Pore-to-Pore Hopping Model for the Interpretation of the Pulsed Gradient Spin Echo Attenuation of Water Diffusion in Cell Suspension Systems

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ABSTRACT A simplified pore-to-pore hopping model for the two-phase diffusion problem is developed for the analysis of the pulsed gradient spin echo (PGSE) attenuation of water diffusion in the condensed cell suspension systems. In this model, the two phases inside and outside the cells are treated as two different kinds of pores, and the spin-bearing molecules perform hopping diffusion between them. The size and the orientations of those two respective pores are considered, and then the diffraction pattern of the PGSE attenuation may be well simulated. Nevertheless, the intensity of the characteristic peak decreases with increasing membrane permeability, from which the exchange time may be estimated. We then analyze the experimental $^1$H PGSE results of the erythrocytes suspension system. The water-residence lifetime in the erythrocyte is obtained to be 10 ms, which is the same as that estimated from the two-region approximation. Furthermore, the PGSE attenuation curve of addition of p-Chloromercuribenzenesulfonate (p-CMBS) is also discussed. It predicts that the alignment of erythrocytes will become normal to the magnetic field direction after the addition of p-CMBS, and inspection using a light microscope confirms that result.

INTRODUCTION

Pulsed field gradient spin echo (PGSE) nuclear magnetic resonance technique has been used to probe the structures of porous materials and the diffusion of the confined spin-bearing molecules (Tanner and Stejskal, 1968; Callaghan, 1991). It is well known that, in q space experiments there are diffraction-like patterns shown in the PGSE attenuation curves for various cases of restricted diffusion (Callaghan et al., 1991; Bafinov et al., 1994; Kuchel et al., 1997; Callaghan, 1995; Codd and Callaghan, 1999). For restricted diffusion in single pores, the characteristic diffraction-like pattern may reflect the size of the pore, whereas, for restricted diffusion among well-separated multipores, the patterns reflect the mean distance between the pores.

Kärger (1985) developed an analytical approach to examining PGSE attenuation through two-region exchange approximation. Because there is exchange between two freely diffusing phases, the calculated double exponential decay profile does not display the diffraction-like pattern as observed in the PGSE experiment on the erythrocyte suspension system. Stanisz et al. (1998) modified Kärger’s interpretation for the PGSE attenuation of the erythrocyte suspension, in which they considered the diffusion within an erythrocyte as one-dimensional restricted diffusion. They also estimated an apparent diffusion coefficient from the PGSE attenuation feature by considering one-dimensional restricted diffusion and then calculated the profile of PGSE attenuation using the two-region exchange model. Similarly, Price et al. (1998) derived the apparent diffusion coefficient from the PGSE attenuation of the restricted diffusion in a spherical pore. Also, Peled et al. (1999) applied the same strategy to studying water diffusion in the frog sciatic nerve and derived the apparent diffusion coefficient from the PGSE attenuation in a cylindrical pore to mimic the restricted diffusion in the nerve cells. All those results involve the usage of the apparent diffusion coefficient to illustrate the effects of the restricted diffusion in the cells and, in turn, to explain the diffraction-like pattern of the PGSE attenuation curve.

Kuchel et al. (1997) explored the PGSE experiments for the erythrocytes suspension system and estimated two physically significant lengths from the two diffraction-like patterns of the attenuation profile. They assigned the two lengths obtained to the size of the erythrocyte and the average distance of extracellular pore spacing. Such a treatment is similar to that adopted by Callaghan et al. (1991) for the pore-like space between the polystyrene spheres. They have used this method to derive the PGSE attenuation for the restricted diffusion among multipores of the same size. Also, they introduced an effective diffusion coefficient to describe self-diffusion for long-range migration between pores. The formulation used by Stanisz et al., (1998), Peled et al., (1999), and Price et al., (1998) can be used to interpret the diffraction-like pattern caused by the restricted diffusion in the erythrocytes, but it cannot be used to explain the first diffusion-like peak observed by Kuchel et al. (1997). It implies that there exists some constraint in extracellular diffusion. Therefore, a new model is needed to include the effects of the size and the arrangement of the erythrocytes and the external pores between the erythrocytes. Because such systems are too complicated to be solved exactly by the general diffusion equation, the modified pore-to-pore
hopping model may be used as an approximation for studying the diffusions in the erythrocyte suspension system.

In the present work, we develop a simplified diffusion model for a two-phase system, represented by the coupled master equations (Haus and Kehr, 1987) with pore-to-pore hopping exchange between two different pores. We take into account the effects of the pore size, the spatial arrangement of the pores, and the variation of water-residing times in each phase on the PGSE attenuation. Then we calculate the PGSE attenuation of diffusion among multipores of the same size and compare the results with those derived by Callaghan (1995). Also, we applied the proposed model to analyzing the results of the PGSE experiments for the erythrocyte suspension system.

**THEORY**

**The general formulation**

The formulation of the PGSE attenuation for the molecular diffusion among multipores can be easily derived based on the “pore equilibrium” condition (Callaghan et al. 1991). This assumption is suitable for a porous medium with well-defined pore-channel structure, i.e., the size of the channels is much smaller than that of the pores. With the pore equilibrium condition and the short gradient pulse approximation (Tanner and Stejskal, 1968; Linse and Söderman, 1995), where the waveform of the gradient pulse is considered to be a δ-function in time domain, the PGSE attenuation of diffusion among multi-identical pores can be expressed in q space by (Callaghan, 1991)

\[
E(q, \Delta) = \sum_{n} \int_{V_0} dr_0 \int_{V_n} dr_0 \rho(r_0) \frac{P(n, \Delta)}{V} \times \exp[i2\pi q \cdot (r_n + R_n - r_0)]
\]

\[
= S(q)S(q)P(q, \Delta),
\]

where \( q = \gamma \delta G / 2\pi \), \( \gamma \) is the gyromagnetic ratio, \( \delta \) is the duration of the magnetic field gradient pulses, \( G \) is the strength of the gradient; and \( \rho(r_0) \) is the density of the spins within the 0th pore initially, and \( \Delta \) is the interval between the two pulsed gradients. The subscript 0 represents the 0th pore, e.g., the pore where the spin is situated at the initial time, and the subscript \( n \) indicates the nth pore where the spin is situated at time \( \Delta \). \( V_0 \) and \( V_n \) are the internal space of the 0th and the nth pores, respectively. \( r_0 \) and \( r_n \) are the position vectors of the pore centers at the 0th and the nth pores, respectively. In Eq. 1, \( S(q) = \int_{V} dr(1/V) \exp(i2\pi q \cdot r) \) is called the “structure factor” of the pore, where \( 1/V \) is the density of the spins, which normalizes the amount of the spins in a pore, and \( V \) is the volume of a single pore. \( P(n, \Delta) \) is the probability of a spin existing in the nth pore at time \( \Delta \),

\( \mathbf{R}_n \) is the position vector of the center of the nth pore relative to that of the 0th pore, and

\[
P(q, \Delta) = \sum_{n} P(n, \Delta) \exp(i2\pi q \cdot \mathbf{R}_n).
\]

Thus \( P(q, \Delta) \) describes the arrangement of the pores as expressed in \( q \) space, and one may derive it in terms of the hopping exchange model described in the master equation in the next section.

**Diffusion among identical pores**

For restricted diffusion in a single pore with a permeable wall, one may treat the boundary as a semi-adsorptive wall (Barzykin et al. 1995),

\[
D \frac{\partial P(r_0|r, t)}{\partial r} + H \cdot P(r_0|r), t_{|t=0} = 0,
\]

where \( D \) is the diffusion coefficient, \( H \) is a constant to represent the transport ability of the boundary; \( r_0 \) and \( r \) are the position vectors; \( P(r_0|r, t) \) is the probability of finding a particle initially at \( r_0 \) and at \( r \) after a time \( t \), and \( a \) represents the position where the boundary exists. By solving the diffusion equation with the boundary condition described by Eq. 3, the total probability within the pore, e.g., \( \int_{V} dr P(r_0|r, t) \), gives an exponential decay form with a characteristic lifetime. By analogy, we adopted the same idea to the case of the exchange diffusion among multipores and take the lifetime as the time needed to travel from one pore to another. Then, the master equation, which is analogous to that of the multisite jump diffusion (Haus and Kehr 1987), can be constructed. The master equation of hopping diffusion among pores of the same size can be written as

\[
\frac{\partial P_i(t)}{\partial t} = \sum_{j=1}^{N} W_{ji}(t) - N W_i(t),
\]

where \( P_i(t) \) is the probability of a spin existing at the \( i \)th pore at time \( t \), \( P_{ji}(t) \) is the joint probability of a spin existing at the \( j \)th pore neighboring to the \( i \)th pore, \( N \) is the number of the first shell of pores, and \( W = 1/N\tau \) is the pore-to-pore exchange rate, where \( \tau \) is the spin residence lifetime in a pore.

In Eq. 2, \( P(q, t = \Delta) \) may be obtained readily by the Fourier–Laplace transform of Eq. 4 and then solved by inverse Laplace transform, which yields

\[
P(q, \Delta) = \exp(-\Delta \cdot N W(1 - A(q)))
\]

\[
= \exp \left[ -\frac{\Delta}{\tau} (1 - A(q)) \right],
\]

\[2494\text{ Jiang et al.}

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Application to cell suspension systems

The formulation may be extended with the help of Eq. 1 to condensed cell suspension systems. As shown in Fig. 1, the condensed cell suspension system can be approximated as a system consisting of two kinds of pores. We denote the cells as pores C. The space enclosed by the cells can be considered as the external pores, which are denoted as pores S. One may suppose that the diffusion of spin-bearing molecules starting from a pore C or a pore S at time can equally be found in pores C initially, and at a later time in pores S, the contribution to the magnetization is given by

\[
M_{C\rightarrow S}(q, \Delta) = S_C(q, \Delta) 
\]

where \( S_C(q, \Delta) = \sum_n S_C(n, \Delta) \exp(i2\pi q \cdot R_{Sn}) \), and where \( S_C(n, \Delta) \) is the probability for a spin diffusing from a pore C initially to the n-th pore S at time \( \Delta \). \( R_{Sn} \) is the position vector at the center of the n-th pore S relative to that of the initial pore C. \( V_C \) and \( V_S \) are the volumes of a single pore C and pore S, respectively. \( r_C \) and \( r_S \) are the position vectors within a pore C and a pore S relative to their pore centers, respectively. The expressions for the other cases, C→C (pore C to pore C), S→C and S→S are similar. Here, the subscript C means the starting point is a pore C, and the subscript S means the starting point is a pore S. There are only three hopping rate constants considered in this system because the cells do not connect to each other directly. Pores S are all connected to their neighboring pores S with a hopping rate of \( W_{SS} \). Pores C are isolated from the other pores C, but are connected to their neighboring pores S with hopping rate constants \( W_{CS} = 1/(N\tau_C) \) and \( W_{SC} = 1/(N\tau_S) \), where \( \tau_C \) and \( \tau_S \) are the spin residence lifetimes of pores C and pores S, respectively.

Furthermore, in the PGSE experiment, the time interval between the two gradient pulses is set shorter than the transverse spin relaxation, and the observed magnitudes of PGSE attenuation for various \( q \) is normalized by the observed value at \( q = 0 \). Thus, for this simplified system, the effect of the transverse spin relaxation processes is cancelled. The master equation may be written without the spin relaxation term as

\[
\frac{\partial C_{n}(t)}{\partial t} = \sum_{i=1}^{N} W_{SC} C_{n}(t) - \sum_{i=1}^{N} W_{CS} C_{n}(t), \quad (8a)
\]

\[
\frac{\partial S_{m}(t)}{\partial t} = \sum_{i=1}^{M} W_{SS} S_{m}(t) + \sum_{i=1}^{N} W_{CS} C_{m}(t) - (NW_{SC} + MW_{SS}) S_{m}(t), \quad (8b)
\]

where \( C_n(t), S_{n}(t), C_{m}(t), \) and \( S_{m}(t) \) are the corresponding probabilities at time \( t \), \( N \) is the coordination number of a pore C surrounded by S pores, and \( M \) is that of a pore S surrounded by other S pores. For simplicity, we set the same value \( N \) as the coordination number of a pore S surrounded by pores C. Then we follow the hopping exchange model and obtain \( C_C(q, \Delta), S_C(q, \Delta), C_S(q, \Delta), \) and \( S_S(q, \Delta) \) by solving the coupled master equation after the Fourier–Laplace transform and the inverse Laplace transform as

FIGURE 1 The pictorial representation of the simplified two-phase model consisting of the cells (solid circle, pores C, solid line represents the membrane) and the pores between the cells (dotted circle, pores S). \( R_C \) and \( R_S \) are the radius of pores C and pores S, respectively. The mean distance between two pores S is \( d \). The mean distance between a pore C and a pore S is \( b \). The hopping rates between pores are also marked by \( W \) with appropriate subscripts to indicated the exchanging species.
derived in the Appendix. \( M_{C \rightarrow S} \) may be calculated in accordance with Eq. 6. By analogy, \( M_{C \rightarrow C} \), \( M_{S \rightarrow C} \), and \( M_{S \rightarrow S} \) can be obtained accordingly. Consequently, for random pore arrangement, we obtain

\[
\begin{align*}
C_c(q, \Delta) &= x_{CC}\exp(\lambda_1\Delta) + y_{CC}\exp(\lambda_2\Delta), \\
S_c(q, \Delta) &= x_{SC}\exp(\lambda_1\Delta) + y_{SC}\exp(\lambda_2\Delta), \\
C_s(q, \Delta) &= x_{CS}\exp(\lambda_1\Delta) + y_{CS}\exp(\lambda_2\Delta), \\
S_s(q, \Delta) &= x_{SS}\exp(\lambda_1\Delta) + y_{SS}\exp(\lambda_2\Delta),
\end{align*}
\]

where the parameters \( x_i \) and \( y_i \) \( (i = CC, SC, CS, \text{and } SS) \) are defined in the Appendix. They are related to the hopping rate and the pore-to-pore distance. The exponent parameters, \( \lambda_1 \), and \( \lambda_2 \), in Eqs. 9 are given by

\[
\lambda_{1,2} = -\frac{(NW_{CS} + \alpha) \pm \sqrt{(NW_{CS} - \alpha)^2 + 4N^2W_{CS}W_{SC}\sin^2(2\pi q d)}}{2} \tag{10}
\]

where \( \alpha \) is defined by

\[
\alpha = NW_{SC} + MW_{SS}[1 - \sin(2\pi q d)]. \tag{11}
\]

On the basis of the completely random arrangement of pore C and pore S, the derivation procedures are readily presented in the Appendix. By summing up the four parts as given in Eq. 9, we obtain the PGSE attenuation

\[
E(q, \Delta) = \frac{1}{V_C + V_S} \left[ \frac{x_{CC}}{V_C}F_C^2 + \left( \frac{x_{SC}}{V_S} + \frac{x_{CS}}{V_C} \right)F_CF_S + \frac{x_{SS}}{V_S}F_S^2 \right] \times \exp(\lambda_1\Delta) + \left[ \frac{y_{CC}}{V_C}F_C^2 + \left( \frac{y_{SC}}{V_S} + \frac{y_{CS}}{V_C} \right)F_CF_S + \frac{y_{SS}}{V_S}F_S^2 \right] \exp(\lambda_2\Delta), \tag{12}
\]

where the structure integral for a single pore C and a single pore S are given by

\[
F_C = \int_{V_C} d\mathbf{r}_C\exp(i2\pi\mathbf{q} \cdot \mathbf{r}_C)
\]

and

\[
F_S = \int_{V_S} d\mathbf{r}_S\exp(i2\pi\mathbf{q} \cdot \mathbf{r}_S),
\]

respectively. \( 1/(V_C + V_S) \) is the density of the spins, which normalizes the amount of the spins in one pair of pore C and pore S.

**MATERIALS AND METHODS**

**Preparation of erythrocyte suspension**

Blood was obtained from a healthy human volunteer. The erythrocytes were centrifugally washed (3000 \( \times g \), 10 min) two times in cold glucose-enriched saline solution (154 mM NaCl, 10 mM glucose, \( 4^\circ \)C). The plasma and the buffy coat were discarded. The appropriate amount of cold glucose-enriched saline solution was then added to form the erythrocyte suspension. All erythrocyte suspensions were gently bubbled with carbon monoxide for 5 min to transform the hemoglobin into a stable low-spin diamagnetic state. For the experiments on inhibiting transmembrane water exchange, p-Chloromercuribenzenesulfonate (p-CMBS) (Sigma, St. Louis, MO) was added (1.9 mg to 1 ml of suspension) and the suspension was kept at \( 37^\circ \)C for 1 hr before doing the PGSE experiments.

**PGSE experiment**

PGSE experiments were performed on a (Bruker Analytik GmbH, Rheinstetten, Germany) MSL-500 spectrometer, operating at a 11.4-T magnetic field, equipped with a Bruker DIFF-25 gradient probe capable of a maximum gradient of 10 T m\(^{-1}\). The use of the actively shielded gradient coil in the probe and the precompensation function of preemphasis unit greatly reduce the effect of eddy current on diffusion measurements. A blanking unit is open 200 \( \mu s \) before the gradient pulse and stays on during the gradient pulse and the ring-down period to allow the preemphasis to work. The eddy current generated after the gradient pulse is less than 2% of the static value of the gradient pulse within 150 \( \mu s \). It rings down to less than 1% after 250 \( \mu s \). In all the experiments, the standard PGSE pulse sequence and phase cycles were used (Kuchel et al., 1997). The duration of the 90° pulse was 25 \( \mu s \); that of the two gradient pulses, \( \delta \), was 1.2 or 2 ms. Because the proton transverse relaxation times inside and outside the erythrocyte (Stanisz et al., 1998) yield 160 and 400 ms, respectively, to achieve significant signal in our experiments, one may set the time interval between the two gradient pulses to be shorter than 160 ms. Here the time interval between the gradient pulses was set to 15 or 40 ms. The relaxation delay between transients was 8 \( s \); and the number of transients per spectrum was 80. The probe temperature was maintained at 298 \( \pm \) 0.3 K to minimize the convection. The \( S/N \) for full magnetization was higher than 2000 in all the experiments.

**Orientation observation**

Gelatin solution was prepared by adding an appropriate amount of gelatin (Sigma, St. Louis, MO) into a saline solution. The erythrocyte suspensions (with or without p-CMBS treatment) were added to the gelatin solution and kept at \( 37^\circ \)C for at least 3 h within the 11.4-T magnetic field to ensure the
Consequently, the attenuation reduces to

\[
E(k, \Delta) = \frac{1}{V_C + V_S} \left[ F_C^2 + \frac{F_S^2}{V_S} \exp(\lambda_2 \Delta) \right],
\]

where the first part, \(F_C^2/V_C\), results solely from the restricted molecular diffusion in pores C, and the second part, \(F_S^2 \exp(\lambda_2 \Delta)/V_S\), from the molecular diffusion in the external pores. Furthermore, one may enhance \(W_{SC}\) and \(W_{CS}\) to the same magnitude of \(W_{SS}\) to investigate the effect of the permeability. However, when \(W_{SC}\) and \(W_{CS}\) are significant, the PGSE attenuation is no longer dominated by the spins in pores C and pores S only. Instead, the effect of spin diffusion from pores C(S) into pores S(C) is considered (see Eq. 9).

In those cases, the \(\exp(\lambda_1 \Delta)\) versus \(qb\) plot is presented in Fig. 2 A, and the \(\exp(\lambda_2 \Delta)\) versus \(qb\) plot is presented in Fig. 2 B. We can clearly see that \(\exp(\lambda_1 \Delta)\) oscillates with the same periods as \(\exp(\lambda_2 \Delta)\) does. The position of the first peak of \(\exp(\lambda_1 \Delta)\) and \(\exp(\lambda_2 \Delta)\) is situated at \(qb = 0.72\), which is close to \(qb = 0.57\) \((qd = q \cdot \sqrt{3} b \approx 1)\). As compared with the PGSE results for the diffusion among pores of the same size, the position is characterized by the length between two pores S, or two pores C.

**RESULTS AND DISCUSSION**

**Permeability effect**

Considering the condensed spherical cell (pores C) suspension systems, the pores (pores S) between pores C may also be approximated to be a sphere, as shown in Fig. 1. For the case with the volume ratio of pore C to pore S to be \(V_C:V_S = 0.7\), we obtain the radius ratio of pore C to pore S, \(R_C: R_S = \sqrt{0.7}\). The mean distance \(b\) between pore C and pore S may be approximated by the sum of their radii because of the compact stacking of the two kinds of pores (see Fig. 1). Moreover, if one considers the 3-dimensional cubic packing of two kinds of pores, the mean distance \(d\) between two S pores may be set to \(\sim \sqrt{3} b\). Here the same density of spins is assigned to each pore, i.e., \(\rho_C/\rho_S = 1.0\), and thus the population ratio of the spins in pore C to pore S gives \(P_C/P_S = V_C/P_S = 0.7\), which also implies the ratio of the two rate constants \(W_{SC}/W_{CS} = P_C/P_S = 0.7\) in accordance with the principle of detailed balance. Then, we set \(\Delta = 1.5/\tau_{SS}\) which is 1.5 times the lifetime of a spin in pore S. In the present work, we first considered a situation in which the spin-bearing molecules may not penetrate the cell membrane, e.g., \(W_{CS} = W_{SC} = 0\), but \(W_{SS} \neq 0\) because there must be connections between pores S. In this case, we obtain \(\exp(\lambda_1 \Delta) = 1\) and \(\lambda_2 = -\tau_{SS}[1 - \sin(qd)]\). Consequently, the attenuation reduces to

\[
\exp(\lambda_2 \Delta),
\]

where the increasing oscillation magnitude of the \(\exp(\lambda_2 \Delta)\) term with increasing \(W_{SC}\) and \(W_{CS}\) shows the effect of the pore arrangement with the exchange process.

The \(E(q, \Delta)\) versus \(qb\) curves with different \(NW_{CS}\) values are plotted in Fig. 3. The individual contribution to the magnetization, e.g., \(M_{C\rightarrow S}\), \(M_{S\rightarrow C}\), and \(M_{S\rightarrow S}\) for three cases, \(NW_{SC}/NW_{SS} = 0, 0.5,\) and 1.0, are shown in Fig. 4 for comparison. Apparently, in Fig. 3, there are characteristic peaks at \(qR_C\) or \(qR_S \approx q \cdot (b/2) \approx 1\) for all of the five curves with various magnitudes of \(NW_{SC}\). The intensity of the characteristic peak decreases as the permeability increases. As shown in Fig. 4, the PGSE attenuation may be analyzed in detail as follows. The characteristic peaks result mainly from \(M_{C\rightarrow C}\), but not from \(M_{S\rightarrow S}\), because the high molecular mobility of the spins in pores S causes more attenuation of \(M_{S\rightarrow S}\) in \(q\) space. Analogously, the intensity of the characteristic peak in \(M_{C\rightarrow C}\) decreases with increasing \(W_{SC}\) (\(W_{CS}\)), which reduces the intensity of the characteristic peak in \(E(q, \Delta)\). The increasing decay rate of \(E(q, \Delta)\) at small \(q\)
with increasing $W_{SC}$ ($W_{CS}$) comes mainly from the change of the relative proportion of each component. As shown in Fig. 4 A, $M_{c-c}$ decreases with increasing $W_{SC}$ ($W_{CS}$), whereas the fast decaying $M_{c-s}$, $M_{s-c}$, and $M_{c-c}$ dominate the shape of the curve at small $q$. In addition, when $W_{SC}$ ($W_{CS}$) is about the same magnitude of $W_{SS}$, i.e., $NW_{SC}/MW_{SS} = 1.0$, represented by the dotted line in Fig. 4 A, there is also a fast decay in $M_{c-c}$ at small $qb$. When $NW_{SC}/MW_{SS} < 0.5$, because $M_{c-c}$ is large, the first diffraction-like pattern of $M_{s-s}$, reflecting the distance between two pores $S$, is under the shadow of $M_{c-c}$, and therefore it is invisible on the $E(q, \Delta)-qb$ plot.

**Orientation effect**

For a nonspherical cell suspension system, the orientations of the cells may affect the PGSE attenuation. As shown in Fig. 5, we may consider the disk-shape cells (pores $C$) suspension system with disk thickness $t$, radius $R_1$, and the spacing between the cells $x$. The cells are randomly packed. We may then simply take the averaged shape of the medium (pores $S$) separating the cells as a disk with radius $R_S$ and thickness $t_s$ estimated from the lattice model shown in Fig. 5 B. The PGSE attenuation for a specific cell orientation $\theta$ (see Fig. 5 A) may be derived as

$$E(q, \Delta, \theta) = \frac{1}{V_C + V_S}$$

$$\times \left\{ \frac{x_{CC}}{V_C} F_C^2(\theta) + \left( \frac{x_{SC}}{V_S} + \frac{x_{SS}}{V_C} \right) F_S(\theta) F_C(\theta) \right.$$

$$\left. + \frac{x_{SS}}{V_S} F_S^2(\theta) \exp(\lambda_1 \Delta) \right.$$

$$+ \left[ \frac{y_{CC}}{V_C} F_C^3(\theta) + \left( \frac{y_{SC}}{V_S} + \frac{y_{SS}}{V_C} \right) F_S(\theta) F_C(\theta) \right.$$

$$\left. + \frac{y_{SS}}{V_S} F_S^3(\theta) \right\} \frac{1}{\sin(2\pi q \cdot r_1)dr_1},$$

where the cell orientation $\theta$ is defined as the angle between the magnetic field and the central axis of the pore $C$. Other parameters have been defined previously for Eq. 9. The structure integral, $F_C(\theta)$, for a pore $C$ with the cell orientation $\theta$ is expressed by

$$F_C(\theta) = \int_{v_C} \exp(i2\pi q \cdot r_1)dr_1$$

$$= \frac{t_s \cdot \sin[(\pi q t_s \cdot \cos \theta)] \cdot 2\pi R_1(2\pi q \sin \theta \cdot R_s)}{2\pi q \sin \theta}.$$
Observation of erythrocyte orientation

As shown in Fig. 7 A, pure erythrocytes are oriented with their disk plane parallel to the magnetic field direction. For the controlled experiments in the absence of magnetic field, the erythrocyte orientations were random. These results were the same as those obtained by Higashi et al. (1993). Besides, Kuchel et al. (2000) used the diffusion tensor method to analyze the PGSE attenuation of the erythrocyte suspension system, and they found that the diffusion tensor component at z-direction (the direction parallel to the magnetic field of the NMR spectrometer) is larger, which also verifies the anisotropic orientations of the erythrocytes. The erythrocytes with p-CMBS added, as shown in Fig. 7 B, are oriented with their disk plane perpendicular to the magnetic field direction.

Applications to the erythrocyte suspension system

Experimental PGSE results

Erythrocyte suspension systems prepared as described in Materials and Methods were considered as model cell suspension systems. Here, we have repeated the PGSE experiments of the erythrocyte suspension system studied by Kuchel et al. (1997) but with enhancement of the magnetic field gradient. The PGSE attenuation curve in the q space plot is shown in Fig. 8. For the pure erythrocyte suspension system, we performed PGSE experiments with δ = 1.2 ms and δ = 2 ms. These two experiments showed the same results in PGSE experiments; short gradient pulse approximation may be applied accordingly. For the system of erythrocyte suspension with p-CMBS added, the PGSE signal attenuates more slowly than that of a pure erythrocyte suspension system. Moreover, there was no characteristic peak found (see Fig. 8). These two features may account for the change of erythrocyte orientation as compared to those of the orientation effects. The results of the erythrocyte suspension system may be applied accordingly.

FIGURE 6 The PGSE attenuation E(α, Δ) versus q plot. The curves a, b, c represent the simulation data of a 51% hematocrit erythrocyte suspension at θ = 90°, θ = 45° and θ = 0°, respectively. The simulation parameters for curve a, a = 3.06 μm, b = 4.12 μm, and d = 8.24 μm; curve b, a = 3.06 μm, b = 3.1 μm, and d = 6.2 μm; and curve c, a = 3.06 μm, b = 1.51 μm, and d = 3.01 μm. The parameters MWCS = 100 s⁻¹ and MWSS = 120 s⁻¹ are for all three curves.

where J₁(2πq sin θ · R₁) is the first-order Bessel function of the first kind. Similarly, the structure integral, F₁(θ), for a pore S with the specific cell orientation θ may be expressed as

\[ F_S(\theta) = \frac{t_s \cdot \text{sinc}(\pi q t_s \cos \theta) \cdot t_s}{2 \pi q \sin \theta} \cdot \frac{2 \pi q J_1(2 \pi q \sin \theta \cdot R_S)}{2 \pi q \sin \theta} \cdot (16) \]

To demonstrate the effect of the cells orientation, we may consider the case of R₁ = 1.5l, t_s = l and x = l/4 where the length, l, is arbitrary. We then set Δ = 1.5/MWSS and NWSC/MWSS. The PGSE attenuation of different cell orientations versus q/l plot is presented in Fig. 6. We can find that the characteristic peak shifts from q/l = 0.56 to a higher q/l value while the cell orientation changes from 90° to 45°. The characteristic peak shifts to an even higher q/l value while θ = 0° and cannot be seen in the low q/l region. Moreover, the PGSE signal attenuates slower in the low q/l region while θ = 0° as compared to the cases of θ = 90° and θ = 45°. The effects of cell orientation merit attention.

PGSE NMR Study of Erythrocyte Solution

FIGURE 5 (a) The cell orientation θ is defined as the angle between the magnetic field direction (q direction) and the central axis of the cell. (b) The lattice model of the equally separated disk-shaped cells with disk radius R₁ and thickness t₁. The averaged shape of the medium (pores S) can be approximated as a disk with radius R₂ and thickness t₂. There is one pair of pore C and pore S in a unit cell. The distance between a pore C and a pore S is denoted by b. The distance between two pores S is denoted by d. The distance between cells is denoted by x. R₂ is the radius of a pore S. b = \sqrt{(b \cos \theta)^2 + (b \sin \theta)^2}, d = \sqrt{(d \cos \theta)^2 + (d \sin \theta)^2}, R₂ = (l₁ + l₂)/2, l₁ = \sqrt{2} h₁ - R₁, l₂ = h₁.

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account the differences in the erythrocytes radii, we then analyze the PGSE experimental results. The erythrocyte suspension model can be used to consider that the radii of erythrocytes follow the distribution

\[ P(R_1) = N_C \exp\left(-\frac{(R_1 - R_m)^2}{2\sigma^2}\right), \tag{17} \]

where \( R_m = 3.85 \, \mu m \) is the mean radius and \( \sigma = 0.5 \, \mu m \) is the standard deviation of radius distribution. Thus, by considering the radius distribution of erythrocytes, the PGSE attenuation can be modified from Eq. 14 as

\[ \bar{E}(q, \Delta, \theta) = \sum_{R_1} E(q, \Delta, \theta, R_1), \tag{18} \]

where

\[
E(q, \Delta, \theta, R_1) = \frac{1}{\rho_w V_C + V_S} \times \left\{ \left[ \frac{\rho_w}{V_C} F_C^2(R_1, \theta) + \left( \frac{\rho_w}{V_S} + \frac{x_{SC}}{V_C} \right) F_C(R_1, \theta) F_S(R_1, \theta) \right] \exp(\lambda_1 \Delta) + \left[ \frac{\rho_w}{V_C} F_C^2(R_1, \theta) + \left( \frac{\rho_w}{V_S} + \frac{x_{SS}}{V_S} \right) F_C(R_1, \theta) F_S(R_1, \theta) \right] \exp(\lambda_2 \Delta) \right\} \tag{19}
\]

Here \( F_S(R_1, \theta) \) means that the size and the structural integral of an external pore depends on the radius of the erythrocyte in each subsystem, because the hematocrit value is set to be constant in all of the subsystems. It is known that the density

orientation were observed by light microscope, confirming this prediction.

**Analysis of the experimental results**

The disk-shaped cell suspension model can be used to analyze the PGSE experimental results. The erythrocytes can be approximated as a biconcave disk (Beck, 1978; Higashi et al. 1993; Kuchel et al., 1997) with diameters ranging from 6.7 to 8.7 \( \mu m \). To take into account the differences in the erythrocytes radii, we then consider the differences in the erythrocytes radii, we then

![FIGURE 7](a) Pure erythrocytes and (b) erythrocytes with p-CMBS added inside a 11.4-T magnetic field. The magnetic field direction is normal to the test plane. Pure erythrocytes were photographed on their edge so that they were orientated with their disk plane parallel to the magnetic field direction. Erythrocytes with p-CMBS added were photographed on their edge so that they were orientated with their disk plane normal to the magnetic field direction. For the controlled experiments in the absence of magnetic field, the erythrocyte orientations were random. The controlled results were the same as those obtained by Higashi et al. (1993).

![FIGURE 8](The experimental PGSE attenuation \( E(q, \Delta) \) versus \( q \) plot and the simulated results. Solid circles and dashed line stand for the experimental and fitted results, respectively, for the 48% hematocrit erythrocyte suspension with p-CMBS added (NMR parameters: \( \delta = 1.2 \, ms \) and \( \Delta = 40 \, ms \)). Open circles and solid squares stand for the experimental result of \( \delta = 2 \, ms \) and \( \delta = 1.2 \, ms \), respectively, for the 51% hematocrit pure erythrocyte suspension. Solid line is the fitted result for 51% hematocrit pure erythrocyte suspension. The time interval of the two gradient pulses was set to 15 ms. Open inverted triangles and dotted line stand for the experimental and fitted results, respectively, for the 40% hematocrit pure erythrocyte suspension (NMR parameters: \( \delta = 2 \, ms \) and \( \Delta = 40 \, ms \)).
of the erythrocyte is almost the same as that of the outer medium, and the weight percentage of water $\rho_w$ in the erythrocyte is about 70\% (Grimes, 1980). In addition, for a constant hematocrit value, as $R_1$ varies according to the Gaussian distribution, Eq. 17, it is noted that the radius $a$ of the external pore follows linearly with the relation, $(a - a_m)/a_m = (R_1 - R_m)/R_m$, where $a_m$ is the mean value of $a$. All other parameters in Eq. 19 have been defined already in the previous sections.

As shown in Fig. 9, considering the shape of erythrocyte, according to the definition of the structure integral for a pore $C$, it can be calculated as

$$F_C(R_1, \theta) = \int_{V_C} \exp(2\pi q \cdot r_C) dr_C$$

$$= \int_{V_1} \exp(2\pi q \cdot r_1) dr_1 - \int_{V_2} \exp(2\pi q \cdot r_2) dr_2$$

$$+ \int_{V_3} \exp(2\pi q \cdot r_3) dr_3$$

$$= t_1 \cdot \sin c[(2\pi q \cdot \cos \theta/2) \cdot t_1] \cdot 2\pi R_1 J_1(2\pi q \cdot \sin \theta R_1)$$

$$- t_1 \cdot \sin c[(2\pi q \cdot \cos \theta/2) \cdot t_1] \cdot 2\pi R_2 J_1(2\pi q \cdot \sin \theta R_2)$$

$$+ t_2 \cdot \sin c[(2\pi q \cdot \cos \theta/2) \cdot t_2] \cdot 2\pi R_3 J_1(2\pi q \cdot \sin \theta R_3).$$

(20)

The fitting parameters are described as follows. The ratio of $W_{CS}$ to $W_{SC}$ is obtained by the principle of detailed balance $P_C W_{CS} = P_S W_{SC}$, where $P_C$ and $P_S$ are the populations of the water inside and outside the erythrocytes, respectively. The ratio is given by $P_C/P_S = \rho_w h (1 - h)$ because the hematocrit value $h$ is defined as the ratio of the volume of a disk and the volume of the total suspension.

Because the shape of the erythrocyte resembles a disk, the mean distance between pore $C$ and pore $S$ and that between two pores are not as easily described as in the case of the spherical-cell suspension. Therefore, we constructed a periodically stacked structure for pore $C$ and pore $S$, as shown in Fig. 5 B, where the distances between all pairs of pores $C$ are the same. By the definition of the hematocrit content, $h = V_C/V_{a.e.}$, the spacing $x$ between the erythrocytes may be evaluated. For 40\% (or 51\%) hematocrit, it yields $x = 0.98 \mu m$ (or 0.54 \mu m). This is about the size of the channel between pores, which is small compared to the diameter of erythrocyte, $R_i = 7.7 \mu m$ and to that of pore $S$, $a = 6.6 \mu m$ (or 6.1 \mu m). The differences between the sizes of channel and the pore confirm the validity of pore equilibrium conditions. Moreover, the estimations of the averaged values of distance parameters $b$ (the distance between the adjacent pore $C$ and pore $S$), $d$ (the distance between two adjacent pores $S$), and the mean size $a_m$ of a pore $S$ can also be made from this model. The results for different erythrocyte solutions are listed in Table 1.

For the pure erythrocyte suspension system, the erythrocytes preferably align with a magnetic field of 11.4 T (Higashi et al. 1993). Then the orientation was determined as $\theta = 90^\circ$. Finally, only the hopping rates, $NW_{SS}$, $NW_{CS}$, and $NW_{SC}$, remain to be determined, and only either $NW_{CS}$ or $NW_{SC}$ needs to be solved, according to the detailed balance principle.

Based on the theoretical analysis, the hopping rate $NW_{SS}$ between pores $S$, which represents the diffusivity of water outside the erythrocytes, can be determined from the slope of the early part, i.e., the fast-decaying part of the curve. The hopping rates $NW_{SS}$ and $NW_{CS}$ between the two phases may be calculated from the intensities of the slow-decaying part of the curve and the characteristic peak. For the erythrocyte suspension with p-CMBS added, as mentioned previously, the erythrocyte orientation becomes normal to the magnetic field direction, so we can set $\theta = 0^\circ$. In the two cases ($\theta = 0^\circ$ and $90^\circ$), the hopping rate $NW_{SS}$ was kept unchanged, and the hopping rate $NW_{SC}$ varies to fit the result, whereas the water residence times $\tau_C$ inside an erythrocyte correspond to the inverse of the fitting parameter $NW_{CS}$.

The fitting results are shown in Fig. 8, and all the fitting parameters are listed in Table 1. In addition, the magnetization contributed from each part of the spins in the 51\% and 40\% hematocrit erythrocyte suspensions are shown in Figs. 10 and 11, respectively. From Figs. 10 and 11, it is obvious that the slow decaying is attributed to both $M_{C \rightarrow C}$.
TABLE 1 Results of fitting the experimental PGSE attenuation curves of pure erythrocyte suspensions and the erythrocyte suspension with p-CMBS added

<table>
<thead>
<tr>
<th>Sample</th>
<th>θ</th>
<th>$a_m*$ (µm)</th>
<th>$b$ (µm)</th>
<th>$d$ (µm)</th>
<th>$MW_{ss}$ (s$^{-1}$)</th>
<th>$NW_{cs}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure erythrocyte suspension (Ht = 51%)</td>
<td>90°</td>
<td>3.05</td>
<td>4.12</td>
<td>8.24</td>
<td>110</td>
<td>90</td>
</tr>
<tr>
<td>Pure erythrocyte suspension (Ht = 40%)</td>
<td>90°</td>
<td>3.31</td>
<td>4.34</td>
<td>8.68</td>
<td>110</td>
<td>110</td>
</tr>
<tr>
<td>Erythrocyte suspension with p-CMBS (Ht = 48%)</td>
<td>0°</td>
<td>3.09</td>
<td>1.54</td>
<td>3.05</td>
<td>140</td>
<td>35</td>
</tr>
</tbody>
</table>

*a_m is the mean radius of an external pore, and the radius of an external pore is proportional to the radius of an erythrocyte in the simulation program.

and $M_{ss\rightarrow ss}$, i.e., the signal intensity from the water inside the erythrocytes and the external pores. That is, the diffraction-like pattern at low $q (\approx 10^3$ m$^{-1}$), which is indicative of the mean distance between two pores $S$ as proposed by Kuchel et al. (1997), is actually caused by the combination of the restricted diffusion between multi-external pores and the restricted diffusion within the erythrocytes. In addition, the lifetime of the water in the erythrocyte $\tau_C$ is equal to $1/NW_{cs}$ according to the definition in Eq. 8a (or 8b), thus $\tau_C$ ranges from 9 to 11 ms, which is close to the mean value of 10 ms obtained from the original two-phase exchange model without considering the restriction effect (Andrasko, 1976) and also close to that obtained from the modified two-phase model when including the restricted effect (Stanisz et al., 1998). In other words, the same exchange rate can be obtained by one of the three models, but the diffraction-like peak caused by the restricted diffusion can only be interpreted by the modified two-phase model and ours. However, our model can be used to investigate the restricted diffusion between external pores connecting to each other, which is especially useful when the cells in a cell suspension are concentrated enough to generate the pore-like external space. The sufficient cell concentration for a cell suspension is necessary if one wants to measure the weak diffraction-like peak and obtain directly the size of the cell from the position of the diffraction-like peak.

APPENDIX

One may solve $C(q, \Delta)$ and $S(q, \Delta)$ with the help of the Fourier–Laplace transform of Eqs. 8a and 8b. We obtain

$$sC[q, s] - C[q, t = 0] = W_{ss} \sum_{i=1}^{N} \exp[i2\pi q \cdot R_{ss}]S[q, s] - NW_{cs}C[q, s],$$

(A1a)

$$sS[q, s] - S[q, t = 0] = W_{ss} \sum_{i=1}^{M} \exp[i2\pi q \cdot R_{ss}]S[q, s]$$

$$+ W_{cs} \sum_{i=1}^{N} \exp[i2\pi q \cdot R_{cs}]C[q, s]$$

$$- (NW_{ss} + MW_{ss})S[q, s],$$

(A1b)

![FIGURE 10](image1.png)  
**FIGURE 10** The magnetization contributed from each part of the spins in the 51% hematocrit erythrocyte suspension, and the experimental PGSE attenuation $E(q, \Delta)$ versus $q$ plot (solid circles) and the simulated results. (a) $E(q, \Delta)$, (b) $M_{ss\rightarrow ss}$, (c) $M_{ss\rightarrow ss}$, (d) $M_{ss\rightarrow ss}$ and $M_{ss\rightarrow ss}$.

![FIGURE 11](image2.png)  
**FIGURE 11** The magnetization contributed from each part of the spins in the 40% hematocrit erythrocyte suspension, and the experimental PGSE attenuation $E(q, \Delta)$ versus $q$ plot (solid circles) and the simulated results. (a) $E(q, \Delta)$, (b) $M_{ss\rightarrow ss}$, (c) $M_{ss\rightarrow ss}$, (d) $M_{ss\rightarrow ss}$ and $M_{ss\rightarrow ss}$. 

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where $\mathbf{R}_{n}(X, Y = S, C)$ is the position vector at the center of the $n$th neighboring pore $X$ relative to that of the central pore $Y$. The arrangement of pores $C$ and pores $S$ must be defined for the calculations of the three configuration integrals in Eqs. A1a and A1b. Considering the nature of a cell suspension system, the stacking of pores $C$ and pores $S$ is presumably random. If the distance between a pair of neighboring pore $C$ and $S$ is $d$ (see Fig. 1), the mean configuration integrals in Eqs. A1a and A1b for the randomly packed pores are

$$\sum_{i=1}^{N} \exp(i2\pi \mathbf{q} \cdot \mathbf{R}_{CSi}) = N \cdot \text{sinc}(2\pi q_d)$$

and

$$\sum_{i=1}^{M} \exp(i2\pi \mathbf{q} \cdot \mathbf{R}_{SSi}) = M \cdot \text{sinc}(2\pi q_d)$$

(Callaghan et al., 1991). If the spin initially exists in a given pore $A$ with its center at origin (case A), the initial condition is

$$C(\mathbf{q}, t = 0) = 1 \cdot \exp(i2\pi \mathbf{q} \cdot 0) = 1,$$

$$S(\mathbf{q}, t = 0) = \sum_{n} 0 \cdot \exp(i2\pi \mathbf{q} \cdot \mathbf{R}_{nS}) = 0$$

according to Eq. 4, where $\mathbf{R}_{nS}$ is the center of the $n$th pore $S$. Similarly, if a spin exists at a pore $S$ at the initial time $t = 0$ (case B), the initial condition yields $C(\mathbf{q}, t = 0) = 0$ and $S(\mathbf{q}, t = 0) = 1$. Because the present system is isotropic, $C(\mathbf{q}, t)$ and $S(\mathbf{q}, t)$ can be replaced by $C(q, t)$ and $S(q, t)$. Eqs. A1a and A1b then reduce to

$$C(q, t = 0) = \begin{cases} 1 & \text{for case A} \\ 0 & \text{for case B} \end{cases}$$

(A2a)

$$S(q, t = 0) = \begin{cases} 0 & \text{for case A} \\ 1 & \text{for case B} \end{cases}$$

(A2b)

where

$$\alpha = NW_{SC} + MW_{SS} [1 - \text{sinc}(2\pi q_d)].$$

(A3)

The solutions of the coupled Eqs. A2a and A2b are given by

$$C_{i}[q, s] = x_{CC} \frac{(s - \lambda_1)}{(s - \lambda_2)} + y_{CC} \frac{(s - \lambda_2)}{(s - \lambda_1)},$$

$$x_{CC} = \frac{\lambda_1 + \alpha}{\lambda_1 - \lambda_2}, \quad y_{CC} = \frac{\lambda_2 + \alpha}{\lambda_2 - \lambda_1},$$

$$S_{i}[q, s] = x_{SC} \frac{(s - \lambda_1)}{(s - \lambda_2)} + y_{SC} \frac{(s - \lambda_2)}{(s - \lambda_1)},$$

$$x_{SC} = \frac{NW_{CS} \cdot \text{sinc}(2\pi q_b)}{\lambda_1 - \lambda_2}, \quad y_{SC} = \frac{NW_{CS} \cdot \text{sinc}(2\pi q_b)}{\lambda_2 - \lambda_1},$$

$$x_{SS} = \frac{\lambda_1 + NW_{CS}}{\lambda_1 - \lambda_2}, \quad y_{SS} = \frac{\lambda_2 + NW_{CS}}{\lambda_2 - \lambda_1},$$

$$\lambda_{1,2} = \frac{-(NW_{CS} + \alpha) \pm \sqrt{(NW_{CS} - \alpha)^2 + 4NW_{CS}W_{CS}W_{SC} \text{sinc}^2(2\pi q_b)}}{2}.$$  \hspace{1cm} \text{(A4c)}

$$C_{i}(q, \Delta), S_{i}(q, \Delta), C_{i}(q, \Delta), \text{and } S_{i}(q, \Delta) \text{ can be obtained by the inverse Laplace transform of } C_{i}[q, s], S_{i}[q, s], C_{i}[q, s], \text{and } S_{i}[q, s]; \text{then the four parts of the magnetizations, } M_{C \rightarrow S}, M_{C \rightarrow S}, M_{S \rightarrow C}, \text{and } M_{S \rightarrow C} \text{ may be calculated in accordance with Eq. 6. By summing the four parts, we obtain the PGSE attenuation } E(q, \Delta) \text{ as given in Eq. 12.}$$

**REFERENCES**


