**MONTE CARLO STUDIES
OF THE MAGNETIC RESONANCE DIFFUSION DECAY**

by

Elena Olariu, B.Sc.

A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfillment of
the requirements for the degree of
Master of Science

Department of Physics

Carleton University
Ottawa, Ontario, Canada

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Chair, Department of Physics

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Date May 6, 2003

Abstract

The brain tissue is considered to be a heterogeneous system containing micro-scale compartments separated by semi-permeable membranes. Due to this structure, the signal decay computed from NMR diffusion experiments is complex and difficult to interpret.

In this study, diffusion decays were computed using analytical and numerical methods for several different cell geometries in order to investigate how reliably the diffusion decay can be analyzed as a sum of exponential decays. In practice it is often assumed that the diffusion decay consists of a contribution from intracellular and extracellular compartments which both decay exponentially even though there is no theoretical justification for such an assumption. It was found that the restricted diffusion in each compartment produces non-exponential signal decay when the b -value range was expanded well beyond the usual range employed in Diffusion-Weighted Imaging applications. Furthermore, the apparent diffusion coefficient D_a values of the extracellular compartment are consistent with measurements in porous medium revealing structural information and diffusion dynamics (e.g. the transition from free to restricted diffusion).

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List of Symbols

μ	magnetic moment
γ	gyromagnetic ratio
B_0	static magnetic field
ω_0	$\omega_0 = \gamma B_0$, resonant frequency of the spin
M	magnetization of spin system
J	nuclear angular momentum
B_1	rf pulse applied to spin system
θ	flip angle
m_z	magnetic quantum number
$E_{+\frac{1}{2}}$	$E_{+\frac{1}{2}} = -\gamma \frac{\hbar}{2} B_0$, lower state energy
$E_{-\frac{1}{2}}$	$E_{-\frac{1}{2}} = \gamma \frac{\hbar}{2} B_0$, upper state energy
N_s	total number of spins in the system
T_1	longitudinal relaxation time
T_2	transverse relaxation time
M_{xy}	transverse magnetization component

M_z	longitudinal magnetization component
r_0	the initial position of molecule
$r(t)$	the position of molecule at time t
$n(r, t)$	number density of particles with a given position r at time t
$\langle (r - r_0)^2 \rangle$	mean-squared displacement
D_0	diffusion coefficient
D_a	$D_a = \frac{\langle (r - r_0)^2 \rangle}{2dt_d}$, apparent diffusion coefficient
t_d	diffusion time
d	dimensionality of the system
G	diffusion gradient amplitude
δ	diffusion gradient duration
Δ	separation time between the gradient pulses
$P(z_2 z_1, \Delta)$	probability for a spin starting at z_1 and to move to z_2 during a period Δ .
$\rho(z_1)$	the initial density of the spins
R	radius of the circular cells
R_m	the parameter of the hexagonal lattice
P	permeability of the cell membrane
a	the length of the side of a square cell
t_r	$t_r = \frac{D_0}{a^2} t_d$ and $t_r = \frac{D_0}{\pi R^2} t_d$, the reduced time for square cell and circular cells, respectively.
q	$q = \gamma \delta G / 2\pi$, wave vector representing the reciprocal q -space
b	$b = 4\pi^2 q^2 t_d$, b -value
φ	residual phase-shift of spins
N	number of trajectories followed in MC simulations
S/S_0	attenuation of the echo-signal due to diffusion

Chapter 1

Introduction

Physicists F. Bloch [1] and E. Purcell [2] independently discovered the phenomenon of nuclear magnetic resonance, NMR, in 1946. Rapid progress in NMR was made in the following years during which theories were developed to explain the observed phenomena of chemical shifts, diffusion effects, spin-spin coupling and spin relaxation [3, 4]. However, with the development of high resolution NMR spectroscopy, NMR became an important non-destructive probe of chemical structure and diffusion in liquids. A major improvement to enhance the usefulness of the spin-echo measurement came in 1965, when Stejskal and Tanner [5] proposed the pulse gradients spin echo method, PGSE. Since then, PGSE has been the most widely used technique for non-invasively studying nuclear spin diffusion and an increasingly important tool, not only to study molecular diffusion in liquids, but also for providing structural information when diffusion is restricted to the NMR time scale [6].

The first published NMR imaging experiment was accomplished by Lauterbur [7] in 1973, showing that by changing the homogeneity of the magnetic field with the addition of a linear field gradient, the resulting shift in the resonant frequency could be used to obtain spatial information. This technique has come to be known as magnetic

resonance imaging, MRI [7]. This was followed by the start of clinical trials [8] as a non-invasive tool to evaluate tissue anatomy, pathology, metabolism and other biological processes [9, 10].

There is considerable interest in using the diffusion characteristics of water in tissue for the characterization of tissue pathology [11]. Le Bihan et al. [12] were the first to report diffusion measurements in the human brain in vivo in 1985. A couple of years later, researchers were able to generate diffusion maps of the human brain. Since then, a rapidly increasing evolution of NMR technology and an increased focus on its clinical applications to brain have led to the development of tools to follow at least some aspects of the evolution of multiple sclerosis [13, 14] lesions and to detect early stroke [15, 16]. For example, Diffusion Weighted MRI, DW-MRI, provides a unique form of NMR contrast that enables molecular motion to be quantitatively measured. Since diffusion is inhibited by the internal boundaries of the tissue where the molecular motion occurs, the measured diffusion coefficient for water in tissue is smaller than it is in bulk water and the apparent diffusion coefficient, D_a , has to be introduced as:

$$D_a = \frac{\langle r^2 \rangle}{2dt_d} \quad (1.1)$$

where $\langle r^2 \rangle$ represents the mean-squared displacement, d gives the dimensionality of the system and t_d is the time allowed for diffusion.

D_a values differ from the free diffusion coefficient, D_0 , because of its diffusion time dependence. When t_d is short such that the molecules cannot diffuse far enough to feel the presence of the internal tissue boundaries, the observed D_a will be the same as that measured for free diffusion. As t_d advances, a certain fraction of molecules will be affected by the boundaries lowering their mean-squared displacement such that $\langle r^2 \rangle$ will not scale with t_d . In the long time limit, all molecules will have interacted with the tissue borders, and thus the measured displacement profiles and D_a become time independent.

Typical diffusion measurements use the Stejskal-Tanner [17] PGSE technique to provide information about the average diffusion and displacement profile of particles in the tissue. The structural information can be derived when the diffusion displacements are related by means of a model to the physical and geometrical properties of the tissue, e.g. permeability of membranes, diffusion coefficients and architecture of the tissue borders. Pathological processes, which are able to alter the tissue integrity [18] or change the membrane permeability, in turn cause abnormal water diffusion. Due to an oriented cellular microstructure [19] the molecular motion in the brain is a three-dimensional process and the molecular displacement is not the same in all directions. In addition, other transport phenomenon [20] and blood perfusion [21] may enhance the diffusional motion reflected by an increase in measured D_a values. All these factors motivate the difficulty in properly understanding the mechanisms at the molecular level responsible for NMR diffusion contrast. Knowledge of the diffusion coefficients is fundamental to the understanding of the tissue at the molecular level. However, D_a values are not readily interpreted unless the measuring conditions are specified in detail. In general D_a [22] is smaller than the unrestricted value of D_0 and decreases monotonically from a value close to D_0 to a smaller limiting value as t_d tends to infinity.

A variety of analytical [23, 24, 25, 26] and numerical studies [27, 28, 29, 30, 31] have been designed to follow the behaviour of water molecules in brain tissue to understand its structure. Most of these studies report multi-component diffusion behaviour. The observed diffusion attenuation has been thought to result from a superposition of the signals from multiple compartments [32, 33]. The brain tissue is considered a heterogeneous system containing micro-scale compartments separated by semi-permeable membranes. This structure makes it difficult to distinguish between the water signals coming from these compartments using NMR. Also, the signal decay computed from the diffusion experiments is complex and difficult to interpret. The free and restricted diffusion components are still hard to resolve. The fast and slow components have often been related with the fraction of spins that diffuse freely and spins that interact with the boundaries respectively. The observed multi-component diffusion

behaviour caused by restricted motion of water and limited by cellular compartments becomes exponential in the long time limit [34].

The complexity of tissue parameters and geometry appears to be the major reason responsible for the multi-exponential diffusion decay. An adequate mathematical model to study such a complex process has to include at least two compartments [35, 36], with size, self-diffusion coefficients and decay rate included as parameters. In addition, the exchange rate and effects of water restriction have very important effects on the diffusion and must be incorporated into a realistic model.

To clarify the contribution of each component to the multi-exponential decay curve, it is essential to check if indeed the restricted diffusion in each compartment produces a mono-exponential decay curve even when diffusion-weighting exceeds the level normally employed in DWI applications (e.g. b -value about 10000 s/mm^2). The availability of new powerful gradient hardware on clinical MR imagers offers the opportunity to study brain water diffusion at much larger b -values than were previously available.

The goal of this thesis was to investigate how reliable the diffusion decay can be analyzed as a sum of exponential decays. It is known in the literature [13, 22, 30, 31] that the diffusion decay in human brain consists of a contribution from intracellular and extracellular compartments and both are considered to decay exponentially. There is no theoretical justification for this assumption but in practice it seems to work reasonably well. The validity of this approach along with any constraints that are appropriate for this problem was analyzed. Elucidation of the correct mechanism has proven to be difficult because intracellular and extracellular water MR signals are difficult to resolve in brain. Toward this goal, a numerical simulation approach was used that sheds some light on the cause of the non-exponential diffusion decay behaviour observed. In this study, the b -value range was expanded well beyond the usual range employed in most of the diffusion-weighted imaging experiments that have been reported to date.

Chapter 2

Theory

2.1 Principle of Nuclear Magnetic Resonance

2.1.1 Classical Description

The physical model of Nuclear Magnetic Resonance, NMR, starts with the property of certain nuclei called “spin”. Nuclei having spin, those with an odd number of protons and/or neutrons, include several that are biologically abundant, such as ^1H , ^{13}C , ^{31}P , ^{23}Na . Modern radiological procedures are primarily concerned with the ^1H nucleus, which is simply an individual proton, because of its large NMR signal and high natural abundance in biological systems.

The charge of a proton can be considered to be distributed and rotating about a central axis as a result of a non-zero angular momentum. The spinning motion of the charge induces a local magnetic field, making each nucleus behave like a tiny magnet characterized by the magnetic moment, μ . The magnetic field of the proton is parallel to

the angular momentum and normal to the plane of charge circulation. In this case, μ is given by:

$$\mu = \gamma J, \quad (2.1)$$

where γ is the gyromagnetic ratio. For a proton, $\gamma \cong \frac{q}{2m}$, where m and q are the proton mass and charge.

An external magnetic field B_0 will exert a torque on a magnetic moment, causing the angular momentum to change at a rate:

$$\tau = \frac{dJ}{dt} = \mu \times B_0, \quad (2.2)$$

or using Eq. (2.1) :

$$\left| \frac{dJ}{dt} \right| = \gamma B_0 \sin \theta. \quad (2.3)$$

Thus, during an infinitesimal time dt , the angular momentum will change by an amount dJ corresponding to a rotation through an angle $d\phi$ in the plane perpendicular to B_0 . As shown in Fig.2.1, this angle is given by:

$$d\phi = \frac{dJ}{J \sin \theta} = \frac{\gamma B_0 \sin \theta dt}{J \sin \theta} = \gamma B_0 dt. \quad (2.4)$$

The frequency of precession of the spin therefore is given by:

$$\omega_0 = \frac{d\phi}{dt} = \gamma B_0. \quad (2.5)$$

Since each nucleus is spinning, the torque on it produced by the magnetic field prevents it from actually aligning with the applied magnetic field. Instead it precesses around the magnetic field. The behaviour is analogous to a gyroscope in a gravitational field. Eq. (2.5) states that the precessional frequency, which is known as the Larmor frequency, is directly proportional to the magnetic field strength. This is the fundamental concept in MRI and is one from which almost everything else can be derived.

In a sample comprised of many nuclei, the net magnetic moment M can be defined to be the vector sum of all of the nuclear magnetic moments. Classical theory is inadequate to describe the behaviour of a single spin because of the quantum nature of

nuclear properties. However, the behaviour of M obeys the classical laws of physics. In a magnetic field B_0 , the magnetization M at equilibrium will be aligned with B_0 . In order to measure M , it must be tilted away from B_0 to produce a component in the xy plane. This is actually the basis of NMR measurements.

Figure 2.1: Precession of the magnetic moment μ with angular velocity ω_0 about the static magnetic field B_0 .

The motion of M under the influence of a magnetic field B_0 can be described by:

$$\frac{dM}{dt} = \gamma M \times B_0. \quad (2.6)$$

Considering a reference frame in Fig. 2.2 rotating with the angular velocity ω in the xy plane, the behaviour of M in the laboratory frame can be related to its behaviour in the rotating frame by:

$$\frac{dM}{dt} = \frac{\partial M}{\partial t} + \omega \times M, \quad (2.7)$$

where $\frac{\partial \mathbf{M}}{\partial t}$ represents the rate of change of $\mathbf{M}$ in the rotating frame [37].

Using Eq. (2.6):

$$\begin{aligned}\frac{\partial \mathbf{M}}{\partial t} &= \gamma \mathbf{M} \times \mathbf{B}_0 + \mathbf{M} \times \boldsymbol{\omega} = \gamma \mathbf{M} \times \left(\mathbf{B}_0 + \frac{\boldsymbol{\omega}}{\gamma} \right) \\ &= \gamma \mathbf{M} \times \mathbf{B}_{eff},\end{aligned}\quad (2.8)$$

where $\mathbf{B}_{eff}$ is the sum of the static field $\mathbf{B}_0$, and a fictitious magnetic field whose direction is the same as $\boldsymbol{\omega}$. The magnetic resonance phenomenon appears when a second magnetic field $\mathbf{B}_1$ of frequency $\omega = -\omega_0 = -\gamma B_0$ is applied perpendicular to $\mathbf{B}_0$.

Figure 2.2: The motion of $\mathbf{M}$ in the laboratory frame showing the components along the z axis and in the xy plane

For magnetic field strengths used in MRI, the resonance frequencies are in the radio frequency range. In the rotating frame, the effective field is:

$$\mathbf{B}_{eff} = \mathbf{B}_0 + \frac{\boldsymbol{\omega}}{\gamma} + \mathbf{B}_1, \quad (2.9)$$

which reduces to $B_{eff} = B_1$, when the resonance condition applies ($\omega = -\omega_0$). The corresponding equation of motion for the magnetization vector $\mathbf{M}$ in the rotating frame is:

$$\frac{\partial \mathbf{M}}{\partial t} = \gamma \mathbf{M} \times \mathbf{B}_1. \quad (2.10)$$

The effect of $\mathbf{B}_1$ is to rotate the magnetization vector $\mathbf{M}$ about its direction, with an angular frequency $\omega_1 = \gamma B_1$. If $\mathbf{B}_1$ is applied on-resonance for a finite time t , the magnetization will rotate through an angle:

$$\theta = \omega_1 t = \gamma B_1 t. \quad (2.11)$$

By varying the amplitude and duration of this radio frequency pulse, any desired angle can be produced.

2.1.2 Quantum Mechanical Description

The NMR signal comes from nuclei that have an intrinsic angular momentum $\mathbf{J}$. The magnitude of the nuclear angular momentum is given by:

$$J = \hbar [I(I+1)]^{1/2}, \quad (2.12)$$

where I is the nuclear spin quantum number and $\hbar = \frac{h}{2\pi}$, with h known as Planck's constant. From the uncertainty principle, the direction of $\mathbf{J}$ cannot be determined precisely. By applying an external magnetic field $\mathbf{B}_0 = B_0 \mathbf{k}$, arbitrarily chosen to be aligned along the z direction, an axis of quantization can be defined.

The component of $\mathbf{J}$ along the z -axis is:

$$J_z = m_z \hbar. \quad (2.13)$$

m_z is called the magnetic quantum number and can take the values $\pm I, (\pm I-1), \dots, 0$ giving $(2I+1)$ possible values [38], each corresponding to a possible orientation for $\mathbf{J}$. For the proton, $I = \frac{1}{2}$ and therefore m_z can only take the values $\pm \frac{1}{2}$. This gives two possible

orientations for the spin, which are referred to as spin up ($m_z = \frac{1}{2}$) and spin down ($m_z = -\frac{1}{2}$).

The potential energy of these states is:

$$E = -\mu B_0 = -\gamma \hbar J_z B_0. \quad (2.14)$$

As shown in Fig. 2.3, the energy levels for a proton are:

$$E_{+\frac{1}{2}} = -\gamma \frac{\hbar}{2} B_0 \text{ called the lower state energy,} \quad (2.15)$$

and

$$E_{-\frac{1}{2}} = \gamma \frac{\hbar}{2} B_0 \text{ called the upper state energy.} \quad (2.16)$$

Figure 2.3: a) The available orientations for J and the corresponding values of m_z , b) Zeeman effect associated with the transition from the lower state to the upper state as a result of absorption of a quantum of energy.

This is an example of the Zeeman effect, where the nuclear magnetic moment of the spin causes the spin states to split into discrete energy levels in the presence of a magnetic field. The energy separation, ΔE , between the two quantum states, gives the magnitude of the energy absorbed or released by the proton spin system following a transition between the lower and higher states. From Eq. (2.15) and Eq. (2.16) it can be seen that:

$$\Delta E = \gamma \hbar B_0 = \hbar \omega_0, \quad (2.17)$$

where ω_0 is the Larmor frequency which was defined in Eq. (2.5) to be $\omega_0 = \gamma B_0$. It takes a specific amount of energy to flip spins between the lower state and upper state.

2.1.3 Statistical Distribution of Spin States

For a large sample containing N_s spins placed in a magnetic field B_0 , quantum mechanics dictates that the magnetic moment of each individual spin must be aligned in the direction of the applied field either with spin up ($m_I = +\frac{1}{2}$), or spin down ($m_I = -\frac{1}{2}$). The population ratio between the two levels, in equilibrium is described by the Boltzmann [39] distribution:

$$\frac{n_{\uparrow}}{n_{\downarrow}} = \exp\left(\frac{\Delta E}{kT}\right) = \exp\left(\frac{\gamma \hbar B_0}{kT}\right), \quad (2.18)$$

where k is Boltzmann's constant, T is the temperature of the spin system, $n_{\downarrow}$ is the number of spins in the spin down state (i.e. higher energy) and $n_{\uparrow}$ is the number of spins in the spin up state. At room temperature, in a field of 1.5T, there are only 10 more protons per million in the lower energy state than in the higher energy state. The difference in population is given by:

$$\begin{aligned}
n_{\uparrow} - n_{\downarrow} &= N_s \frac{1 - \exp\left(\frac{-\Delta E}{kT}\right)}{1 + \exp\left(\frac{-\Delta E}{kT}\right)} \\
&= \frac{N_s}{2} \frac{\Delta E}{kT} = \frac{N_s}{2} \frac{\gamma \hbar B_0}{kT}
\end{aligned} \tag{2.19}$$

where $N_s = n_{\uparrow} + n_{\downarrow}$.

The net equilibrium magnetization, considering the difference of population between these two levels, can be written as:

$$M_0 = (n_{\uparrow} - n_{\downarrow}) \gamma \hbar \frac{1}{2} = \frac{N_s \gamma^2 \hbar^2}{4kT} B_0. \tag{2.20}$$

The intensity of the NMR signal depends on the difference in population between the spin states, which is determined by the magnetic field strength B_0 and the temperature T . The energy difference between the states varies linearly with the applied magnetic field. All the signals generated in MRI are based on the small population difference between the two states, explaining why MR techniques are limited by signal strength.

2.2 Bloch's Equation and Relaxation

When RF energy is no longer being supplied to the nuclei, they begin to give-off the absorbed energy and revert back to their equilibrium state. The molecular environment is reflected in the time-variation of the signal amplitude. The release of energy occurs in two ways. Energy is given off by the spin system to the other energy reservoirs in the material being studied and normally manifests itself as heat. This process is known as spin-lattice or longitudinal relaxation and can be characterised by the exponential time constant, T_1 . The other energy transfer process involve the exchange of energy among the nuclear spins, such that the total energy of the spin system remains unchanged. This process is referred to as spin-spin or transverse relaxation, and in fluids, can be characterized by the exponential time constant, T_2 . The relaxation process by

which the thermal equilibrium is re-established following the application of a RF pulse can be modelled phenomenologically by Bloch's equations.

When the "motion" due to relaxation is superimposed on the precessional motion of the free spins under the influence of a static field and a much smaller RF field, the rate of change of the magnetization is given by [40]:

$$\frac{d\mathbf{M}}{dt} = \gamma \mathbf{M} \times \mathbf{B} - \left(\frac{M_x}{T_2} \mathbf{i} + \frac{M_y}{T_2} \mathbf{j} \right) - \frac{(M_0 - M_z)}{T_1} \mathbf{k}, \quad (2.21)$$

where $\mathbf{i}, \mathbf{j}, \mathbf{k}$ are unit vectors in the laboratory frame. Eq (2.21) is known as Bloch's equation. From Bloch's equation, it can be seen that the longitudinal magnetization displays an exponential form of evolution to its equilibrium value M_0 given by

$$M_z = M_0(1 - e^{-t/T_1}), \quad (2.22)$$

and for the precessing transverse component in the rotating frame:

$$M_{xy} = M_0 e^{-i\gamma B_1 t - t/T_2}. \quad (2.23)$$

The observed signal in a MR experiment comes from the detection of the emf generated by the precessing transverse magnetization. Once a component of the magnetization is tipped into the xy plane, a free induction decay, FID, signal is generated if a suitable coil is used. This characteristic signal, plotted in Fig. 2.4, has a frequency of oscillation equal to the Larmor frequency. In an ideal system with a perfectly uniform magnetic field, the exponential envelope of the FID signal in the xy plane decays according to Eq. (2.23). In reality, field inhomogeneities cause the signal to decay more quickly, masking the true relaxation characteristics of the tissue. This observed relaxation in tissue is called T_2^* . The decay of transverse magnetization occurs because of dephasing of the spins as they precess. Several different effects can cause this dephasing. When it is a result of different spins experiencing different magnetic fields due to inhomogeneities in the applied field then the dephasing can be reversed. This can be done by using a 180° RF pulse. This in turn causes the transverse magnetization to reappear and form an echo event, which produces a detectable RF signal. The intensity of the echo signal is proportional to the level of transverse magnetization, which is determined by the relaxation rate T_2 .

Figure 2.4: The resultant FID signal that can be measured from the xy component of the sample magnetization.

2.3 Spatial Encoding of the NMR Signal

During the MR image formation process, the volume that is analyzed is “subdivided” to form a matrix of individual tissue voxels. The RF signal coming from each individual voxel must be distinguished from all of the others and its intensity displayed in the corresponding image field. During the acquisition phase, RF signals are emitted by the tissue and received by the coils. The result of the image acquisition process is a large amount of data collected by using magnetic field gradients that give in turn to the RF signal their frequency and phase characteristics. The signal from all voxels are produced simultaneously and are emitted mixed together to form a single, composite signal. The reconstruction process converts the collected data into an actual image. The

signals are sorted out using Fourier transform methods and delivered to the correct pixel address.

2.4 Diffusion Theory

This section describes approaches for dealing with translational motion in molecular ensembles in a fluid state. In general, the random thermal motion of molecules can be characterized by a time-dependent displacement $r(t)$ due to Brownian motion. In order to adopt the language of statistics, this function is considered to vary in a random way from molecule to molecule.

In equilibrium, the number density of particles with a given position r at time t denoted $n(r, t)$ is independent of position. In the case of an initially non-uniform molecular distribution, the system will tend to increase its entropy by a net motion of the molecules to make the concentration $n(r, t)$ more uniform.

The classical description of diffusion is via Fick's law where particle flux $J(r, t)$ is considered to be proportional to the particle concentration gradient [41].

$$J(r, t) = -D \nabla n(r, t), \quad (2.24)$$

where D is defined to be the diffusion coefficient. Because there is no mass motion of the system as a whole, the continuity theorem applies and [42]:

$$\nabla J(r, t) + \frac{\partial n(r, t)}{\partial t} = 0. \quad (2.25)$$

Combining Eq. (2.24) and Eq. (2.25) we obtain the diffusion equation known as Fick's second law:

$$\nabla^2 n(r, t) - \frac{1}{D} \frac{\partial n(r, t)}{\partial t} = 0. \quad (2.26)$$

For a given distribution of N molecules the initial condition

$$n(r, 0) = \delta(r - r_0) \quad (2.27)$$

applies.

The solution to Eq. (2.26), with the appropriate boundary condition $n(r, t) \rightarrow 0$ as $r \rightarrow \infty$, satisfying the initial condition of Eq. (2.27), follows a Gaussian distribution:

$$n(r, t) = \frac{N_s}{(4\pi Dt)^{3/2}} \exp\left[-\frac{(r-r_0)^2}{4Dt}\right]. \quad (2.28)$$

In fact, $n(r, t)$ depends only on the net displacement $(r-r_0)$. This displacement causes an attenuation of the measured NMR signal, which as a result, can be used to evaluate the mean displacement of the spins in the material. Using the conservation theory of a fluid:

$$\int_0^\infty n(r, t) 4\pi r^2 dr = N_s. \quad (2.29)$$

The average squared displacement, variance, which gives the measure of how the distribution is spread about the mean value r_0 , can be determined based on the probability of moving from position r to r_0 during time t :

$$\langle (r-r_0)^2 \rangle = \frac{4\pi}{N_s} \int_0^\infty n(r, t) (r-r_0)^2 dr = 2dDt, \quad (2.30)$$

where d represents the dimensionality of the system. Eq. (2.30) is known as Einstein's equation.

However, for in vivo measurements, the diffusion coefficient is no longer a constant with time, because the translational motion of molecules is influenced or restricted by different biological structures. Also the diffusional motion may be enhanced by other transport phenomena such as blood motion or cerebrospinal fluid, CSF, flow [21]. Consequently, the parameter used to characterize the mean squared displacement in tissue is normally referred to as apparent diffusion coefficient, D_a [43]. To measure D_a experimentally a fraction of molecules must be labelled and their movement along the direction of the diffusion-sensitizing gradient followed for a period of time t_d . By analogy with Eq. (2.30) D_a is defined as:

$$D_a = \frac{\langle (r-r_0)^2 \rangle}{2dt_d}. \quad (2.31)$$

Several techniques exist for the experimental determination of D_a in liquids and gases using radioactive and fluorescent tracers, but these cannot be used for in vivo studies in humans. NMR is well suited to study the diffusion of water molecules in

human tissues in vivo. In principle, it is possible to give a spatial label to nuclei at one time and then to monitor their movement using magnetic field gradients. What is then measured, in effect, is the absolute phase of the spins, which is related to their displacement.

The pulsed-gradient-spin-echo sequence, PGSE, is the most commonly used MR sequence for measuring diffusion [6] and is appropriate not only for studying molecular diffusion but can also provide structural information when diffusion is restricted on the NMR time-scale.

The PGSE experiment consists, in its simplest form, of two magnetic fields-gradient pulses of magnitude G , duration δ and separation Δ , inserted into an ordinary NMR spin-echo experiment on either side of the 180° pulse. This sequence is represented schematically in Fig. 2.5. The molecules are "phase" tagged by the effect of the first gradient pulse and their displacement is determined by the second pulse.

The effect of the first gradient is to introduce a phase-shift, φ_1 , as a function of the position of the spin, z_1 :

$$\varphi_1 = \int_0^\delta \omega dt = \gamma \int_0^\delta Gz dt = \gamma G \delta z_1. \quad (2.32)$$

It is assumed that the gradient pulse duration is short enough that any motion of the spins during the gradient pulse can be ignored. This phase-shift φ_1 is subsequently inverted by the 180° pulse applied, so that φ_1 becomes $-\varphi_1$.

The second gradient is applied to rephase the spins, introducing an additional phase shift, φ_2 proportional with the position of the spin, z_2 along z -axis:

$$\varphi_2 = \gamma \int_{\Delta}^{\delta+\Delta} Gz dt = \gamma G \delta z_2. \quad (2.33)$$

The net dephasing is given by:

$$\varphi = \varphi_2 - \varphi_1 = \gamma G \delta (z_2 - z_1). \quad (2.34)$$

Figure 2.5: The diffusion sequence (PGSE) with the basic components. After the 90° pulse, the first diffusion gradient tags the spin by dephasing. The application of the same diffusion gradient after the 180° pulse partially rephases the spins. Depending on the net displacement during δ , the incomplete rephasing will cause attenuation of the signal.

If the spins are stationary, a perfectly refocused echo will occur (i.e. $\phi = 0$). But any motion of the spins in the direction of the magnetic field gradient will cause phase shifts in their contribution to the echo.

The total signal is a superposition of transverse magnetizations, in which each phase term is weighted by the probability $P(z_2 | z_1, \Delta)$ for a spin to begin at z_1 and to move to z_2 during a period Δ . Then:

$$M = M_0 \sum_j \exp(i\phi_j), \quad (2.35)$$

where M_0 stands for the total magnetization at the beginning of the experiment at time $t=0$. For spins diffusing within a free homogeneous system, the attenuation of the transverse magnetization is given by [44]:

$$M/M_0 = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \exp[i\gamma G \delta(z_1 - z_2)] \rho(z_1) P(z_2 | z_1, \Delta) dz_1 dz_2, \quad (2.36)$$

where $\rho(z_1)$ represents the initial density of the spins. But since $\rho(z_1)$ is constant, Eq. (2.36) reduces to:

$$M/M_0 = \int_{-\infty}^{\infty} \exp[i\gamma G \delta(z_1 - z_2)] P(z_2 | z_1, \Delta) dz_2. \quad (2.37)$$

For free diffusion in a fluid, $P(z_2 | z_1, \Delta)$ can be equated with $\frac{n(r, t)}{N_s}$. This gives according to Eq. (2.28), the following:

$$P(z_2 | z_1, \Delta) = (4\pi Dt)^{-1/2} \exp\left[-\frac{(z_2 - z_1)^2}{4Dt}\right]. \quad (2.38)$$

Substituting this into Eq. (2.36),

$$\begin{aligned} M/M_0 &= (4\pi Dt)^{-1/2} \int_{-\infty}^{\infty} \exp[i\gamma G \delta(z_1 - z_2)] \exp\left[-\frac{(z_2 - z_1)^2}{4Dt}\right] dz_2 \\ &= (4\pi Dt)^{-1/2} \int_{-\infty}^{\infty} \cos[\gamma G \delta(z_1 - z_2)] \exp\left[-\frac{(z_2 - z_1)^2}{4Dt}\right] dz_2 \\ &= \exp[-(\gamma G \delta)^2 D \Delta]. \end{aligned} \quad (2.39)$$

Using Eq. (2.30), this equation becomes:

$$M/M_0 = \exp\left[-\frac{(\gamma G \delta)^2 \langle \Delta z^2 \rangle}{2}\right]. \quad (2.40)$$

Eq. (2.40) constitutes the key for all MR diffusion measurements. However, there are two comments to make regarding this expression. First, δ is not negligible with respect to Δ , so that diffusion during the application of the gradient pulses is not always negligible. Also, the imaging gradients may also affect the spin dephasing and rephasing but this is not considered in the derivation of Eq. (2.40).

In NMR experiments, the diffusive transport of magnetization is accounted for by modifying the Bloch equation to include a term, which describes the diffusion [45]:

$$\frac{d\mathbf{M}}{dt} = \gamma \mathbf{M} \times \mathbf{B} - \left(\frac{M_x}{T_2} \mathbf{x} + \frac{M_y}{T_2} \mathbf{y} \right) + \frac{(M_0 - M_z)}{T_1} \mathbf{z} + D \nabla \cdot (\nabla \mathbf{M}). \quad (2.41)$$

In the case of a PGSE experiment, the spins experience the main static field $\mathbf{B}_0$, assumed here to be along the z direction, superimposed with the linear gradient $\mathbf{G}$, so that

$$\mathbf{B}(\mathbf{r}, t) = \mathbf{B}_0 + \mathbf{G} \cdot \mathbf{r}, \quad (2.42)$$

where $\mathbf{r}$ represents the position vector. Also, the transverse magnetization $M_{x,y}(\mathbf{r}, t)$, can be written as:

$$M_{x,y}(\mathbf{r}, t) = M_x(\mathbf{r}, t) + iM_y(\mathbf{r}, t). \quad (2.43)$$

By combining Eq. (2.42) with Eq. (2.43) and ignoring the relaxation terms, the equation of motion in the rotating frame becomes:

$$\frac{dM_{xy}(\mathbf{r}, t)}{dt} = -i\gamma \mathbf{G} \cdot \mathbf{r} + D \nabla \cdot (\nabla M_{xy}(\mathbf{r}, t)), \quad (2.44)$$

This equation has the following solution when D is set to zero:

$$M_{xy}(\mathbf{r}, t) = A \exp(-i\gamma \mathbf{r} \cdot \int_0^t \mathbf{G}(t') dt'). \quad (2.45)$$

For $D \neq 0$, $A \rightarrow A(t)$ for spatially invariant diffusion. The exponential term gives the phase shift of a spin at position $\mathbf{r}$ checked at time t .

$$\ln A(t) = -D\gamma^2 \left[\int_0^{TE} \left(\int_0^{t'} \int_0^{t''} \mathbf{G}(t''') dt''' \right)^2 dt' \right]. \quad (2.46)$$

Finally it can be shown that:

$$\begin{aligned}
 A(t) \rightarrow M_{xy}(t) &= M_0 \exp \left[-D\gamma^2 \int_0^{TE} \left(\int_0^{t'} G(t'') dt'' \right)^2 dt' \right] \\
 &= M_0 \exp[-bD].
 \end{aligned} \tag{2.47}$$

Here the gradient factor b , which is often called the b -value, was introduced as [37]:

$$b = \gamma^2 \int_0^{TE} \left(\int_0^{t'} G(t'') dt'' \right)^2 dt'. \tag{2.48}$$

Eq. (2.45) represents the magnetization attenuation due to the diffusion of spins. The diffusion integral of the b -value can be solved numerically for various NMR sequences and diffusion gradient pulse profiles. The b -value gives a measure of the gradient strength in the diffusion sequence.

2.5 Brain Cellular Structure

The most common molecule in the brain is water, but there are also many special molecules such as proteins, RNA, DNA and simple inorganic substances such as sodium, potassium and chloride ions. However the brain's compartmentalisation of intracellular and extracellular water results in an inhomogeneous system.

Brain tissue is composed of two main types of cells, neurons and glial cells. The human brain contains more than 10^{12} neurons and there are 10 to 50 times more glial cells than neurons [9]. Glial cells come in two varieties: astrocytes and oligodendrocytes.

Astrocytes occur in white matter at the surface of the central nervous system, CNS, to provide structural and metabolic support for the nervous system [46]. They are also found in close affiliation with blood vessels to form a part of the blood brain barrier. The major function of the oligodendrocytes is the formation of myelin segments that encase the axons. Numerous axons are connected to each oligodendrocyte in this way. Myelin consists of spirally wrapped glial membranes that serve as an electrical insulator that increases the speed and the efficiency of neural transmission. The functioning of the nervous system is dependent on the precise anatomical arrangement and interconnectivity

of the nerve cells. Nerve cells have three distinct regions: the dendritic tree, the cell body and the axons.

Because of the large amount of insulating myelin in areas of the brain where axons predominate, these areas are more opaque than regions where cell bodies predominate, giving the axon-containing regions a whiter appearance [47]. Thus the part of the brain that contains mainly axonal fibre tracts is called white matter while the neuronal cell body containing regions is called gray matter. Each fibre tract consists of a bundle of axons, responsible for connecting one part of the brain with another. These tracts have a clear organization and their directional orientation is the main cause of the anisotropic behaviour seen in MR diffusion experiments of white matter [48].

The principle source of nourishment for the nervous system is glucose, which is delivered to the brain through the cerebrovascular system [49]. The amount of NMR signal coming from the water inside the major blood vessels of the brain is generally considered to be about 4% of the total signal [50]. Thus the bulk of the NMR signal in the brain comes from intracellular and interstitial water with intravascular water contributing relatively little.

Currently, both normal and abnormal conditions of white matter are far better demonstrated by MR than by any other imaging modality. The incorporation of PGSE into conventional MRI sequences has been shown to introduce diffusion-weighted contrast in the acquired images. The interpretations of these images are based on phenomenological models of diffusion in tissue, which may be traced back to an analysis of D_a , which in turn can be associated with pathological changes in white matter. Apparent diffusion coefficients are reduced by 50% within acute ischemic lesions [51]. The mechanism of decreased diffusion in ischemia is thought to be linked to energy failure and loss of neuronal and glial membrane function [51]. The brain requires a constant supply of glucose and oxygen, which is delivered by cerebral blood flow at normal rates of about 55 ml/mg/min [15]. The brain has no significant reserves of energy. Under normal conditions the brain is a highly aerobic organ. However, when cerebral blood flow drops, the extraction fractions of oxygen and glucose increase in order to compensate for the decreased delivery. Since there is no oxygen reserve in the brain, the

central nervous system tissue shifts to anaerobic glycolysis. Glucose stores cannot maintain cerebral functions and irreversible damage will follow within minutes after the onset of cerebral ischemia. Also with increasing time, after an ischemic episode, cell membrane Na^+ / K^+ ATP-ase begin to fail and ion transport homeostasis is disrupted leading to dissipative efflux of cellular potassium and inflow of sodium, calcium and water [16]. Cell swelling and edema occur within minutes of energy failure. Diffusion weighted MRI is sensitive to these effects.

It has been argued that the changes in D_a following cellular swelling are due to a lower effective cellular diffusion, caused by an increased tortuosity of the paths available for diffusion in the extracellular space [47]. Also it has been speculated that the decrease of D_a within a few hours of the onset of a stroke is due to a reduction in cell membrane permeability associated with the shutdown of energy dependent membrane pumps [16]. This is thought to further restrict the motion of water molecules in the tissue.

Besides the interest in applying diffusion weighting to MRI studies of the neuro-radiological evolution of stroke, a lot has also been done to determine the structural components of white matter and its diseases. [13] In a white matter disease such as multiple sclerosis, MS, which has a high degree of variability of clinical signs and symptoms over time and between individuals, with no current biological markers of their progression, MR techniques may be able to provide a direct indication of disease pathology. MS is the most common form of demyelinating disease characterized by preferential damage to the myelin and relative preservation of axons. The clinical deficits are due to the effect of myelin loss on the transmission of electrical impulses along axons and the inability of the central nervous system to regenerate myelin. White matter diffusion is extremely variable and dependent on the relative orientation of the axonal tracts. The myelin sheath hinders the motion of the molecule in the direction perpendicular to the membrane. Damage to these structures is presumed to allow less restricted diffusion leading to a raised D_a value. The pathological effects of MS have the potential to alter the permeability or geometry of structural barriers to water molecular diffusion in the brain. Consistent with this, results of preliminary studies [52] found that water diffusion in MS lesions is higher than in white matter of healthy volunteers.

Currently, MRI is considered a very important tool in monitoring the efficacy of new therapies in MS.

2.6 Restricted Diffusion

Diffusion is the microscopic random translational motion of molecules. Water molecular diffusion can be measured in vivo using diffusion-weighted imaging. Because the diffusion is affected by the properties of the medium in which the molecular motion occurs, measurements of diffusion inside biological tissues can provide information about tissue structure at a microscopic level. The motion of water molecules can be hindered by the presence of structural barriers at a cellular or molecular level. In addition, diffusion is inherently a three-dimensional process, and in tissues with an oriented microstructure, such as brain white matter, the molecular mobility is anisotropic.

Figure 2.6: Tissue model of human white matter.

White matter fibre tracts, such as those shown diagrammatically in Fig. 2.6, consist of aligned myelinated axons. In such a system, diffusion across the axon fibres is much less than along the fibres. Also by changing the duration and the amplitude of the gradient pulses, the sensitivity of the sequence to diffusion can be changed. By measuring the signal attenuation caused by diffusion as a function of the gradient strength, D_a can be evaluated. The value of D_a will be affected by many things such as the permeability of membranes that the water encounters during the measurement. Also for short diffusion times, most molecules can be considered to diffuse freely, without reaching the barriers.

With increasing time, such that the diffusion distance has the same order as the cell size, D_a decreases until, for long diffusion times it reaches an asymptotic value [43].

Very early in the development of NMR it was realized that the capability of monitoring whether the diffusion is Gaussian or hindered, using magnetic field gradients, could be of enormous chemical benefit. Despite the rapidly increasing evolution of MR technology, studies of hindered diffusion have been rather limited in number until recently. Several theoretical models that deal with restricted diffusion were published in the 1960's by Stejskal and Tanner [5]. The expression they derived for the echo decay of molecules diffusing within a volume defined by infinite planar boundaries is valid in the short-gradient-pulse, SGP, limit. This approximation refers to diffusion of the molecules during the application of the pulsed gradients, which is considered to be negligible in the limit $\delta \rightarrow 0$ and $G \rightarrow \infty$ such that $G\delta$ remains constant. The motion that does occur during the two gradient pulses contributes to a reduction in the resolution of the experiment. Fig. 2.7 shows a homogeneous fluid, characterized by a intrinsic diffusion coefficient D_0 and confined between two infinite, parallel barriers. The factor R by which diffusion will attenuate the spin-echo amplitude is given by:

$$R = \exp(-\gamma^2 \delta^2 G_{\parallel}^2 \Delta D_0) \left[\frac{2[1 - \cos(\gamma \delta G_{\perp} a)]}{(\gamma \delta G_{\perp} a)^2} + 4(\gamma \delta G_{\perp} a)^2 \sum_{N=1}^{\infty} \exp\left(-\frac{n^2 \pi^2 D_0 \Delta}{a^2}\right) \frac{1 - (-1)^n \cos(\gamma \delta G_{\perp} a)}{[(\gamma \delta G_{\perp} a)^2 - (n\pi)^2]^2} \right] \quad (2.51)$$

The expression above reveals its anisotropic character, being dependent upon the direction of G relative to the layer. The motion perpendicular to the barriers can be evaluated by setting $G_{\perp} \rightarrow G$ and $G_{\parallel} = 0$. Laminar systems, such as this one, may be the most uncomplicated examples of restricted diffusion. The behaviour in the long time limit, is given by:

$$R_{\infty} = \lim_{\Delta \rightarrow \infty} R = \frac{2[1 - \cos(\gamma \delta G a)]}{(\gamma \delta G a)^2}, \quad (2.52)$$

when R reaches the asymptotic value. The asymptotic behaviour is characteristic for a completely trapped particle.

Figure 2.7: Diffusion in between infinite parallel planes with a separation a and non-permeable walls.

Later on, Tanner derived a more useful expression, which gives the apparent diffusion coefficient as a function of wall permeability, P [17]:

$$D_a(\Delta \rightarrow \infty) = \left[\frac{1}{D_0} + \frac{1}{aP} \right]^{-1}. \quad (2.53)$$

Measurements of coefficient D_0 and $D_a(\infty)$ according to Eq. (2.53) give information on the cellular environment, the permeability of the barrier and the dimensions of the system.

It was to take another 20 years before additional progress was made. Soderman et al. [24] developed an expression for hindered diffusion within a cylinder of length L with perfectly reflecting walls and radius R (see Fig. 2.8) which is valid in the SGP approximation. Between encounters with the walls of the cylinder, the water molecules diffuse with the free diffusion coefficient D_0 .

The attenuation of the NMR signal due to diffusion perpendicular to the cylinder axis is:

$$\frac{M(\gamma G \delta, \Delta)}{M(0, \Delta)} = 4(\gamma G \delta R)^2 \sum_{k=1}^{\infty} \sum_{m=0}^{\infty} \frac{k_m \alpha_{km}^2 [J'_m(\gamma G \delta R)]^2}{[\alpha_{km}^2 - (\gamma G \delta R)^2]^2 (\alpha_{km}^2 - m^2)} \exp\left[-\left(\frac{\alpha_{km}}{R}\right)^2 D_0 \Delta\right], \quad (2.54)$$

where $k_m = 1$, for $m=0$ and $k_m = 2$, for $m \neq 0$. The quantities α_{km} are the roots of the Bessel equation $J'_m(\alpha) = 0$. The attenuation of the NMR signal no longer has an exponential dependence on $(\gamma G \delta)^2$.

Figure 2.8: Schematic diagram of diffusion in a cylinder. The cylinder is of length L and radius R with the symmetry axis of the cylinder subtending an angle θ with the direction of the gradient. When $\theta = \pi/2$, the spin-echo attenuation can be described by Eq. (2.54).

The apparent diffusion coefficient can be calculated from the initial slope as:

$$D_a = \lim_{G\delta \rightarrow 0} -\frac{1}{2\Delta} \left[\frac{d\left(\frac{M}{M_0}\right)}{d(\gamma G \delta)^2} \right]_{G\delta \rightarrow 0}. \quad (2.55)$$

Later on, Mitra et al. [53], Callaghan [54], Price et al. [26] derived expressions for diffusion within spherical geometries in the SGP limit. In the scenario when the SGP approximation is valid, the manipulation of the relaxation properties of the system to achieve the partially absorbing wall condition is more mathematical tractable.

The analysis of the data provides structural information on the restricting interface and also the exchange rate of the transporting species together with the topology of the domains between which they are transported. Using the proper boundary conditions at the interface, a more rigorous relationship between the transport and the experimental variables can be derived for the situation where the molecules are only partially restricted and have a finite probability of diffusing from one compartment to another (a simple case of a two-compartment model is shown in Fig. 2.9).

Figure 2.9: Schematic representation of spherical interface in which the species undergoes free diffusion in each of the two equivalent compartments. In analyzing the case of exchange through the interface, one domain is referred to as the intracellular phase, D_1 and the other as the extracellular phase, D_2 .

The analytical solution relating the echo attenuation $E(\Delta)$ to the experimental variables gives a measure of the signal from each phase separately, where the observables are defined as follows [26]:

$$\left\{ \begin{array}{l} E_1(\Delta) = E_{11}(\Delta) + \frac{1-\varphi}{\varphi k} E_{21}(\Delta) , \\ E_2(\Delta) = E_{22}(\Delta) + \frac{1-\varphi}{\varphi k} E_{21}(\Delta) , \end{array} \right. \quad (2.56)$$

$$\left\{ \begin{array}{l} E_1(\Delta) = E_{11}(\Delta) + \frac{1-\varphi}{\varphi k} E_{21}(\Delta) , \\ E_2(\Delta) = E_{22}(\Delta) + \frac{1-\varphi}{\varphi k} E_{21}(\Delta) , \end{array} \right. \quad (2.57)$$

where E_1 and E_2 are the signal attenuation for spins inside and outside the compartment, respectively. With E_{ij} the first subscript indicates whether the initial position of the particle is inside ($i=1$) or outside ($i=2$) and the second bears information on the current spatial coordinate r . Also $k = \frac{C_1}{C_2}$, where C_1 and C_2 are the concentrations of particles in domains 1 and 2, respectively, and φ represents the volume fraction of the intracellular space.

While the use of diffusion weighted NMR to measure boundary geometry was first demonstrated by Tanner and Stejskal [5], there has recently been renewed interest in its use to study restricted diffusion in porous media. Lots of theories [55, 56, 57] of PGSE-NMR of molecules trapped within pores exist and some have been extended to include wall-relaxation effects. Expressions for the echo-attenuation have been derived under the short-gradient-pulse condition for rectangular, cylindrical and spherical pores. Within this framework, Callaghan et al. [44] presented a model of weakly coupled identical pores to interpret their data. In this work he proved that PGSE measurements could provide an effective measure of pore structure and connectivity. The method relies on the measurement of restricted motion of liquid molecules that fill the pores and channels.

For a configuration such as this (see Fig. 2.10) the connecting channels enable the molecules originating in one location to end up anywhere in the structure when the diffusion time is sufficiently long. The connectivity array gives a useful formalism for dealing with the diffusion problem between pores and the calculation of the finite time echo attenuation. As time advances, the probability of being in neighbouring pores gradually increases according to a spreading diffusion probability characterized by the Gaussian distribution. The spatial Fourier transform of these profiles provides a clear illustration of the structural information present in the signal. This model shows

agreement with calculations made by Tanner [17] of diffusive profiles in one-dimensional systems partitioned by permeable membranes. Also for the long distance scale the model is probed for all possible structures and the utility of the Gaussian-envelope assumption gives a nice physical picture of all the structures represented by the signal decay.

Figure 2.10: Representation of a general array of pores and interconnecting channels.

The idea that D_a measured using a PGSE sequence is characterized by a term described as the mean squared displacement divided by diffusion time is only valid when the gradient applied is weak such that $q = \gamma \delta G$ is small compared with reciprocal of the barrier spacing. Is it interested indeed to evaluate the behaviour of $S(q)$ in the long time limit. Tanner's model of rectangular boxes, (see Eq. 2.51) represents one of the few exact solutions to the problem, dealing with restricted diffusion within a specified geometry. On a long time scale compared with that needed to diffuse the box distance, a , all molecules interact many times with the walls, such that they lose the memory of their initial positions. In the long time scale limit, such that $t_d \gg \frac{a^2}{D_0}$, Eq. 2.51 becomes:

$$E(q) = \frac{2\{1 - \cos(2\pi qa)\}}{(2\pi qa)^2}. \quad (2.58)$$

The behaviour of Eq. 2.51 is shown in Fig. 2.11. Note the zeros of the echo signal when qa is an integer.

Figure 2.11: Signal attenuation for diffusion within square cells, shown for t_d both small and comparable with the mean time taken to diffuse across the cell. For larger values of t_d the behaviour becomes time independent and equivalent to the one shown for $t_d = \frac{a^2}{D_0}$.

This result has an optical analogue, the diffraction pattern, where the phase cancellation arises from different slit elements. This long time-scale experiment gives diffusion signal attenuation dependent on the confined geometry. The Fourier transform of the signal gives the profile of the object.

A rather powerful approach to estimate the MR signal decay in tissue, in the presence of diffusion is to use Monte Carlo simulations. These methods are involved in assessing tissue characteristics from experimental data. It seems that it is hardly possible to get exact analytical results for restricted diffusion when gradient pulses of finite duration are employed, and therefore, only numerical simulations are available [58, 59]. Stanisiz et al. [29] used numerical simulations to estimate D_a in spinal cord, taking into account the distribution of axon diameters and permeability and the relative volume occupied by the axons. More recent work by Szafer et al. [30] presented a MC simulation of diffusion in a two-pool model with permeable membranes and showed that tissue can be regarded as two independent compartments, intracellular and extracellular.

Gates presented a Monte Carlo analysis [60] of water diffusion in human tissue and its results suggested that extracellular fluid motion at the cellular level (assumed to originate from cerebrospinal fluid pressure) was a main factor affecting the motion of water molecules. Also, his experimental findings showed that, at short diffusion time tissue anisotropy could be removed at short diffusion times. A phantom study was used by Gauthier to model the axonal system of white matter [61] also using a Monte Carlo analysis to explain his results.

Chapter 3

Methods

The research presented in this thesis consists of diffusion decays computed either from analytical solutions presented in the literature or from Monte Carlo simulations and the analysis of these decays. These computations were performed on Personal Computers using programs written in Visual C++ version 6.0 and the Interactive Data Language, IDL, version 5.0.

3.1 Diffusion Decays Computed From Analytical Solutions

Theoretical models for water diffusion in square cells (see Eq. 2.51) and circular cells (see Eq. 2.54) were used to compute the diffusion decays for various cell sizes and diffusion times. The original code for both of these models was written by Yvan Gauthier [61] although some modifications were required in order to obtain accurate diffusion decays for the full range of b -values and diffusion times investigated in this thesis. The derivatives of the Bessel functions and the roots of the Bessel functions in Eq. (2.54) for the circular cell model were computed using routines obtained from Numerical Recipes [62].

For more complex situations (e.g. restricted diffusion within tortuous extracellular compartment) analytical solutions do not exist in the literature. In this case, the Monte Carlo model became a valuable tool for the prediction of MR diffusion decay behaviour.

3.2 Diffusion Decays Computed from Monte Carlo Simulations

A 2D Monte Carlo simulation model was used to compute the diffusion attenuated MR signal and to study the diffusion time development of D_a for a white matter tissue model at the cellular level. For a given set of parameters that describes the white matter tissue wall, the MR signal is computed as a function of any desired gradient strength for a given diffusion time t_d .

White matter tissue was modelled as a set of axons viewed as long cylinders of radius R parallel to each other and organized in a periodic hexagonal lattice, with lattice spacing R_m , as shown in Fig. 3.1. Each axon is surrounded by a membrane of permeability P . Intracellular water molecules have a diffusion coefficient D_i and their transverse magnetization relaxes with a characteristic time $(T_2)_i$. These two parameters summarize phenomenologically the interaction of water with the organelles, cytoskeleton and proteins in the cell. The axons are surrounded by extracellular water, characterized by the diffusion coefficient D_E and spin-spin relaxation rate $(T_2)_E$.

The 2D Monte Carlo model is based on a model developed by Szafer et al. [30] but for circular geometry. The program used for the Monte Carlo simulation was originally written by Yvan Gauthier [61], however, he used it only to determine the initial slope of the decay curves. Since for the research presented in this thesis, the full diffusion decay was used, the code had to be carefully tested. During this testing, several bugs were discovered in the code and had to be fixed. These bugs only affect the diffusion decay once the signal gets small and do not affect the results presented by Gauthier. In the Monte Carlo model used, the axons are regarded as circles of radius R placed inside a

hexagonal cell of side equal to $\frac{2R_m}{\sqrt{3}}$ (see Fig. 3.1). Since the geometrical symmetry of the model is considered, boundary conditions can be applied for each hexagonal cell.

Figure 3.1: Two-dimensional hexagonal geometry used in MC simulations where the cells are represented by circles of radius R surrounded by a membrane of permeability P . Intracellular water molecules diffuse with diffusion coefficient D_I and relaxation rate $(T_2)_I$. Extracellular water molecules are characterized by the diffusion coefficient D_E and relaxation rate $(T_2)_E$.

The input parameters that characterize the tissue can be changed according to different situations considered. These parameters are $D_I, D_E, (T_2)_I, (T_2)_E, P$ and N , the number of trajectories simulated.

The starting position for each molecule was clearly assigned to either the intracellular or extracellular compartment. At time $t_d = 0$, the position within the chosen compartment is selected randomly from a uniform distribution.

The contribution to m_j , the transverse magnetization from particle j , has real and imaginary parts and can be written in the form:

$$m_j(t) = \mu_j(t) \exp[i\phi_j(t)]. \quad (3.1)$$

The 90° RF pulse applied to the system tips and aligns the m_j such that all spins are initially in phase with each other. Immediately after the RF pulse, at time $t=0^+$ the diffusion process is simulated. After the first diffusion step, a phase increment proportional to the x -coordinate of the particle at that moment is given according to:

$$m_j(0^+) = \exp[iG\gamma\delta x_j], \quad (3.2)$$

as a result of the first gradient pulse, where γ is the gyromagnetic ratio, δ is the diffusion gradient duration and G is the amplitude of the gradient pulse.(see Fig. 2.5)

At this level, all spins are well described by their initial phase and position, which are clearly defined. At this stage, the program starts to simulate the Brownian motion of the spins during a period of time t_d which is divided into a series of small diffusion steps of duration dt . The step size used was chosen to be small due to the necessity of keeping track of the possibility of multiple reflections. The position and the magnetization are updated every dt . The evolution describes a random segmented walk and the magnetization decay during each diffusion step depends on the T_2 relaxation time and the appropriate diffusion coefficient of the compartment where the particle is found at that moment. Each step size has two components x and y , each of which follows a Gaussian distribution with a standard deviation $\sigma_i = \sqrt{2D_i dt}$ representing the intracellular displacement and $\sigma_e = \sqrt{2D_e dt}$ representing the extracellular displacement, then the magnetization evolves according to:

$$m_j(t + dt) = m_j(t) \exp\left(\frac{-dt}{T_2}\right), \quad (3.3)$$

The model also treats the cell membranes as being semi-permeable. Every time a particle reaches the membrane, the probability p of being transmitted or reflected is given by [30]:

$$p = \frac{4P}{v} \quad (3.4)$$

;where v represents the velocity of a particle in the medium.

For a particle that reaches the border of the cell and eventually goes through the membrane, the length of the diffusion step is rescaled considering the diffusion coefficients in both environments. In the scenario where a particle inside the cell collides with the membrane after $dt_I < dt$ and is transmitted, the step size is calculated according to

$v_I dt_I + v_E dt_E$ where $v_I = \sqrt{\frac{2D_I}{dt_I}}$ and $v_E = \sqrt{\frac{2D_E}{dt_E}}$. The final position in the

extracellular space x_E, y_E is then given by the following expressions:

$$x_E = x_w + \sqrt{\frac{D_E}{D_I}}(x_E - x_w), \quad (3.5)$$

$$\text{and } y_E = y_w + \sqrt{\frac{D_E}{D_I}}(y_E - y_w) \quad (3.6)$$

;where x_w and y_w are x and y coordinates of the position on the wall at which the interaction took place. The signal attenuation for each spin m_j is rescaled taking into account the interval of time spent in the intracellular space, dt_I and in the extracellular space, $dt_E = dt - dt_I$. The magnetization m_j evolves as:

$$m_j(t + dt) = m_j(t) \exp\left[-\frac{dt_I}{(T_2)_I}\right] \exp\left[-\frac{dt - dt_I}{(T_2)_E}\right] \quad (3.7)$$

Finally, after the desired diffusion time t_d , the phase increment due to the application of the second diffusion sensitive gradient is proportional to the position of the particle. The magnetization for each spin is given by:

$$m_j(t_d^+) = m_j(t_d^-) \exp[-iG\gamma\delta x_j(t_d)]. \quad (3.8)$$

where t_d^+ and t_d^- are immediately before and after t_d , respectively. In the expression above the net phase is proportional to the particle net displacement. The echo signal is calculated by adding the m_j for all of the particles:

$$M(q, t_d) = \left| \sum_{j=1}^N m_j(t_d) \right| \quad (3.9)$$

;where N is the number of trajectories simulated and the wave vector q is equal to $\gamma G \delta / 2\pi$. The MR signal was normalized to the signal in the absence of the diffusion gradient.

This mathematical model, based on a simplified geometry of white matter tissue is expected to shed light on the causes of the non-exponential diffusion attenuation for situations where analytical solutions are unavailable. Toward this goal, in each MC simulation the signal amplitude was computed for 250 b -values, which were increased linearly for 0 to 10000 s/mm^2 . The diffusion step size was set to: $dt = t_d / 10^4$. To mimic water diffusion in the extracellular compartment, simulations have been performed for the two arrangements shown in Fig. 3.2:

Figure 3.2: Cylindrical arrangements representing: a) a hexagonal close-packed lattice of abutting cells of radius R with lattice spacing $R_m = R$; b) a hexagonal lattice of non-abutting cells of radius R with lattice spacing $R_m > R$.

The other input parameters used were: $D_0 = 2 \times 10^{-3} \text{ mm}^2 / \text{s}$ and $(T_2)_I = (T_2)_E = 250 \text{ ms}$. The number of particles followed during simulations was $N=500000$. A large number of particles was required so that the results would not change due to lack of iterations.

3.3 Analysis of Decay Curves

Once the diffusion decays were generated, the available information was extracted from the curves by using fitting routines. D_a was calculated from the negative of the initial slope of the decay curve. This is equivalent to the assumption that the decay can be written as:

$$S / S_0 = \exp(-bD_a). \quad (3.10)$$

S / S_0 represents the magnetization of the sample normalized by the total magnetization at $b=0$ with no applied gradients. To evaluate D_a , the initial slope of the curves was calculated from the signal strengths at $b=0$ and $b=500 \text{ s/mm}^2$.

However in most of the cases, for b -values greater than 500 s/mm^2 , the diffusion decay deviate drastically from exponential behaviour. In some cases, the decay curve could be fitted to a summation of two exponential functions using a generalized least squares analysis. The bi-exponential model is formalized as:

$$S / S_0 = f_{\text{slow}} \exp(-bD_{a,\text{slow}}) + f_{\text{fast}} \exp(-bD_{a,\text{fast}}), \quad (3.11)$$

where $D_{a,\text{slow}}$ and $D_{a,\text{fast}}$ represent the slow diffusion coefficient and the fast diffusing coefficient, respectively. The deviation from the mono-exponential behaviour is a result of the restricted nature of the diffusional motion. These parameters are treated as phenomenological ones being useful in characterizing the attenuation curve. They will not necessarily be equal to the apparent diffusion coefficients of any of the compartments in the model.

Chapter 4

Results

The results presented in this chapter are from Monte Carlo simulations of particles diffusing in cellular models consisting of a system of circles or squares and analytical solutions in corresponding geometries. The diffusion decays are computed for $b=0$ to $b=10000 \text{ s/mm}^2$. As a first step, the theoretical expressions given by Tanner [5] and Soderman et al. [24] were used to compute the signal decay $S(b)$ within the specified geometry, using a broad variation of parameters and the results were compared, where possible, with Monte Carlo, MC, simulation results. The observations were then extended with the MC simulations to situations that could not be studied using theoretical expressions found in the literature [22]. These results, along with some tests of convergence will be presented in this chapter.

For very short diffusion times t_d , a negligible number of particles will be able to diffuse far enough to encounter the walls of the cells. For these short diffusion times the diffusion is essentially the same as free diffusion. On the other hand, when the diffusion time is very long, all of the particles will have had many collisions with the cell walls. The diffusion decays computed in this thesis cover this full range of behaviour.

4.1 Intracellular Diffusion

4.1.1 Diffusion in Square Cells (Tanner's model)

Tanner has presented an analytical solution to the diffusion of particles in square cells with impenetrable walls (see Eq. 2.51). The diffusion decays shown in Fig. 4.1 were calculated using Tanner's model where the length of the side was $a=5\text{ }\mu\text{m}$ for a wide range of diffusion times between $30\text{ }\mu\text{s}$ and 1000 ms (see also Table 4.1).

Figure 4.1: Diffusion attenuation curves for square cells of side $a=5\text{ }\mu\text{m}$. Each curve was obtained using Eq. 2.51 for $30\text{ }\mu\text{s} < t_d < 1000\text{ ms}$. The magnitude of the initial slope of the curves monotonically decreases with increasing t_d . For a complete list of the diffusion times for the decay curves plotted here, refer to Table 4.1.

The complicated shape of the diffusion decay curves for some diffusion times made it difficult to characterize these curves unambiguously in terms of a few simple parameters (e.g. slope). However, as a first attempt, the well-established procedure of determining the initial slope was used for all of the curves.

$t_d (ms)$	$D_a (10^{-3} mm^2 / s)$	N	$b_{min} (s / mm^2)$	$q_{max} a$
0.03	1.823	19		14.52
0.05	1.775	15		11.248
0.07	1.736	12		9.5
0.1	1.689	10		7.95
0.3	1.488	6		4.59
0.5	1.36	5		3.55
0.7	1.262	4		3.006
0.9	1.18	4		2.651
1	1.143	3		2.515
2	0.847	3	3700	1.77
3	0.646	2	5100	1.452
5	0.417	2	8000	1.124
7	0.301	1		0.95
10	0.21	1		0.795
20	0.105	1		0.562
30	0.07	1		0.459
50	0.042	1		0.355
100	0.021	1		0.251
300	0.007	1		0.145
500	0.004	1		0.112
700	0.003	1		0.095
900	0.002	1		0.083
1000	0.002	1		0.079

Table 4.1: Results of D_a obtained from the initial slope of the attenuation curves for square cells (Tanner's model) as a function of t_d . Also, N is the number of terms used in the summation of Eq. (2.51), b_{min} is the position at which the relative minimum values of the curves occurred and $q_{max} a$, where q_{max} is the maximum q value (see Section 2.7) used in the computation and a is the barrier separation.

These results are given in Table 4.1 and plotted as a function of t_d in Fig. 4.2 where D_a was set to the negative of the initial slope of the decay curves (see Eq. 2.55). It can be seen from Fig. 4.1 that D_a has a strong dependence on t_d . To investigate the effect of such variations, D_a values are plotted as a function of t_d in Fig. 4.2. The dependence of the diffusion-weighted signal on t_d is reflected in D_a , which declines quickly from 1.823 to $0.002 \cdot 10^{-3} \text{ mm}^2/\text{s}$ for the diffusion times considered.

Figure 4.2: Relative apparent diffusion coefficient, D_a / D_0 plotted as function of t_d for water diffusing with $D_0 = 2 \times 10^{-3} \text{ mm}^2/\text{s}$ within square cells.

Until recently, with the introduction of fast computers, Tanner's equation has been difficult to deal with because it is an infinite sum of trigonometric terms. As a result, for very small diffusion times, the computation time was often very long, since the convergence of this expression was slow. The number of terms required to get convergence for each diffusion time is given in column three of Table 4.1. On a 1GHz

PC, the computation time for the diffusion decays (for 1000 b -values) at $t_d=30\ \mu s$ was about 5 minutes.

It can also be seen from Fig. 4.1 that the behaviour of the diffusion decay seems to fall into three different zones based on its shape. For diffusion times below $t_d=2\ ms$ the decay seems to be a bi-exponential decay with the more slowly decaying component corresponding to a very small fraction of the signal, (a few percent) which increases with increasing diffusion time. Between $t_d=2\ ms$ and $t_d=20\ ms$ the decay changes to a non-exponential decay with several broad extrema at larger b -values. The approximate positions of the observed minima are given in column four of Table 4.1. For $t_d>20\ ms$ the decay appears to be a single exponential decay for b -values up to $10000\ s/mm^2$. These zones will be referred to as the weak ($t_d<2\ ms$), intermediate ($2\ ms<t_d<20\ ms$) and strong ($t_d>20\ ms$) collision limits, respectively.

4.1.2 Diffusion in Circular Cells (Soderman's Model)

An analytical expression for the diffusion decays for particles diffusing in a circle with non-permeable walls has been presented by Soderman (see Eq. 2.54). The resulting decay curves for $R=5\ \mu m$ are shown in Fig. 4.3. The diffusion decays were computed using Soderman's model for circles of radius $R=1, 5, 10, 20$ and $30\ \mu m$ and for t_d values between $30\ \mu s$ and $1500\ ms$.

A comparison of Fig. 4.1 with Fig. 4.3 shows that the shapes of the diffusion decay curves calculated using Tanner's equation are very similar to those calculated using Soderman's equation even though they are for cells of different geometry. The weak, intermediate and strong collision limits are apparent and the extrema at intermediate t_d 's are again observed. The values of D_a calculated from the initial slopes of the curves along with b_{min} values are given in Table 4.2.

Figure 4.3: Computed diffusion decays as a function of the diffusion-weighting factor b for circular cells of radius $R=5\text{ }\mu\text{m}$ using t_d values from $30\text{ }\mu\text{s}$ to 1500 ms . The magnitude of the initial slopes of the curves monotonically decreases with increasing t_d . For a complete list of the diffusion times for the decay curves plotted here, refer to Table 4.2.

As with Tanner's model, the convergence of Soderman's equation at small values of t_d can be slow. The number of terms used in the computation of the diffusion decay using Soderman's model when $R=5\text{ }\mu\text{m}$ is given in Table 4.2 for each corresponding diffusion time. The number of terms used in the two summations in Eq. (2.54) was arbitrarily chosen to be equal. The computation time on a 1GHz computer for the diffusion decay (1000 b -value) at $t_d=30\text{ }\mu\text{s}$ was about 30 minutes.

t_d (ms)	$D_a(10^{-3} \text{ mm}^2 / \text{s})$	$m=k$	$b_{\min} (\text{s} / \text{mm}^2)$	$q_{\max} R$
0.03	1.913	200		14.52
0.05	1.888	60		11.248
0.07	1.868	50		9.5
0.1	1.842	40		7.95
0.3	1.729	30		4.59
0.5	1.652	20		3.55
0.7	1.59	12		3.006
0.9	1.537	12		2.651
1	1.512	12		2.515
2	1.319	12		1.77
3	1.173	12		1.452
5	0.947	12	4300	1.124
7	0.777	12	5100	0.95
10	0.597	12	6400	0.795
20	0.315	12		0.562
30	0.210	12		0.459
50	0.126	12		0.355
100	0.063	12		0.251
300	0.021	12		0.145
500	0.013	12		0.112
700	0.009	12		0.095
900	0.007	12		0.083
1000	0.006	12		0.079
1500	0.004	12		0.052

Table 4.2: Results for D_a obtained from the initial slope of the attenuation curves for $R=5 \mu\text{m}$ (Soderman's model). Also given are the number of terms $m=k$ used in the double summation of Eq. (2.54), $b_{\min}$, the position at which the relative minimum values of the curves occurred and $q_{\max} R$, where $q_{\max}$ is the maximum q value (see Section 2.7) used in the computation and R is the cell radius.

To study the dependence of D_a as a function of t_d , D_a values were calculated from the initial slope of the diffusion decays computed for circles of radius $R=1, 5, 10, 20$ and $30 \mu m$ and plotted vs. t_d in Fig. 4.4. The effect of increased intracellular volume is manifested primarily in a shift of the curve to higher t_d values with the curve maintaining the same shape. For $R=1 \mu m$ at $t_d=10 \mu s$ for example, the effect of highly restricted diffusion is reflected in a lower value for D_a . As the size of the cell increases the effect of restriction can be seen to occur at larger diffusion times.

Figure 4.4: The relationship between D_a and t_d . At short diffusion times, D_a almost equals the free diffusion value, $D_0 = 2 \times 10^{-3} s/mm^2$. All D_a values were obtained for $30 \mu s < t_d < 1500 ms$.

$t_d (ms)$	$D_a (10^{-3} mm^2 / s)$				
	$R=1 \mu m$	$R=5 \mu m$	$R=10 \mu m$	$R=20 \mu m$	$R=30 \mu m$
0.03	1.576	1.913	1.957	1.978	1.985
0.05	1.457	1.888	1.944	1.972	1.981
0.07	1.361	1.868	1.934	1.967	1.978
0.1	1.242	1.842	1.921	1.96	1.974
0.3	0.742	1.729	1.863	1.932	1.954
0.5	0.493	1.652	1.824	1.912	1.941
0.7	0.359	1.59	1.792	1.895	1.93
0.9	0.28	1.537	1.765	1.882	1.921
1	0.252	1.512	1.752	1.875	1.917
2	0.126	1.319	1.652	1.824	1.882
3	0.084	1.173	1.576	1.785	1.856
5	0.051	0.947	1.457	1.724	1.815
7	0.036	0.777	1.361	1.674	1.781
10	0.025	0.597	1.242	1.612	1.739
20	0.013	0.315	0.947	1.457	1.634
30	0.0089	0.210	0.742	1.340	1.554
50	0.0057	0.126	0.493	1.157	1.429
100	0.003	0.063	0.252	0.835	1.203
300	0.0011	0.021	0.0841	0.336	0.688
500	0.00029	0.013	0.05	0.202	0.448
700	0.0002	0.009	0.036	0.144	0.324
900	0.0001	0.007	0.028	0.112	0.252
1000	0.00002	0.006	0.025	0.1	0.227
1500	0.000012	0.004	0.017	0.067	0.091

Table 4.3. Computed D_a values for $R=1, 5, 10, 20$ and $30 \mu m$ measured from the initial slope of the diffusion decay curves generated using Soderman's equation for circular cells with $30 \mu s < t_d < 1500 ms$.

4.1.3 Monte Carlo Simulations

Diffusion decays were computed using the Monte Carlo model described in Section 3.2. For the purpose of comparison with the results presented in the previous section, the decay curves presented in Fig. 4.5 were computed for a circle of radius $R=5\text{ }\mu\text{m}$ and diffusion times ranging from $10\text{ }\mu\text{s}$ to 1000 ms . A similar set of curves was also computed for $R=2.5, 8$ and $10\text{ }\mu\text{m}$. To ensure that the results do not change due to a lack of iterations, $N=500000$ particles was used (See also Section 4.1.4). A comparison between Fig. 4.5, with Fig. 4.1 and Fig. 4.3 shows that the shapes of the decay curves obtained from Monte Carlo simulations are very similar to those obtained with Tanner's and Soderman's models, down to about 1%.

Figure 4.5: Simulated diffusion attenuation curves for $N=500000$ particles using a wide range of t_d from $10\text{ }\mu\text{s}$ to 1000 ms . The radius of the cell is $R=5\text{ }\mu\text{m}$. For a complete list of diffusion times for the decay curves plotted here, refer to Table 4.4. The 1% level is shown for reference as a broken line.

The weak, intermediate and strong collision limits are seen and the broad extrema at intermediate diffusion times are again observed. D_a values calculated from the initial slope of the simulated decay curves are given in Table 4.4 and plotted as a function of t_d in Fig. 4.6 for cell radii ranging from 2.5 to 10 μm .

$t_d (ms)$	$D_a (10^{-3} mm^2 / s)$			
	$R=2.5 \mu$	$R=5 \mu m$	$R=8 \mu m$	$R=10 \mu m$
0.01	1.913	1.955	1.978	1.987
0.03	1.842	1.927	1.949	1.966
0.05	1.794	1.902	1.935	1.954
0.07	1.754	1.882	1.924	1.944
0.1	1.712	1.86	1.913	1.929
0.3	1.487	1.748	1.845	1.882
0.5	1.331	1.668	1.800	1.842
0.7	1.203	1.602	1.758	1.817
0.9	1.095	1.559	1.731	1.788
1	1.053	1.533	1.715	1.78
2	0.703	1.331	1.587	1.678
3	0.509	1.173	1.488	1.593
5	0.308	0.9365	1.333	1.473
7	0.2245	0.7694	1.209	1.377
10	0.1627	0.5936	1.064	1.248
20	0.0837	0.3136	0.7119	0.94
30	0.0423	0.2205	0.516	0.7295
50	0.0177	0.1182	0.318	0.487
100	0.0128	0.0577	0.1547	0.246
300	0.0143	0.0339	0.0544	0.08123
500	0.013	0.0216	0.0465	0.06163
700	0.012	0.011	0.0115	0.02697
900	0.011	0.0135	0.0131	0.02529
1000	0.009	0.0126	0.0129	0.01892

Table 4.4: Calculated D_a values from MC simulations as a function of t_d using different cell sizes, $R=2.5, 5, 8$ and $10 \mu m$. The long t_d values at which D_a starts to behave asymptotically is a function of cell dimensions.

Figure 4.6: Normalized D_a values obtained from the initial slope of simulated decay curves with $R=2.5$, 5, 8, and 10 μm cell radius. As the diffusion time increases, the response of D_a depends on the cell size.

It should be noticed that almost all of the t_d dependence of D_a occurs between 1 and 100 ms with only minimal changes for long t_d .

A point by point comparison of the decays obtained from the Monte Carlo simulations with those obtained using Soderman's model reveals that the curves are almost identical. The maximum deviation values, calculated as the maximum difference when the normalized signal strength for the two decays at corresponding b -values are subtracted, are given in Table 4.5 for $R=5 \mu m$ and $R=10 \mu m$ and each diffusion time considered. In all cases the maximum deviation is within 2.5 %. However, in most cases it is less than 1%.

t_d (ms)	max_deviation	
	R=5 μm	R=10 μm
0.03	0.00108	0.002620
0.05	0.002690	0.00125
0.07	0.00272	0.00165
0.1	0.00945	0.00268
0.3	0.00111	0.00297
0.5	0.0047	0.001510
0.7	0.00129	0.00204
0.9	0.00148	0.00110
1	0.00147	0.00960
2	0.00153	0.00182
3	0.00125	0.00147
5	0.00102	0.00158
7	0.003192	0.0168
10	0.0023	0.00169
20	0.00131	0.02377
30	0.0017	0.000758
50	0.00134	0.00154
100	0.0029	0.002410
300	0.00295	0.000617
500	0.0169	0.0019
700	0.00424	0.00183
900	0.00695	0.0011
1000	0.0077830	0.00783

Table 4.5: Maximum deviation computed as a difference point by point between Soderman's curve and the simulated curve generated with $N=500000$, both obtained using $R=5 \mu m$ and $R=10 \mu m$ for each t_d used. The maximum deviation values greater than 1% are shown in bold.

4.1.4 Investigations of Convergence

With all of the decay curves presented in this thesis, the issue of whether enough terms in the series expansions, or enough particles in the simulations, have been used must be considered. Empirically, convergence could be seen by adding more terms or particles to the computation and observing the effect. This leads to a systematic, monotonic convergence in all cases.

The comparison of the decay curves obtained with Soderman's model with those from the Monte Carlo computations based on maximum deviation values (See Section 4.1.2) gives confidence that enough terms and particles to get convergence have been used. A direct comparison of this sort between Tanner's decays and those obtained from either the Monte Carlo model or from Soderman's model is not appropriate since they correspond to different geometries. However, the close qualitative similarities between Tanner's curves and Soderman's curves, along with the fact that adding more terms is the expansion beyond what was taken as the convergence limit does not appear to change the decay curve, gives confidence that convergence with Tanner's decays was obtained as well. To investigate this further, the convergence of the Monte Carlo simulation decay curves was studied as a function of the number of particles used in the simulation and compared with the decay curve calculated from Soderman's model as the reference curve. By subtracting the two curves b -value by b -value, a maximum deviation was computed. This value is plotted as a function of N for several values of R and t_d in Fig. 4.7. It can be seen that the simulated decay curves converge to be essentially identical with the Soderman's decay curves by about 300000 particles. This gives further confidence that both, the Monte Carlo decay curves and the Soderman's decay curves reported here are accurate.

a).

b).

c).

Figure 4.7: Maximum deviation vs. number of iterations, N . The parameters used to compute the decay curves were: a) $R=5\mu m$, $t_d=10ms$ b) $R=3\mu m$, $t_d=300\mu s$ and c) $R=10\mu m$, $t_d=4ms$. These are typical curves.

4.1.5 Distribution of Cell Sizes

The results presented so far have been for situations where the cells are all one size. However, in biology, the restricting compartments (e.g. axons), are not all the same size. In brain tissue, there is a range of axonal diameters. To investigate the effect of such a distribution on the diffusion decay, a composite diffusion decay curve corresponding to a Gaussian distribution of cell radii was considered.

Figure 4.8: Diffusion attenuation curves computed using Soderman's equation. Broken lines represent the decay curves computed using the Gaussian distribution for: a) mean $R=3 \mu\text{m}$ and $t_d=700 \mu\text{s}$ b) mean $R=4 \mu\text{m}$ and $t_d=2 \text{ ms}$ c) mean $R=5 \mu\text{m}$ and $t_d=7.5 \text{ ms}$ d) mean $R=9.5 \mu\text{m}$ and $t_d=80 \text{ ms}$ and e) mean $R=6.5 \mu\text{m}$ and $t_d=300 \text{ ms}$. The standard deviation for the distribution was $0.5 \mu\text{m}$ in all cases. The solid lines represent the corresponding curves computed for the mean values of R and the appropriate t_d .

For each distribution, the composite diffusion decay represents a combination of the several cell radii in proportions representing their relative contribution. Gaussian distributions with the mean radii set to be $R=3, 4, 5, 6.5, 9.5 \mu m$ were considered. The standard deviation for the distribution was $0.5 \mu m$ in all cases. The resulting decays are shown in Fig. 4.8 compared with the decay curve for the mean of the distribution in each case. The broken lines represent the computed diffusion decay for the distribution and the solid lines depict the corresponding diffusion decays computed only for the mean cell size.

It can be seen that the decay curve for the distribution does not differ substantially from the decay for the mean. The transition from free to restricted diffusion remains characteristic. Overall, the composite curve appears to mimic the behaviour of the corresponding mean cell radius curve. However, in the intermediate collision limit, the distribution has the effect of smoothing out the bumps to a certain extent.

4.2 Extracellular Diffusion

4.2.1 MC Simulations for Close-Packed Configuration of Cells

In order to investigate the diffusion process within the extracellular space, Monte Carlo simulations were performed assuming that the particles were diffusing in the space between the circles for a hexagonal close-packed configuration. The circular cells were packed together such that each cell was touching its neighbours (See Fig. 3.2a). In this way the extracellular compartment is totally restricted with a roughly triangular shape. The radius of the cell considered for this specific geometry was $R=5 \mu m$. Fig. 4.9 shows the computed signal versus b with t_d as a parameter, when the bulk signal is only from particles diffusing in the extracellular space. The classical method for detection of restricted diffusion is based on the reduction of D_a as t_d increases. The diffusion process

Figure 4.9: MC decay curves for extracellular diffusion with $30 \mu\text{s} < t_d < 1000 \text{ ms}$ and $R = 5 \mu\text{m}$ b). The relationship between D_a and t_d .

was analyzed in terms of the initial slope of the extracellular decay curves with the results summarized in Table 4.6 and plotted against t_d in Fig. 4.9b. For each of these simulations the number of particles considered was $N=500000$.

$t_d (ms)$	$D_a (10^{-3} mm^2 / s)$
0.03	1.529
0.05	1.423
0.07	1.353
0.1	1.271
0.3	0.96
0.5	0.7755
0.7	0.6457
0.9	0.5494
1	0.5079
2	0.2838
3	0.1906
5	0.1031
7	0.0804
10	0.066
20	0.03076
30	0.0277
50	0.01364
100	0.00813
300	0.01583
500	0.0198
700	0.0231
900	0.02201
1000	0.02127

Table 4.6: Reported D_a values as measured from the initial slope of the decay curves displayed in Fig. 4.9 for each t_d used between 30 μs and 1000 ms .

4.2.2 Distribution of Extracellular Compartment Sizes

Due to a distribution of axonal radii, the extracellular spaces cover a wide range of sizes. Assuming a gaussian distribution of the intracellular compartment with a mean of $5 \mu m$ and a standard deviation of $0.5 \mu m$, the echo signal decay was computed as a weighted average of the signals coming from each extracellular compartment. Fig. 4.10 shows the resulting signal compared with the corresponding signal decay for the mean. The diffusion time used was $t_d = 70 \mu s$. The two decays in the figure are almost identical, however, it should be noted that the curve for the distribution is a bit smoother than the decay computed for the mean radius.

Figure 4.10: Diffusion-attenuation curve for a distribution of cell sizes with a mean of $5 \mu m$ and a standard deviation of $0.5 \mu m$ (dotted line) compared with the attenuation curve for the corresponding mean (solid line).

4.2.3 Extracellular Diffusion for Hexagonal Configurations of Cells

A set of MC simulations was run to find out the t_d -dependence of D_a for the situation where the cells don't abut. In this scenario, the extracellular space is modeled as a tortuous, infinitely wide compartment in which diffusion is partially restricted (see Fig. 3.2). The D_a changes are related to alterations in the restriction of the extracellular water associated with changes in the extracellular volume fraction. The size of the extracellular space was modified by increasing the hexagonal unit cell by setting $R_m=5, 5.02, 5.1, 5.3, 6.5$ and $12 \mu m$ while keeping the cell radius fixed at $R=5 \mu m$ (see Fig. 3.1).

Figure 4.11: The relationship between measured D_a and t_d for different cell sizes when $30 \mu s < t_d < 1500 ms$

The corresponding extracellular volume fractions are: 0.09, 0.1, 0.13, 0.19, 0.46 and 0.84. The D_a values corresponding to each configuration are presented in Table 4.7 for $30 \mu s < t_d < 1500 ms$ and are plotted vs. t_d in Fig. 4. 11.

$t_d (ms)$	$D_a (10^{-3} mm^2 / s)$					
	$R_m = 5 \mu m$	$R_m = 5.02 \mu m$	$R_m = 5.1 \mu m$	$R_m = 5.3 \mu m$	$R_m = 6.5 \mu m$	$R_m = 12 \mu m$
0.03	1.529	1.488	1.546	1.686	1.91	1.997
0.05	1.423	1.45	1.493	1.586	1.862	1.96
0.07	1.353	1.386	1.379	1.575	1.855	1.975
0.1	1.271	1.297	1.351	1.469	1.835	1.962
0.3	0.96	1.034	1.147	1.268	1.732	1.917
0.5	0.7755	0.906	1.072	1.197	1.652	1.944
0.7	0.6457	0.7891	0.984	1.14	1.606	1.887
0.9	0.5494	0.7528	0.9277	1.151	1.537	1.844
1	0.5079	0.747	0.9096	1.09	1.559	1.9
2	0.2838	0.702	0.8322	1.031	1.39	1.922
3	0.1906	0.6453	0.844	1.019	1.362	1.875
5	0.1031	0.6574	0.8413	1.046	1.351	1.834
7	0.0804	0.7072	0.8569	1.05	1.293	1.812
10	0.066	0.7323	0.9176	1.098	1.327	1.762
20	0.03076	0.8098	0.9852	1.106	1.301	1.766
30	0.0277	0.8526	0.9892	1.123	1.339	1.742
50	0.01364	0.8624	1.09	1.156	1.338	1.709
100	0.00813	0.731	1.185	1.233	1.373	1.718
300	0.01583	0.4333	1.326	1.324	1.426	1.726
500	0.0198	0.2809	1.229	1.397	1.463	1.765
700	0.0231	0.1765	1.026	1.398	1.434	1.75
900	0.02201	0.1171	0.8035	1.318	1.505	1.76
1000	0.02127	0.09857	0.7163	1.32	1.476	1.793
1200	0.0206	0.07424	0.5309	1.217	1.506	1.774
1500	0.01805	0.0539	0.407	1.084	1.534	1.796

Table 4.7: D_a values calculated from the initial slope of the diffusion decays with $R_m = 5, 5.02, 5.1, 5.3, 6.5$ and $12 \mu m$ as a parameter of the hexagonal configuration.

The MC simulations show evidence that variations in the effective extracellular compartment have a significant impact on D_a . An increase in D_a due to the increasing separation between cells is manifested in the plots by the presence of maxima.

Chapter 5

Discussions

The diffusion of water molecules in pure bulk water is a well-understood problem. The process is characterized by a Gaussian displacement distribution and the average displacement is given by the Einstein equation (see Eq. 2.31). The particles are in constant motion as a result of their thermal energy and the resulting trajectories correspond to random walks, which are influenced only by interactions with other water molecules. The diffusion weighted MR signal for such a system, such as would be obtained with a Stejskal-Tanner experiment, corresponds to an exponential decay as a function of the b -values.

The diffusion of water molecules in biological tissue is much more complicated. The motion of the molecules is still affected by collisions with other water molecules but it is also influenced by interactions with many other structures such as macromolecules and cell membranes. In some cases the diffusion decay for such a system can be expected to be a multi-exponential decay but in others it may look more like a diffraction pattern [63]. In general the diffusion decay can be expected to be the superposition of diffusion decays of various forms from a variety of sources. To develop a detailed understanding of the diffusion of water molecules in tissue is a very complex problem. However, it should

still be possible to extract useful information from an analysis of the diffusion decay in tissue by considering appropriate model systems.

The goal of this thesis was to perform a systematic study of diffusion in model systems using analytical solutions where they are available and MC simulations where they are not, to study several aspects of the problem of diffusion in tissue. Intracellular and extracellular diffusion decays were considered for cells of various sizes and shapes over a wide range of diffusion times and b -values. Distributions of cell sizes were also considered.

In all of the computations discussed in this chapter it was assumed that there was a linear magnetic field gradient in the plane of the 2D cell. It is only diffusion in this direction that contributes to the diffusion decay. Between encounters with the restricting barriers the particles are assumed to diffuse with the free diffusion coefficient $D_0 = 2 \times 10^{-3} \text{ mm}^2 / \text{s}$. The decay curves are computed for b -values ranging from 0 to 10000 s/mm^2 . Most of the diffusion decays reported in the literature to date have considered a much smaller range of b -values [64] but there are a few that have appeared recently that have used values as high as 10000 s/mm^2 [65]. It is anticipated that the range of b -values used here will become much more common in the future. A typical value of the apparent diffusion coefficient in the brain is about $1.0 \times 10^{-3} \text{ mm}^2 / \text{s}$.

In order to properly measure the corresponding decay curve, the attenuation must be followed over as large a range of b -values as possible. This would not be necessary if the diffusion decay was always exponential but this is not the case. This is why it was decided to study the diffusion decay from 0 to 10000 s/mm^2 . For most clinical MR imagers today it is still difficult to study diffusion for b -values greater than 10000 s/mm^2 due to the gradient power restrictions and signal-to-noise ratio limitations.

5.1 Intracellular Diffusion

5.1.1 Initial Slope

The classical method for the detection of restricted diffusion (as opposed to free

diffusion) is based on the observed diffusion time dependence of D_a . If restriction occurs, the intracellular water diffusivity will be modified by the interaction with the walls which results in a decrease in D_a with increasing diffusion time and D_a asymptotically approaches a constant value for long t_d . For free diffusion, D_0 , is independent of t_d .

To observe this effect, diffusion decays were generated for square and circular geometries from analytical results in the literature and for circular cells using Monte Carlo simulations. These computations were done as a function of t_d for a range of cell sizes and D_a was calculated for each of the curves from the initial slope of the decay. These results are given in Tables 4.1, 4.3 and 4.4 and in Figs. 4.2, 4.4 and 4.6. The values of R were chosen to be in the range applicable to organelles and cells. At short diffusion times, the D_a values for all the large radius values equal the unrestricted value, D_0 . As the diffusion time increases, the response in D_a depends on the cell size. The effect of decreased intracellular volume is manifested primarily as a decrease in D_a at shorter diffusion times. For smaller volumes, the particles interact with the boundaries more readily causing D_a to decrease sooner. For big radii such as $R=10, 20$ and $30\mu m$, the upper region corresponding to free diffusion is very apparent. For the smallest radii, $R=1$ and $2.5\mu m$, decreases in D_a occur earlier and more dramatically. For intermediate t_d , D_a shows a strong dependence on cell size.

These results can be summarized by introducing a unitless parameter known as the reduced time, t_r . For square cells the reduced time is given by $t_r = \frac{D_0}{a^2} t_d$ whereas for circular cells it is given by $t_r = \frac{D_0}{\pi R^2} t_d$, where a is the length of a side of the square and R is the radius of the circle [25].

In Fig. 5.1a, D_a values calculated from the decay curves computed from Soderman's equation for circular cells are plotted as a function of t_r for all of the diameters that have been considered in this thesis. The D_a values obtained from MC

Figure 5.1: Normalized D_a values plotted as a function of reduced time, a) data obtained using Soderman's model for $30 \mu s < t_d < 1500 ms$, b) data obtained using MC simulations for $10 \mu s < t_d < 1000 ms$.

simulations are shown in Fig. 5.1b. It is clear in both cases that the results for the different diameters fall on the same curve when plotted against t_r . This is to be expected since t_d and R always appear together in Eq. (2.54), however, it is a useful check to make sure that the computed decay curves are accurate.

In Fig. 5.2 the results of D_a vs. t_r , computed from Tanner's model, Soderman's model and the Monte Carlo simulations are compared. Within the limits of the plot there is no perceivable difference between the curve for the simulation and the curves for Soderman's results, however, Tanner's D_a values are systematically a few percent lower for $D_a/D_0 > 0.5$.

Figure 5.2: Normalized D_a values corresponding to different geometry, squares of side $a=5 \mu m$ and circles of radius $R=5 \mu m$. The curves overlap when are plotted vs. t_r .

It is gratifying to see that the results for Soderman's model agree so well with the simulations. The small difference between the results from Tanner's model and Soderman's model is consistent with the fact that the geometry is different and is not unexpected. Indeed, it is a pleasant surprise that the two curves are in such close agreement.

5.1.2 Full Decay Curve

The initial slope can be a very useful parameter but it can also be misleading and can lead to erroneous interpretations of the data. It suggests that the diffusion decay is exponential and, as has been shown in Chapter 4, this may be far from the truth. The interpretation of MR diffusion data for tissue is complicated due to the presence of boundaries such as cell membranes. In a finite volume, the displacement is limited by boundaries and, for long t_d , the displacement will be determined by the dimensions of the studied volume rather than the free diffusion coefficient. In this section an analysis of the full diffusion decay is presented.

The shape of the diffusion decay changes significantly with diffusion time. In the weak collision limit ($t_r < 0.25$) it appears to be a bi-exponential decay. For the intermediate collision limit ($0.25 < t_r < 2.5$) the decay has some resemblance to a diffraction pattern. In the strong collision limit ($t_r > 2.5$) the decay appears to be exponential. Since the decay is substantially different in these three domains they will be considered separately.

a). Weak Collision Domain

In this limit, the diffusion decay appears to be bi-exponential with one of the exponential terms being associated with a very small fraction (~1%) of the total decay. Since in this case the diffusion times are very short, most particles will not have diffused far enough to feel the presence of the boundaries. The measured diffusion coefficient for these particles can be expected to be close to the diffusion coefficient for free diffusion. The small second component presumably corresponds to the fraction of spins that have

been reflected from the walls. This fraction increases with increasing t_d consistent with this interpretation.

In an attempt to characterize these curves in a more reliable way than using just the initial slope, the decay curves were fit to a bi-exponential function using a least-squares algorithm. These results are summarized in Table 5.1 and a typical fit is shown in Fig. 5.3.

$t_d (ms)$	$D_1 (\times 10^{-3} mm^2 / s)$	$D_2 (\times 10^{-3} mm^2 / s)$	f	Chi-squared ($\times e^{-5}$)
0.03	1.822	0.1963	0.0178	1.71
0.05	1.7786	0.1956	0.0229	2.16
0.07	1.7436	0.19456	0.027	2.47
0.1	1.7012	0.1932	0.032	2.78
0.3	1.5268	0.18845	0.0521	3.22
0.5	1.42118	0.1773	0.0635	2.84
0.7	1.3428	0.171	0.0714	2.06
0.9	1.2712	0.1698	0.0747	4.95
1	1.253	0.163	0.0798	7.45

a).

$t_d (ms)$	$D_1 (\times 10^{-3} mm^2 / s)$	$D_2 (\times 10^{-3} mm^2 / s)$	f	Chi-squared ($\times e^{-5}$)
0.03	1.90485	0.29625	0.00593	0.78
0.05	1.87670	0.2974	0.00776	0.58
0.07	1.85980	0.297336	0.009253	0.94
0.1	1.83488	0.2969	0.011103	1.14
0.3	1.72220	0.282803	0.017838	1.64
0.5	1.65647	0.285096	0.024128	1.97
0.7	1.60052	0.279261	0.027914	1.96
0.9	1.55309	0.273747	0.030902	1.89
1	1.53170	0.271099	0.032184	0.18
2	1.36665	0.247136	0.040342	0.97
3	1.24755	0.223299	0.043104	0.88

b).

$t_d (ms)$	$D_1 (\times 10^{-3} mm^2 / s)$	$D_2 (\times 10^{-3} mm^2 / s)$	f	Chi-squared ($\times e^{-5}$)
0.3	1.695	0.157	0.0099	2.34
0.5	1.645	0.265	0.0257	5.03
0.7	1.6296	0.2843	0.03143	0.28
0.9	1.5829	0.3918	0.04811	1.88
1	1.551	0.2636	0.0369	0.65
2	1.36	0.2472	0.043	2.2
3	1.2437	0.248	0.046	5.94

c).

Table 5.1: The fitted model parameters for: a) Tanner's b) Soderman's and c) MC considered models where D_1, D_2 represent the fast and slow decaying coefficients, respectively and f represents the fraction corresponding to the slow component. As anticipated, this fraction increases with t_d . The *chi-squared* values are also reported.

Figure 5.3: An example of a bi-exponential fit to the decay curve (Soderman's model, $R=5 \mu m$) showing its fast (dotted line) and slow (broken line) components.

The fraction of particles associated with the slowly decaying component grows with increasing diffusion time from less than 1% to almost 5% for the circles and almost 8% for the square cells. The second diffusion coefficient is smaller than the first by about an order of magnitude but decreases slightly as diffusion time increases.

If our suggestion that the fast decaying component corresponds to particles undergoing free diffusion while the slow component is for particles that have been reflected from the cell wall is correct, then it is to be expected that D_1 and D_2 should both be independent of diffusion time and that D_1 would be equal to D_0 . The small deviation from this prediction for D_2 is likely associated with a distribution of behaviours for particles that have been reflected from the wall. Many of these will have had a single reflection but some will have undergone multiple reflections. The number experiencing multiple reflections will increase with t_d . The small t_d dependence observed for D_1 is likely a result of the inaccurate characterization of the D_2 due to the multiple reflections for some of the particles.

In spite of all these small inconsistencies, the decay curves in this domain can be well characterized in terms of a bi-exponential decay curve and can be interpreted (with caution) in terms of one fraction of particles that has interacted with the barrier and one that has not. It may be instructive to analyze these decays using a fitting method, such as the Non Negative Least Squares algorithm [68], that does not restrict the number of components in the decay since the second component appears to be associated with a distribution of diffusion coefficients. However, such an analysis will not be presented here.

b). Strong Collision Limit

For very long diffusion times such that $t_d \gg \frac{a^2}{D_0}$ or $t_d \gg \frac{R^2}{D_0}$, the shape of the diffusion decay is determined by the structure of the bounding region rather than the coefficient of free diffusion with which the particles diffuse between collisions with the wall. When the diffusion decay in this limit is plotted against the wave vector $q = \gamma \delta G$ the decay has a $\text{sinc}^2(x)$ form (see Fig. 2.11). For planar geometry, the minima occur at

$q=n/a$ for $n=1,2,3,\dots$, whereas for spherical geometry the minima occur at $q=n/R$ where the first two values of n are 0.61 and 1.12 [66]. These minima, shown in Fig. 5.4 are an additional source of information and they can be used to extract information about the size and structure of the region in which the particles are contained.

The b -value is related to q by:

$$b = 4\pi^2 q^2 t_d \quad (5.1)$$

Thus, for decay curves as a function of b we would expect to see minima at $b = 4\pi^2 \frac{n^2}{a^2} t_d$ and $b = 4\pi^2 \frac{n^2}{R^2} t_d$ respectively. For cells with $a=5\mu m$ or $R=5\mu m$ this corresponds to b -values of $b=1579 t_d \text{ s/mm}^2$ or $b=2350 t_d \text{ s/mm}^2$, respectively. For the first minimum these b -values are well beyond the range of b -values considered for most of the diffusion times used in this research. This can be seen from the $q_{max} a$ and $q_{max} R$ values Table 4.1 and Table 4.2, respectively. It is also well beyond the range that is accessible with a state of the art clinical MR imager for accessible t_d 's.

Fig. 5.4 and Fig. 5.5 show the echo amplitude for square and circular geometry, respectively. For the cases when t_d is small, the decay is monotonic whereas for large t_d prominent minima depending on the size of the system occur. The position of the first minimum for circular geometry is small compared with the square geometry, because the average distance traveled by the particles between the walls in the direction of the gradient is smaller in the first case than for the latter. It is interesting to see that for large t_d the position of the minima is shifted toward larger b -values and the signal drop at the minima is more dramatic. As the diffusion time increases, the effect of the impenetrable barriers becomes more prominent.

For circular geometry, the position of the minima converged with respect to variations in diffusion times. Thus, the position of the first minimum should be used when information about the restricted volume is extracted from this diffraction pattern. In this case, the t_d has to be adjusted such that at least one minimum can be detected.

Figure 5.4: Echo-attenuation for spins trapped between parallel planes separated by a distance $a=5 \text{ } \mu\text{m}$ for $t_d = 0.01 \frac{a^2}{D_0}$, $t_d = 0.1 \frac{a^2}{D_0}$ and $t_d = \frac{a^2}{D_0}$, plotted vs. qa and b -value.

The diffraction pattern depends on the size of the restricting volume.

Figure 5.5: Echo-attenuation for spins trapped in a circle of radius $R=5 \mu\text{m}$ for $t_d = 0.01 \frac{R^2}{D_0}$, $t_d = 0.1 \frac{R^2}{D_0}$ and $t_d = \frac{R^2}{D_0}$, plotted vs. qa and b -value. The diffraction pattern depends on the size of the restricting volume.

The diffusion decays shown in Figs. 4.1, 4.3 and 4.5 for large diffusion times appear to be exponential. However, from the above discussion it should be clear that this is just the small b -value behaviour. It is a reasonable approximation in this case for the b -values considered, but it must be realized that this is only valid for small b -values. If the decay is measured for larger b -values, a diffraction-like pattern will be the result with minima occurring as discussed above.

c). Intermediate Collision Domain

For intermediate diffusion times, $t_d \sim \frac{a^2}{D_0}$ or $t_d \sim \frac{R^2}{D_0}$, a systematic deviation from the exponential behaviour was observed. Although non-exponential diffusion decays may arise from restricted diffusion, this will not necessarily give rise to a bi-exponential decay or even a multi-exponential decay. The computed curves show a clear upward curvature for b -values larger than b_{min} . Strong interaction effects are evident when t_d is comparable to $\frac{R^2}{D_0}$ or $\frac{a^2}{D_0}$. For this particular range of diffusion times, a significant fraction of the particles will feel the effects of the boundaries. As a response of these interactions the mean squared displacement along the gradient axis does not scale linearly with t_d , thus the diffusion coefficient will appear to be observation time dependent. The structural information contained in the decay curve due to the fact that the molecules suffer collisions with the boundaries, is the major reason for the non-exponential behaviour.

For this range of diffusion times the behaviour of the diffusion decay displays aspects of both the weak and strong collision limits. There is an indication of the minima expected for the strong collision limit but they are very broad and they are not necessarily located at the expected b -values. For the decays computed from Soderman's equation the minima occur at: $qR=0.73$ for $t_d=5$ ms, $qR=0.67$ for $t_d=7$ ms and $qR=0.63$ for $t_d=10$ ms, which are in reasonable agreement with the value of $qR=0.61$ expected for the strong collision limit [24] for circular cells. For the decays calculated from Tanner's equation, the minima occur at $qa=1.082$ for $t_d=2$ ms, $qa=1.037$ for $t_d=3$ ms and $qa=1.006$ for

$t_d = 5 \text{ ms}$, which are in very good agreement with the value of $qa=1$ expected for the strong collision limit for square cells. These small differences are not unexpected and are simply a result of not being in the strong collision limit where these predictions would be valid.

Fig. 5.6 shows the computed signals plotted vs. b using Tanner's and Soderman's theoretical expressions. The solid line represents the decay curve as calculated using Tanner's equation, assuming the particles are diffusing between planes separated by a distance $a=10\mu\text{m}$. The input parameters for this calculation are $t_d=9.5\text{ms}$, $D_0 = 2 \times 10^{-3} \text{ mm}^2 / \text{s}$ and $N=20$. The dotted line displays the attenuation curve using Soderman's equation. For the purpose of comparison, the radius of the cylinder is set to $R=10 \mu\text{m}$ and $t_d=30 \text{ ms}$ so that the two calculations correspond to the same reduced time $t_r=0.19$. After a sufficient number of iterations, $n=k=20$ the curve converges rapidly. In Tanner's and Soderman's attenuation curves, the first feature one would notice is the very good agreement in terms of the initial slope. D_a is $0.748 \times 10^{-3} \text{ mm}^2 / \text{s}$ for Tanner's and $0.727 \times 10^{-3} \text{ mm}^2 / \text{s}$ for Soderman's curve. The main difference is that the minimum in Tanner's curve occurs at a lower b -value than for Soderman's. This is expected from theory. Despite of a high degree of mismatch at the end of the curves, the shape of the curves is about the same.

The numerical results obtained using Tanner's and Soderman's equations to compute the signal decay are self-consistent. A pleasing thing about these curves is that they converge after only a few iterations for all but very small t_d . Validation of numerical convergence data holds to a high degree of accuracy. It was found that the number of terms chosen in the summation included in the theoretical expressions to compute the attenuation curves needs to exceed a critical value in order to obtain meaningful and accurate results. After this critical number of iterations is exceeded the convergence occurs rapidly.

The analysis of convergence for the simulated signal decay curves allows the validity of the MC results to be checked. The geometry for the convergence tests was chosen such that the results of the simulation can be compared with known analytical

solutions. The main disadvantage of the MC simulations is the fact that the results suffer from statistical uncertainties, the magnitude of which is controlled by the number of the trajectories simulated.

Figure 5.6: Representative decay curves computed using the analytical expressions given by Tanner (solid line) and Soderman (broken line) for $t_r=0.19$.

To trust the Monte Carlo algorithm presented in Section 3.2, the number of particles used in the simulations has to be beyond the value determined to be the limit of convergence. In order to be valid, the results are not allowed to change due to a lack of iterations. All the data obtained from simulations used $N=500000$ particles. It can be seen in Fig. 4.7, that after a sufficient number of particle iterations, the size of misfit goes down below 0.5%. The limit of convergence was estimated to be approximately 300000. This result confirms that 500000 particles are well beyond the convergence limit. The simulated decay curves have also been checked for consistency with the corresponding

Soderman curves to make sure that the data are accurate. The maximum deviation was found to be less than 3%.

5.1.3 Distribution of Cell Sizes

In human white matter the axons are not all the same size. For example, in the corticospinal tract, 90% of the 700,000 axonal fibers are between 1 and 4 microns [9]. Most of the remaining fibres are between 5 and 10 microns and a small fraction are between 10 and 22 μm . To investigate the effect of such a distribution of axon's sizes, the distribution was modelled as a Gaussian distribution with a standard deviation of 0.5 μm . The intracellular MR signal is a function of the cross-sectional area of the axons. Soderman's equation was used to compute the intracellular diffusion decay for each axon size considered in this extended model. The composite curves were calculated as a weighted-mean from:

$$S_{sum}(b, t_d) = \sum_i w(R_i) S(b, t_d, R_i), \quad (5.1)$$

where $w(R_i)$ gives the relative contribution to the composite signal from each axon radius considered in the distribution computed as a Gaussian probability. In computing these composite curves, thirteen cell radii were used, each composite curve with mean values of $R=3, 4, 5, 6.5$ and $9.5 \mu m$.

From Fig. 4.8 it can be seen that the decay curves for such distributions do not differ substantially from the decay curves for the distribution mean. The main effect seems to be that any structure observed in the curves is smoothed out to a certain extent. Thus, to a first approximation the distribution of axonal fibres can be ignored and the analysis can be done assuming that all of the cells have the mean size.

The smoothed curve for the distribution is more amenable to characterization using a bi-exponential fit. For these diffusion times where it was possible to obtain such a fit, the fitted model parameters are listed in Table 5.2 along with D_a values measured from the initial slope of the decay curves plotted in Fig. 4.8. It is convenient to be able to characterize these curves in this way, however, since the diffusion times chosen for this analysis correspond to the intermediate collision domain, the values for D_1, D_2 and f

given in Table 5.2 should be treated simply as fitting parameters. They should not be expected to relate in any meaningful way to separate groups of particles, as was proposed for the weak collision limit.

R (μm)	With distribution			
	$D_1(\times 10^{-3} \text{mm}^2 / \text{s})$	$D_2(\times 10^{-3} \text{mm}^2 / \text{s})$	f	$D_a(\times 10^{-3} \text{mm}^2 / \text{s})$
3	1.35562	0.2449	0.0421	1.353
4	1.2752	0.22123	0.0434	1.154
5				0.727
6.5				0.03553
9.5				0.2823

a).

R (μm)	Without distribution			
	$D_1(\times 10^{-3} \text{mm}^2 / \text{s})$	$D_2(\times 10^{-3} \text{mm}^2 / \text{s})$	f	$D_a(\times 10^{-3} \text{mm}^2 / \text{s})$
3	1.324	0.2483	0.044	1.34
4	1.2256	0.219	0.0438	1.14
5				0.7216
6.5				0.03551
9.5				0.2816

b).

Table 5.2: Results obtained by performing a bi-exponential Least-Squares fit to curves plotted in Fig. 4.8 along with measured D_a values calculated from the initial slope: a) only for curves where the size distribution was considered b) for curves calculated for the distribution mean.

The behaviour seen in the computed diffusion decay considering a Gaussian distribution of cell sizes correlates with the geometry of the restricting boundaries. By comparing the results, the transition from free diffusion to restricted diffusion remains characteristic. Overall, the composite curves appear to closely mimic the behaviour of their mean.

5.2 Extracellular Diffusion for Close-Packed Configuration of Cells

5.2.1 Initial Slope

The motion of water molecules within the extracellular compartment is restricted by the presence of the cells. In fact, in real tissue the extracellular motion is characterized by a tortuosity coefficient as a result of the cells and other organelles partially obstructing the water motion [27] with the degree of tortuosity depending on how closely packed the cells are. In this section, diffusion in the situation where the cells are in contact is considered. In this limit the water diffusion in the extracellular compartment is totally restricted and the water molecules are confined to a single extracellular compartment. This situation is shown in Fig. 3.2a. The model is analogous to the restricted intracellular diffusion considered in previous sections except that the geometry is different. To be able to observe the effect of the cell walls on the restricted extracellular diffusion the diffusion times must be long enough to ensure that most of the particles interact with the cell barriers. The more general case of extracellular diffusion shown in Fig. 3.2b, where the particles can move from one extracellular compartment to the next, is considered in the next section.

The diffusion decay curves obtained from this series of simulations are shown in Fig. 4.9a. These curves were first analyzed in terms of the initial slope. The decrease of D_a with increasing t_d is visible even for short t_d . On this time scale, the diffusion length is relatively small compared with the restricted dimensions. Those molecules reflected from the cell membranes cause the decrease in D_a as t_d increases. The curvature of the semi-log plots of the diffusion decays for short t_d seen in Fig. 4.9a indicates non-exponential behaviour. The most reasonable interpretation for the two components is associated with the groups of spins, one that diffuses freely and the other one interacting with the walls. Although difficult to quantify analytically, the effect of restricted motion causes a significant decrease in the initial slope.

In Section 5.1 the D_a values for diffusion in circular and square cells were compared by plotting D_a vs. t_r , where $t_r = \frac{D_0}{A} t_d$ and A is the area of the cell in which the particles are diffusing. The area of the triangle-like extracellular compartment can be expressed in terms of the intracellular radius R as $A=0.161 R^2$. With this expression t_r values can be obtained which allow us to compare the time dependence of D_a for the extracellular space with D_a values obtained using the MC model for the circular intracellular space (see Fig. 5.7).

Figure 5.7: D_a vs. t_d for intracellular and extracellular diffusion in a lattice of circular close-packed cells where the intracellular volume fraction was $f_I=0.9$ and the extracellular volume fraction $f_E=0.1$. The parameters used are $R=5 \mu m$ and $D_0 = 2 \times 10^{-3} mm^2 / s$.

The two curves display the characteristic relationship between D_a and t_r . Qualitatively the curves are very similar but quantitatively there are some differences. The plots are not expected to overlap, and the difference observed is not surprising due to geometric difference between the two compartments. The deviation may be due in part to a different probability of reflection when the particles reach the confined borders.

From Fig. 5.7 is apparent that, even for the shortest diffusion times considered for the extracellular diffusion simulations, the behaviour of D_a suggests that the diffusion is in the intermediate collision domain. However, when the full diffusion decays are considered, the behaviour for extracellular diffusion differs from that which was observed for circular and square cells.

In the weak collision limit, based on the behaviour seen with the square and circular cells, a bi-exponential decay curve is anticipated with the contribution from the slowly decaying component contributing only a few percent. It could be argued that the curves at the lowest diffusion times considered are indeed bi-exponential but the slowly decaying fraction corresponds to 10% or more for these curves. The diffraction pattern expected for the intermediate diffusion times is also not apparent.

A suggested explanation for this observed behaviour for the extracellular diffusion is that the weak collision limit occurs at diffusion times smaller than $10 \mu s$ and the decay curves shown in Fig. 4.9 are all in the intermediate and strong collision limits. Furthermore, the expected b_{min} values may occur at values greater than $10000 s / mm^2$. This is consistent with the small size of the triangle-like extracellular compartments. The shape of the decay curves for $t_d=30\mu s$ and $t_d=70\mu s$ may be an indication of the presence of minima at b -values greater than $10000 s / mm^2$.

In an attempt to characterize the full diffusion decay the curves were fit to a bi-exponential function using a Least-Squares algorithm. The results are summarized in Table 5.3. In all cases the bi-exponential curves are in reasonable agreement with the simulated decays.

$t_d (ms)$	$D_1 (\times 10^{-3} mm^2 / s)$	$D_2 (\times 10^{-3} mm^2 / s)$	f	<i>Chi-squared</i> ($\times e^{-5}$)
0.03	1.593	0.346	0.0831	2.73
0.05	1.49	0.31	0.0923	0.76
0.07	1.4423	0.307	0.1102	0.83
0.1	1.3496	0.2669	0.111	2.04
0.3	1.066	0.2083	0.1538	1.51
0.5	0.9149	0.1785	0.1783	2.13
0.7	0.142	0.142	0.18026	1.89
0.9	0.091	0.65	0.1558	3.5
1	0.0849	0.594	0.1467	2.7

Table 5.3: The fast D_1 , slow D_2 decaying coefficients and the intercept f , obtained from a bi-exponential fit to the decay curves displayed in Fig. 4.9 along with the corresponding D_a values. The *chi-squared* value is also reported.

For small values of t_d it is tempting to interpret f as the fraction of molecules that interact with the structures. This fraction is expected to increase with increasing t_d . In contrast, for long t_d , long diffusion paths will quickly bring spins into contact with restricting barriers. When the diffusive motion becomes totally restricted this fraction ideally should be one. Under these circumstances, the dependence of D_a on tissue geometry will be at the highest level. In the long time limit, D_a reaches a constant value, which approaches zero.

5.2.2 Distribution of Extracellular Compartment Size

It is more realistic to consider a distribution of sizes for the extracellular compartments due to a distribution of intracellular radii. The resulting curve shown in Fig. 4.10 represents a Gaussian-weighted combination of thirteen different sizes for the extracellular space (see Section 5.1.3). This study confirms that the diffusion decay of a Gaussian distribution of extracellular spaces of different sizes is well represented by the behaviour of the mean.

This decay curve was also analyzed by fitting it to a bi-exponential function. The parameters obtained by performing a Least-Squares fit to the signal decay curve are listed in Table 5.4, along with D_a values.

With distribution				
$t_d (ms)$	$D_1 (mm^2 / s)$	$D_2 (mm^2 / s)$	f	$D_a (mm^2 / s)$
0.07	1.3503	0.2491	0.0809	1.353

a).

Without distribution				
$t_d (ms)$	$D_1 (mm^2 / s)$	$D_2 (mm^2 / s)$	f	$D_a (mm^2 / s)$
0.07	1.3785	0.2653	0.0896	1.351

b).

Table 5.4: The fast and slow components D_1 and D_2 as extracted by the fitting procedure from the decay curves shown in Fig. 4.10: a) considering the distribution b) ignoring the distribution. The intercept f of the slowly diffusing component along with D_a values is also given.

The assignment of the slowly diffusing fraction to the spins that interact with the wall may be further complicated, due to a complex diffusion process. The resolution between those two fractions of spins, one that diffuses freely and the other one that interacts with the walls could not be established due to a distribution of reflections that one particle may suffer.

5.3 Extracellular Diffusion for Hexagonal Configuration of Cells

5.3.1 Initial slope

As the literature already reported [67], water diffusion in the interstitial space of the tissue is partially hindered, but the particles may have rather long diffusion pathways.

To test this approach, the tissue was modelled as a porous medium, a wide collection of spherical pores with interconnecting channels. The non-abutting cells define the small extracellular compartments as being connected with one another. The results of the simulations for this specified geometry reveal information about their size and connectivity. In this context, the model considered here could be approximated as a porous medium.

As expected, D_a is affected by cell separation as a consequence of the effectively infinite but still hindered diffusion paths in the extracellular space as opposed to the restricted spaces when the cells are in contact with each other. The results presented in Fig. 4.11 indicate that the relative volume of the extracellular space is the primary factor responsible for the observed changes in D_a . The plots show an increase in D_a when the separation distance between cells is increased. When t_d is small, the spins from one pocket diffuse a limited distance from their starting position. As time increases, more and more particles will diffuse to the adjacent compartments and beyond. For large t_d , a spin originating in any location will have the possibility of ending up anywhere in the extracellular space. This is reflected in bumps in the t_d dependence, corresponding to the fraction of spins that pass through the small channels connecting different extracellular compartments and, as a result, increasing their molecular displacement. Under these circumstances, D_a will increase once enough particles have passed through the connecting channels. Thus, measurements of this sort reveal structural information about porous medium. The diffusion dynamics reflected in variations of D_a as a function of t_d and R_m reveal connectivity of the pore spaces and a transition from very strong hindered diffusion to weakly hindered diffusion, which is close to free diffusion.

Chapter 6

Conclusions and Future Directions

6.1 Conclusions

Cumulatively, all of the observations led to the conclusion that the restricted diffusion of water molecules in a single compartment (e.g. intracellular or extracellular) results in a complicated signal decay. In some cases the decay resembles a mono- or bi-exponential decay while in others it showed definite minima and looked more like a diffraction pattern. The diffusion decays were studied for square and circular cells as well as a triangular-like extracellular space. The initial slopes of these curves were in close agreement with each other when plotted against the reduced diffusion time, t_r . The decay curves for the different geometries differed at higher b -values, consistent with the different shaped cells.

These results suggest that the initial slope of the diffusion decay, when analyzed in terms of the reduced time is independent of the actual shape of the cell. This is important since cells in the body are, in general, not square or circular or any other regular geometric shape.

The diffusion decay is very sensitive to the size of the area in which the particles are diffusing. Furthermore, in human tissue the size of the cells is not uniform: there is a

range of cell sizes. It is reasonable to expect that this could complicate the analysis of the diffusion decay considerably. However, the results in this thesis show that the diffusion decay for a gaussian distribution of cell sizes differs very little from the decay for the mean of the distribution. Thus, even though this distribution exists, the decays can be meaningfully analyzed in terms of the mean size.

The shape of the diffusion decays for restricted diffusion are, in general, very complicated and do not lend themselves to easy experimental interpretation. In an attempt to characterize the curves using multi-exponential fitting, a least squares analysis was used. A good fit to the data was found for short diffusion times using a bi-exponential function, while for long diffusion times were fit well to an exponential decay. For intermediate times, the curves did not lend themselves to this type of analysis. In general, the diffusion coefficients and signal fractions obtained from this sort of analysis must be treated as fitting parameters rather than physical constants. However, for short diffusion times it appears reasonable to associate one of the components with spins that are diffusing freely and the other with spins that have had interactions with the walls of the cell. The characterization of the diffusion decays in terms of simple functions such as mono- or bi-exponential decays rather than complicated general expressions such as those derivated by Tanner [5] and Soderman [24], could be very useful in the interpretation of experimental data from human subjects.

Monte Carlo simulations of diffusion in the extracellular space showed that the diffusion decay is very different for tightly packed cells, where the diffusion is similar to intracellular restricted diffusion, as compared to loosely packed cells where the situation is more like free diffusion. For intermediate packing, maxima appear in the diffusion time dependence of D_a . The position of these maxima is a function of the extracellular geometry and could be useful in characterizing tissues.

A proper interpretation of the diffusion decay curve requires the consideration of all possible physiological processes that can affect the decay. A systematic study of this problem, considering some of the most important contributions to the diffusion decay, has been presented in this thesis. This work can form the foundation on which further

work that incorporates additional complicating factors such as exchange of water molecules between intracellular and extracellular compartments, can be based.

6.2 Future Directions

Although MRI measurements cannot provide the full range of data used in the analysis given in this thesis, the D_a results presented here provide a basic platform to give measured D_a values a more physical meaning. A complete biophysical explanation of water diffusion in brain tissue requires a solid framework to assess the contribution of the different mechanism responsible for tissue D_a changes. Cell structures such as membranes, influence of a multi-compartment system and capillary network [21] as well as contribution from tissue perfusion [50] contribute to a complex diffusional process.

In order to characterize the overall water diffusion in brain tissue, it is desirable to achieve first a complete separation between the contributions of the intracellular and extracellular components. The Monte Carlo model was used to compute the diffusion of water in both intracellular and extracellular volumes with no exchange between them. The radius of the cell was set to $R=5\mu m$ and the relative fraction of the intracellular compartment was 0.95. This curve was then analyzed using a bi-exponential function assuming that both intracellular and extracellular diffusion decays are exponential. This is the approach commonly used in the literature. Comparison of the fitted parameters shows significant differences with the expected volume fraction of the two compartments appearing to challenge the validity of the assumption regarding the contributions from each compartment. The non-exponential signal attenuation curves indicate that a complete description of water diffusion within such a heterogeneous system cannot be explained by the weighted-average of the exponential intracellular and extracellular contributions.

The simulations may be further refined, by including the possibility that the intracellular and extracellular compartments have different T_2 values. Assaf et al. [52] and Mulkern et al. [33] pointed out the possibility that T_2 relaxation weighting could

influence the behaviour of the diffusion decay. If this hypothesis is true, then an echo-time dependence in the estimated intracellular and extracellular volume fractions of the diffusion attenuation curve can be expected to occur [47].

A problem with our model is that the exchange between compartments was totally ignored, while in real tissues the cell membranes are semi-permeable. Water exchange between compartments is expected to correlate with membrane permeability. It has been speculated that the water exchange critically affects the apparent fractions of diffusion components and is a likely cause for the mismatch between the apparent diffusion component fractions and tissue compartment volume fractions [31]. The changes in the cell membrane permeability to water may be exclusively responsible for tissue D_a changes. This kind of study can be used to assess the tissue permeability and in turn, the permeability-weighted MR images which have proven their value in the reliable clinical diagnosis of stroke [15] or to assess the pathological elements of brain tissue, e.g. multiple sclerosis [13].

It can be concluded that considerable work remains to be done to properly interpret a non-exponential diffusion decay of water in the human brain.

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