxation rate constants and σ_{IS} is the cross-relaxation rate constant. The Solomon transition rate constants (Section 5.1.2) are

$$\begin{aligned}W_0 &= j(\omega_I - \omega_S)/24, \\ W_I &= j(\omega_I)/16, \\ W_S &= j(\omega_S)/16, \\ W_2 &= j(\omega_I + \omega_S)/4.\end{aligned}\quad [5.118]$$

Next, relaxation of the transverse I^+ operator is considered. The double commutators $[\mathbf{A}_{2p}^{-q}, [\mathbf{A}_{2p}^q, I^+]]$ are calculated as follows for each

combination of p and q in Table 5.3:

$$\begin{aligned}
 [A_{20}^0, [A_{20}^0, I^+]] &= (2/3)[I_z S_z, [I_z S_z, I^+]] = (1/6)I^+, \\
 [A_{21}^0, [A_{21}^0, I^+]] &= (1/24)[I^- S^+, [I^+ S^-, I^+]] = 0, \\
 [A_{2-1}^0, [A_{2-1}^0, I^+]] &= -(1/24)[I^+ S^-, [I^- S^+, I^+]] \\
 &= -(1/12)[I^+ S^-, I_z S^+] = (1/24)I^+, \\
 [A_{20}^{-1}, [A_{20}^1, I^+]] &= -(1/4)[I_z S^-, [I_z S^+, I^+]] = (1/8)[I_z S^-, I^+ S^+] \\
 &= -(1/8)I^+, \\
 [A_{20}^1, [A_{20}^{-1}, I^+]] &= -(1/4)[I_z S^+, [I_z S^-, I^+]] = -(1/8)[I_z S^+, I^+ S^-] \\
 &= -(1/8)I^+, \\
 [A_{21}^{-1}, [A_{21}^1, I^+]] &= -(1/4)[I^- S_z, [I^+ S_z, I^+]] = 0, \\
 [A_{21}^1, [A_{21}^{-1}, I^+]] &= -(1/4)[I^+ S_z, [I^- S_z, I^+]] = (1/4)S_z^2[I^+, I_z] = -(1/8)I^+, \\
 [A_{20}^{-2}, [A_{20}^2, I^+]] &= (1/4)[I^- S^-, [I^+ S^+, I^+]] = 0, \\
 [A_{20}^2, [A_{20}^{-2}, I^+]] &= (1/4)[I^+ S^+, [I^- S^-, I^+]] = -(1/4)[I^+ S^+, I_z S^-] \\
 &= (1/4)I^+.
 \end{aligned} \tag{5.119}$$

All nonzero results are proportional to I^+ ; therefore, because the operator basis is orthogonal, no operator cross-relaxes with I^+ . This result is a consequence of the secular approximation. For autorelaxation of the I^+ operator, the operators in [5.119] are premultiplied by I^+ and the trace operation is performed:

$$\begin{aligned}
 \langle I^+ | I^+ \rangle &= \langle I^- | I^+ \rangle = \{ \langle \alpha\alpha | I^- I^+ | \alpha\alpha \rangle + \langle \alpha\beta | I^- I^+ | \alpha\beta \rangle \\
 &\quad + \langle \beta\alpha | I^- I^+ | \beta\alpha \rangle + \langle \beta\beta | I^- I^+ | \beta\beta \rangle \} = 2.
 \end{aligned} \tag{5.120}$$

This same factor is the normalization in the denominator of [5.73].

Thus,

$$R_{2I} = (1/48) \{ 4j(0) + j(\omega_I - \omega_S) + 3j(\omega_I) + 6j(\omega_S) + 6j(\omega_I + \omega_S) \} \tag{5.121}$$

TABLE 5.5
Relaxation Rate Constants for IS Dipolar Interaction

Coherence level	Operator	Relaxation rate constant ^a
Populations 0	I_z	$(d_{00}/4) \{J(\omega_I - \omega_S) + 3J(\omega_I) + 6J(\omega_I + \omega_S)\}$
	S_z	$(d_{00}/4) \{J(\omega_I - \omega_S) + 3J(\omega_S) + 6J(\omega_I + \omega_S)\}$
	$I_z \longleftrightarrow S_z^b$	$(d_{00}/4) \{-J(\omega_I - \omega_S) + 6J(\omega_I + \omega_S)\}$
	$2I_z S_z$	$(3d_{00}/4) \{J(\omega_I) + J(\omega_S)\}$
	ZQ_x, ZQ_y	$(d_{00}/8) \{2J(\omega_I - \omega_S) + 3J(\omega_I) + 3J(\omega_S)\}$
± 1	I^+, I^-	$(d_{00}/8) \{4J(0) + J(\omega_I - \omega_S) + 3J(\omega_I) + 6J(\omega_S) + 6J(\omega_I + \omega_S)\}$
	S^+, S^-	$(d_{00}/8) \{4J(0) + J(\omega_I - \omega_S) + 3J(\omega_S) + 6J(\omega_I) + 6J(\omega_I + \omega_S)\}$
	$2I^+ S_z, 2I^- S_z$	$(d_{00}/8) \{4J(0) + J(\omega_I - \omega_S) + 3J(\omega_I) + 6J(\omega_I + \omega_S)\}$
± 2	$2I_z S^+, 2I_z S^-$	$(d_{00}/8) \{4J(0) + J(\omega_I - \omega_S) + 3J(\omega_S) + 6J(\omega_I + \omega_S)\}$
	DQ_x, DQ_y	$(d_{00}/8) \{3J(\omega_I) + 3J(\omega_S) + 12J(\omega_I + \omega_S)\}$

^a $d_{00} = (\mu_0/4\pi)^2 \hbar^2 \gamma_I^2 \gamma_S^2 r_{IS}^{-6}$.

^bCross-relaxation only occurs between I_z and S_z .

and

$$d\langle I^+ \rangle / dt = (-i\omega_I - R_{2I})\langle I^+ \rangle. \quad [5.122]$$

If r_{IS} is constant,

$$R_{2I} = (d_{00}/8) \{4J(0) + J(\omega_I - \omega_S) + 3J(\omega_I) + 6J(\omega_S) + 6J(\omega_I + \omega_S)\}. \quad [5.123]$$

Analogous equations are written by inspection for the I^- , S^+ , and S^- operators. The complete set of dipolar relaxation rate constants for the basis operators for the two spin system is given in Table 5.5.

The dependence of R_1 and R_2 on τ_c for a rigid molecule is illustrated in Fig. 5.8. Notice that R_1 has a maximum for $\omega_0\tau_c = 1$ while R_2 increases monotonically with τ_c .

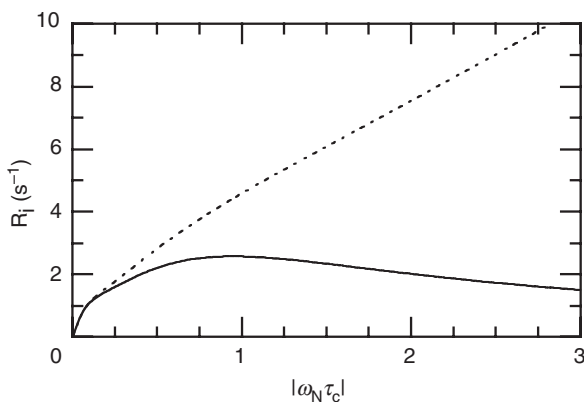


FIGURE 5.8 Relaxation rate constants for an ^1H - ^{15}N dipolar spin system. (—) ^{15}N R_1 spin-lattice rate constants; (···) ^{15}N R_2 spin-spin rate constants. Calculations were performed using expressions given in Table 5.5 together with [5.116] and [5.98]. Parameters used were $B_0 = 11.74 \text{ T}$, $\gamma_I = 2.675 \times 10^8 \text{ T}^{-1} \text{ s}^{-1}$ (^1H), $\gamma_S = -2.712 \times 10^7 \text{ T}^{-1} \text{ s}^{-1}$ (^{15}N), and $r_{IS} = 1.02 \text{ \AA}$.

5.4.2 INTRAMOLECULAR DIPOLAR RELAXATION FOR SCALAR-COUPLED IS SPIN SYSTEM

The I_z and S_z operators both commute with the scalar coupling Hamiltonian; consequently, dipolar spin-lattice relaxation is unaffected by the scalar coupling interaction and the expressions given in [5.114] and [5.117] remain valid. The in-phase and antiphase transverse operators, I^+ and $2I^+S_z$, are coupled together by the scalar coupling Hamiltonian. Applying [5.71] yields the following equations (assuming the I spin is on-resonance):

$$\begin{aligned} d\langle I^+ \rangle(t)/dt &= -i\pi J_{IS} \langle 2I^+S_z \rangle(t) - R_{2I} \langle I^+ \rangle(t), \\ d\langle 2I^+S_z \rangle(t)/dt &= -i\pi J_{IS} \langle I^+ \rangle(t) - R_{2IS} \langle 2I^+S_z \rangle(t), \end{aligned} \quad [5.124]$$

in which R_{2I} and R_{2IS} are given in Table 5.5 and $\omega_I = 0$ is assumed for simplicity. These equations are written in matrix form as

$$\frac{d}{dt} \begin{bmatrix} \langle I^+ \rangle(t) \\ \langle 2I^+S_z \rangle(t) \end{bmatrix} = - \begin{bmatrix} R_{2I} & i\pi J_{IS} \\ i\pi J_{IS} & R_{2IS} \end{bmatrix} \begin{bmatrix} \langle I^+ \rangle(t) \\ \langle 2I^+S_z \rangle(t) \end{bmatrix} \quad [5.125]$$

and are solved by analogy to [5.15] to yield

$$\begin{aligned}
 \langle I^+ \rangle(t) &= \frac{1}{2} \left[\left(1 - \frac{R_{2I} - R_{2IS}}{(\lambda_+ - \lambda_-)} \right) \exp(-\lambda_- t) \right. \\
 &\quad \left. + \left(1 + \frac{R_{2I} - R_{2IS}}{(\lambda_+ - \lambda_-)} \right) \exp(-\lambda_+ t) \right] \langle I^+ \rangle(0) \\
 &\quad - \frac{i\pi J_{IS}}{(\lambda_+ - \lambda_-)} [\exp(-\lambda_- t) - \exp(-\lambda_+ t)] \langle 2I^+ S_z \rangle(0), \\
 \langle 2I^+ S_z \rangle(t) &= \frac{1}{2} \left[\left(1 + \frac{R_{2I} - R_{2IS}}{(\lambda_+ - \lambda_-)} \right) \exp(-\lambda_- t) \right. \\
 &\quad \left. + \left(1 - \frac{R_{2I} - R_{2IS}}{(\lambda_+ - \lambda_-)} \right) \exp(-\lambda_+ t) \right] \langle 2I^+ S_z \rangle(0) \\
 &\quad - \frac{i\pi J_{IS}}{(\lambda_+ - \lambda_-)} [\exp(-\lambda_- t) - \exp(-\lambda_+ t)] \langle I^+ \rangle(0),
 \end{aligned} \tag{5.126}$$

in which

$$\lambda_{\pm} = \left\{ (R_{2I} + R_{2IS})/2 \pm [((R_{2I} - R_{2IS})/2)^2 - (\pi J_{IS})^2]^{1/2} \right\}. \tag{5.127}$$

If $(2\pi J_{IS})^2 \gg (R_{2I} - R_{2IS})^2$, then

$$\begin{aligned}
 \langle I^+ \rangle(t) &= \frac{1}{2} [\exp\{-(-i\pi J_{IS} + \bar{R}_2)t\} \\
 &\quad + \exp\{-(i\pi J_{IS} + \bar{R}_2)t\}] \langle I^+ \rangle(0) \\
 &\quad - [\exp\{-(-i\pi J_{IS} + \bar{R}_2)t\} \\
 &\quad - \exp\{-(i\pi J_{IS} + \bar{R}_2)t\}] \langle 2I^+ S_z \rangle(0), \\
 \langle 2I^+ S_z \rangle(t) &= \frac{1}{2} [\exp\{-(-i\pi J_{IS} + \bar{R}_2)t\} \\
 &\quad + \exp\{-(i\pi J_{IS} + \bar{R}_2)t\}] \langle 2I^+ S_z \rangle(0) \\
 &\quad - [\exp\{-(-i\pi J_{IS} + \bar{R}_2)t\} \\
 &\quad - \exp\{-(i\pi J_{IS} + \bar{R}_2)t\}] \langle I^+ \rangle(0),
 \end{aligned} \tag{5.128}$$

with

$$\begin{aligned}
 \bar{R}_2 &= (R_{2I} + R_{2IS})/2 \\
 &= (d_{00}/8) \{ 4J(0) + J(\omega_I - \omega_S) + 3J(\omega_S) + 3J(\omega_I) + 6J(\omega_I + \omega_S) \}.
 \end{aligned} \tag{5.129}$$

Equation [5.128] predicts that the signal arising from I^+ has the expected form of a doublet with linewidth \bar{R}_2/π . The doublet is in-phase if $\langle 2I^+S_z \rangle(0)=0$ and antiphase if $\langle I^+ \rangle(0)=0$. Evolution of the scalar coupling interaction on a faster time scale compared to the relaxation processes averages the two relaxation rate constants, R_{2I} and R_{2IS} , because coherence is rapidly exchanged between the I^+ and $2I^+S_z$ operators.

An alternative viewpoint, consistent with the presentation of Section 5.2.2, is obtained by transforming to a new operator basis using the following transformation matrix,

$$\mathbf{U} = \frac{1}{\sqrt{2}} \begin{bmatrix} 1 & 1 \\ 1 & -1 \end{bmatrix}, \quad [5.130]$$

to give

$$\begin{aligned} \mathbf{U} \frac{d}{dt} \begin{bmatrix} \langle I^+ \rangle(t) \\ \langle 2I^+S_z \rangle(t) \end{bmatrix} &= -\mathbf{U} \begin{bmatrix} R_{2I} & i\pi J_{IS} \\ i\pi J_{IS} & R_{2IS} \end{bmatrix} \mathbf{U}^{-1} \mathbf{U} \begin{bmatrix} \langle I^+ \rangle(t) \\ \langle 2I^+S_z \rangle(t) \end{bmatrix}, \\ \frac{1}{\sqrt{2}} \frac{d}{dt} \begin{bmatrix} \langle I^+ \rangle(t) + \langle 2I^+S_z \rangle(t) \\ \langle I^+ \rangle(t) - \langle 2I^+S_z \rangle(t) \end{bmatrix} & \quad [5.131] \\ &= -\frac{1}{\sqrt{2}} \begin{bmatrix} i\pi J_{IS} + \bar{R}_2 & (R_{2I} - R_{2IS})/2 \\ (R_{2I} - R_{2IS})/2 & -i\pi J_{IS} + \bar{R}_2 \end{bmatrix} \begin{bmatrix} \langle I^+ \rangle(t) + \langle 2I^+S_z \rangle(t) \\ \langle I^+ \rangle(t) - \langle 2I^+S_z \rangle(t) \end{bmatrix}, \\ \frac{d}{dt} \begin{bmatrix} \langle I^+S^\alpha \rangle(t) \\ \langle I^+S^\beta \rangle(t) \end{bmatrix} &= -\begin{bmatrix} i\pi J_{IS} + \bar{R}_2 & (R_{2I} - R_{2IS})/2 \\ (R_{2I} - R_{2IS})/2 & -i\pi J_{IS} + \bar{R}_2 \end{bmatrix} \begin{bmatrix} \langle I^+S^\alpha \rangle(t) \\ \langle I^+S^\beta \rangle(t) \end{bmatrix}. \end{aligned}$$

In the new basis, which is the eigenbasis of the scalar coupling Hamiltonian, all precession terms are diagonal elements of the Hamiltonian. The term $\langle (I^+S^\alpha)(t) \rangle = \langle (I^+ + 2I^+S_z)/2 \rangle(t)$ represents the multiplet component of the scalar-coupled doublet with frequency $J/2$ Hz and the term $\langle (I^+S^\beta)(t) \rangle = \langle (I^+ - 2I^+S_z)/2 \rangle(t)$ represents the multiplet component of the scalar-coupled doublet with frequency $-J/2$ Hz. When $(2\pi J_{IS})^2 \gg (R_{2I} - R_{2IS})^2$, the off-diagonal elements can be neglected and the two multiplet components relax independently with identical relaxation rate constants. In this limit, the doublet components are nonsecular with respect to each other and cross-relaxation is quenched. The off-diagonal terms in [5.131] illustrate the effect of the breakdown of the secular approximation as πJ_{IS} and $(R_{2I} - R_{2IS})/2$ become comparable.

For the purely dipolar IS interaction in the spin diffusion limit,

$$R_{2I} - R_{2IS} = 3d_{00}J(\omega_S)/4 = \frac{3\mu_0^2\hbar^2\gamma_I^2}{160\pi^2B_0^2r_{IS}^6\tau_c} \quad [5.132]$$

normally is quite small. For example, if $I = {}^{15}\text{N}$, $S = {}^1\text{H}^{\text{N}}$, and $\tau_c = 5$ ns, then $R_{2I} - R_{2IS} = 0.031 \text{ s}^{-1}$, compared with $J_{IS} = 92 \text{ Hz}$. However, the S_z operator may have relaxation pathways other than the IS dipolar interaction. In the cited example, the S_z operator would be dipolar coupled to other ${}^1\text{H}$ spins, and the relaxation rate constant for the $2I^+S_z$ operator contains a contribution, R_{ext} , from ${}^1\text{H}$ dipolar longitudinal relaxation. Ignoring cross-correlation and cross-relaxation effects, R_{ext} is simply additive to R_{2IS} . The additional contribution from R_{ext} has two important effects. First, \bar{R}_2 is increased by $R_{\text{ext}}/2$. Practical consequences of the increased linewidth in heteronuclear NMR spectra are discussed in Section 7.1.2.4. Second, if R_{ext} is sufficiently large, then $(R_{2I} - R_{2IS} - R_{\text{ext}})^2 \approx R_{\text{ext}}^2 \gg (2\pi J_{IS})^2$, $\lambda_+ = R_{2I}$, $\lambda_- = R_{2IS} + R_{\text{ext}}$, and [5.126] reduces to

$$\begin{aligned} \langle I^+ \rangle(t) &= \langle I^+ \rangle(0) \exp[-R_{2I}t], \\ \langle 2I^+S_z \rangle(t) &= \langle 2I^+S_z \rangle(0) \exp[-(R_{2IS} + R_{\text{ext}})t]. \end{aligned} \quad [5.133]$$

The expected doublet has been reduced to a singlet resonance in a process called *self-decoupling*, which is similar both to scalar relaxation of the second kind (Section 5.4.5) and to chemical exchange (Section 5.6.2). For $(R_{2I} - R_{2IS} - R_{\text{ext}})^2 \approx (2\pi J_{IS})^2$, the doublet is partially decoupled and broadened, as for intermediate chemical exchange (Section 5.6.1). Self-decoupling can complicate the measurement of scalar coupling constants (Section 6.2.1.5, 6.3.3, and 7.5) (39).

A similar set of equations is obtained for the S^+ and $2S^+I_z$ coherences by interchanging the I and S labels. For an uncoupled IS spin system, $R_{2I} \neq R_{2S}$, but for a scalar-coupled spin system undergoing free precession, \bar{R}_2 is identical for the I and S spins.

5.4.3 INTRAMOLECULAR DIPOLAR RELAXATION FOR IS SPIN SYSTEM IN THE ROTATING FRAME

An IS homonuclear spin system, in which the two spins interact through the dipolar interaction, but are not scalar coupled, is treated here. The spin lock field is assumed to be applied with x -phase. The autorelaxation rate constant of the I'_z operator and the cross-relaxation rate constant between the I'_z and S'_z operators are calculated in the

tilted rotating frame. As discussed in Section 5.2.3, in the presence of the spin lock field, the I and S spins are treated as like spins (alternatively, the relaxation rate constants are calculated using [5.84] without invoking the secular hypothesis); thus, the components of the dipolar interaction listed in Table 5.3 are redefined according to [5.78] as

$$\begin{aligned} \mathbf{A}_2^0 &= \mathbf{A}_{20}^0 + \mathbf{A}_{2-1}^0 + \mathbf{A}_{21}^0, \\ \mathbf{A}_2^{\pm 1} &= \mathbf{A}_{20}^{\pm 1} + \mathbf{A}_{21}^{\pm 1}, \quad \mathbf{A}_2^{\pm 2} = \mathbf{A}_{20}^{\pm 2}. \end{aligned} \quad [5.134]$$

From [5.89],

$$\begin{aligned} I'_z &= \sin\theta_I I_x + \cos\theta_I I_z, \\ S'_z &= \sin\theta_S S_x + \cos\theta_S S_z. \end{aligned} \quad [5.135]$$

Applying [5.88], the double commutators $[\mathbf{A}_2^{-q}, [\mathbf{A}_2^q, I'_z]]$ are calculated first. Straightforward, but tedious, calculations yield

$$\begin{aligned} [\mathbf{A}_2^0, [\mathbf{A}_2^0, I'_z]] &= \sin\theta_I (5I_x + 4S_x)/24 + \cos\theta_I (I_z - S_z)/6, \\ [\mathbf{A}_2^{-1}, [\mathbf{A}_2^1, I'_z]] &= [\mathbf{A}_2^1 [\mathbf{A}_2^{-1}, I'_z]] = -\sin\theta_I (2I_x + 2S_x + 2I^-)/8 \\ &\quad - \cos\theta_I I_z/8, \\ [\mathbf{A}_2^{-2}, [\mathbf{A}_2^2, I'_z]] &= [\mathbf{A}_2^2, [\mathbf{A}_2^{-2}, I'_z]] = -\sin\theta_I I^-/8 - \cos\theta_I (I_z + S_z)/8. \end{aligned} \quad [5.136]$$

The autorelaxation rate constant is determined by premultiplying the preceding expressions by I'_z and forming the trace:

$$\begin{aligned} &\langle \sin\theta_I I_x + \cos\theta_I I_z | \sin\theta_I (5I_x + 4S_x)/24 + \cos\theta_I (I_z - S_z)/6 \rangle \\ &= (5/24) \sin^2\theta_I + (1/12) \cos^2\theta_I, \\ &\langle \sin\theta_I I_x + \cos\theta_I I_z | -\sin\theta_I (2I_x + 2S_x + 2I^-)/8 - \cos\theta_I I_z/8 \rangle \\ &= -(3/16) \sin^2\theta_I - (1/8) \cos^2\theta_I, \\ &\langle \sin\theta_I I_x + \cos\theta_I I_z | -\sin\theta_I I^-/8 - \cos\theta_I (I_z + S_z)/8 \rangle \\ &= (1/8) \sin^2\theta_I + (1/4) \cos^2\theta_I. \end{aligned} \quad [5.137]$$

Thus, the autorelaxation rate, $R_1(\theta_I)$ (which commonly is called $R_{1\rho}$) is given by

$$\begin{aligned} R_I(\theta_I) &= (1/48) \{ (2 \cos^2\theta_I + 5 \sin^2\theta_I) j(0) \\ &\quad + (6 \cos^2\theta_I + 9 \sin^2\theta_I) j(\omega_0) + (12 \cos^2\theta_I + 6 \sin^2\theta_I) j(2\omega_0) \} \\ &= R_{1I} \cos^2\theta_I + R_{2I} \sin^2\theta_I. \end{aligned} \quad [5.138]$$

Similarly, the cross-relaxation rate constant is found by premultiplying the expressions in [5.136] by S'_z and forming the trace:

$$\begin{aligned}
 & \langle \sin\theta_S S_x + \cos\theta_S S_z | \sin\theta_I (5I_x + 4S_x)/24 + \cos\theta_I (I_z - S_z)/6 \rangle \\
 & \quad = (1/6) \sin\theta_S \sin\theta_I - (1/12) \cos\theta_S \cos\theta_I, \\
 & \langle \sin\theta_S S_x + \cos\theta_S S_z | -\sin\theta_I (2I_x + 2S_x + 2I^-)/8 - \cos\theta_I I_z/8 \rangle \\
 & \quad = -(1/8) \sin\theta_S \sin\theta_I, \\
 & \langle \sin\theta_S S_x + \cos\theta_S S_z | -\sin\theta_I I^-/8 - \cos\theta_I (I_z + S_z)/8 \rangle \\
 & \quad = (1/4) \cos\theta_S \cos\theta_I.
 \end{aligned} \tag{5.139}$$

Thus, the cross-relaxation rate, $R_{IS}(\theta_I, \theta_S)$ is given by

$$\begin{aligned}
 R_{IS}(\theta_I, \theta_S) &= (1/24) \{ (-\cos\theta_S \cos\theta_I + 2 \sin\theta_S \sin\theta_I) j(0) \\
 & \quad + 3 \sin\theta_S \sin\theta_I j(\omega_0) + 6 \cos\theta_S \cos\theta_I j(2\omega_0) \} \\
 &= \cos\theta_I \cos\theta_S \sigma_{IS}^{\text{NOE}} + \sin\theta_I \sin\theta_S \sigma_{IS}^{\text{ROE}},
 \end{aligned} \tag{5.140}$$

in which the pure laboratory-frame cross-relaxation rate constant, σ_{IS}^{NOE} , is given in [5.114] and the pure rotating-frame cross-relaxation rate constant is given by (40)

$$\sigma_{IS}^{\text{ROE}} = (1/24) \{ 2j(0) + 3j(\omega_0) \}. \tag{5.141}$$

For both autorelaxation and cross-relaxation, the effect of the tilted field is to average the relaxation rate constants of the laboratory (longitudinal) and rotating frames (transverse) by the projection of the spin operators onto the tilted reference frame.

5.4.4 CHEMICAL SHIFT ANISOTROPY AND QUADRUPOLEAR RELAXATION

Chemical shifts are reflections of the electronic environments that modify the local magnetic fields experienced by different nuclei (Section 1.5). These local fields are anisotropic; consequently, the components of the local fields in the laboratory reference frame vary as the molecule reorients due to molecular motion. These varying magnetic fields are a source of relaxation. Very approximately, the maximum CSA for a particular nucleus is of the order of the chemical shift range for the nucleus. Consequently, CSA is most important as a relaxation mechanism for nuclei with large chemical shift ranges. In the NMR spectroscopy of biological molecules, carbon, nitrogen, and phosphorous have significant CSA contributions to relaxation. CSA is generally a

small effect for ^1H relaxation, except at very large static magnetic field strengths. CSA relaxation rate constants have a quadratic dependence on the applied magnetic field strength. Thus, use of higher magnetic field strengths does not always increase the achievable signal-to-noise ratio as much as anticipated, because increased CSA relaxation broadens resonance linewidths. This effect is particularly significant for ^{13}C spins in carbonyl groups and for ^{31}P .

Nuclei with $I > 1/2$ also possess nuclear electric quadrupole moments. The quadrupole moment is a characteristic of the particular nucleus and represents a departure of the nuclear charge distribution from spherical symmetry. The interactions of the quadrupole moment with local oscillating electric field gradients (due to electrons) provide a relaxation mechanism. Quadrupolar interactions can be very large and efficient for promoting relaxation. Quadrupolar nuclei display broad resonance lines in NMR spectra, unless the nuclei are in a highly symmetric electronic environment (which reduces the magnitudes of the electric field gradients at the locations of the nuclei). As discussed in more detail elsewhere, Bloch spin-lattice and spin-spin relaxation rate constants can only be defined for quadrupolar nuclei under extreme narrowing conditions or for quadrupolar nuclei with $I = 1$ (2).

The terms \mathbf{A}_{2p}^q for the CSA and quadrupolar interactions are given in Tables 5.6 and 5.7, respectively. The spherical harmonic and spatial functions for the different interactions are given in Tables 5.1 and 5.2. Relaxation rate constants for the CSA and quadrupolar interactions are calculated by the same procedure as for the dipolar interactions and are given in Tables 5.8 and 5.9, respectively. The results are calculated for axially symmetric chemical shift and electric field gradient tensors (i.e., $\sigma_{xx} = \sigma_{yy} \neq \sigma_{zz}$ and $V_{xx} = V_{yy} \neq V_{zz}$). Extensions to these results for anisotropic tensors are given elsewhere (2).

TABLE 5.6
Tensor Operators for the CSA Interaction

q	p	\mathbf{A}_{2p}^q	$\mathbf{A}_{2p}^{-q} = (-1)^q \mathbf{A}_{2p}^{q\dagger}$	ω_p^q
0	0	$(2/\sqrt{6})I_z$	$(2/\sqrt{6})I_z$	0
1	0	$-(1/2) I^+$	$(1/2) I^-$	ω_I
2	0	—	—	$2\omega_I$

TABLE 5.7
Tensor Operators for the Spin-1 Quadrupolar Interaction

q	p	A_{2p}^q	$A_{2p}^{-q} = (-1)^q A_{2p}^{q\dagger}$	ω_p^q
0	0	$(1/\sqrt{6})(3I_z^2 - 2)$	$(1/\sqrt{6})(3I_z^2 - 2)$	0
1	0	$-(1/2)(I_z I^+ + I^+ I_z)$	$(1/2)(I_z I^- + I^- I_z)$	ω_I
2	0	$(1/2) I^+ I^+$	$(1/2) I^- I^-$	$2\omega_I$

TABLE 5.8
CSA Relaxation Rate Constants

Coherence level	Operator	Relaxation rate constant ^a
Populations	I_z	$d_{00} J(\omega_I)$
± 1	I^+, I^-	$(d_{00}/6) \{4J(0) + 3J(\omega_I)\}$

$$^a d_{00} = (\Delta\sigma\gamma_I B_0)^2/3 = \Delta\sigma^2\omega_I^2/3.$$

TABLE 5.9
Relaxation Rate Constants for the Spin-1 Quadrupolar Interaction

Coherence level	Operator	Relaxation rate constant ^a
Populations	I_z	$3d_{00} \{J(\omega_I) + 4J(2\omega_I)\}$
0	$3I_z^2 - 2$	$9d_{00} J(\omega_I)$
± 1	I^+, I^-	$(3d_{00}/2) \{3J(0) + 5J(\omega_I) + 2J(2\omega_I)\}$
	$I^+ I_z + 2I_z I^+, I^- I_z + 2I_z I^-$	$(3d_{00}/2) \{3J(0) + J(\omega_I) + 2J(2\omega_I)\}$
± 2	$I^+ I^+, I^- I^-$	$3d_{00} \{J(\omega_I) + 2J(2\omega_I)\}$

$$^a d_{00} = [e^2 q Q / (4\hbar)]^2.$$

5.4.5 RELAXATION INTERFERENCE

As discussed in Section 5.2.1, correlations between two stochastic Hamiltonians results in cross-correlated relaxation or relaxation interference. The principal cause of the correlation between the Hamiltonians is that the same molecular motions affect each Hamiltonian. The interference between the dipolar and chemical shift anisotropy relaxation

mechanisms has been recognized for many years [see Goldman (21) and references therein]. The hallmark of this phenomenon is that the two lines in a scalar-coupled doublet have different linewidths. Dipole–CSA relaxation interference has long been regarded as a curiosity or a complication to be suppressed experimentally (41–43). In the past few years, a number of new applications have emerged that make use of relaxation interference for line narrowing in heteronuclear correlation spectroscopy (44), for measuring chemical shift anisotropies in solution (45, 46), and for investigating dynamic properties of macromolecules (45).

The present discussion is based on the treatment of Goldman (21). A two-spin system is considered. The I spin has a dipolar relaxation interaction with the S spin and has a significant chemical shift anisotropy. The evolution of longitudinal components of the density operator is given by

$$\begin{aligned}\frac{d\langle I_z \rangle(t)}{dt} &= -(R_{1I}^{\text{DD}} + R_{1I}^{\text{CSA}})(\langle I_z \rangle(t) - \langle I_z^0 \rangle) - \sigma_{IS}(\langle S_z \rangle(t) - \langle S_z^0 \rangle) \\ &\quad - \eta_z \langle 2I_z S_z \rangle(t), \\ \frac{d\langle S_z \rangle(t)}{dt} &= -R_{1S}^{\text{DD}}(\langle S_z \rangle(t) - \langle S_z^0 \rangle) - \sigma_{IS}(\langle I_z \rangle(t) - \langle I_z^0 \rangle), \\ \frac{d\langle 2I_z S_z \rangle(t)}{dt} &= -(R_{1IS}^{\text{DD}} + R_{1I}^{\text{CSA}})\langle 2I_z S_z \rangle(t) - \eta_z(\langle I_z \rangle(t) - \langle I_z^0 \rangle).\end{aligned}\tag{5.142}$$

The evolution of the transverse components of the density operator is given by

$$\begin{aligned}\frac{d\langle I^+ \rangle(t)}{dt} &= -i\pi J_{IS}\langle 2I^+ S_z \rangle(t) - (R_{2I}^{\text{DD}} + R_{2I}^{\text{CSA}})\langle I^+ \rangle(t) - \eta_{xy}\langle 2I^+ S_z \rangle(t) \\ \frac{d\langle 2I^+ S_z \rangle(t)}{dt} &= -i\pi J_{IS}\langle I^+ \rangle(t) - (R_{2IS}^{\text{DD}} + R_{2I}^{\text{CSA}})\langle 2I^+ S_z \rangle(t) \\ &\quad - \eta_{xy}\langle I^+ \rangle(t),\end{aligned}\tag{5.143}$$

in which the superscripts refer to the dipole–dipole (DD) and CSA relaxation mechanisms, respectively. All terms other than η_z and η_{xy} are relaxation rate constants determined earlier. If $\eta_z = 0$, the first two equations in [5.142] are simply the Solomon equations. For an axially symmetric chemical shift tensor and a rigid spherical molecule, the interference rate constants are given by

$$\eta_z = \sqrt{3}cdP_2(\cos\theta)J(\omega_I),\tag{5.144}$$

$$\eta_{xy} = \frac{\sqrt{3}}{6} cdP_2(\cos\theta)\{4J(0) + 3J(\omega_I)\}, \quad [5.145]$$

in which $d = -(\mu_0\hbar\gamma_I\gamma_S)/(4\pi r_{IS}^3)$ and $c = \gamma_I(\sigma_{||} - \sigma_{\perp})B_0/\sqrt{3}$ are obtained from Table 5.2, and θ is the angle between the symmetry axis of the CSA principal axis system and the vector connecting the I and S spins. These results are derived using [5.76].

The meaning of [5.143] is seen more easily by transforming using [5.130] to yield

$$\frac{d}{dt} \begin{bmatrix} \langle I^+ S^\alpha \rangle(t) \\ \langle I^+ S^\beta \rangle(t) \end{bmatrix} = - \begin{bmatrix} i\pi J_{IS} + \bar{R}_2 + \eta_{xy} & (R_{2I}^{DD} - R_{2IS}^{DD})/2 \\ (R_{2I}^{DD} - R_{2IS}^{DD})/2 & -i\pi J_{IS} + \bar{R}_2 - \eta_{xy} \end{bmatrix} \begin{bmatrix} \langle I^+ S^\alpha \rangle(t) \\ \langle I^+ S^\beta \rangle(t) \end{bmatrix}, \quad [5.146]$$

in which $\bar{R}_2 = (R_{2I}^{DD} + R_{2IS}^{DD})/2 + R_{2I}^{CSA}$. As discussed in Section 5.4.2, the off-diagonal terms are unimportant provided that $(2\pi J_{IS}^2) \gg (R_{2I}^{DD} - R_{2IS}^{DD})^2$. These terms represent cross-relaxation between the two doublet components and can become important in multipulse experiments that suppress the effects of the scalar coupling interaction. The effect of relaxation interference is that the interference term adds to the relaxation rate (and hence the linewidth) of the doublet component with frequency $J_{IS}/2$ Hz and subtracts from the relaxation rate (and hence the linewidth) of the doublet component with frequency $-J_{IS}/2$ Hz. This result is the basis of the TROSY technique for line narrowing at high static magnetic field strengths, for which the magnitude of η_{xy} can approach \bar{R}_2 due to the field dependence of the chemical shift anisotropy (44).

5.4.6 SCALAR RELAXATION

As discussed in Sections 1.6 and 2.5.2, the isotropic scalar coupling Hamiltonian, $\mathcal{H}_J = 2\pi J_{IS} \mathbf{I} \cdot \mathbf{S}$, slightly perturbs the Zeeman energy levels of the coupled spins; the resonances thereby are split into characteristic multiplet patterns. Spin I experiences a local magnetic field that depends on the value of the coupling constant and the state of spin S . The local magnetic field becomes time dependent if the value of J_{IS} is time dependent or if state of the S spin varies rapidly. The former relaxation mechanism is termed *scalar relaxation of the first kind*; the latter mechanism is termed *scalar relaxation of the second kind*. Scalar relaxation of the first kind results from transitions of the spin system between environments with different values of J_{IS} . For example, the

three-bond scalar coupling constant for a pair of ^1H spins depends upon the intervening dihedral angle according to the Karplus relationship (see Chapter 9, [9.2]). If the dihedral angle is time dependent, the consequent time dependence of J_{IS} can lead to scalar relaxation. Scalar relaxation of the second kind results if the S spin relaxes rapidly (e.g., S is a quadrupolar nucleus) or is involved in rapid chemical exchange. Scalar relaxation of the second kind also can be significant for ^1H S spins in macromolecules, in which case the homonuclear relaxation rate constants (reflecting the dipolar interaction of the S spins with ^1H spins other than the I spin) are large. Normally, field fluctuations produced by this mechanism are not fast enough for effective longitudinal relaxation, but transverse relaxation may be induced (Section 5.4.2).

For relaxation of the I spin, expressions for R_1^{sc} and R_2^{sc} are given by (2)

$$\begin{aligned} R_1^{\text{sc}} &= \frac{2A^2}{3} S(S+1) \frac{\tau_2}{1 + (\omega_I - \omega_S)^2 \tau_2^2}, \\ R_2^{\text{sc}} &= \frac{A^2}{3} S(S+1) \left[\frac{\tau_2}{1 + (\omega_I - \omega_S)^2 \tau_2^2} + \tau_1 \right], \end{aligned} \quad [5.147]$$

assuming that the scalar relaxation mechanism is fast enough to result in a single, broadened resonance, rather than a resolved multiplet. For scalar relaxation of the first kind, $A = 2\pi(p_1 p_2)^{1/2}(J_1 - J_2)$, in which J_1 and J_2 are the scalar coupling constants in the two environments; p_1 and p_2 are the site populations, $\tau_1 = \tau_2 = \tau_e$, and τ_e is the exchange time constant for transitions between the two environments. For scalar relaxation of the second kind, $A = 2\pi J_{IS}$, and τ_1 and τ_2 are the spin-lattice and spin-spin relaxation time constants for the S spin, respectively. If the S spin is a quadrupolar nucleus, then the relaxation time constants are calculated using the expressions given in Table 5.9. The secular contribution to R_2^{sc} can be calculated using the random-phase model for transverse relaxation (Section 5.1.3). A more general treatment of scalar relaxation applicable to all time scales has been given by London (47).

5.5 Nuclear Overhauser Effect

The nuclear Overhauser effect (NOE) is a manifestation of the prediction [5.117] that dipolar-coupled spins do not relax independently. The NOE is without doubt one of the most important effects in NMR spectroscopy and more detailed discussions are found in monographs

devoted to the subject (48, 49). The Solomon equations ([5.11]) are extremely useful for explication of NOE experiments. The NOE is characterized by the cross-relaxation rate constant, σ_{IS}^{NOE} , defined by [5.114], or the steady-state *NOE enhancement*, η_{IS} , which is defined in the following discussion. These two quantities naturally arise in transient or steady-state NOE experiments, respectively.

The steady-state NOE experiment is illustrated here by using a dipole-coupled two-spin system as an example. If the *S* spin is irradiated by a weak rf field (so as not to perturb the *I* spin) for a period of time $t \gg 1/\rho_S, 1/\rho_I$, then the average populations across the *S* spin transitions are equalized and the *I* spin magnetization evolves to a steady-state value, $\langle I_z^{\text{ss}} \rangle$. In this situation, the *S* spins are said to be saturated. Setting $d\Delta I_z(t)/dt = 0$ and $\langle S_z \rangle(t) = 0$ in [5.11] and solving for $\langle I_z^{\text{ss}} \rangle / \langle I_z^0 \rangle$ yields

$$\begin{aligned} \frac{d\langle I_z^{\text{ss}} \rangle}{dt} &= -\rho_I (\langle I_z^{\text{ss}} \rangle - \langle I_z^0 \rangle) + \sigma_{IS}^{\text{NOE}} \langle S_z^0 \rangle = 0, \\ \langle I_z^{\text{ss}} \rangle / \langle I_z^0 \rangle &= 1 + \sigma_{IS}^{\text{NOE}} \langle S_z^0 \rangle / (\rho_I \langle I_z^0 \rangle). \end{aligned} \quad [5.148]$$

Using $\langle S_z^0 \rangle / \langle I_z^0 \rangle = \gamma_S / \gamma_I$ yields

$$\langle I_z^{\text{ss}} \rangle / \langle I_z^0 \rangle = 1 + \frac{\sigma_{IS}^{\text{NOE}} \gamma_S}{\rho_I \gamma_I} = 1 + \eta_{IS}, \quad [5.149]$$

in which

$$\eta_{IS} \equiv \frac{\sigma_{IS}^{\text{NOE}} \gamma_S}{\rho_I \gamma_I}. \quad [5.150]$$

As shown, the value of the longitudinal magnetization (or population difference) for the *I* spin is altered by saturating (equalizing the population difference) the *S* spin. If η_{IS} is positive, then the population differences across the *I* spin transitions are increased by reducing the population differences across the *S* spin transitions. Because the equilibrium population differences are inversely proportional to temperature, this result appears to indicate that heating the *S* spins (reducing the population difference) has the effect of cooling the *I* spins (increasing the population difference). This conclusion would appear to violate the Second Law of Thermodynamics; however, if coupling between the spin system and the lattice is properly taken into account, then no inconsistency with thermodynamics exists.

The value of the NOE enhancement, η_{IS} , is measured using the *steady-state NOE difference experiment*. In this experiment, two spectra are recorded. In the first spectrum, the *S* spin is saturated for a period of

time sufficient to establish the NOE enhancement of the I spin, a 90° pulse is applied to the system, and the FID is recorded. The intensity of the I spin resonance in the spectrum is proportional to $\langle I_z^{ss} \rangle$. In the second experiment, the S spin is not saturated. Instead a 90° pulse is applied to the system at equilibrium and the FID is recorded. The intensity of the I spin resonance in this spectrum is proportional to $\langle I_z^0 \rangle$. The value of η_{IS} is calculated from [5.150]. In practice, the steady-state NOE difference experiment is performed somewhat differently than described in order to maximize the accuracy of the results; such issues are not relevant to the present discussion and are discussed elsewhere (49).

Measurements of σ_{IS}^{NOE} are made by use of the one-dimensional *transient NOE experiment*, discussed in Section 5.1.2, or the two-dimensional *NOESY experiment* (Section, 6.6.1). These laboratory-frame relaxation transient NOE experiments have rotating-frame analogs: the *transient ROE experiment* and the two-dimensional *ROESY experiment* (Section 6.6.2) in which the rotating-frame cross-relaxation rate constant, σ_{IS}^{ROE} , is given by [5.141].

Using the isotropic rotor spectral density function [5.98], the cross-relaxation rate constants for a homonuclear spin system ($\gamma_I = \gamma_S = \gamma$) are given by

$$\sigma_{IS}^{\text{NOE}} = \frac{\hbar^2 \mu_0^2 \gamma^4 \tau_c}{160 \pi^2 r_{IS}^6} \left\{ -1 + \frac{6}{1 + 4\omega_0^2 \tau_c^2} \right\}, \quad [5.151]$$

$$\sigma_{IS}^{\text{ROE}} = \frac{\hbar^2 \mu_0^2 \gamma^4 \tau_c}{160 \pi^2 r_{IS}^6} \left\{ 2 + \frac{3}{1 + \omega_0^2 \tau_c^2} \right\},$$

and the NOE enhancement is given by

$$\eta_{IS} = \left\{ -1 + \frac{6}{1 + 4\omega_0^2 \tau_c^2} \right\} / \left\{ 1 + \frac{3}{1 + \omega_0^2 \tau_c^2} + \frac{6}{1 + 4\omega_0^2 \tau_c^2} \right\}. \quad [5.152]$$

The cross-relaxation rate constants are proportional to the inverse sixth power of the distance between the two dipolar interacting spins, r_{IS} , but the enhancement η_{IS} does not depend upon r_{IS} . Thus, a measurement of η_{IS} indicates whether two spins are close enough in space to experience dipolar cross-relaxation, but a quantitative estimate of the distance separating the spins cannot be obtained. To estimate the distance between two nuclei, σ_{IS}^{NOE} or σ_{IS}^{ROE} must be measured directly (or η_{IS} measured in one experiment and ρ_I in a second experiment).

In the extreme narrowing limit ($\omega_0\tau_c \ll 1$), [5.151] and [5.152] reduce to

$$\sigma_{IS}^{\text{NOE}} = \sigma_{IS}^{\text{ROE}} = \frac{\hbar^2 \mu_0^2 \gamma^4 \tau_c}{32\pi^2 r_{IS}^6}, \quad \eta_{IS} = \frac{1}{2}, \quad [5.153]$$

and in the spin diffusion limit ($\omega_0\tau_c \gg 1$),

$$\sigma_{IS}^{\text{NOE}} = -\frac{\hbar^2 \mu_0^2 \gamma^4 \tau_c}{160\pi^2 r_{IS}^6}, \quad \sigma_{IS}^{\text{ROE}} = \frac{\hbar^2 \mu_0^2 \gamma^4 \tau_c}{80\pi^2 r_{IS}^6}, \quad \eta_{IS} = -1. \quad [5.154]$$

In the slow tumbling regime, the laboratory- and rotating-frame cross-relaxation rate constants are related by

$$\sigma_{\text{ROE}} = -2\sigma_{\text{NOE}}. \quad [5.155]$$

This relationship has been used to compensate approximately for cross-relaxation effects in NMR spectra (50, 51). The values of σ_{IS}^{NOE} and η_{IS} are zero if $\omega\tau_c = 1.12$, whereas, $\sigma_{IS}^{\text{ROE}} > 0$ for all τ_c .

The principal use of the NOE in biological NMR spectroscopy is the determination of distances between pairs of ^1H spins (52). The NOE enhancements of interest arise from slowly tumbling biological macromolecules in the spin diffusion limit. For such molecules, relatively large transient homonuclear ^1H NOE (or ROE) enhancements build up quickly and are detected most effectively by transient NOE and NOESY (or transient ROE and ROESY) methods (Section 6.6).

5.6 Chemical Exchange Effects in NMR Spectroscopy

NMR spectroscopy provides an extremely powerful and convenient method for monitoring the *exchange* of a nucleus between environments due to chemical reactions or conformational transitions. In the first instance, the nucleus exchanges intermolecularly between sites in different molecules; in the second, the nucleus exchanges intramolecularly between conformations. The exchange process can be monitored by NMR spectroscopy even if the sites are chemically equivalent, provided that the sites are magnetically distinct. Nuclear spins can be manipulated during the NMR experiment without affecting the chemical states of the system, because of the weak coupling between the spin system and the lattice. Thus, chemical reactions and conformational exchange processes can be studied by NMR spectroscopy while the system remains in chemical equilibrium.

To establish a qualitative picture of the effects of exchange on an NMR spectrum, suppose that a given nucleus exchanges with rate constant k between two magnetically distinct sites with resonance frequencies that differ by $\Delta\omega$. On average, the resonance frequency of the spin in each site can only be observed for a time of the order of $1/k$ before the spin jumps to the other site and begins to precess with a different frequency. The finite observation time places a lower limit on the magnitude of $\Delta\omega$ required to distinguish the two sites. If the exchange rate is *slow* ($k \ll \Delta\omega$), then distinct signals are observed from the nuclei in the two sites; in contrast, if the exchange rate is *fast* ($k \gg \Delta\omega$), then a single resonance is observed at the population-weighted average chemical shift of the nuclei in the two sites. *The NMR chemical shift time scale is defined by the difference between the frequencies of spins in the two sites.*

5.6.1 CHEMICAL EXCHANGE FOR ISOLATED SPINS

For simplicity, only the case of chemical exchange in spin systems without scalar coupling interactions is treated here. In this situation, the exchange process is treated using an extension of the Bloch equations (Section 1.2). The results obtained using the Bloch equations are applicable to scalar-coupled spin systems if relaxation processes other than exchange are in the macromolecular limit and scalar coupling constants are not modified by the exchange process (53, 54). If scalar coupling constants are modified by the exchange process, as might be observed for $^1\text{H}^3J_{\text{HH}}$ scalar coupling interactions, then a complete treatment based on the density operator formalism is required (55).

A first-order chemical reaction (or two-site chemical exchange) between two chemical species, A_1 and A_2 , is described by the reaction



in which k_1 is the reaction rate constant for the forward reaction and k_{-1} is the reaction rate constant for the reverse reaction. The chemical kinetic rate laws for this system are written in matrix form as

$$\frac{d}{dt} \begin{bmatrix} [A_1](t) \\ [A_2](t) \end{bmatrix} = \begin{bmatrix} -k_1 & k_{-1} \\ k_1 & -k_{-1} \end{bmatrix} \begin{bmatrix} [A_1](t) \\ [A_2](t) \end{bmatrix}. \quad [5.157]$$

For a coupled set of N first-order chemical reactions between N chemical species, this equation is generalized to

$$\frac{d\mathbf{A}(t)}{dt} = \mathbf{K}\mathbf{A}(t), \quad [5.158]$$

in which the matrix elements of the rate matrix, \mathbf{K} , are given by

$$\begin{aligned} K_{ij} &= k_{ji} \quad (i \neq j), \\ K_{ii} &= - \sum_{\substack{j=1 \\ j \neq i}}^N k_{ij}, \end{aligned} \quad [5.159]$$

and the chemical reaction between the i th and j th species is



The modified Bloch equations are written in matrix form for the j th chemical species as

$$\begin{aligned} \frac{dM_{jx}(t)}{dt} &= \gamma(1 - \sigma_j)[\mathbf{M}_j(t) \times \mathbf{B}(t)]_x - R_{2j}M_{jx}(t) + \sum_{k=1}^N K_{jk}M_{kx}(t), \\ \frac{dM_{jy}(t)}{dt} &= \gamma(1 - \sigma_j)[\mathbf{M}_j(t) \times \mathbf{B}(t)]_y - R_{2j}M_{jy}(t) + \sum_{k=1}^N K_{jk}M_{ky}(t), \\ \frac{dM_{jz}(t)}{dt} &= \gamma(1 - \sigma_j)[\mathbf{M}_j(t) \times \mathbf{B}(t)]_z - R_{1j}\{M_{jz}(t) - M_{j0}(t)\} + \sum_{k=1}^N K_{jk}M_{kz}(t), \end{aligned} \quad [5.161]$$

with

$$M_{j0}(t) = M_0[A_j](t) / \sum_{j=1}^N [A_j](t). \quad [5.162]$$

The Bloch equations modified for chemical reactions are called the McConnell equations (56). The index j in [5.161] and [5.162] refers to the same spin in different chemical environments, not to different nuclear spins (cf. Section 1.2). If the system is in chemical equilibrium, then $[A_j](t) = [A_j]$ and

$$\sum_{k=1}^N K_{jk}M_{k0}(t) = 0. \quad [5.163]$$

Using this result, and defining $M_{k0} = M_{k0}(t)$, the expression for the evolution of longitudinal magnetization in [5.161] is expressed as

$$\begin{aligned} \frac{dM_{jz}(t)}{dt} = & \gamma(1 - \sigma_j)[\mathbf{M}_j(t) \times \mathbf{B}(t)]_z - R_{1j}\{M_{jz}(t) - M_{j0}\} \\ & + \sum_{k=1}^N K_{jk}[M_{kz}(t) - M_{k0}]. \end{aligned} \quad [5.164]$$

The preceding equations are generalized to the case of higher order chemical reactions by defining the pseudo-first-order rate constants:

$$k_{ij} = \frac{\dot{\xi}_{ij}(t)}{[A_i](t)}, \quad [5.165]$$

in which $\dot{\xi}_{ij}(t)$ is the rate law for conversion of the i th species containing the nuclear spin of interest into the j th species containing the nuclear spin of interest. The effect of the chemical reactions is to shift the spin of interest between molecular environments. For example, consider the elementary reaction



in which a spin in species A_1 is transferred to species A_2 as a result of the chemical reaction. The chemical kinetic rate laws for this system are

$$\frac{d}{dt} \begin{bmatrix} [A_1](t) \\ [A_2](t) \end{bmatrix} = \begin{bmatrix} -k_1[B](t) & k_{-1}[C](t) \\ k_1[B](t) & -k_{-1}[C](t) \end{bmatrix} \begin{bmatrix} [A_1](t) \\ [A_2](t) \end{bmatrix}, \quad [5.167]$$

which has the same form as [5.158] in which the elements of \mathbf{K} are defined using [5.159] and [5.165]. Notice that the rate expressions for $[B](t)$ and $[C](t)$ are not included in [5.167] because the spin of interest is not contained in either species.

In the absence of applied rf fields, the equation governing the evolution of longitudinal magnetization becomes

$$\frac{dM_{jz}(t)}{dt} = -R_{1j}\{M_{jz}(t) - M_{j0}(t)\} + \sum_{k=1}^N K_{jk}M_{kz}(t). \quad [5.168]$$

Defining

$$\mathbf{M}_z(t) = \begin{bmatrix} M_{1z}(t) \\ \vdots \\ M_{Nz}(t) \end{bmatrix} \quad [5.169]$$

yields the compact expression,

$$\frac{d\mathbf{M}_z(t)}{dt} = (-\mathbf{R} + \mathbf{K})\{\mathbf{M}_z(t) - \mathbf{M}_0(t)\} + \mathbf{K}\mathbf{M}_0(t), \quad [5.170]$$

in which the elements of \mathbf{R} are given by $R_{ij} = \delta_{ij}R_{1j}$. For simplicity, the possibility of simultaneous dipolar cross-relaxation and chemical exchange is not considered. If the system is in chemical equilibrium, $\mathbf{K}\mathbf{M}_0(t) = \mathbf{K}\mathbf{M}_0 = 0$ and defining $\Delta\mathbf{M}_z(t) = \mathbf{M}_z(t) - \mathbf{M}_0$

$$\frac{d\Delta\mathbf{M}_z(t)}{dt} = (-\mathbf{R} + \mathbf{K})\Delta\mathbf{M}_z(t). \quad [5.171]$$

By similar reasoning, the equation of motion for the transverse magnetization is written in the rotating frame as

$$\frac{d\mathbf{M}^+(t)}{dt} = (i\mathbf{\Omega} - \mathbf{R} + \mathbf{K})\mathbf{M}^+(t), \quad [5.172]$$

in which the elements of $\mathbf{\Omega}$ are given by $\Omega_{ij} = \delta_{ij}\Omega_j$, and the elements of \mathbf{R} are given by $R_{ij} = \delta_{ij}R_{2j}$.

Equations [5.171] and [5.172] have the same functional form as [5.14] and are solved by the same methods ([5.15]). For example, the rate matrix for longitudinal relaxation in a system undergoing two-site exchange is given by

$$\mathbf{R} - \mathbf{K} = \begin{bmatrix} \rho_1 + k_1 & -k_{-1} \\ -k_1 & \rho_2 + k_{-1} \end{bmatrix} \quad [5.173]$$

with eigenvalues

$$\lambda_{\pm} = \frac{1}{2} \left\{ (\rho_1 + \rho_2 + k_1 + k_{-1}) \pm [(\rho_1 - \rho_2 + k_1 - k_{-1})^2 + 4k_1k_{-1}]^{1/2} \right\}. \quad [5.174]$$

The time course of the magnetization is given by

$$\begin{bmatrix} \Delta M_{1z}(t) \\ \Delta M_{2z}(t) \end{bmatrix} = \begin{bmatrix} a_{11}(t) & a_{12}(t) \\ a_{21}(t) & a_{22}(t) \end{bmatrix} \begin{bmatrix} \Delta M_{1z}(0) \\ \Delta M_{2z}(0) \end{bmatrix}, \quad [5.175]$$

in which

$$\begin{aligned} a_{11}(t) &= \frac{1}{2} \left[\left(1 - \frac{\rho_1 - \rho_2 + k_1 - k_{-1}}{(\lambda_+ - \lambda_-)} \right) \exp(-\lambda_- t) \right. \\ &\quad \left. + \left(1 + \frac{\rho_1 - \rho_2 + k_1 - k_{-1}}{(\lambda_+ - \lambda_-)} \right) \exp(-\lambda_+ t) \right], \\ a_{22}(t) &= \frac{1}{2} \left[\left(1 + \frac{\rho_1 - \rho_2 + k_1 - k_{-1}}{(\lambda_+ - \lambda_-)} \right) \exp(-\lambda_- t) \right. \\ &\quad \left. + \left(1 - \frac{\rho_1 - \rho_2 + k_1 - k_{-1}}{(\lambda_+ - \lambda_-)} \right) \exp(-\lambda_+ t) \right], \\ a_{12}(t) &= \frac{k_{-1}}{(\lambda_+ - \lambda_-)} [\exp(-\lambda_- t) - \exp(-\lambda_+ t)], \\ a_{21}(t) &= \frac{k_1}{(\lambda_+ - \lambda_-)} [\exp(-\lambda_- t) - \exp(-\lambda_+ t)]. \end{aligned} \quad [5.176]$$

If the initial perturbation is nonselective, so that $\Delta M_{1z}(0) \propto p_1$ and $\Delta M_{2z}(0) \propto p_2$, in which p_1 and p_2 are the equilibrium fractional site populations for sites A_1 and A_2 , and if $\rho_1 = \rho_2$, then longitudinal relaxation is not affected by the exchange process. In addition, if these conditions are not met, but exchange is fast on the chemical shift time scale and $|k_{\text{ex}}(p_1 - p_2)| \gg |\rho_1 - \rho_2|$, in which $k_{\text{ex}} = k_1 + k_{-1}$, then longitudinal relaxation similarly is unaffected and the population-averaged longitudinal relaxation rate constant is observed. If these conditions are not satisfied, that is, if exchange is slow on the chemical shift time scale and either a selective perturbation is applied to the spins in the two sites or $\rho_1 \neq \rho_2$, then the exchange process transfers longitudinal magnetization between sites.

To obtain some insight into the form of these equations, assume that $\rho_1 = \rho_2 = \rho$. Under these conditions, the time dependence of the

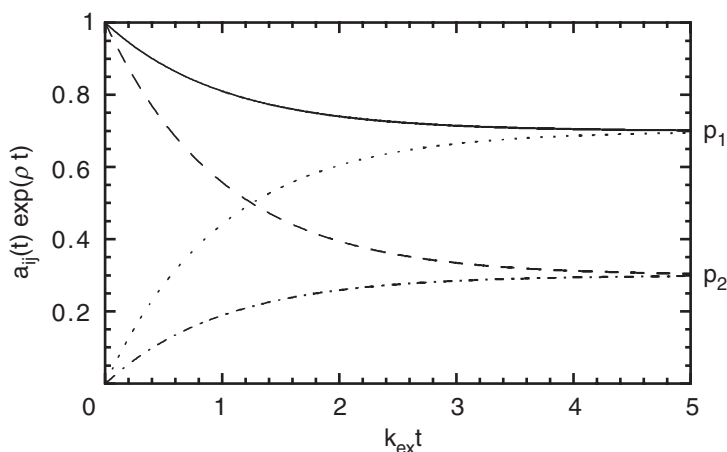


FIGURE 5.9 Population transfer due to chemical exchange. The transfer function amplitudes (—) $a_{11}(t)$, (---) $a_{22}(t)$, (···) $a_{12}(t)$, and (-·-) $a_{21}(t)$ calculated using [5.177].

longitudinal magnetization is given by

$$\begin{aligned}
 a_{11}(t) &= [p_1 + p_2 \exp(-2k_{\text{ex}}t)] \exp(-\rho t), \\
 a_{22}(t) &= [p_2 + p_1 \exp(-2k_{\text{ex}}t)] \exp(-\rho t), \\
 a_{12}(t) &= p_1 [1 - \exp(-2k_{\text{ex}}t)] \exp(-\rho t), \\
 a_{21}(t) &= p_2 [1 - \exp(-2k_{\text{ex}}t)] \exp(-\rho t).
 \end{aligned}
 \tag{5.177}$$

The time dependence of the transfer amplitudes, $a_{ij}(t)$, given in [5.177] is shown in Fig. 5.9. The homology between [5.22] and [5.177] illustrates the similarity between the effects of cross-relaxation and chemical exchange on longitudinal magnetization. Indeed, similar experimental techniques are utilized to study both phenomena (such as NOESY and ROESY experiments, Section 6.6).

The rate matrix for transverse relaxation in a system undergoing two-site exchange is given by

$$-i\mathbf{\Omega} + \mathbf{R} - \mathbf{K} = \begin{bmatrix} -i\Omega_1 + \rho_1 + k_1 & -k_{-1} \\ -k_1 & -i\Omega_2 + \rho_2 + k_{-1} \end{bmatrix}, \tag{5.178}$$

with eigenvalues

$$\lambda_{\pm} = \frac{1}{2} \left\{ (-i\Omega_1 - i\Omega_2 + \rho_1 + \rho_2 + k_1 + k_{-1}) \pm [(-i\Omega_1 + i\Omega_2 + \rho_1 - \rho_2 + k_1 - k_{-1})^2 + 4k_1k_{-1}]^{1/2} \right\}. \quad [5.179]$$

The time course of the magnetization is given by

$$\begin{bmatrix} M_1^+(t) \\ M_2^+(t) \end{bmatrix} = \begin{bmatrix} a_{11}(t) & a_{12}(t) \\ a_{21}(t) & a_{22}(t) \end{bmatrix} \begin{bmatrix} M_1^+(0) \\ M_2^+(0) \end{bmatrix}, \quad [5.180]$$

in which

$$\begin{aligned} a_{11}(t) &= \frac{1}{2} \left[\left(1 - \frac{-i\Omega_1 + i\Omega_2 + \rho_1 - \rho_2 + k_1 - k_{-1}}{(\lambda_+ - \lambda_-)} \right) \exp(-\lambda_- t) \right. \\ &\quad \left. + \left(1 + \frac{-i\Omega_1 + i\Omega_2 + \rho_1 - \rho_2 + k_1 - k_{-1}}{(\lambda_+ - \lambda_-)} \right) \exp(-\lambda_+ t) \right], \\ a_{22}(t) &= \frac{1}{2} \left[\left(1 + \frac{-i\Omega_1 + i\Omega_2 + \rho_1 - \rho_2 + k_1 - k_{-1}}{(\lambda_+ - \lambda_-)} \right) \exp(-\lambda_- t) \right. \\ &\quad \left. + \left(1 - \frac{-i\Omega_1 + i\Omega_2 + \rho_1 - \rho_2 + k_1 - k_{-1}}{(\lambda_+ - \lambda_-)} \right) \exp(-\lambda_+ t) \right], \end{aligned} \quad [5.181]$$

$$a_{12}(t) = \frac{k_{-1}}{(\lambda_+ - \lambda_-)} [\exp(-\lambda_- t) - \exp(-\lambda_+ t)],$$

$$a_{21}(t) = \frac{k_1}{(\lambda_+ - \lambda_-)} [\exp(-\lambda_- t) - \exp(-\lambda_+ t)].$$

The NMR spectrum is given by the Fourier transformation of $M_1^+(t) + M_2^+(t)$.

The eigenvalues are resolved into real and imaginary parts, corresponding to the relaxation rates and precession frequencies, by using the identity

$$(a + ib)^{1/2} = \frac{1}{\sqrt{2}} [a + (a^2 + b^2)^{1/2}]^{1/2} + \frac{i}{\sqrt{2}} [-a + (a^2 + b^2)^{1/2}]^{1/2}. \quad [5.182]$$

If $\rho_1 = \rho_2 = \rho$ is assumed for simplicity, then $\lambda_{\pm} = i\Omega - R_2$, in which (57, 58)

$$R_2 = \rho + \frac{k_{\text{ex}}}{2} \pm \frac{1}{\sqrt{8}} \left\{ k_{\text{ex}}^2 - \Delta\omega^2 + \left[(k_{\text{ex}}^2 + \Delta\omega^2)^2 - 16p_1p_2\Delta\omega^2k_{\text{ex}}^2 \right]^{1/2} \right\}^{1/2}, \quad [5.183]$$

$$\Omega = \frac{\Omega_1 + \Omega_2}{2} \pm \frac{1}{\sqrt{8}} \left\{ \Delta\omega^2 - k_{\text{ex}}^2 + \left[(k_{\text{ex}}^2 + \Delta\omega^2)^2 - 16p_1p_2\Delta\omega^2k_{\text{ex}}^2 \right]^{1/2} \right\}^{1/2}, \quad [5.184]$$

and $\Delta\omega = \Omega_2 - \Omega_1$.

When exchange is slow, $k_{\text{ex}} < |\Delta\omega|$, the magnetization components for the two sites are nonsecular with respect to each other and the off-diagonal terms in [5.178] can be neglected. In this case, the magnetization components in the two sites evolve independently with

$$\begin{aligned} M_1^+(t) &= M_1^+(0) \exp[-(i\Omega_1 + \rho_1 + k_1)t], \\ M_2^+(t) &= M_2^+(0) \exp[-(i\Omega_2 + \rho_2 + k_{-1})t]. \end{aligned} \quad [5.185]$$

When exchange is fast, $k_{\text{ex}} > \Delta\omega$, only the averaged magnetization $M^+(t) = M_1^+(t) + M_2^+(t)$ is observable. The evolution of the averaged magnetization is derived simply by using the random-phase model for transverse relaxation (Section 5.1.3) together with the identification $\tau_c = 1/k_{\text{ex}}$. The averaged resonance evolves as

$$M^+(t) = M^+(0) \exp[-(i\bar{\Omega} + \bar{\rho} + p_1p_2\Delta\omega^2/k_{\text{ex}})t], \quad [5.186]$$

in which the average resonance offset is $\bar{\Omega} = p_1\Omega_1 + p_2\Omega_2$ and $\bar{\rho} = p_1\rho_1 + p_2\rho_2$.

Simulated spectra are shown in Fig. 5.10 for two situations: the first is symmetric unimolecular exchange in which $p_1 = p_2 = 0.5$; the second is skewed unimolecular exchange in which $p_1 = 3p_2$. In the absence of exchange, spectra f and l in Fig. 5.10 show that resolved lines are observed for the two sites with resonance frequencies Ω_1 and Ω_2 , and relaxation decay constants ρ_1 and ρ_2 . As the exchange rate increases, the resonance lines broaden, as shown in spectra e and k, and the evolution is described by [5.185]. When the exchange rate is of the order of the chemical shift separation between the two sites, the lines become very broad and begin to coalesce when $k_{\text{ex}} \approx \Delta\omega$ (spectra c and i). This is known as the *intermediate exchange* regime, or *coalescence*. Intermediate exchange processes can cause peaks to disappear in spectra because the broadening becomes so great that the resonance line becomes indistinguishable from the baseline noise. Increasing the exchange rate for the system above the coalescence point pushes the system into fast exchange, $k_{\text{ex}} > \Delta\omega$. A single averaged resonance line is observed at the population-weighted average shift and evolution is described by [5.186], as shown in spectra b and h in Fig. 5.10. As the

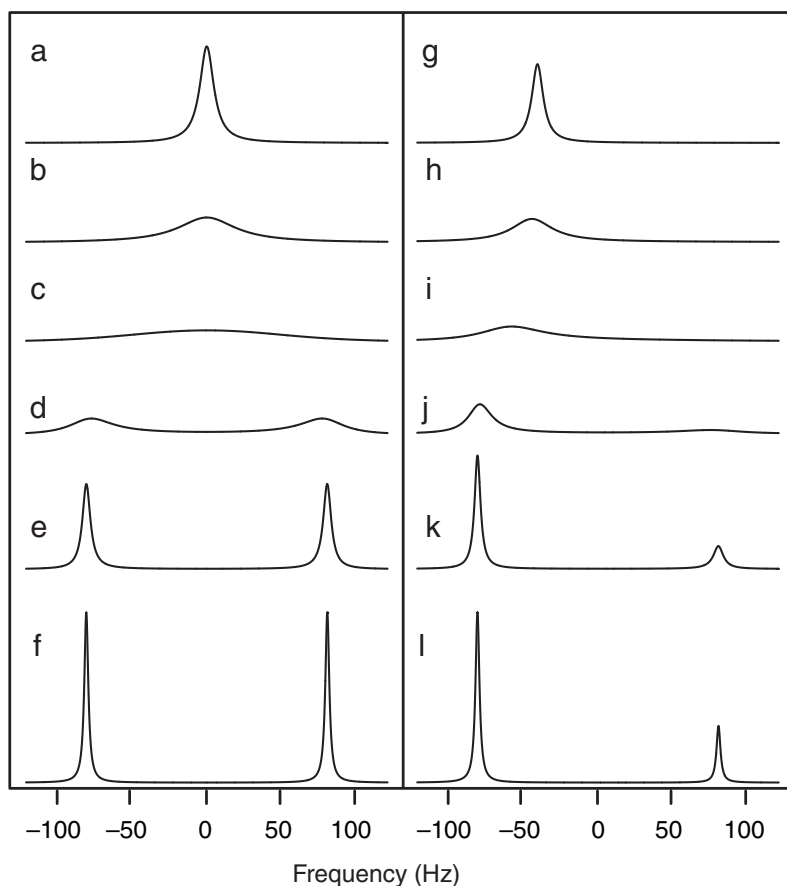


FIGURE 5.10 Chemical exchange for a two-site system. Shown are the Fourier transformations of FIDs calculated by using [5.181]. The calculations used $\Delta\omega/2\pi = 180 \text{ Hz}$ and $\rho_1 = \rho_2 = 10 \text{ s}^{-1}$. In (a–f), $p_1 = p_2 = 0.5$; in g–l, $p_1 = 3p_2$. Calculations were performed for values of the exchange rate, k_{ex} , equal to (a, g) 10000 s^{-1} , (b, h) 2000 s^{-1} , (c, i) 900 s^{-1} , (d, j) 200 s^{-1} , (e, k) 20 s^{-1} , and (f, l) 0 s^{-1} .

exchange rate continues to increase, the resonance lineshape becomes increasingly narrow until, in the limit $k_{\text{ex}} \rightarrow \infty$, the relaxation decay constant is given by $\bar{\rho}$. Equations [5.185] and [5.186] confirm the qualitative conclusions about the slow and fast exchange regimes stated herein.

5.6.2 QUALITATIVE EFFECTS OF CHEMICAL EXCHANGE IN SCALAR-COUPLED SYSTEMS

Multiplet structure due to scalar couplings is affected by chemical exchange. Detailed theoretical treatment using the density matrix formalism is beyond the subject matter of this text (59); instead, the discussion here presents qualitatively the most important effects. Formally, scalar relaxation (Section 5.4.6) and chemical exchange in scalar-coupled systems are homologous. Two different cases must be considered: intermolecular (homologous to scalar relaxation of the second kind) and intramolecular exchange (homologous to scalar relaxation of the first kind).

Intermolecular chemical exchange in scalar-coupled systems is encountered frequently in biological NMR applications. For example, exchange between labile amide and solvent protons perturbs the $^1\text{H}^{\text{N}}$ to $^1\text{H}^{\alpha}$ scalar coupling interaction. In an IS spin system, the I spin resonance is a doublet, with the lines separated by J_{IS} . One line of the doublet is associated with the S spin in the α state, and the other line is associated with the S spin in the β state. Suppose that a given I spin is coupled to an S spin in the α state. If the S spin exchanges with another S spin originating from the solvent (intermolecular exchange), then after the exchange, the I spin has equal probability of being coupled to an S spin in the α and β states because the incoming spin has a 50% chance of either being in its α state or in its β state. Similar considerations hold for an I spin initially coupled to an S spin in the β state. Consequently, the I spin sees the S spin state constantly changing due to exchange and thus the frequency of the I spin resonance constantly changes between the frequencies of the two lines of the doublet. This phenomenon constitutes a two-site exchange process and exhibits properties of slow, intermediate, and fast exchange. If the exchange is fast compared to the difference in frequency between the two lines (i.e., compared to the scalar coupling constant), a single line is observed at the mean frequency (the Larmor frequency of the I spin). Because homonuclear scalar coupling constants tend to be small, relatively slow exchange processes, which would minimally perturb the chemical shifts of the exchanging spins, can result in collapse of multiplet structure. Indeed, the broadening of multiplets and the disappearance of multiplet structure are the first clues to the existence of exchange phenomena in NMR spectra.

Intramolecular exchange constitutes a slightly different situation. Consider a system in which spin I is scalar coupled to spin S , but due to the presence of multiple conformers, spin S experiences n environments,

S^1, S^2, \dots, S^n , with different scalar coupling constants. For simplicity, the chemical shift of the I spin is assumed to be identical in all conformers. If the conformers interconvert on a time scale that is long compared to the scalar coupling constants, the I spin multiplet is a superposition of n doublets arising from the IS^1, IS^2, \dots, IS^n scalar coupling interactions. On the other hand, if the conformers interconvert at a rate much larger than the scalar coupling constants, the I spin resonance is a doublet with an effective scalar coupling constant that is a population-weighted average of the n scalar coupling constants. An example of this effect arises for the scalar coupling between $^1\text{H}^\alpha$ and $^1\text{H}^\beta$ spins in amino acids. If the conformations of the H^β nuclei are fixed relative to the H^α nuclei, then the H^α multiplet is split by two coupling constants, one from each of the $^1\text{H}^\beta$ spins to the $^1\text{H}^\alpha$ spin (e.g., 12 and 3 Hz, respectively, for a trans and gauche conformation). On the other hand, if the H^β nuclei exchange between trans, gauche⁺, and gauche⁻ rotameric sites due to free rotation about the $\text{C}^\alpha\text{--C}^\beta$ bond, then the H^α multiplet is split by a single average coupling constant, with a value $(12 + 3 + 3)/3 = 6$ Hz, due to the $^1\text{H}^\beta$ spins.

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