JOURNAL OF MAGNETIC RESONANCE, Series A **114**, 248–254 (1995)

Influence of Internal Exchange Processes and Nuclear Relaxation on the CW Saturation EPR of Spin Populations Undergoing Two-Site Exchange

DEREK MARSH

Max-Planck-Institut für biophysikalische Chemie, Abteilung Spektroskopie, D-37077 Göttingen, Germany

Received October 19, 1994; revised January 10, 1995

As is well known, at least in NMR, the effective spin-lattice relaxation rates of spin systems undergoing two-site exchange are dependent on the exchange rates between them, when these are comparable to the intrinsic spin–lattice relaxation rates. Recently, this effect has been exploited to determine the exchange rates of spin-labeled lipids at the intramembranous surface of integral proteins by using CW saturation EPR (*1*). The analogous problem of determining the frequency of Heisenberg exchange between spin-labeled lipids and spin-labeled proteins, and hence their mutual accessibilities in membranes, has been treated by similar methods (*2*). In these studies, a straightforward two-site exchange model was used to extract the exchange rates by assuming that all internal exchange processes (and nuclear relaxation) within the individual component populations could be approximated by a single effective spin–lattice relaxation time for each isolated component (for a review of the methods, see Ref. *3*). This approach has the advantage of both experimental and theoretical simplicity and is applicable to a wide range of systems of biological interest. The purpose of the present paper is to investigate the conditions under which the simple approximation is valid, in order that the method may be exploited fully.

The situation considered is illustrated in Fig. 1. The ensembles of spins undergoing two-site exchange are designated b and f, and the corresponding rates of transfer from these sites are τ_b^{-1} and τ_f^{-1}, respectively. Each ensemble is composed of different spin systems, i, which, in a practical case, would normally correspond to the individual molecular orientations contributing to a powder-pattern spectrum. The individual spin systems, i, may be coupled by internal exchange with all the other systems, j, within a given ensemble. This coupling could be by Heisenberg exchange, in the case of a localized region of high spin concentration, but more generally by rotational diffusion. It is characterized by an effective second-order rate constant, K_x. If the internal exchange is very rapid, e.g., in the case of conventional motional narrowing, then the approximation of a single effective T_1 is valid. Otherwise, it is necessary to consider the coupling between the internal and two-site exchange processes. This is done here.

The CW saturation experiment can be analyzed in terms of the rate equations for the spin-population differences (*4*), an approach which follows that introduced by Yin and Hyde (*5*) for saturation recovery and ELDOR experiments. The results for internal Heisenberg exchange (*4*), simple two-site physical exchange (*1*), and simple two-site Heisenberg exchange (*2*) have been presented previously. It has been shown that Heisenberg exchange and physical or chemical exchange are formally equivalent in this context (*4*). Thus the Heisenberg spin-exchange formalism for the internal process can also be used, with appropriate definition of the exchange rates, to represent the physical exchange process of rotational diffusion (cf. Ref. *6*). This is the convention that will be used throughout the following.

The notation of Slichter (*7*) is used, as was done previously (*4*). The populations of the $M_{S_i} = \pm\frac{1}{2}$ energy levels for the ith spin system in ensemble b are denoted by $N_{b,i}^{\pm}$, and the population difference in this spin system by $n_{b,i} = N_{b,i}^{-} - N_{b,i}^{+}$. The population difference corresponding to Boltzmann equilibrium is denoted by $n_{b,i}^{\circ}$. The total population of the ith spin system in ensemble b is further given by $N_{b,i} = N_{b,i}^{-} + N_{b,i}^{+}$. In addition, the total population summed over all spin systems in ensemble b is given by $N_b = \sum N_{b,i}$, and correspondingly for the population differences $n_b = \sum n_{b,i}$, where the summation is over all i. Similar definitions hold for the ensemble f. The condition of detailed balance for the two-site exchange can be expressed in terms of equilibrium spin population differences as

$$n_b^{\circ}\tau_b^{-1} = n_f^{\circ}\tau_f^{-1}. \qquad [1]$$

A further useful relation for the spin-population differences at Boltzmann equilibrium is

$$n_{b,i}^{\circ} = \frac{N_{b,i}}{N_b} \times n_b^{\circ}. \qquad [2]$$

These relations will be used extensively below.

1064-1858/95 $6.00

For a simple two-level system ($S_{b,k} = \frac{1}{2}$), the standard expression for the steady-state population difference under CW irradiation is (*7*)

$$n_{b,k} = \frac{n^{\circ}_{b,k}}{1 + 2WT_1}, \qquad [3]$$

where W is the rate of transitions induced by the H_1 field and T_1 is the spin–lattice relaxation time. In two ensembles of multispin systems, the kth (i.e., saturated) spin system in ensemble b is coupled by internal exchange with the other spin systems, i, in ensemble b, and by two-site exchange with those in ensemble f. For the present analysis, the dependence of $n_{b,k}$ on W given by Eq. [3] provides a definition of the effective spin–lattice relaxation time, $T^{\mathrm{eff}}_{1,b,k}$, as determined in CW saturation studies for these more complex spin systems (*4*).

The first case treated is that of restricted two-site exchange with internal exchange at one site. Exchange between the two ensembles of spins b and f is considered in which the spin systems, i, in ensemble b are coupled by Heisenberg exchange (or equivalently by rotational diffusion). For simplicity, the spin systems in ensemble f are assumed to be identical but not to be coupled with each other. The latter situation would obtain, for instance, in the case of rapid motional averaging at a rate far exceeding that of the processes considered here. Initially, a restricted form is assumed for the two-site exchange in which spin system i in ensemble b exchanges only with the corresponding spin system i in ensemble f. Within the context of anisotropic powder patterns, this would correspond to preservation of angular orientation on exchange. With this model for one-to-one two-site exchange, material balance for a given system, i, requires that

$$n^{\circ}_{b,i}\tau_b^{-1} = n^{\circ}_{f,i}\tau_f^{-1}. \qquad [4]$$

Summing this equation over all i yields the general, less restrictive condition given by Eq. [1]; as for consistency it must.

Transitions are induced by the microwave H_1 field between the kth pair of electron spin levels in ensemble b; i.e., it is the saturation of this transition that is considered. The steady-state rate equation for the spin-population difference of the kth transition in ensemble b undergoing internal exchange with all the spin systems i and two-site exchange with ensemble f is then given by (see Refs. *1*, *4*)

$$\frac{dn_{b,k}}{dt} = -2W_{e,b}(n_{b,k} - n^{\circ}_{b,k}) - K_{x,b}(N_b n_{b,k} - N_{b,k} n_b) - n_{b,k}\tau_b^{-1} + n_{f,k}\tau_f^{-1} - 2Wn_{b,k} = 0, \qquad [5]$$

where $2W_{e,b} = 1/T^{\circ}_{1,b}$ is the electron spin–lattice relaxation rate (assumed to be the same for all transitions in ensemble b), and $K_{x,b}$ is the rate constant for internal exchange in ensemble b. The corresponding steady-state rate equation for the spin-population difference of the kth transition in ensemble f that is coupled to ensemble b, but is not undergoing internal exchange and is not irradiated, is given by

$$\frac{dn_{f,k}}{dt} = -2W_{e,f}(n_{f,k} - n^{\circ}_{f,k}) - n_{f,k}\tau_f^{-1} + n_{b,k}\tau_b^{-1} = 0, \qquad [6]$$

where $2W_{e,f} = 1/T^{\circ}_{1,f}$ is the electron spin–lattice relaxation rate for transitions in ensemble f.

Eliminating $n_{f,k}$ between Eqs. [5] and [6] yields

$$-\left(2W_{e,b} + \frac{2W_{e,f}\tau_b^{-1}}{2W_{e,f} + \tau_f^{-1}} + K_{x,b}N_b + 2W\right)n_{b,k} + K_{x,b}N_{b,k}n_b + \left(2W_{e,b} + \frac{2W_{e,f}\tau_b^{-1}}{2W_{e,f} + \tau_f^{-1}}\right)n^{\circ}_{b,k} = 0, \qquad [7]$$

where use has also been made of Eq. [4]. Summing Eq. [7] and its equivalents over all spin systems i in ensemble b, where only the kth system is irradiated, then yields

$$\left(2W_{e,b} + \frac{2W_{e,f}\tau_b^{-1}}{2W_{e,f} + \tau_f^{-1}}\right)(n^{\circ}_b - n_b) - 2Wn_{b,k} = 0. \qquad [8]$$

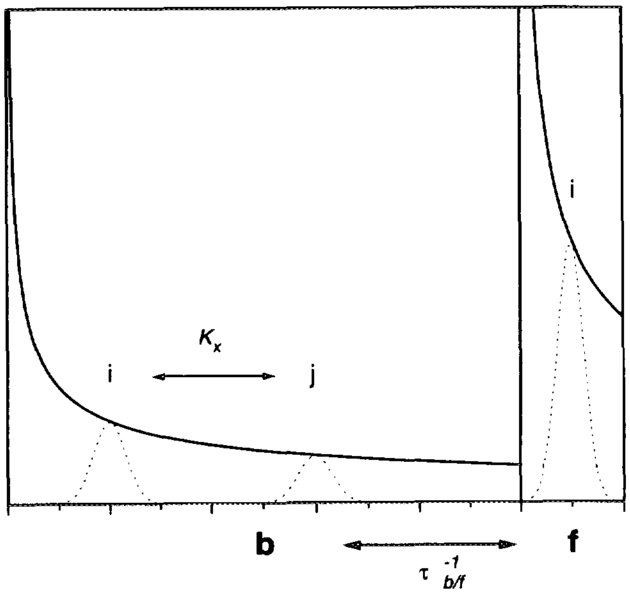

FIG. 1. Schematic indication of the spectra (heavy lines) of two ensembles of spins, b and f, which are undergoing mutual two-site exchange and in one of which (ensemble b) the individual spin systems, i, are undergoing internal exchange (Heisenberg exchange, rotational diffusion, etc.) with the other spin systems, j. The two-site exchange is characterized by the transfer rates τ_b^{-1} and τ_f^{-1}, and the internal exchange within ensemble b by the rate constant, K_x.

Solving Eqs. [7] and [8] for $n_{b,k}$ and making use of Eq. [2] then yields an expression that is identical in form to Eq. [3] for the saturation of the kth spin system in ensemble b. The effective spin–lattice relaxation time is then given by

$$T^{\mathrm{eff}}_{1,b,k} = \frac{2W_{e,b} + 2W_{e,f}\tau_b^{-1}/(2W_{e,f} + \tau_f^{-1}) + K_{x,b}N_{b,k}}{[2W_{e,b} + 2W_{e,f}\tau_b^{-1}/(2W_{e,f} + \tau_f^{-1})] \times [2W_{e,b} + 2W_{e,f}\tau_b^{-1}/(2W_{e,f} + \tau_f^{-1}) + K_{x,b}N_b]} . \qquad [9]$$

In the absence of two-site exchange (i.e., $\tau_b^{-1} = 0 = \tau_f^{-1}$), Eq. [9] yields the standard expression for the effective spin–lattice relaxation time, $T^{(x)}_{1,b,k}$, in the presence of coupling between spin systems solely by internal Heisenberg exchange (cf. Ref. *4*),

$$T^{(x)}_{1,b,k} = \frac{1 + Z_{b,k}T^{\circ}_{1,b}\tau^{-1}_{ex,b}}{1 + T^{\circ}_{1,b}\tau^{-1}_{ex,b}} \cdot T^{\circ}_{1,b} \qquad [10]$$

where $Z_{b,k} = N_{b,k}/N_b$ is the fractional population (or degeneracy) of the kth spin system, and $\tau^{-1}_{ex,b}$ is the Heisenberg exchange frequency, in ensemble b. The latter is given in the usual bimolecular formalism (i.e., $\tau^{-1}_{ex,b} = K_{x,b}N_b$) and is proportional to the total spin concentration in ensemble b (*4*).

In the absence of internal exchange (i.e., $K_{x,b} = 0$), Eq. [9] yields the standard expression for the effective spin–lattice relaxation time, $T^{(2si)}_{1,b}$, in the presence of exchange between ensembles b and f (cf. Ref. *1*),

$$\frac{T^{\circ}_{1,b}}{T^{(2si)}_{1,b}} = 1 + \frac{T^{\circ}_{1,b}\tau_b^{-1}}{1 + T^{\circ}_{1,f}\tau_f^{-1}} , \qquad [11]$$

which for this model of the two-site exchange it must.

The results given above make it possible to explore the validity of the simple two-site exchange formalism. Examination of Eq. [9] reveals the conditions under which simplified expressions of the form of Eq. [11] may be used to determine rates of two-site exchange in the presence of Heisenberg exchange (or of other internal exchange process such as rotational diffusion) within one of the ensembles. First, for relatively slow two-site exchange, given by the condition $2W_{e,b} + K_{x,b}N_{b,k} \gg 2W_{e,f}\tau_b^{-1}/(2W_{e,f} + \tau_f^{-1})$, the effective spin–lattice relaxation rate is approximated by

$$\frac{T^{(x)}_{1,b,k}}{T^{\mathrm{eff}}_{1,b,k}} = \frac{T^{\circ}_{1,b}}{T^{(2si)}_{1,b}} . \qquad [12]$$

Hence, the normalized exchange rates defined by Eq. [11] can be obtained from measurements of the experimentally accessible quantities $T^{(x)}_{1,b}$ and $T^{\mathrm{eff}}_{1,b}$ which correspond to the effective spin–lattice relaxation times for the one-component and two-component systems, respectively. This condition is most usefully fulfilled by fast rates of Heisenberg exchange (or rotational diffusion) relative to the intrinsic spin–lattice relaxation rate. It is also fulfilled for slow two-site exchange relative to the intrinsic spin–lattice relaxation rate, but in these circumstances the sensitivity to the two-site exchange is relatively low.

Second, for relatively slow Heisenberg exchange (or rotational diffusion), given by the condition $2W_{e,b} + 2W_{e,f}\tau_b^{-1}/(2W_{e,f} + \tau_f^{-1}) \gg K_{x,b}N_b$, the effective spin–lattice relaxation time is given by Eq. [11] for simple two-site exchange, as would be expected. In this case, determination from the experimental parameters according to Eq. [12] is also valid because then $T^{(x)}_{1,b} \approx T^{\circ}_{1,b}$.

It is possible to rewrite Eq. [9] for the effective spin–lattice relaxation time, in terms of the relaxation times defined by Eqs. [10] and [11] for the internal (Heisenberg) exchange and two-site exchange alone, respectively:

$$T^{\mathrm{eff}}_{1,b,k} = \frac{1 + Z_{b,k}T^{(2si)}_{1,b} \times \tau^{-1}_{ex,b}}{1 + T^{(2si)}_{1,b} \times \tau^{-1}_{ex,b}} \times T^{(2si)}_{1,b} . \qquad [13]$$

Interestingly, this latter expression is of exactly the same form as that for Heisenberg exchange (or rotational diffusion, etc.) alone (cf. Eq. [10]). For this model of two-site exchange, therefore, the Heisenberg exchange rates may be extracted in the normal way (cf. Ref. *8*), yielding values, $T^{(2si)}_{1,b}\tau^{-1}_{ex,b}$, that are normalized by the effective spin–lattice relaxation time for two-site exchange in the absence of Heisenberg exchange.

Equation [13] may be used to analyze more closely the range of validity of Eqs. [11] and [12] for determining two-site exchange rates. The relaxation rate enhancement, $T^{(x)}_{1,b}/T^{\mathrm{eff}}_{1,b}$, predicted by Eq. [9] and normalized relative to the value, $T^{\circ}_{1,b}/T^{(2si)}_{1,b}$, given by the approximation in Eq. [11], is given as a function of the normalized internal exchange rate, $T^{\circ}_{1,b}\tau^{-1}_{ex,b}$, in Fig. 2. Plots are given for various values of the degeneracy factor, $Z_{b,k}$, and the degree of relaxation enhancement by two-site exchange, $T^{\circ}_{1,b}/T^{(2si)}_{1,b}$. The values chosen for the latter span, or even exceed, those likely for these parameters. Validity of the approximate Eq. [12] requires that $(T^{(x)}_{1,b}/T^{\mathrm{eff}}_{1,b})/(T^{\circ}_{1,b}/T^{(2si)}_{1,b}) \approx 1$. From Fig. 2, it is seen that this condition is approximately fulfilled at low exchange rates, $T^{\circ}_{1,b}\tau^{-1}_{ex} \leqslant 1$, and at high exchange rates, $T^{\circ}_{1,b}\tau^{-1}_{ex} \geqslant 30$–$40$, depending on the values of $Z_{b,k}$ and $T^{\circ}_{1,b}/T^{(2si)}_{1,b}$. In terms of rotational diffusion, this implies that the correlation times should lie either in the slow motional regime (or faster) of conventional spin label EPR spectroscopy ($\leqslant 10^{-8}$ s) or in the slower regime of saturation transfer EPR spectroscopy ($\geqslant 10^{-4}$ s) (cf. Refs. *9*, *10*). In particular, the fast-exchange condition for validity of the simple approximation is easily fulfilled for spin-label spectra in the conventional motional narrowing regime (i.e., $\tau_R \leqslant 10^{-9}$

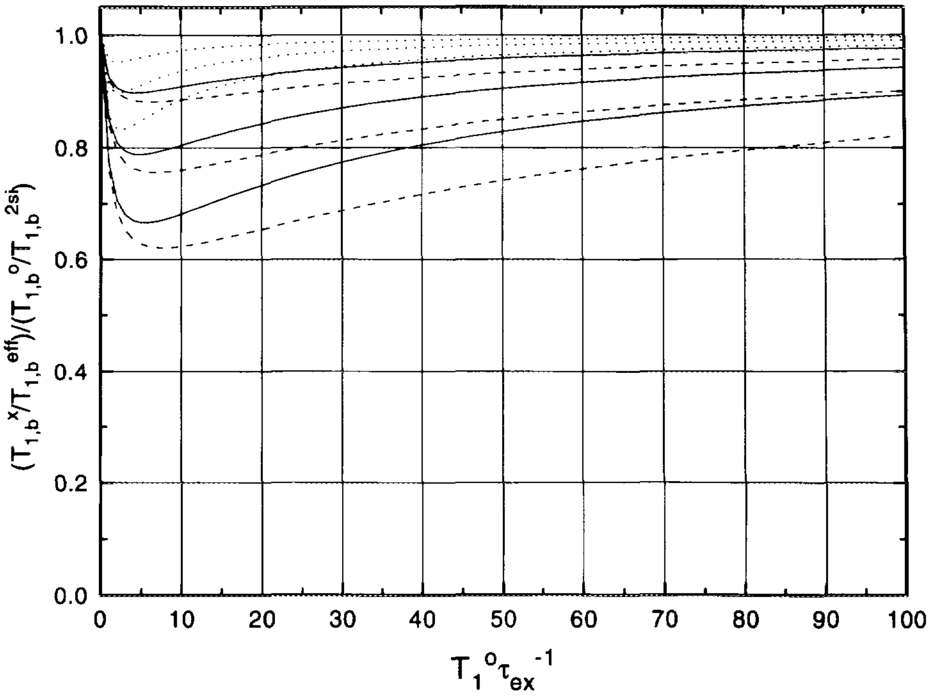

FIG. 2. Dependence of the effective spin–lattice relaxation rate, $1/T_{1,b}^{eff}$, for two-site exchange in the presence of internal exchange, relative to that for a simple two-site exchange model, $1/T_{1,b}^{(2si)}$, on the normalized internal exchange frequency, $T_{1,b}^{o}\tau_{ex,b}^{-1}$. The effective relaxation rates, $1/T_{1,b}^{eff}$, and $1/T_{1,b}^{(2si)}$, are obtained from Eqs. [9] and [11], respectively, and are normalized to the corresponding values ($1/T_{1,b}^{(x)}$ and $1/T_{1,b}^{o}$, respectively) in the absence of two-site exchange. (---) $Z_{b,k} = \frac{1}{30}$, (—) $Z_{b,k} = \frac{1}{15}$, and (· · ·) $Z_{b,k} = \frac{1}{3}$. From upper to lower, each set of curves corresponds to $T_{1,b}^{o}/T_{1,b}^{(2si)} = 1.2$, 1.5, and 2.0.

s—see Refs. *11, 12*). Equation [12] may or may not be a reasonable approximation at intermediate exchange rates. The criterion for a reasonable approximation is that the deviation of $(T_{1,b}^{(x)}/T_{1,b}^{eff})/(T_{1,b}^{o}/T_{1,b}^{(2si)})$ from unity be much less than that of $T_{1,b}^{o}/T_{1,b}^{(2si)}$. In general, the approximation therefore can be expected to have a reasonably broad range of validity (see also later).

Because of the asymmetry in the above two-site exchange model, in that only ensemble b is subject to internal exchange, the saturation of component f will be characterized by an effective spin–lattice relaxation time that differs in form from that for ensemble b. If for simplicity, it is assumed that the ensemble f is motionally narrowed, all component subsystems will be irradiated simultaneously. The steady-state rate equations for the population differences, summed over the component spin systems in each ensemble, are then identical to those for a simple two-site model (cf. Ref. *1*), and the effective spin–lattice relaxation rate for ensemble f is given exactly by Eq. [11] with the indices b and f permuted. Under these circumstances, the saturation of the ensemble f is unaffected by the internal exchange processes in ensemble b.

The second case treated is that of random two-site exchange with internal exchange at both sites. This model is an alternative to, and less restrictive than, that given above and can be envisaged realistically for the two-site exchange process. In this situation, each spin system in ensemble f exchanges with all of those in ensemble b (e.g., a scrambling of orientation on exchange), rather than a restricted one-to-one exchange. Simultaneously, the restriction that internal exchange takes place in only one of the two ensembles may also be lifted. In this more general case, a fraction $N_{b,k}/N_b$ of the total f ensemble on average will exchange with the kth spin system in the b ensemble. The steady-state rate equation corresponding to Eq. [5] is then

$$\frac{dn_{b,k}}{dt} = -2W_{e,b}(n_{b,k} - n_{b,k}^{o}) - K_{x,b}(N_b n_{b,k} - N_{b,k} n_b) - n_{b,k}\tau_b^{-1} + \frac{N_{b,k}}{N_b} n_f \tau_f^{-1} - 2W n_{b,k} = 0, \qquad [14]$$

and that corresponding to the version of Eq. [6] summed over all i is

$$\frac{dn_f}{dt} = -2W_{e,f}(n_f - n_f^{o}) - n_f\tau_f^{-1} + n_b\tau_b^{-1} = 0. \qquad [15]$$

This latter equation is unchanged in the presence of internal exchange within ensemble f because these internal terms drop out on summation.

The solution of Eqs. [14] and [15] with the summed version of Eq. [14], and using Eqs. [1] and [2], yields an expression for $n_{\mathrm{b},k}$ that is identical in form to Eq. [3], with the effective spin–lattice relaxation time given by

$$T_{1,\mathrm{b},k}^{\mathrm{eff}} = \frac{\begin{array}{r} 2W_{\mathrm{e,b}} + 2W_{\mathrm{e,f}}\tau_{\mathrm{b}}^{-1}/(2W_{\mathrm{e,f}} + \tau_{\mathrm{f}}^{-1}) + K_{\mathrm{x,b}}N_{\mathrm{b},k} \\ + (N_{\mathrm{b},k}/N_{\mathrm{b}})\tau_{\mathrm{b}}^{-1}\tau_{\mathrm{f}}^{-1}/(2W_{\mathrm{e,f}} + \tau_{\mathrm{f}}^{-1}) \end{array}}{\begin{array}{r} [2W_{\mathrm{e,b}} + 2W_{\mathrm{e,f}}\tau_{\mathrm{b}}^{-1}/(2W_{\mathrm{e,f}} + \tau_{\mathrm{f}}^{-1})] \\ \times (2W_{\mathrm{e,b}} + \tau_{\mathrm{b}}^{-1} + K_{\mathrm{x,b}}N_{\mathrm{b}}) \end{array}}. \quad [16]$$

This expression is somewhat more complicated than that of the previous model. It leads to similar conclusions regarding the validity of the approximate Eq. [12], but with slightly less stringent conditions. It may be rewritten in the same form as Eq. [13], except that $\tau_{\mathrm{ex,b}}^{-1}$ is augmented by a term that depends on the two-site exchange. The modified effective Heisenberg exchange rate is then

$$\tau_{\mathrm{ex,b}}^{*-1} = \tau_{\mathrm{ex,b}}^{-1} + \frac{\tau_{\mathrm{f}}^{-1}\tau_{\mathrm{b}}^{-1}}{2W_{\mathrm{e,f}} + \tau_{\mathrm{f}}^{-1}}. \quad [17]$$

For this model of the two-site exchange, the expression given by Eq. [17] replaces $\tau_{\mathrm{ex,b}}^{-1}$ at all occurrences in Eq. [13]. Therefore the condition for the validity of the approximation given by Eqs. [11] and [12] is achieved more readily than for the restricted one-to-one exchange model.

Because of the inherent symmetry in this more general model, the effective spin–lattice relaxation time on irradiating spin system k in ensemble f is now given simply by permutation of the indices b and f in Eq. [16], and similar considerations for the validity of the approximations apply.

The final case considered is that of two-site exchange in the presence of nuclear relaxation. This situation with nuclear relaxation between the hyperfine manifolds within a given component (b or f) is closely related to that of internal exchange. The nuclear transition probability for a component, say b, with nuclear spin I and nuclear magnetic quantum number M_{I} is given by

$$\begin{aligned} &W_{\mathrm{b}}(M_{\mathrm{S}}, M_{\mathrm{I}} \rightarrow M_{\mathrm{S}}, M_{\mathrm{I}} \pm 1) \\ &\quad = W_{\mathrm{n,b}}[I(I+1) - M_{\mathrm{I}}(M_{\mathrm{I}} \pm 1)], \end{aligned} \quad [18]$$

where an electron–nuclear dipolar (END) mechanism is assumed and the selection rules for nuclear transitions are $\Delta M_{\mathrm{I}} = \pm 1$. This is a considerably more restrictive selection rule than that for internal exchange which was treated above.

An $S = \frac{1}{2}$ electron spin system with nuclear spin $I = 1$ is assumed, as appropriate to ^{14}N-nitroxyl spin labels. Internal exchange within a component is neglected, for simplicity. The steady-state rate equations for the spin-population differences in the $M_{\mathrm{I}} = 0$, and ±1 manifolds of component b in two-site exchange with component f are then, respectively (see Refs. *1*, *4*),

$$\begin{aligned} \frac{dn_{\mathrm{b},0}}{dt} &= -(2W_{\mathrm{e,b}} + 4W_{\mathrm{n,b}} + \tau_{\mathrm{b}}^{-1} + 2W\delta_{0,\mathrm{m}})n_{\mathrm{b},0} \\ &\quad + 2W_{\mathrm{n,b}}(n_{\mathrm{b},+1} + n_{\mathrm{b},-1}) \\ &\quad + n_{\mathrm{f},0}\tau_{\mathrm{f}}^{-1} + 2W_{\mathrm{e,b}}n_{\mathrm{b},0}^{\circ} = 0 \end{aligned} \quad [19]$$

$$\begin{aligned} \frac{dn_{\mathrm{b},\pm1}}{dt} &= -(2W_{\mathrm{e,b}} + 2W_{\mathrm{n,b}} + \tau_{\mathrm{b}}^{-1} + 2W\delta_{\pm1,\mathrm{m}})n_{\mathrm{b},\pm1} \\ &\quad + 2W_{\mathrm{n,b}}n_{\mathrm{b},0} + n_{\mathrm{f},\pm1}\tau_{\mathrm{f}}^{-1} \\ &\quad + 2W_{\mathrm{e,b}}n_{\mathrm{b},\pm1}^{\circ} = 0, \end{aligned} \quad [20]$$

where the second subscripts for n now indicate the values of M_{I}. The equilibrium population differences are assumed to be the same for a particular spin system in the three manifolds, i.e., $n_{\mathrm{b},0}^{\circ} = n_{\mathrm{b},+1}^{\circ} = n_{\mathrm{b},-1}^{\circ}$. Transitions are taken to be induced by the H_1 field within the manifold for which $M_{\mathrm{I}} = m$ (i.e., $\delta_{M_{\mathrm{I}},m} = 1$ for $M_{\mathrm{I}} = m$, and 0 otherwise).

The component f is not irradiated. For convenience, nuclear relaxation in this component is neglected. This would correspond either to very fast or to very slow nuclear relaxation, relative to the two-site exchange. The steady-state rate equation for the spin-population difference in hyperfine manifold M_{I} of component f is then (cf. Eq. [6])

$$\begin{aligned} \frac{dn_{\mathrm{f},M_{\mathrm{I}}}}{dt} &= (2W_{\mathrm{e,f}} + \tau_{\mathrm{f}}^{-1})n_{\mathrm{f},M_{\mathrm{I}}} \\ &\quad + n_{\mathrm{b},M_{\mathrm{I}}}\tau_{\mathrm{b}}^{-1} + 2W_{\mathrm{e,f}}n_{\mathrm{f},M_{\mathrm{I}}}^{\circ} = 0. \end{aligned} \quad [21]$$

The condition for material balance at equilibrium two-site exchange is, in this case, given by an equation similar to Eq. [4] above, with $i \equiv M_{\mathrm{I}}$.

Solution of Eqs. [19]–[21] for saturation in the $M_{\mathrm{I}} = 0$ or ±1 manifolds of component b, together with Eq. [4], yields results of the same form as Eq. [3]. The corresponding values for the effective electron spin–lattice relaxation times are, respectively

$$\begin{aligned} T_{1,\mathrm{b}}^{\mathrm{eff}}(0) &= \frac{1 + T_{1,\mathrm{b}}^{\circ}\tau_{\mathrm{b}}^{-1}/(1 + T_{1,\mathrm{f}}^{\circ}\tau_{\mathrm{f}}^{-1}) + \mathrm{b}}{1 + T_{1,\mathrm{b}}^{\circ}\tau_{\mathrm{b}}^{-1}/(1 + T_{1,\mathrm{f}}^{\circ}\tau_{\mathrm{f}}^{-1}) + 3b} \\ &\quad \times \frac{T_{1,\mathrm{b}}^{\circ}}{1 + T_{1,\mathrm{b}}^{\circ}\tau_{\mathrm{b}}^{-1}/(1 + T_{1,\mathrm{f}}^{\circ}\tau_{\mathrm{f}}^{-1})} \end{aligned} \quad [22]$$

and

$$\begin{aligned} T_{1,\mathrm{b}}^{\mathrm{eff}}(\pm1) &= T_{1,\mathrm{b}}^{\mathrm{eff}}(0) \\ &\quad + \frac{bT_{1,\mathrm{b}}^{\circ}}{(1 + b)[1 + T_{1,\mathrm{b}}^{\circ}\tau_{\mathrm{b}}^{-1}/(1 + T_{1,\mathrm{f}}^{\circ}\tau_{\mathrm{f}}^{-1}) + 3b]}, \end{aligned} \quad [23]$$

where $b = W_{\mathrm{n,b}}/W_{\mathrm{e,b}}$ is the ratio of the nuclear to the electron spin–lattice relaxation rates.

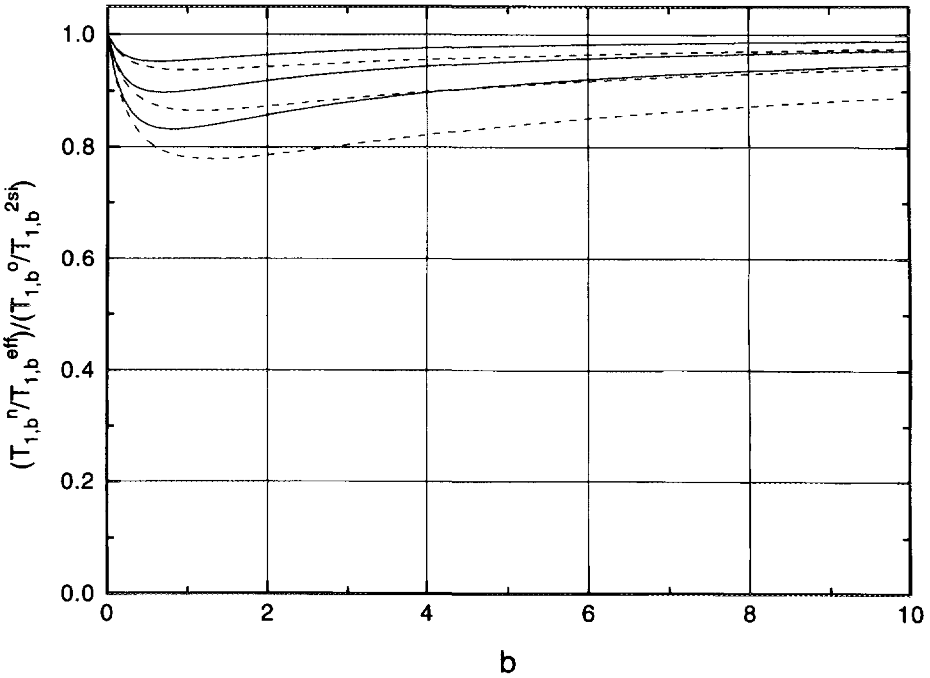

FIG. 3. Dependence of the effective electron spin-lattice relaxation rate, $1/T_{1,b}^{\mathrm{eff}}$, for two-site exchange in the presence of nuclear relaxation, relative to that for a simple two-site exchange model, $1/T_{1,b}^{(2si)}$, on the normalized nuclear relaxation rate, b, for $I = 1$. The effective relaxation rates, $1/T_{1,b}^{\mathrm{eff}}$ and $1/T_{1,b}^{(2si)}$, are obtained from Eqs. [22] or [23] and [11], respectively, and are normalized to the corresponding values ($1/T_{1,b}^{(n)}$ and $1/T_{1,b}^{o}$, respectively) in the absence of two-site exchange. (—) $M_I = 0$, and (---) $M_I = \pm 1$. From upper to lower, each set of curves corresponds to $T_{1,b}^{o}/T_{1,b}^{(2si)} = 1.2$, 1.5, and 2.0.

In the absence of nuclear relaxation ($b = 0$), Eqs. [22] and [23] both reduce to the expression for the simple two-site exchange model, i.e., Eq. [11], as they should. In the absence of two-site exchange ($\tau_b^{-1} = 0 = \tau_f^{-1}$), they yield the standard expressions for a single component with $I = 1$ in the presence of nuclear relaxation (cf. Ref. *13*),

$$T_{1,b}^{(n)}(0) = \frac{1 + b}{1 + 3b} \times T_{1,b}^{o} \qquad [24]$$

and

$$T_{1,b}^{(n)}(\pm 1) = \frac{1 + 3b + b^2}{(1 + b)(1 + 3b)} \times T_{1,b}^{o} \qquad [25]$$

for the $M_I = 0$ and ± 1 hyperfine manifolds, respectively.

Conditions for the validity of the simple expression for two-site exchange given by Eq. [11] in the presence of nuclear relaxation may be deduced from Eqs. [22] and [23]. Here the principal requirement is that the nuclear relaxation should either be slow, $3b \ll 1 + T_{1,b}^{o}\tau_b^{-1}/(1 + T_{1,f}^{o}\tau_f^{-1})$, or fast, $b \gg 1 + T_{1,b}^{o}\tau_b^{-1}/(1 + T_{1,f}^{o}\tau_f^{-1})$, relative to the two-site exchange rate. The relaxation enhancement, $T_{1,b}^{(n)}/T_{1,b}^{\mathrm{eff}}$, from Eqs. [22]–[25], normalized to that given by the simple two-site approximation, $T_{1,b}^{o}/T_{1,b}^{(2si)}$, is given in Fig. 3 as a function of the normalized nuclear relaxation rate, b. The effects are less than those for internal exchange, presumably because of the more restricted selection rules for nuclear relaxation. It would appear that over most of the likely range of nuclear relaxation rates (*14, 15*), the simple model provides a reasonable approximation.

In conclusion it is found that only over limited ranges is the simple two-site exchange approximation subject to appreciable error. The maximum deviations are for correlation times in the region of 5 μs for rotational diffusion/Heisenberg exchange (cf. Fig. 2) and for $b \sim 1$–2 for nuclear relaxation (cf. Fig. 3). The latter correspond to rotational correlation times in the region of 10^{-10} s (*14*). In these cases, corrections may be made by using Eqs. [9] and [16], and Eqs. [22] and [23], respectively. For this purpose, a correspondence between the rotational correlation time, as determined by saturation-transfer EPR experiments, and the effective exchange/spin diffusion rate has been established in Ref. (*6*). It will be noted that the correlation time regimes for which rotational diffusion and nuclear relaxation, respectively, have their largest effects are quite different. Therefore, there is little or no reinforcement of the two combined effects. In particular, nuclear relaxation is expected to have only a limited effect in anisotropic powder patterns. This is because of the very restricted selection rules for the latter, as compared with the general case of two-site exchange with reorientation.

REFERENCES

1. L. I. Horváth, P. J. Brophy, and D. Marsh, *Biophys. J.* **64,** 622 (1993).
2. M. M. E. Snel and D. Marsh, *Biophys. J.* **67,** 737 (1994).
3. D. Marsh, *Chem. Soc. Rev.* **22,** 329 (1993).
4. D. Marsh, *J. Magn. Reson.* **99,** 332 (1992).
5. J.-J. Yin and J. S. Hyde, *J. Magn. Reson.* **74,** 82 (1987).
6. D. Marsh and L. I. Horváth, *J. Magn. Reson.* **99,** 323 (1992).
7. C. P. Slichter, "Principles of Magnetic Resonance," 2nd ed., Springer-Verlag, Berlin/New York, 1978.
8. D. Marsh and L. I. Horváth, *J. Magn. Reson.* **97,** 13 (1992).
9. D. D. Thomas, L. R. Dalton, and J. S. Hyde, *J. Chem. Phys.* **65,** 3006 (1976).
10. J. S. Hyde and L. R. Dalton, in "Spin Labeling II, Theory and Applications" (L. J. Berliner, Ed.) p. 1, Academic Press, New York, 1979.
11. J. S. Hwang, R. P. Mason, L.-P. Hwang, and J. H. Freed, *J. Phys. Chem.* **79,** 489 (1975).
12. P. W. Percival and J. S. Hyde, *J. Magn. Reson.* **23,** 249 (1976).
13. J. S. Hyde, J. C. W. Chien, and J. H. Freed, *J. Chem. Phys.* **48,** 4211 (1968).
14. J. S. Hyde, W. Froncisz, and C. Mottley, *Chem. Phys. Lett.* **110,** 621 (1984).
15. C. A. Popp and J. S. Hyde, *Proc. Natl. Acad. Sci. USA* **79,** 2559 (1982).