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Theory of Paramagnetic Contrast Agents in Liposome Systems

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We develop a theoretical description of nuclear spin relaxation mediated by MRI contrast agents and transport processes in liposome systems. Such systems compartmentalize the physical space such that paramagnetic contrast agents, which enhance relaxation, are trapped in some subvolume. Due to diffusive transport across compartmental barriers, i.e., across liposome membranes, nuclear spins in the whole volume exhibit fast relaxation. The description developed is based on the diffusion–Bloch equations for the nuclear magnetization with appropriate boundary and continuity conditions. From this set of equations a new inhomogeneous differential equation for the local relaxation times is derived. For simple geometries of compartmentalized spaces the equation can be solved analytically. A simple formula for average relaxation times in liposome systems is presented. The resulting relaxation times agree well with observations.

1. INTRODUCTION

The availability of image contrast is a fundamental issue in magnetic resonance imaging (MRI). For some applications image contrast can be enhanced through MRI contrast agents—paramagnetic molecules or superparamagnetic substances which shorten signal decay times. This paper presents research that extends earlier work on the mechanisms of MRI contrast agents (*3*). That work demonstrated the important role of diffusive transport on the enhancement of nuclear spin relaxation through paramagnetic and superparamagnetic contrast agents, e.g., for the intravascular system and for Kupffer cells of the liver. An understanding of the action of contrast agents should provide new insights into the effective administration of contrast agents and aid in the development of new agents. Moreover, in the developing field of MRI microscopy, a novel observational capability is unfolding as it becomes possible to label individual cells or groups of cells with a contrast agent (*15*).

In the present study we consider a paramagnetic contrast agent, e.g., Gd-DTPA (*23*), which is either contained in or excluded from liposomes in solution. Liposomes are formed by a lipid bilayer membrane which encloses an aqueous interior and which is rather permeable to water and under certain conditions impermeable to Gd-DTPA. With these features liposomes provide a simple model for compartmentalized tissues which contain contrast agents in a small fraction of the tissue, examples of which include Kupffer cells of the liver and erythrocytes (See Ref. (*10*) and references therein). In addition to their use as a model for compartmentalized tissue, liposomes containing contrast agents have recently been used in animals to improve MRI contrast between

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normal liver and tumors (*1, 13, 22*). The advantages of such contrast agent delivery systems include the protection of the contrast agent from binding to plasma proteins, modulation of water access, and site-specific distribution for certain applications. (For these and other aspects of liposomes see Refs. (*5, 11, 14, 19, 21*).)

For appropriately constructed liposome systems, it is known that the contrast agent is strictly confined to the liposome interior (*1, 22*). It is also accepted that paramagnetic molecules in free solution, i.e., the contrast agent, can directly relax only adjacent water protons (*4*). Hence, we suggest that the increased relaxation in the bulk water of liposome systems is primarily due to proton transport across liposomal membranes on a time scale shorter than that of the native nuclear spin relaxation. In addition, due to the higher susceptibility of the contrast agent, the magnetic field in the immediate vicinity of the liposomes will be perturbed, possibly contributing to faster relaxation (*7*). Yet for longitudinal relaxation this effect should be of minor significance, especially for small diameter (60 to 400 nm) liposomes as are considered here (*10*), a point to which we will later return. By the transport mechanism one expects that the factors which influence the rate at which an average water molecule can enter a liposome play a role in contrast enhancement. Those factors are: (1) the average size of the liposomes, (2) the number of liposomes per unit volume, and (3) the permeability of the liposomal membrane to water. An important advantage of the liposome system is that these factors can be experimentally controlled. In fact, Refs. (*1, 16, 20*) provide evidence that a variation of these properties changes relaxation rates of bulk water. In the study presented here we developed a simple theoretical formulation which relates properties (1–3) to relaxation rates in liposomal systems. We will show that the theoretical expressions for relaxation rates derived are in excellent agreement with experimental data.

Our approach below rests on the theoretical formulation developed in Ref. (*3*). The approach involves solutions of Bloch equations to which diffusion terms have been added to account for the transport of water. In Section 2 we derive simple mathematical expressions which relate relaxation rates to liposome radius and concentration as well as to water diffusion coefficient and to membrane permeability. In Section 3 we compare the results to observations reported in Refs. (*16, 20*).

2. THEORY OF TRANSPORT-MEDIATED NUCLEAR SPIN RELAXATION

Physical Model of Liposomes Entrapping Contrast Agent

We first consider a system of liposomes which confine a paramagnetic MRI contrast agent to their interior and which are surrounded by bulk water without contrast agent. In water doped with a paramagnetic contrast agent, a nuclear spin must diffuse into the immediate vicinity of a paramagnetic molecule in order to experience a faster relaxation of its magnetization, than would otherwise occur. Even more restricting is the situation of a chelated agent, such as Gd-DTPA, where only one coordination site is available for the water protons (*9*). Consequently, since the MRI contrast agent is located solely within the liposome interior, and because susceptibility effects should not be significant for small liposomes, we assume that water protons must enter the interior in order to be relaxed at a faster than native rate.

The liposome system investigated in Ref. (*20*) contains the paramagnetic contrast agent Gd-DTPA. In water doped with this agent, the relaxation times T_1 and T_2 are

much shorter than in plain water; for example, T_1 for water protons in 2 mM Gd-DTPA is about 30 times shorter than in water. Since the concentration of the agent in liposomes is about 670 mM, one expects extremely short relaxation times in the liposome interior. In our description we will make the simplifying assumption that water protons entering the liposome interior lose their magnetization immediately.

The (bulk) water outside of the liposomes can be parcelled into imaginary cells of equal volume V for each liposome, and one can consider the diffusion of water to be confined to within these cells, i.e., as if the bulk consisted of cells separated by *impenetrable* walls. This situation is physically equivalent to the real system because the effect on a water molecule visiting two *different* liposomes at times t_1 and t_2 cannot be distinguished from the effect of a water molecule visiting the *same* liposome at times t_1 and t_2. Therefore, we model the entire proton diffusion space by a single, spherical cell of volume V which contains a single liposome as a spherical compartment at the center. The cell is divided into three concentric, spherical regions (Fig. 1): a liposome interior of radius R_e (I) where water spins relax very rapidly due to the contrast agent, a membrane wall (II) which is marked by low diffusivity, and an outer region (III) of radius R_a representing bulk water. The outer radius R_a is related to the cell volume $V = (4\pi/3)R_a^3$ where V = total volume/number of liposomes.

The spaces defined differ with respect to the local diffusion coefficients of water and with respect to the local relaxation rates. We will assume that the diffusion coefficient for water inside and outside of the liposome is simply equal to the self-diffusion coefficient of water D_w, but takes on a different value D_m in the region of the membrane wall. D_m will be treated as a parameter which will be chosen in order to match calculated and observed relaxation rates. Affected only by the composition and condition (e.g.,

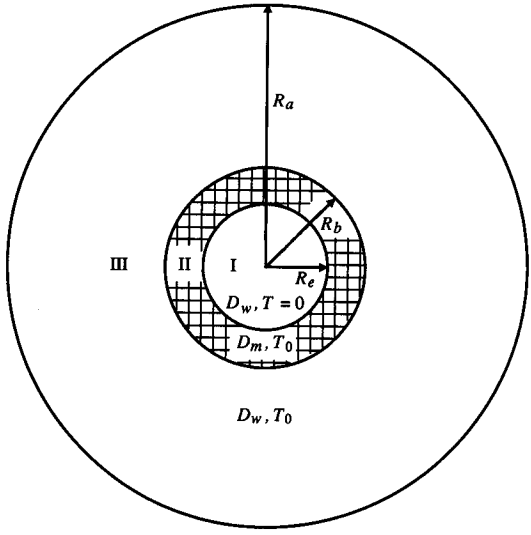

FIG. 1. Geometry and physical properties of the liposome system. Shown are the borders of regions with different physical properties (not to scale). Region I is the interior of the liposome, region II is the liposome membrane, and region III is the bulk water phase. D_w and D_m are the diffusion coefficients of water in water and in the membrane, respectively. T_0 is the native relaxation rate (for either longitudinal or transverse relaxation). $T = 0$ denotes our assumption of immediate relaxation in the liposome interior (region I).

temperature) of the liposomes, D_m should remain constant for liposomes of different size and for variable liposome concentrations and will be several orders of magnitude smaller than the value of D_w. For the relaxation rates we will assume a value of $T_{1,0}^{-1} = 0.2\ s^{-1}$ for the bulk water and an infinite rate in the liposome interior. For simplicity we assume that the relaxation rate in the membrane wall, which ordinarily occupies less than one percent of the total volume, is just that of the bulk water even though it is probably somewhat greater due to the lipid.

At this point we introduce a consideration which will be cardinal to the theory. Assuming that nuclear spin relaxation can be described by the model encompassed in Fig. 1, an observation of nuclear spin relaxation at some position $\mathbf{r}$ in the bulk water, i.e., region III of Fig. 1, will yield a relaxation rate which is larger than the *native* rate $T_{1,0}^{-1}$ or $T_{2,0}^{-1}$. This is due to an additional relaxation process parallel to the *native* relaxation which arises through transport into and out of the liposome: water molecules diffuse through the liposome membrane into the liposome interior, experience rapid relaxation through the contrast agent confined there, leave the liposome interior, and at the instance of observation appear at position $\mathbf{r}$. In our description of nuclear spin relaxation we will make the simplifying assumption [1] of monoexponential decays of magnetization. In this approximation the decay constant T^{-1} for the combined *native* and *transport-mediated* decay processes is

$$T^{-1}(\mathbf{r}) = T_0^{-1} + \tau^{-1}(\mathbf{r}) \qquad [1]$$

where $\tau(\mathbf{r})$ is the relaxation time of magnetization at position $\mathbf{r}$, casting also this process into the form of a monoexponential decay. For T_0 one can choose either $T_{1,0}$ or $T_{2,0}$ resulting in $T(\mathbf{r})$ being either the observed T_1 or T_2, respectively. This interchangeability results from the relaxation processes inside a liposome, i.e., in the presence of a contrast agent, acting almost immediately when compared with the native relaxation, whether the relaxation be transverse or longitudinal. If, in an experimental setting, there is *another* relaxation enhancement mechanism (e.g., especially for T_2 susceptibility effects), then the effect of that mechanism can be incorporated into $T_{1,0}$ or $T_{2,0}$ in the same way that T_2 goes to T_2^*.

The experimental observables are in fact spatial averages of these rates, written as $1/T$ and $1/\langle\tau\rangle$. By the previous discussion $\langle\tau\rangle$ is an exchange time of membrane transport. The main goal of our paper is to provide a mathematical expression for $\langle\tau\rangle$ and to demonstrate its validity in describing observed relaxation rates.

The quantity which will be evaluated below is the magnetization $\mathbf{m}(\mathbf{r}, t)$ of nuclear spins at location $\mathbf{r}$ and at time t. It is more convenient to consider separately the transverse and longitudinal components of this vector, defined in cylindrical coordinates through $\mathbf{m}(\mathbf{r}, t) = m_{\text{long}}(\mathbf{r}, t)\hat{z} + m_{\text{trans}}(\mathbf{r}, t)\hat{\rho}$ where $\hat{z}$ is the direction of the static, external magnetic field. Henceforth, $m(\mathbf{r}, t)$, with neither a subscript nor a vector symbol, will indicate either $m_{\text{long}}(\mathbf{r}, t)$ or $m_{\text{trans}}(\mathbf{r}, t)$.

Since magnetization is linked to water molecules, it is subject to the same diffusion law as the water molecules themselves. Hence, in order to describe each component

[1] This monoexponential assumption can be relaxed if one employs a more general version of the *mean relaxation time theory* to be presented in Section 2. Such a generalization can be found, for example, in Refs. (*6, 18*). Since the analysis of raw data in MRI presupposes monoexponential relaxation, the assumption made here should be satisfactory. Nonetheless, it appears to be most interesting to consider the possibility of improved image contrast by exploiting multiexponential decays (2).

$m(\mathbf{r}, t)$ we will use the Bloch equations to which we add a term $\nabla \cdot D(\mathbf{r})\nabla m(\mathbf{r}, t)$. The components of the local nuclear magnetization $m(\mathbf{r}, t)$ are then governed by

$$\partial_t \begin{pmatrix} m_{\text{trans}}(\mathbf{r}, t) \\ m_{\text{long}}(\mathbf{r}, t) \end{pmatrix} = \left[\nabla \cdot D(\mathbf{r})\nabla - \begin{pmatrix} i\omega_0 + T_{2,0}^{-1} & \\ & T_{1,0}^{-1} \end{pmatrix} \right] \begin{pmatrix} m_{\text{trans}}(\mathbf{r}, t) \\ m_{\text{long}}(\mathbf{r}, t) \end{pmatrix}, \quad [2]$$

where $D(\mathbf{r})$ is the local diffusion coefficient, ω_0 is the Larmor frequency, and $T_{2,0}^{-1}$, $T_{1,0}^{-1}$ are the transverse and longitudinal native, i.e. bulk water, relaxation rates, respectively, which can be a function of position.

The solution of Eq. [2] requires specification of the initial and boundary conditions on the magnetization components $m(\mathbf{r}, t)$. We assume that the magnetization is initially uniform and at a maximum absolute value, i.e.,

$$m(\mathbf{r}, t = 0) = m_0, \quad [3]$$

as occurs, for example, for m_{trans} after a simple 90° pulse. The condition at R_a is implied by the impenetrability of the surface: the flux normal to the surface $D(\mathbf{r})\nabla m(\mathbf{r}, t)$ must vanish. The boundary condition is then

$$\hat{r} \cdot D(\mathbf{r})\nabla m(\mathbf{r}, t) = 0 \qquad \text{at } r = R_a. \quad [4]$$

Because of the spherical shape of the boundary, this reads, for $D(\mathbf{r}) \neq 0$,

$$\partial_r m(\mathbf{r}, t) = 0 \qquad \text{at } r = R_a. \quad [5]$$

The boundary condition at R_e is due to the assumption that inside the liposome the relaxation is infinitely fast. This corresponds to the assumption that $m(\mathbf{r}, t)$ vanishes everywhere inside the liposome, in particular, at $r = R_e$, i.e.,

$$m(\mathbf{r}, t) = 0 \qquad \text{at } r = R_e, t > 0. \quad [6]$$

Hence, the diffusion space is effectively limited to the region between the radii R_e and R_a and the magnetization components $m(\mathbf{r}, t)$ need to be evaluated only in this region.

Since the Larmor frequency and the native relaxation rate are spatially independent in the space $R_e < r < R_a$, one can separate out the native relaxation (and Larmor precession for the transverse component) in Eq. [2] so that each component satisfies the simple diffusion equation

$$\partial_t \mu(\mathbf{r}, t) = \nabla \cdot D(\mathbf{r})\nabla \mu(\mathbf{r}, t), \quad [7]$$

where $\mu(\mathbf{r}, t)$ describes either the longitudinal or the transverse component of the magnetization and is connected with m_{trans} and m_{long} through the substitutions

$$m_{\text{trans}} = e^{-i\omega_0 t - t/T_{2,0}} \mu(\mathbf{r}, t) \qquad \text{or} \qquad m_{\text{long}} = e^{-t/T_{1,0}} \mu(\mathbf{r}, t). \quad [8]$$

The prefactors in this substitution account for the relaxation described by the first term in Eq. [1]. Thus, the result of these transformations is a diffusion equation which describes the transport-mediated component of nuclear spin relaxation around liposomes.

The boundary conditions on $\mu(\mathbf{r}, t)$ follow trivially from those on $m(\mathbf{r}, t)$. The solution of Eq. [7] must be continuous and flux conserving across the surface of the liposome at $r = R_b$ where the diffusion jumps from a value D_w to D_m. This implies

$$\mu_<(r = R_b, t) = \mu_>(r = R_b, t) \quad [9]$$

$$D_m \partial_r \mu_<(\mathbf{r}, t)|_{r=R_b} = D_w \partial_r \mu_>(\mathbf{r}, t)|_{r=R_b} \quad [10]$$

where the subscripts $<$ and $>$ denote that the limit $\lim_{r \to R_b} \mu(\mathbf{r}, t)$ is taken from the left and right of R_b, respectively. The spherical symmetry of the diffusion space implies that $\mu(\mathbf{r}, t)$ will also be spherically symmetric. In spherical coordinates Eq. [7] becomes

$$\partial_t \mu(r, t) = \frac{1}{r^2} \partial_r r^2 D(r) \partial_r \mu(r, t). \quad [11]$$

Mean Relaxation Time Approximation

As pointed out above we actually seek to determine the relaxation time $\tau(r)$ which is defined as the time constant which approximates the local magnetization $\mu(r, t)$ in terms of a single exponential decay

$$\mu(r, t) \approx \mu_0 e^{-t/\tau(r)}. \quad [12]$$

This approximation, however, is not unique. It has been demonstrated for a broad number of transport-mediated processes that the following realization of the above approximation (Eq. [12]) often leads to satisfactory results (see Ref. (*18*) and references therein):

$$\mu_0 = \mu(r, t = 0), \qquad \int_0^\infty dt \mu_0 e^{-t/\tau(r)} = \int_0^\infty dt \mu(r, t). \quad [13]$$

The significance of these conditions is that the function $\mu_0 e^{-t/\tau(r)}$ is chosen to match the initial conditions and, rather than match the initial *slope,* which may be due to a small, but fast component of the decay, the time *integral* of the function should match that of $\mu(r, t)$. This latter function represents the total contribution of the nonnative components to the overall relaxation. In this way $\tau(r)$ is a more accurate indicator of the overall relaxation behavior.

Equation [13] is equivalent to the following definition of $\tau(r)$,

$$\tau(r) = \frac{1}{\mu_0} \int_0^\infty dt \mu(r, t). \quad [14]$$

$\tau(r)$ is referred to as the *mean relaxation time* (*18*). By applying Eqs. [13] to Eq. [11], one can show that $\tau(r)$ obeys the inhomogeneous differential equation

$$\frac{1}{r^2} \partial_r r^2 D(r) \partial_r \tau(r) = -1. \quad [15]$$

This differential equation must be complemented by spatial boundary and continuity conditions which follow from those on $\mu(\mathbf{r}, t)$ together with Eq. [14].

Equation [15], with conditions 5–10, can be integrated analytically to reveal

$$\tau(r) = \begin{cases} 0 & r \leq R_e \\ \dfrac{1}{3D_m}\left[\left(\dfrac{R_a^3}{R_e} + \dfrac{R_e^2}{2}\right) - \left(\dfrac{R_a^3}{r} + \dfrac{r^2}{2}\right)\right] & R_e < r \leq R_b \\ \tau(r = R_b) + \dfrac{1}{3D_w}\left[\left(\dfrac{R_a^3}{R_b} + \dfrac{R_b^2}{2}\right) - \left(\dfrac{R_a^3}{r} + \dfrac{r^2}{2}\right)\right] & R_b < r < R_a \end{cases} \quad [16]$$

NMR observations of liposome systems, e.g., those of Ref. (*20*), cannot resolve spatial details on the scale of the mean distance between liposomes, so the observable which determines relaxation rates is the average magnetization

$$\mathbf{M}(t) = \frac{1}{V}\int_V d^3r\mathbf{m}(r, t), \qquad [17]$$

where the integration is over the sphere of radius R_a around a liposome ($V = (4\pi/3)R_a^3$). Employing Eq. [8] for either the longitudinal or transverse component of the local magnetization (with T_0 in place of $T_{1,0}$ or $T_{2,0}$ and ignoring $e^{-i\omega t}$) one obtains for the respective component of $\mathbf{M}(t)$,

$$M(t) = \frac{1}{V}\int_V d^3r\mu(\mathbf{r}, t)e^{-t/T_0}. \qquad [18]$$

In keeping with the analysis of raw data in observations we assume again a mono-exponential decay of the observable: $M(t) \simeq M_0 e^{-t/T}$ for either the transverse or longitudinal magnetization, where $M_0 = m_0 = \mu_0$ is the initial magnetization.[2] Plugging this approximation into Eq. [18], applying approximation [12], and integrating both sides over all time one obtains

$$T = \frac{1}{V}\int_V d^3r\left(\frac{1}{\tau(r)} + \frac{1}{T_0}\right)^{-1}. \qquad [19]$$

To obtain T we approximate further

$$\frac{1}{T} \simeq \frac{1}{T_0} + \frac{1}{\langle\tau\rangle} \qquad \text{where } \langle\tau\rangle = \frac{1}{V}\int_V d^3r\tau(r). \qquad [20]$$

The expression [20] would be exact if $\tau(r)$ were constant in the region $R_e < r < R_a$. Fortunately, for the small ratio $D_m/D_w(\sim 10^{-5}$ is typical; see Section 3) and for $d \ll R_a$, Eq. [16] is in fact nearly constant (deviation less than 0.1%) over the region $R_b < r \leqslant R_a$ which is by far the greater part, namely 99%, of the region of integration for Eq. [19]. The constant contribution to $\tau(r)$ can be interpreted as the exchange time across the liposome membrane. The deviation in $\tau(r)$ describes the time water molecules require to reach the liposome surface. The contribution to the integral [19] from the small (1%) volume $R_e \leqslant r \leqslant R_b$ is negligible, unless $\tau(r)$ becomes very large. The latter possibility is not realized, for in this range $\tau(r)$ varies monotonically (nearly linearly) from a value $\tau(R_e) = 0$ to a maximum value at $r = R_b$, the latter corresponding to the exchange time across the liposome membrane. Hence, in this case the approximation involved in Eq. [20] is nearly exact.

By $M(t) \simeq M_0 e^{-t/T}$, the overall relaxation rate is given by $1/T$, and Eq. [20] implies that $1/\langle\tau\rangle$ describes the increase in the native relaxation rate due to exchange across the liposome membrane. For $\langle\tau\rangle$ one obtains

[2] That the actual decay may be of a form $M(t) = M_0(1 - 2e^{-t/T})$, or may oscillate by $e^{-i\omega t}$ is of no consequence here; we are only concerned with the envelope of the decay.

$$\langle \tau \rangle = \frac{1}{3D_m}\left[R_a^3\left(\frac{1}{R_e}+\frac{D_m-D_w}{D_w}\frac{1}{R_b}\right)+R_b^2\frac{D_m-D_w}{D_w}\right.$$
$$\left.+R_e^2-\frac{1}{5R_a^3}\left(R_b^5\frac{D_m-D_w}{D_w}+R_e^5\right)-R_a^2\frac{9D_m}{5D_w}\right]. \quad [21]$$

The term proportional to R_a^3 gives the main contribution to this expression, the remaining terms being of order $(R_b/R_a)^2$, $(R_b/R_a)(D_m/D_w)$ or smaller. The leading contribution to the exchange time is then

$$\langle \tau \rangle \simeq \frac{R_a^3}{3D_m}\left(\frac{1}{R_e}-\frac{1}{R_b}\right). \quad [22]$$

The corresponding exchange rate can be expressed in terms of the permeability $P = D_m/d$, total volume $V = (4\pi/3)R_a^3$ (which is almost entirely *external* volume) and an effective surface $S = 4\pi R_b R_e$ in the expected way,

$$\frac{1}{\langle \tau \rangle} \simeq P\frac{S}{V}, \quad [23]$$

where $d = R_b - R_e$. Here the radius for the effective surface S is the geometric mean of R_e and R_b. Thus, in the low concentration regime the relaxation rate is proportional to the permeability of surface area of the membrane and inversely proportional to the water (mostly) free of contrast agent.

In Ref. (*20*) values of $1/T$ have been reported as a function of liposome density. The contrast agent is entrapped at a concentration C_0 within the liposomes. If one defines C_{eff} as the concentration of contrast agent which would be measured if the entrapped material were spread uniformly over the whole volume, i.e., bulk water and liposomes, then the relationship holds

$$\frac{\text{volume of liposomes}}{\text{total volume}} = \left(\frac{C_{\text{eff}}}{C_0}\right) = \left(\frac{R_e}{R_a}\right)^3. \quad [24]$$

For the rather low concentrations ($C_{\text{eff}} \ll C_0$) normally employed for experimental or clinical purposes, and typical membrane diffusion coefficients ($D_m \ll D_w$), approximation [22] holds and [21] simplifies to

$$\frac{1}{\langle \tau \rangle} \simeq \frac{3D_m R_b}{d(R_b-d)^2}\frac{C_{\text{eff}}}{C_0} = P\frac{3R_b}{(R_b-d)^2}\frac{C_{\text{eff}}}{C_0}, \quad [25]$$

where for typical values ($C_{\text{eff}}/C_0 \simeq 0.01$) the approximation is within about 3% of the value given by the full expression. Equation [25] illustrates that the exchange rate $1/\langle \tau \rangle$ depends linearly on both the permeability P of the membrane and the concentration ratio C_{eff}/C_0. The parameters, d, C_0, and C_{eff}, as well as the liposome outer radius R_b, are empirically determined.

Liposomes Excluding Contrast Agent

We consider now the situation where the bulk water contains contrast agent and liposomes, but the liposomes do not contain contrast agent. Liposomes are manufactured to contain only water and are then placed in a solution of Gd-DTPA, after

which the MRI experiments are performed on this admixture. The system is analogous to erythrocytes in a plasma doped with Mn^{2+} (*8, 17*).

The geometry of such system is inverse to the one presented in Fig. 1. In the space exterior to the liposomes, the nuclear spins are exposed to the contrast agent and experience fast relaxation. Because most of the water is external to the liposomes, the relaxation outside the liposomes constitutes the largest component of the decay of magnetization. The bimodal relaxation is identical to that of the erythrocyte system investigated in Refs. (*8, 17*). Here we consider only the smaller, slower component of relaxation due to the entrapped water.

The magnetization due to the water in the liposome interior and liposome membrane obeys again the diffusion–Bloch equation [2]. Transformation [8] yields the diffusion equation [7]. We again assume that water nuclear spins relax instantaneously in the presence of Gd-DTPA—in this case, in the bulk water. In the resulting system the boundary conditions to be obeyed are different from those in Section 2. Instantaneous relaxation in the liposome exterior implies $\mu(r > R_b, t < 0) \equiv 0$, and conservation of particles, i.e., vanishing of diffusive flux, at the origin implies the second boundary condition $\partial_r \mu(r = 0, t) \equiv 0$. The magnetization and the flux of particles across the interior side ($r = R_e$) of the membrane are continuous.

Again the local relaxation time $\tau(r)$ defined through Eqs. [12] and [13] satisfies Eq. [15]. The boundary and continuity conditions for $\tau(r)$ are derived by applying the above conditions to Eq. [12]. The ensuing problem can be solved analytically yielding

$$\tau(r) = \begin{cases} \dfrac{1}{6D_w}(R_e^2 - r^2) + \dfrac{1}{6D_m}(R_b^2 - R_e^2) & r \leqslant R_e \\ \dfrac{1}{6D_m}(R_b^2 - r^2) & R_e < r \leqslant R_b \cdot \\ 0 & R_b < r < R_a \end{cases} \qquad [26]$$

A component $M(t)$ of the average magnetization $\mathbf{M}(t)$, as defined by Eq. [17], is the physical observable. In the present setting the observable describes only the nuclei in the liposomes (including membranes). The initial magnetization M_0 is then given by the relation (M_0/total initial magnetization) = (volume of liposomes/volume of liposomes plus bulk). We assume again a monoexponential decay $M(t) \simeq M_0 e^{-t/T}$. In order to obtain a description of the *total* magnetization, a biexponential treatment is indispensable for this geometry where, unlike that of Section 2, a large fraction of the total volume is characterized by a rapid exponential decay (*2*).

As in Section 2, the terms proportional to D_m^{-1} make the dominant contribution to $\tau(r)$. Since these terms exhibit only a weak dependence on r the exchange rate is again given by Eq. [20]. We obtain in the present case

$$\langle \tau \rangle = \frac{1}{15D_m}\left[\frac{R_b^5 - (R_b - d)^5}{R_b^3} + \frac{D_m}{D_w}\frac{(R_b - d)^5}{R_b^3}\right]. \qquad [27]$$

The leading contribution to this expression is

$$\langle \tau \rangle = \frac{dR_b}{3D_m} = \left[P\frac{S}{V}\right]^{-1}. \qquad [28]$$

Here, $P = D_m/d$, $S = 4\pi R_b^2$, but, in contrast to Eq. [23], $V = (4\pi/3)R_b^3$ is the inside

volume, i.e., again the volume which is free of contrast agent. This expression agrees with that used in, e.g., Refs. (*8, 12, 16*).

3. COMPARISON WITH EXPERIMENT

Here we compare the results obtained above with available observations. We consider first liposomes containing a contrast agent such a Gd-DTPA. At low effective concentrations one can apply Eq. [25] for different values of R_b and C_{eff}. In all comparisons we assume a membrane thickness d of 5 nm. We assume a coefficient describing the self-diffusion of water $D_w = 10^{-5}$ cm^2/s; for the reasons stated above the self-diffusion of water contributes negligibly to the relaxation rate.

Using the values for R_b, C_0, and C_{eff} given by Tilcock *et al.* (*20*), we are able to reproduce the experimental results of these authors for egg-PC liposomes with and without cholesterol after adjusting D_m for each composition: the choices which agree well with the data are $D_m = 0.47 \times 10^{-9}$ cm^2/s for liposomes with cholesterol and $D_m = 1.4 \times 10^{-9}$ cm^2/s for those without. These values are in the expected range for such membrane compositions (*16*). The predictions resulting from Eqs. [20] and [21] are compared in Fig. 2 with data of Ref. (*20*). The ordinate axis corresponds to the spatially averaged, overall relaxation rate $1/T$; the intercept is the native rate $T_{1,0}^{-1}$ or $T_{2,0}^{-1}$. (See Eq. [1] and discussion that follows.) At the low effective concentrations of the observations, the linear approximation [25] is within about 3% of the complete expression [21], yet the two expressions give noticeably different results for concentrations C_{eff} above 10 mM (not shown).

While we found excellent agreement between theory and experiment for liposomes with radii $r \leq 100$ nm, a significant discrepancy is evident in Fig. 2 for liposomes with

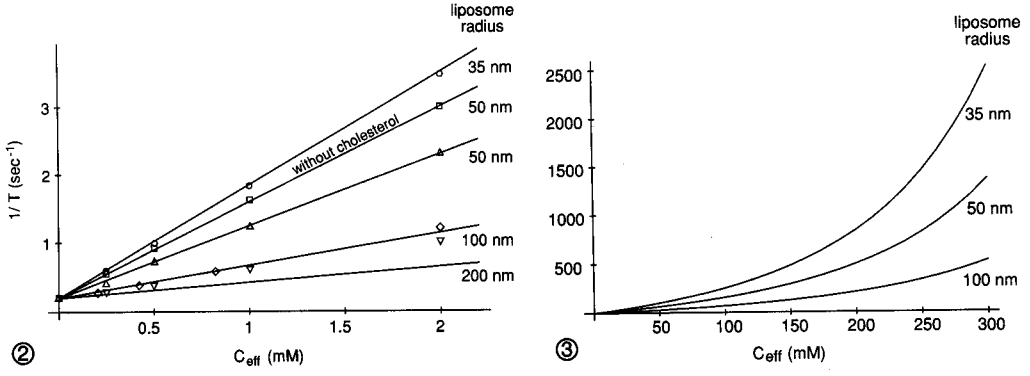

FIG. 2. Relaxation rate vs effective concentration of Gd-DTPA. The results in solid lines are plotted from Eqs. [25] and [20], assuming $d = 5 \times 10^{-7}$ cm and $C_0 = 670$ mM (corresponding to experiment) and $D_m = 0.47 \times 10^{-9}$ cm^2/s (matched value). The individual symbols represent experimental T_1^{-1} relaxation rates for liposomes of radius 35 (○), 50 (△), 100 (◇), and 200 (▽) cm $\times 10^{-7}$, respectively, at a field strength of 1.5 T. (Source: Tilcock *et al.* (*20*).) Also recorded are liposomes of radius 50×10^{-7} cm (□) that, in contrast to the others, are cholesterol-free, i.e., more permeable to water. To match these data, a diffusion coefficient $D_m = 1.4 \times 10^{-9}$ cm^2/s has been assumed. The value for $1/T$ at $C_{\text{eff}} = 0$ is just the native relaxation rate for water, i.e., 0.2 s^{-1} (*20*).

FIG. 3. Extension of Fig. 2 for large effective concentrations. Notice that the relaxation rate takes on a markedly nonlinear increase with concentration. Here also $D_m = 0.47 \times 10^{-9}$ cm^2/s.

a radius of 200 nm. Although we expect that the monoexponential decay approximation applies especially well for liposomes with a large radius, it is possible that the susceptibility effects mentioned in the introduction also contribute to the overall relaxation enhancement, since the measurements must entail *some* transverso relaxation.

For effective concentrations larger than 2 mM the relaxation rates predicted by means of Eqs. [20] and [21] exhibit a nonlinear dependence on C_{eff}. This behavior is presented in Fig. 3. While the concentration range in Fig. 3 may not be clinically relevant, it should nevertheless be possible to test this dependence on C_{eff} experimentally.

Smaller liposomes are more effective than larger liposomes in enhancing relaxation for a given effective concentration of contrast agent. The observations (*20*) indicate that the *relaxivity* increases linearly with the surface to volume ratio. From the approximate expression [22] we find to first order in d/R_b

$$\textit{relaxivity} \equiv (1/T)/C_{\text{eff}} \simeq (S/V)\text{PC}_0, \qquad [29]$$

where $V = (4\pi/3)R_b^3$ is the internal volume and $S/V = 3/R_b$. In Fig. 4 the dependence on the S/V ratio is shown for relaxivities evaluated according to Eqs. [20] and [21]. For low effective concentrations, i.e., on the order of 1 mM, Eq. [21] and the approximation thereof [22] give the same results; i.e., graphs of the two expressions are indistinguishable on the scale of Fig. 4. It can be seen from Fig. 4 that the predictions agree well with the observations, except for the datum corresponding to R_b = 150 nm. This again suggests that for the larger liposomes an additional relaxation enhancement mechanism, most likely involving susceptibility effects, is involved.

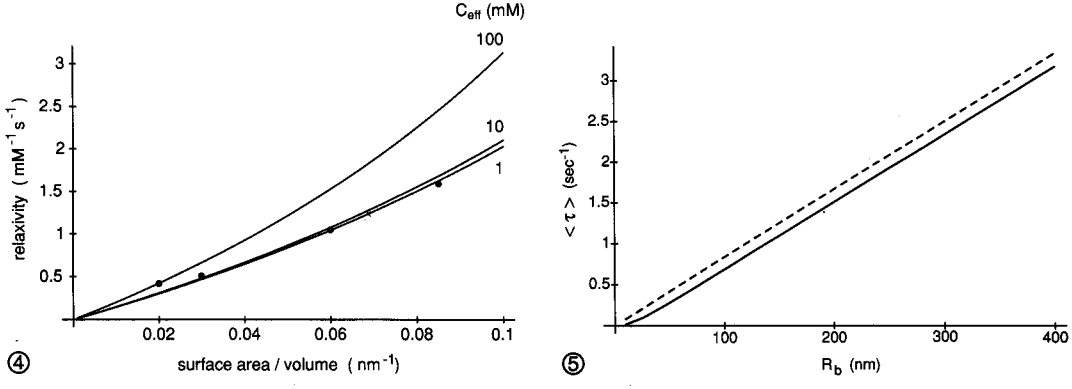

FIG. 4. Relaxivity (s^{-1} mM^{-1}) as a function of the surface to volume ratio plotted (solid lines) from Eqs. [20] and [21] for liposomes of radius 30 nm and larger. An analogous plot using Eq. [25] instead of Eq. [21] is indistinguishable from the curve corresponding to 1.0 mM effective concentration. Observations (dots) are of T_1 relaxivity (from Tilcock *et al.* (*20*)) for liposomes of radii 35, 50, 100, and 150 nm, from right to left. (Again, $D_m = 0.47 \times 10^{-9}$ cm^2/s).

FIG. 5. $\langle \tau \rangle$ versus liposome radius R_b. The dashed line has been plotted from Eq. [28]; the solid line, from Eq. [27]. These values are obtained assuming a reasonable membrane thickness d of 5 nm, and assuming $D_w = 10^{-5}$ cm^2/s and $D_m = 2 \times 10^{-13}$ cm^2/s. The overall relaxation rate for entrapped water can be computed via Eq. [20]. See text for explanation of D_m value.

Liposomes Excluding Gd-DTPA in Solution

We consider now liposome systems which in their interior exclude contrast agent present in the exterior. In Fig. 5 we compare the theoretical relaxation rates resulting from Eq. [27] and the linear approximation [28]. The two expressions are found to agree within 5% for radii somewhat greater than 200 nm. For liposomes with radii of 50 nm or smaller, however, the disagreement between the expressions is larger than 20%, and the former expression should be employed.

The present theory is supported by recent observations by Magin *et al.* (*16*). These authors used liposome suspensions, with Gd-DTPA as an external relaxation agent, to measure D_m by assuming Eq. [28]. When the magnetization decay data of such experiments are plotted on a semilog scale, two relaxation rates can be abstracted from the observed biexponential decay. The fast rate is due to relaxation enhanced by Gd-DTPA in the bulk. The smaller rate is due to relaxation in the liposome interior. The latter rate, at 1.5 s^{-1}, is larger than that in pure water, indicating relaxation due to exchange across the liposome membrane. One can then plug this rate into Eq. [28] or Eq. [27], along with the liposome radii, to determine the membrane permeability.

For their liposomes Magin *et al.* find $D_m \simeq 10^{-12}$ cm^2/s. They show that their results compare well with other methods of determining D_m. One difficulty in such comparisons is that small variations in liposome composition and temperature (esp. near the gel to liquid transition) have an enormous effect on D_m. This effect, however, can be used to great advantage, e.g., in the use of liposomes as a delivery system for contrast agents (*1*).

4. CONCLUSION

We have presented a formalism, the mean relaxation time approximation, which allows a systematic description of transport-mediated relaxation processes of nuclear spins. The theory is found to describe accurately relaxation due to contrast agents entrapped in liposomes, a system for which systematic observations exist. The theory is also used to describe relaxation in systems of bulk water with contrast agent and with liposomes which exclude the contrast agent in their interior. The main results are simple expressions for the overall relaxation times in these systems which reduce in certain limits to the conventional formula:

$$\text{rate} = (\text{permeability})\,\frac{\text{surface}}{\text{volume}}$$

where the volume is that which is free of contrast agent.

In the present paper the problems could be solved in terms of analytical expressions because they allowed reduction of the diffusion-Bloch equation to a single effective spatial dimension with a single compartment of interest. In a forthcoming work (*2*) we show how the mean relaxation time formalism introduced here provides an efficient route to numerical descriptions in situations for which such a reduction cannot be achieved, e.g., a contrast agent present only on the surface of a liposome, and how the formalism can describe relaxation in other compartmentalized systems (e.g., capillaries). There we show through simple applications such as those presented above how the formalism can be extended to more complex geometries and biexponential approximations, providing a suitable tool for modeling important aspects of MRI.

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Note added in proof. After submitting this paper we found that the description presented in the section entitled "Liposomes Excluding Contrast Agent" is similar to the model presented in K. R. Brownstein and C. E. Tarr, *Phys. Rev. A* **19**(6), 2446, 1979. It has recently been predicted that diffusion effects in liposome systems, and in other systems with compartmental boundaries, should play a decisive role in MRI at microscopic resolution (Benno Pütz, Daniel Barsky, and Klaus Schulten, *Chem. Phys. Lett.* **183,** 391, 1991; *J. Magn. Res.*, in press). Equation [27] in the present work allows one to obtain the permeabilities of liposomes to water; i.e., the quantity pertinent to diffusional effects in MRI microscopy.

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