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**MEASUREMENT OF THE APPARENT DIFFUSION COEFFICIENT
OF WATER IN WHITE MATTER USING MAGNETIC
RESONANCE IMAGING: A PHANTOM STUDY**

by

Yvan Gauthier

B.Sc. Université de Sherbrooke

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

Carleton University
Ottawa, Ontario
January, 2000
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Dr. Ian Cameron, Thesis Supervisor

Chair

Physics Department

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January, 2000

Abstract

The apparent diffusion coefficient, D_a , was measured inside a hollow fibre phantom which was used to model the axonal system of white matter. The anisotropic values of D_a are reported for diffusion times ranging between 50 ms and 1020 ms, and are compared with the results of Monte-Carlo simulations. In the presence of a slow pulsed flow in the extraluminal volume of the phantom, the diffusion decay of the transverse magnetization that was observed experimentally has a multi-exponential shape. A similar multi-exponential behaviour of the diffusion decay was also observed *in vivo* within the white matter of 4 normal human subjects. This behaviour could possibly be associated with movement of the extracellular fluid between the axons.

À la mémoire de mon père.

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Chapter 1 Introduction

1.1. Historical Background

In 1945, the groups of Bloch [1] and Purcell [2] independently measured, for the first time, the phenomenon of nuclear magnetic resonance (NMR) absorption in bulk materials. After this important discovery, it was rapidly realized that NMR constitutes a unique tool for the measurement of diffusion and flow [3, 4]. However, through the 50's and 60's, NMR was almost exclusively used by chemists and physicists for spectroscopy purposes. At this time, the most satisfactory method for measuring diffusion was the *constant field gradient spin-echo* method, but this technique suffered from several limitations and was very demanding on the hardware [5]. A major improvement in the diffusion measurement methods occurred in 1965 when Stejskal and Tanner [6] proposed the *pulsed gradient spin-echo* (PGSE) technique. This technique, which is still the most widely used method for measuring diffusion by NMR, made the diffusion measurements technically easy to perform and opened the door to widespread applications in several branches of physics, chemistry, and biology where diffusion processes are under study.

In the early stages, NMR spectrometers did not provide spatial localization of the signal. Spatial encoding of the NMR signal was first accomplished by Lauterbur [7] in 1972. By utilising the shift in resonant frequency resulting from the imposition of magnetic field gradients, his group produced the first images of an inhomogeneous object - two tubes of water - laying the foundations of magnetic resonance imaging (MRI).

At the time, Lauterbur [7] suggested that MRI could eventually be used to produce two- or three-dimensional images displaying the diffusion coefficients of objects. But it was only in 1982 that Mansfield [8] really established the basics of

diffusion imaging for biomedical applications by proving that the combination of MRI techniques and pulsed gradient methods was, in theory, feasible. Three years later, researchers were able to generate diffusion maps of phantoms [9] and human subjects [10, 11]. The investigation of the diffusional processes rapidly became an important topic in the field of MRI. Measuring molecular displacements in biological tissues *in vivo* and non-invasively has led to several clinical applications in the last decade. For example, diffusion-weighted imaging (DWI) is now used for detecting brain pathologies, such as the early stage of infarction caused by stroke [12], or the progression of Multiple Sclerosis plaques [13]. DWI can also be utilized for determining nerve fibre orientation [14] or for monitoring laser surgery [15].

All these applications of DWI are based on the spatial mapping of the *apparent diffusion coefficient*, D_a , of water molecules, which is defined by

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

where $\langle r^2 \rangle$ is the mean square displacement of the molecules and d is the dimensionality of the system. The apparent diffusion coefficient D_a differs from the usual diffusion coefficient D by the fact that T_d , which is the time during which the diffusion process is observed for a particular measurement, is not considered infinitely long. Thus, the value of D_a measured experimentally will depend on the value of T_d . For example, in brain tissues, molecular diffusion is generally restricted in space because the molecules encounter cellular membranes, the myelin sheath or other biological structures. If the observation time T_d is very short, the movement of the molecules is not hindered by these structures and the molecules diffuse freely. However, as T_d gets longer, an increasing fraction of the molecules hit the biological structures, lowering the mean square displacement $\langle r^2 \rangle$ of the molecules and thus lowering D_a . Diffusion-weighted images of the same slice of the brain can look very different from each other if the diffusion time is not the same for each of them.

In addition to T_d , the value of D_a is affected by numerous aspects of the cellular environment. In an anisotropic environment, D_a will depend on the direction of the measurement. If water transport or blood perfusion happen in the region of interest,

these phenomena will tend to increase $\langle r^2 \rangle$, and thus artificially increase the value of D_a . Furthermore, tissues may contain several types of cells and fluids, with different values of D_a within each of them. All these factors can sometimes make it difficult to understand the mechanisms at the cellular level that are responsible for the observed diffusion contrast. However, this also means that DWI has the potential to become a powerful method for probing tissues at the cellular level.

To date, DWI have been used with the most success in the white matter of the brain, where the tissues consist predominantly of myelinated neuronal axons bundled into large tracts [16]. Each axon can be thought of as a long cylindrical fibre with a diameter of about $5 \mu\text{m}$ covered by a myelin sheath which is about $1 \mu\text{m}$ thick. Accordingly, cerebral white matter can often be considered locally as a bundle of parallel fibres with circular cross-sections. But despite the fact that the structure of white matter seems relatively simple, the interpretation of the *in vivo* measurements of D_a in the human brain remains a complicated task. Firstly, it is often difficult to isolate or control the processes affecting D_a at the cellular level. Moreover, the diffusion measurements are extremely sensitive to bulk motion, such as unintentional patient motion, blood pulsations or cerebrospinal fluid (CSF) flow. The duration of the NMR scan must also be kept reasonable to ensure patient comfort and minimize unintentional motions.

Another problematic aspect concerning the evaluation of D_a is that the NMR sequences currently used in clinical applications and in most research studies calculate D_a assuming that the dependence of the NMR signal as a function of the b -value – a measure of the strength of the diffusion gradient – decreases exponentially with the b -value. This is rigorously true only in the case of free diffusion, where the relative attenuation $M/M(0)$ of the NMR signal can be used to evaluate the diffusion coefficient D from the equation

$$\frac{M(b)}{M(0)} = \exp(-bD), \quad (1.2)$$

which will be derived later in Section 2.2. However, when diffusion is restricted in space, the shape of the *diffusion decay* of M as a function of b differs from the

exponential behaviour. If the system under study is composed of several fluids with different diffusion coefficients, or if the fluid(s) are flowing, then the diffusion decay may also deviate from an exponential shape. Recent studies [17, 18, 19] about diffusion in the brain have concluded that the shape of the diffusion decay in the white matter is multi-exponential, as is confirmed in the present work. Hence, a monoexponential fit performed on *in vivo* data may give inaccurate values of D_a . The solution to this problem resides in the identification of the source of each component of the multi-exponential diffusion decay. To date, there is no consensus in the literature on the causes of this multi-exponential behaviour.

1.2. Thesis Overview

One possible explanation of the deviation of the diffusion decay from the exponential behaviour *in vivo* is the pulsatile movement of the extracellular fluid between the axons. Indeed, when the blood pulses through the brain vasculature, it creates a hydrostatic pressure on the cerebral tissues that can cause a pulsation of the extracellular fluid between the nervous fibres. In order to assess how such a motion can affect the shape of the diffusion decays, and ultimately get a better understanding of the water diffusion in human brain *in vivo*, this biological phenomenon was reproduced artificially in a phantom modelling the cerebral white matter. In a phantom study, the number of unknowns is considerably less than in an *in vivo* study and most of the parameters can be modified or controlled. The problems of scan duration or patient motion are also avoided by the use of a phantom. Therefore, a phantom can simplify the investigation of some diffusion effects observed in the white matter.

The phantom used in the present research is made of hundreds of parallel hollow fibres made of cellulose acetate. Different fluids can fill the intra- and extraluminal volumes of the fibres, or flow within them such that an adequate model of the white matter axons can be created. In this work, D_a is first measured as a function of T_d for water inside and/or outside the phantom fibres, and for diffusion sensitization parallel and perpendicular to the fibre orientation. Monte-Carlo (MC) simulations

and analytical equations are then used to analyze the results of these experiments. Finally, the multi-exponential shape of the diffusion decays observed *in vivo* in the cerebral white matter of human volunteers is reproduced in different phantom experiments where water was forced to flow in the extraluminal compartment using a diaphragm pump.

Chapter 2 Theory

2.1. Diffusion Theory

Consider a fluid consisting of similar molecules in Brownian motion. Let the symbol $n(\mathbf{r}, t)$ denote the number density of particles at position $\mathbf{r}$ at time t . In an equilibrium situation, $n(\mathbf{r}, t)$ is independent of the position. However, if the molecular distribution is not uniform then it will cause a motion of the particles tending to increase the entropy and make the concentration $n(\mathbf{r}, t)$ more nearly uniform. The resulting flux of molecules $\mathbf{J}(\mathbf{r}, t)$ due to this motion is defined, according to Fick's law, as

$$\mathbf{J}(\mathbf{r}, t) = -D\nabla n(\mathbf{r}, t), \quad (2.1)$$

where D is the *diffusion coefficient* of the fluid.

Although $\mathbf{J}$ represents the current density of the particles, the *total* mean number of molecules per unit of volume remains constant and there is no mass motion of the whole substance [20]. This conservation of the mass is ensured by the *equation of continuity*, which may be written as [21]

$$\nabla \cdot \mathbf{J}(\mathbf{r}, t) + \frac{\partial n(\mathbf{r}, t)}{\partial t} = 0. \quad (2.2)$$

Substituting equation 2.1 into equation 2.2, we obtain the *diffusion equation*:

$$\nabla^2 n(\mathbf{r}, t) - \frac{1}{D} \frac{\partial n(\mathbf{r}, t)}{\partial t} = 0. \quad (2.3)$$

The solution of this equation for molecules satisfying the initial condition $n(\mathbf{r}, 0) = \delta(\mathbf{r} - \mathbf{r}_0)$, with the boundary condition $n(\mathbf{r}, t) \rightarrow 0$ as $\mathbf{r} \rightarrow \infty$, follows a Gaussian distribution:

$$n(\mathbf{r}, t) = \frac{N}{(4\pi Dt)^{3/2}} \exp \left[\frac{-(\mathbf{r} - \mathbf{r}_0)^2}{4Dt} \right], \quad (2.4)$$

where N is the total number of molecules. Conservation of N in the fluid requires that

$$\int_0^\infty n(\mathbf{r}, t) \cdot 4\pi r^2 dr = N \quad (2.5)$$

at all times. From Eq. 2.4, the probability that a molecule initially at $\mathbf{r}_0$ will diffuse to $\mathbf{r}$ during the time t can be determined. The coefficient of diffusion is related to the mean square displacement of the molecules which, using Eq. 2.4, can be written as

$$\begin{aligned} \langle (\mathbf{r} - \mathbf{r}_0)^2 \rangle &= \frac{4\pi}{N} \int_0^\infty n(\mathbf{r}, t) (\mathbf{r} - \mathbf{r}_0)^2 dr \\ &= 2dDt, \end{aligned} \quad (2.6)$$

where d is the dimensionality of the molecular motion [21]. This equation is generally used for simple homogeneous systems under equilibrium conditions, i.e. when t is long enough that D remains constant as a function of time. However, for *in vivo* measurements, the displacement of the molecules may be affected by other processes in addition to the Brownian movement of the molecules. For instance, the molecules may be impeded by obstacles present in the system or their displacement may be enhanced by other transport processes in addition to diffusion. Moreover, the observation time T_d is sometimes very short experimentally. This explains why in the NMR literature [5, 17, 18, 22, 23] care is taken to distinguish between the measured value of the diffusion coefficient, called the *apparent diffusion coefficient*, D_a , and the usual diffusion coefficient D . By analogy with Eq. 2.6, D_a is often defined as:

$$D_a = \frac{\langle (\mathbf{r} - \mathbf{r}_0)^2 \rangle}{2dT_d}. \quad (2.7)$$

This equation allows the determination of D_a in a straightforward manner by measuring the mean square displacement of the molecules. However, in order to achieve this task experimentally, a certain fraction of the molecules must be labelled and followed for a given period T_d . Traditional techniques are based on the insertion of radioisotopes or chemical tracers into the system under study. But the invasive nature of these procedures makes them impractical for *in vivo* measurements. On

the other hand, NMR field gradient techniques allow molecules to be labelled non-invasively, without changing their mobility, and permit the measurement of D_a with excellent precision.

2.2. Diffusion Measurement by NMR

Basic concepts of NMR – classical description

All atomic nuclei formed of an odd number of protons or neutrons, i.e. about two-thirds of all the stable atomic nuclei, have a non-zero intrinsic angular momentum $\mathbf{J}$ or a (dimensionless) nuclear spin $\mathbf{I} = \mathbf{J}/\hbar$ [24]. To understand how particles with spin behave in a magnetic field, consider for example an isolated proton with a charge $+e$ and a mass m . The rotation of the distributed proton charge around the axis of the angular momentum $\mathbf{J}$ gives rise to a magnetic dipole moment $\boldsymbol{\mu}$:

$$\boldsymbol{\mu} = \gamma \mathbf{J}, \quad (2.8)$$

where the proportionality constant $\gamma = e/2m$ is the *gyromagnetic ratio* of the proton [25]. If the proton is subjected to a magnetic field $\mathbf{B}_0$, the coupling between $\boldsymbol{\mu}$ and $\mathbf{B}_0$ creates a torque $\boldsymbol{\tau}$ that causes $\boldsymbol{\mu}$ to precess about $\mathbf{B}_0$ at an angle θ (see Fig. 2.1). This torque, which can also be defined as the rate of change of the angular momentum, is given by

$$\boldsymbol{\tau} \equiv \frac{d\mathbf{J}}{dt} = \boldsymbol{\mu} \times \mathbf{B}_0, \quad (2.9)$$

or using Eq. 2.8,

$$\left| \frac{d\mathbf{J}}{dt} \right| = \gamma J B_0 \sin \theta. \quad (2.10)$$

As shown in Fig. 2.1, an infinitesimal change $d\mathbf{J}$ of the angular momentum during the time interval dt corresponds to a rotation through an angle $d\phi$ in the plane perpendicular to $\mathbf{B}_0$. This angle can be expressed as

$$\begin{aligned} d\phi &= \frac{dJ}{J \sin \theta} \\ &= \frac{\gamma J B_0 \sin \theta dt}{J \sin \theta} \\ &= \gamma B_0 dt, \end{aligned} \quad (2.11)$$

Figure 2.1: Coupling of the magnetic moment μ with the magnetic field B_0 , resulting in the precession of μ with angular velocity ω_0 about B_0 .

and therefore the frequency of precession ω_0 of the magnetic moment about B_0 , or the *Larmor frequency*, is simply given by:

$$\omega_0 = \frac{d\phi}{dt} = \gamma B_0. \quad (2.12)$$

The behaviour of single spins cannot be described adequately using classical theory, because of the quantum nature of nuclear properties. But no distinction can be made between classical and quantum descriptions when a large ensemble of spins is considered. In an externally applied magnetic field of flux density B_0 , the bulk magnetization $\mathbf{M}$ – the vector sum of the individual magnetic moments – is aligned parallel to B_0 under a thermal equilibrium situation. From Eq. 2.8, the macroscopic angular momentum vector of the spin system is simply $\mathbf{M}/\gamma$. As in

the case of Eq. 2.9 for a single proton, the motion of $\mathbf{M}$ can be described by the equation

$$\frac{d\mathbf{M}}{dt} = \gamma \mathbf{M} \times \mathbf{B}_0 \quad (2.13)$$

for which the solution corresponds to a precession of the magnetization about $\mathbf{B}_0$ at the Larmor frequency, ω_0 .

The magnetic resonance phenomenon results from the application of a second magnetic field, perpendicular to $\mathbf{B}_0$, and oscillating at ω_0 . The behaviour of $\mathbf{M}$ in this situation can be more easily described by considering a reference frame rotating with the angular velocity ω in the plane perpendicular to $\mathbf{B}_0$. The motion of $\mathbf{M}$ in this rotating frame can be related to the laboratory frame by

$$\frac{d\mathbf{M}}{dt} = \frac{\partial \mathbf{M}}{\partial t} + \omega \times \mathbf{M}, \quad (2.14)$$

where $\frac{\partial \mathbf{M}}{\partial t}$ represents the time derivative of $\mathbf{M}$ in the rotating frame of reference. Using Eq. 2.13, we obtain

$$\begin{aligned} \frac{\partial \mathbf{M}}{\partial t} &= \gamma \mathbf{M} \times \mathbf{B}_0 + \mathbf{M} \times \omega \\ &= \gamma \mathbf{M} \times (\mathbf{B}_0 + \omega/\gamma), \end{aligned} \quad (2.15)$$

which is equivalent to Eq. 2.13, except that $\mathbf{B}_0$ has been replaced by the *effective* field $(\mathbf{B}_0 + \omega/\gamma)$, i.e. the sum of the static field of the laboratory $\mathbf{B}_0$ and a fictitious field ω/γ .

Now suppose that a circularly polarized radio-frequency (*rf*) pulse of frequency ω and with magnetic flux density $\mathbf{B}_1$ is applied in the plane perpendicular to $\mathbf{B}_0$. The effective field $\mathbf{B}_{eff}$ in the rotating frame of reference becomes

$$\mathbf{B}_{eff} = \mathbf{B}_0 + \omega/\gamma + \mathbf{B}_1. \quad (2.16)$$

The effect of $\mathbf{B}_1$ will be to create an additional torque on $\mathbf{M}$. For example, if the frame is rotating at the Larmor frequency, $\omega = \omega_0 = -\gamma B_0$, then Eq. 2.15 simplifies to

$$\frac{\partial \mathbf{M}}{\partial t} = \gamma \mathbf{M} \times \mathbf{B}_1, \quad (2.17)$$

Figure 2.2: The evolution of $\mathbf{M}$ in the presence of a longitudinal magnetic field $\mathbf{B}_0$ and a transverse rotating field $\mathbf{B}_1$ a) in the rotating frame where $\mathbf{B}_1$ is stationary and b) in the laboratory frame.

which describe the motion of $\mathbf{M}$ rotating about the direction of $\mathbf{B}_1$ with an angular frequency $\omega_1 = \gamma B_1$. This phenomenon is illustrated in Fig. 2.2, for both rotating and static frames of reference.

Consequently, a *rf* pulse of duration t will cause $\mathbf{M}$ to rotate through an angle $\theta = \omega_1 t = \gamma B_1 t$. By choosing the appropriate values of B_1 and t , it is possible to rotate the magnetization into the plane perpendicular to $\mathbf{B}_0$ ($\theta = 90^\circ$, hence a 90° pulse) or in the direction antiparallel to $\mathbf{B}_0$ (180° pulse).

Quantum-mechanical description

As mentioned previously, the classical representation is not appropriate to describe the properties of isolated nuclei, and for individual nuclei we must resort to quantum-mechanical theory. The angular momentum $\mathbf{J}$ of a single nucleus is given by

$$\mathbf{J} = \hbar \mathbf{I}, \quad (2.18)$$

where $\mathbf{I}$ is the spin angular momentum and $\hbar$ is Plank's constant. From the uncertainty principle, the direction of $\mathbf{I}$ is unknown. An axis of quantization can be defined by placing the nucleus in a static magnetic field, $\mathbf{B}_0$, arbitrarily aligned along the z -direction. The nuclear dipole moment will have the potential energy given by the Hamiltonian, $\mathcal{H}$:

$$\begin{aligned}\mathcal{H} &= -\boldsymbol{\mu} \cdot \mathbf{B}_0 \\ &= -\gamma\hbar\mathbf{I} \cdot \mathbf{B}_0 \\ &= -\gamma\hbar I_z B_0.\end{aligned}\tag{2.19}$$

The eigenvalues of this Hamiltonian are simply the multiples ($\gamma\hbar B_0$) of the eigenvalues of I_z and the allowed energies are [25]

$$E_m = -\gamma\hbar B_0 m \quad (m = I, I-1, \dots, -I+1, -I),\tag{2.20}$$

giving $2I+1$ discrete energy states, or possible orientations of the spin. For the proton, $I = \frac{1}{2}$, and thus m can only take the values $\pm\frac{1}{2}$. As shown in Fig. 2.3, the two allowed quantum states of the proton have the following energies:

$$E_{+\frac{1}{2}} = -\gamma\frac{\hbar}{2}B_0\tag{2.21}$$

$$E_{-\frac{1}{2}} = \gamma\frac{\hbar}{2}B_0.\tag{2.22}$$

The separation ΔE between these two so-called Zeeman energies is the energy required by the proton for a transition from the lower state to the upper state. This transition can take place via spectral absorption, but to satisfy the conservation of energy, the photon energy must be supplied at the Larmor frequency ω_0 such that

$$\Delta E = \hbar\omega_0 = \gamma\hbar B_0.\tag{2.23}$$

Figure 2.3: a) Possible orientations of the nuclear spin $I = \frac{1}{2}$ in the static magnetic field B_0 , and b) the Zeeman splitting of the energy levels for a nuclear spin $I = \frac{1}{2}$ as a function of B_0 .

Statistical distribution of spin states

Now consider a large ensemble of N nuclei of spin $\frac{1}{2}$. If the spins are placed in a static magnetic field B_0 , they will be distributed between the two possible states, spin up ($m = \frac{1}{2}$) and spin down ($m = -\frac{1}{2}$), with the spin up state being more favoured because of its lower potential energy. At equilibrium, the population ratio of spins in each state is given by the Boltzmann distribution:

$$\frac{n_+}{n_-} = \exp\left(\frac{-\Delta E}{kT}\right). \quad (2.24)$$

where n_+ and $n_- = N - n_+$ are respectively the number of spins in the spin up and spin down states, k is the Boltzmann constant, and T is the temperature of the spin system. From this ratio, the population difference at thermal equilibrium can be expressed as

$$n_+ - n_- = N \frac{1 - \exp\left(\frac{-\Delta E}{kT}\right)}{1 + \exp\left(\frac{-\Delta E}{kT}\right)}. \quad (2.25)$$

At room temperature, the energy difference $\Delta E = \gamma \hbar B_0$ for a typical magnet ($B_0 = 1.5$ T) is over five orders of magnitude smaller than the Boltzmann energy kT . In consequence, Eq. 2.25 can be approximated by

$$n_+ - n_- = \frac{N \Delta E}{2 kT} \quad (2.26)$$

$$= \frac{N \gamma \hbar B_0}{2 kT}. \quad (2.27)$$

The total magnetization M_0 of the spins along the direction of B_0 , which is proportional to the difference in population of the two levels, may then be written as

$$\begin{aligned} M_0 &= (n_+ - n_-) \gamma \hbar \frac{1}{2} \\ &= \frac{N \gamma^2 \hbar^2}{4kT} B_0. \end{aligned} \quad (2.28)$$

Consequently, the intensity of the NMR signal will be proportional to the number of spins, or the volume of the region under study, and the intensity of the field B_0 .

Experimental approaches for diffusion measurements

The basic principle of diffusion measurement by NMR is to label a given fraction of the spins using a *rf* pulse in order to monitor their movement as in classical tracer methods. This diffusion monitoring can be achieved via different field gradient techniques. For example, consider a linear magnetic field gradient of strength G and duration δ , applied along the x -direction. The effect of the gradient is to change the phase of the spin's transverse magnetization as a function of the position along x by

$$\varphi_1 = \int_0^\delta \omega dt \quad (2.29)$$

$$\begin{aligned} &= \gamma \int_0^\delta Gx dt \\ &= \gamma G \delta x_1, \end{aligned} \quad (2.30)$$

assuming that the spin stays at the position x_1 during the application of the gradient. Now suppose that a 180° *rf* pulse is applied. Its effect will be to transform this phase shift into $-\varphi_1$. The application of a second magnetic field gradient of duration δ and strength G , at time Δ after the onset of the first gradient, produces another phase shift

$$\begin{aligned} \varphi_2 &= \gamma \int_\Delta^{\Delta+\delta} Gx dt \\ &= \gamma G \delta x_2 \end{aligned} \quad (2.31)$$

that counteracts the phase shift induced by the first gradient pulse, as shown in Fig. 2.4. This refocusing of the spins via a 180° *rf* pulse is called *spin-echo*.

The net dephasing after the application of the two gradients is

$$\varphi = \varphi_2 - \varphi_1 \quad (2.32)$$

$$= \gamma G \delta (x_2 - x_1), \quad (2.33)$$

and has a non-zero value if the spin moves along x during the interval Δ between the pulses.

Figure 2.4: Basic components of the pulsed gradient spin-echo (PGSE) sequence. After the 90° pulse, the first gradient pulse induces a phase shift which depends on the spin position. The application of the same gradient after a 180° pulse completely rephases the spins, unless they have moved during or between the application of the gradients.

In a population of spins, all the magnetic moments may not have the same movement. Consequently, the total spin transverse magnetization M is given by

$$M = M_0 \sum_j \exp(i\varphi_j). \quad (2.34)$$

where M_0 stands for the total transverse magnetization at the beginning of the experiment and the summation is over all particles. Assuming that the spins are diffusing freely in a homogeneous fluid, equation 2.4 implies that the probability $P(x_2|x_1, \Delta)$ of finding a spin, initially at a position x_1 , at position x_2 after a time interval Δ is

$$P(x_2|x_1, \Delta) = (4\pi Dt)^{-1/2} \exp \left[\frac{-(x_2 - x_1)^2}{4Dt} \right]. \quad (2.35)$$

In the general case, the attenuation of transverse magnetization due to diffusion is expressed as [23]

$$M/M_0 = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \exp [i\gamma G \delta (x_1 - x_2)] p(x_1) P(x_2|x_1, \Delta) dx_1 dx_2. \quad (2.36)$$

where $p(x_1)$ represents the initial density of spins. For free diffusion, since the fluid

is homogeneous. $p(x_1)$ is a constant and consequently

$$\begin{aligned}
M/M_0 &= \int_{-\infty}^{\infty} \exp[i\gamma G\delta(x_1 - x_2)] P(x_2|x_1, \Delta) dx_2 \\
&= (4\pi Dt)^{-1/2} \int_{-\infty}^{\infty} \exp[i\gamma G\delta(x_1 - x_2)] \exp\left[\frac{-(x_2 - x_1)^2}{4Dt}\right] dx_2 \\
&= (4\pi Dt)^{-1/2} \int_{-\infty}^{\infty} \cos[\gamma G\delta(x_1 - x_2)] \exp\left[\frac{-(x_2 - x_1)^2}{4Dt}\right] dx_2 \\
&= \exp[-(\gamma G\delta)^2 D\Delta].
\end{aligned} \tag{2.37}$$

or, using equation 2.6,

$$M/M_0 = \exp\left[\frac{-(\gamma G\delta)^2 \langle x^2(\Delta) \rangle}{2}\right]. \tag{2.38}$$

This result shows that it is possible to evaluate D (or $\langle x^2 \rangle$), simply by varying G or δ and by performing an exponential fit on the curve of the signal intensity M as a function of $(\gamma G\delta)^2$. However, this approach is problematic for two reasons [23]. Firstly, the spins are assumed to be static during the application of the gradients. But the gradient duration δ is often significant in comparison with Δ , and thus cannot be neglected. The second problem is that imaging gradients or background residual gradients may also affect the spin dephasing and rephasing. A more accurate expression for M/M_0 has been found by Stejskal and Tanner [6] by solving the Bloch-Torrey equations which describe the diffusion effects on the NMR signal.

2.3. The Bloch Equations

The Bloch equations describe the relaxation process by which the thermal equilibrium of a set of spins is re-established after the application of a *rf* pulse. This set of equations governs the behaviour of the nuclear magnetization, $\mathbf{M}(t)$, in a sample of minimally interacting spins, such as a liquid. According to these equations, in the presence of a magnetic field $\mathbf{B}(t)$, the parallel and perpendicular components of the magnetization tend to their equilibrium states, M_0 and 0 respectively, at a rate

proportional to their respective deviation from equilibrium:

$$\frac{dM_z}{dt} = \gamma(\mathbf{M} \times \mathbf{B})_z + (M_0 - M_z)/T_1 \quad (2.39)$$

$$\frac{dM_x}{dt} = \gamma(\mathbf{M} \times \mathbf{B})_x - M_x/T_2 \quad (2.40)$$

$$\frac{dM_y}{dt} = \gamma(\mathbf{M} \times \mathbf{B})_y - M_y/T_2. \quad (2.41)$$

Here, T_1 is the longitudinal relaxation time with which the magnetization along the direction of the main field is restored. T_1 is also called the *spin-lattice relaxation time constant* because it is associated with the transfer of energy to or from the spin system. The transverse relaxation time T_2 , often known as the *spin-spin relaxation time constant*, describes the decay of the transverse component of $\mathbf{M}$ resulting from the local field inhomogeneities that are caused by neighbouring spins. From the Bloch equations for the longitudinal component of the magnetization, we see that [24]

$$M_z = M_0(1 - e^{-t/T_1}), \quad (2.42)$$

and for the precessing transverse component:

$$M_{xy} = M_0 e^{-i\omega_B t - t/T_2}. \quad (2.43)$$

In NMR, the detected signal is the voltage induced in a radio-frequency coil by the precessing transverse magnetization M_{xy} . Thus, following a 90° pulse, there is an exponentially decreasing signal induced in the receiver coil, with a frequency of oscillation equal to the Larmor frequency. This characteristic signal, plotted in Fig.2.5, is called the *free induction decay* (FID). In an ideal system with a perfectly uniform magnetic field, the rate of the FID signal decay would be equal to the T_2 relaxation rate. Unfortunately, imperfections in the magnets cause field inhomogeneities that accelerate the natural dephasing created by the spin system itself. As a consequence, the FID decays at a rate faster than the true T_2 relaxation time. This accelerated or effective transverse relaxation time is called T_2^* .

Nevertheless, the accelerated dephasing of spins precessing in an inhomogeneous magnetic field is a reversible process: if, after the FID, the component of the magnetization perpendicular to $\mathbf{B}_1$ is flipped by the application of a 180° rf pulse, then

Figure 2.5: Free induction decay of the NMR signal following the application of a 90° rf pulse.

the previously diverging magnetization will converge again. This signal recovery, or Hahn spin-echo [3], is the same phenomenon that is illustrated in Fig. 2.4, except that the diffusion gradients can be considered here as any background gradients.

Treatment of the Bloch equations in the presence of diffusion

The usual way to describe theoretically the signal coming from spins diffusing within an homogeneous system is to combine the Bloch equations with Fick's second law. When this is done, one obtains the Bloch-Torrey equation [26]:

$$\frac{d\mathbf{M}}{dt} = \gamma \mathbf{M} \times \mathbf{B} - \frac{M_x \hat{x} + M_y \hat{y}}{T_2} + \frac{(M_0 - M_z) \hat{z}}{T_1} + D \nabla \cdot (\nabla \mathbf{M}), \quad (2.44)$$

where $\mathbf{B}$ will be assumed here to be along the z -direction. The key in diffusion measurements using NMR is the superposition of a linear gradient $g(t)$ and the main static field such that

$$\mathbf{B}(\mathbf{r}, t) = \mathbf{B}_0 + \mathbf{g} \cdot \mathbf{r}, \quad (2.45)$$

where $\mathbf{r}$ is the position vector. By inserting Eq. 2.45 into Eq. 2.44, it follows [5] that for the quantity M_{xy} , described by Eq. 2.43, the differential equation becomes

$$\frac{dM_{xy}}{dt} = -i\gamma \mathbf{g} \cdot \mathbf{r} M_{xy} + D \nabla \cdot (\nabla M_{xy}). \quad (2.46)$$

This equation is solved by setting

$$M_{xy}(\mathbf{r}, t) = M_{xy}(t) \exp \left(-i\gamma \mathbf{r} \cdot \int_0^t \mathbf{g}(t') dt' \right), \quad (2.47)$$

where the argument of the exponential term is simply the phase shift of a spin at position $\mathbf{r}$, accumulated during the time interval t . Finally, one can show that the solution of Eq. 2.46 is

$$\begin{aligned} M_{xy}(t) &= M_0 \exp \left[-D\gamma^2 \int_0^{TE} \left(\int_0^{t'} \mathbf{g}(t'') dt'' \right)^2 dt' \right] \\ &= M_0 \exp [-bD] \end{aligned} \quad (2.48)$$

where we defined the 'gradient factor' b , often referred to as the *b-value*, as

$$b = \gamma^2 \int_0^{TE} \left(\int_0^{t'} \mathbf{g}(t'') dt'' \right)^2 dt'. \quad (2.49)$$

The integrals leading to the b -value can be easily performed numerically for any type of NMR sequence, and the effect of the applied gradients can therefore be easily taken into account.

2.4. Spatial Encoding of the NMR Signal and Slice Selection

The NMR signal detected by the receiver coil comes from the entire imaging volume and some means are necessary to localize the magnetization. Spatial information on the signal is provided by a linear *readout gradient* of magnetic field, $\mathbf{G}_{READ}$, which is superimposed onto the main magnetic field $\mathbf{B}_0$. This gradient produces a frequency distribution of the FID along the gradient axis because

$$\omega(\mathbf{r}) = \gamma (\mathbf{r} \cdot \mathbf{G}_{READ} + B_0). \quad (2.50)$$

from Eqs. 2.12 and 2.45. Thus, the detected signal contains a broad range of frequencies for which the individual amplitudes can be calculated via a Fourier transform. This Fourier transform of the FID yields a 1-D projection of the signal amplitude along the direction of $\mathbf{G}_{READ}$.

On the other hand, slice selection is also accomplished by applying a linear magnetic field gradient, but this gradient must be combined with the application of a *rf* pulse. Remember that the magnetization can be flipped into the transverse plane only if the frequency of the *rf* pulse is the same as the Larmor frequency. According to Eq. 2.50, a spatial variation of the Larmor frequency is easily obtained by the application of a magnetic field gradient. Consequently, in the presence of such a gradient, the spectrum of the *rf* pulse will determine the position and the thickness of the slice, which will be perpendicular to the gradient orientation. The position of the slice will be a function of the central frequency of the pulse, while the thickness of the slice will depend on the bandwidth of the pulse and the steepness of the slice selection gradient.

Figure 2.6: Basic components of a) a PGSE sequence and b) a PGSTE sequence.

2.5. Pulsed Gradient Spin-Echo Method

The pulsed gradient spin-echo (PGSE) technique, used as an example in section 2.2.4, is illustrated in Fig. 2.6a. The PGSE sequences are still widely used for diffusion measurement by NMR. Remember that Eq. 2.37, previously derived for the magnetization attenuation, was an approximation which is only true for $\delta \ll \Delta$. Using Eq. 2.49, the exact solution [23] for M/M_0 is

$$M/M_0 = \exp \left[-(\gamma G \delta)^2 D(\Delta - \delta/3) \right]. \quad (2.51)$$

assuming that the field gradient pulses are sufficiently intense to neglect any residual background gradient.

The experimental accuracy of the PGSE method is guaranteed only if there is no mismatch between the gradient pulses, i.e. if $G_1 \delta_1 = G_2 \delta_2$. Otherwise, the difference between the two gradient pulses leads to an unwanted dephasing, causing an additional damping of M/M_0 which can be misinterpreted as being due to diffusion.

2.6. Pulsed Gradient Stimulated Echo Method

The pulsed gradient stimulated echo (PGSTE) sequence is shown in Fig. 2.6b. The main difference from the PGSE sequence is that the 180° pulse has been replaced by two 90° pulses, separated by a time interval τ_2 . The advantage of this type of sequence is that during this τ_2 period, the magnetization is stored as longitudinal magnetization, parallel to B_0 . During this time it is insensitive to field inhomogeneities and is subjected to a T_1 relaxation process instead of T_2 relaxation. This is particularly useful because the longitudinal relaxation time T_1 is normally much longer than the transverse relaxation time T_2 . The stimulated echo may then be detected for considerably longer values of Δ and hence for longer values of the diffusion time.

Eq. 2.51 still applies for PGSTE sequences. However, the signal amplitude is reduced by one-half compared to the PGSE signal, because only one component of the magnetization in the transverse plane can be stored along the direction of $\mathbf{B}_0$ during the τ_2 interval [23].

2.7. Theoretical Expressions for Diffusion Decays

In the 1960's, Stejskal and Tanner [22, 27, 28] published a series of papers in which they presented different theoretical expressions describing the diffusional attenuation of M/M_0 that should be obtained using the PGSE technique for spins diffusing within a volume defined by planar boundaries. More recently, Söderman and Jönsson [29] derived the same kind of expressions, but for diffusion of spins within a cylinder.

Consider the PGSE sequence presented in Fig. 2.6a, where two diffusion-gradient pulses of magnitude G , duration δ , and separation Δ are inserted on either side of the 180° pulse. Consider also that the short-gradient-pulse (SGP) approximation applies, i.e. that there is a negligible amount of motion of the molecules during the time that the diffusion gradients are applied. This situation corresponds to the limit where $\delta \rightarrow 0$ while $G \rightarrow \infty$ such that $\gamma G \delta$ stays constant. Within the SGP

limit, the amplitude of the echo attenuation takes the form of Eq. 2.36:

$$\frac{M(\gamma G \delta, \Delta)}{M(0, \Delta)} = \int \int \exp [i \gamma \delta \mathbf{G} \cdot (\mathbf{r} - \mathbf{r}_0)] p(\mathbf{r}_0) P(\mathbf{r} | \mathbf{r}_0, \Delta) d\mathbf{r} d\mathbf{r}_0. \quad (2.52)$$

where $p(\mathbf{r}_0)$ corresponds to the initial density of molecules and the *propagator* $P(\mathbf{r} | \mathbf{r}_0, \Delta)$ yields the probability that a molecule originally at position $\mathbf{r}_0$ diffuses to position $\mathbf{r}$ during the time interval Δ .

For diffusion within a cylinder of radius R with perfectly reflecting walls, the propagator is given by [30]:

$$P(\mathbf{r} | \mathbf{r}_0, \Delta) = \frac{1}{\pi R^2} + \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} \frac{2}{\pi R^2 (1 - n^2 / \alpha_m^2 R^2) [J_n(\alpha_m R)]^2} \times J_n(\alpha_m r_0) J_n(\alpha_m r) (\cos n\theta_0 \cos n\theta + \sin n\theta_0 \sin n\theta) \exp [-D \alpha_m^2 \Delta], \quad (2.53)$$

where D is the free diffusion coefficient of the fluid, and θ is the angle between r and the direction of the gradient. J_n is the n th order Bessel function and α_m is the m th root of $J'_n(\alpha_m R) = 0$. Using this propagator, the result of Eq. 2.52 found by Söderman and Jönsson for a uniform initial density of molecules within the cylinder and diffusion sensitization perpendicular to the cylinder axis is:

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

where

$$\begin{cases} K_m = 1 & \text{if } m = 0 \\ K_m = 2 & \text{if } m \neq 0 \end{cases}.$$

and α_{km} represents the k th root of the first derivative of the Bessel function $J_m(\alpha)$. Note that Eq. 2.54 does not yield an exponential dependence of M/M_0 on the value of $(\gamma G \delta)^2$ as was the case for unrestricted diffusion (Eq. 2.37). Accordingly, the slope of a semi-log plot of Eq. 2.54 will not be a constant. In such a case, the apparent diffusion coefficient will then be defined as the initial slope of $\log [M(b)/M_0]$ or, from the Eq. 2.37, as [5]:

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

Figure 2.7: Preparation of cellulose triacetate.

A natural question is how the SGP approximation affects the validity of Eq. 2.54. This has been tested by Linse and Söderman [31] by performing Brownian dynamic simulations for molecules trapped inside a cylinder. They found that for a cylinder of radius R , if the unitless factors $q^* \equiv (\gamma G \delta) R / \pi$ and $\Delta^* \equiv D \Delta / 4 R^2$ are respecting certain conditions, i.e. $q^* \lesssim 0.25$ and $\Delta^* \gtrsim 0.2$, then the SGP limit is an accurate representation of the real diffusion decay.

2.8. Phantom Membranes

In the present study, dialysis filters containing hundreds of parallel hollow fibres are used as a model of the white matter axons. These filters were originally designed for blood purification via *ultrafiltration*, a process by which a porous permeable membrane differentiates between solutes of different sizes. Ultrafiltration membranes are generally made of polymer and have a porosity of about 80%, with an average pore size ranging between $0.1 \mu\text{m}$ and $1 \mu\text{m}$ [32]. However, the pore constriction within the polymer matrix reduces considerably the effective pore size.

The membranes used in our study are made of cellulose acetate (CA). CA is prepared by hydrolyzing the triester of cellulose triacetate (CTA), which is produced according to the reaction shown in Fig. 2.7 [33]. Hydrolyzation of CTA is done to replace some of its acetyl groups by hydroxyl (OH) groups. The hydroxyl groups of

cellulose esters are hydrophilic sites which form hydrogen bonds with water and are therefore responsible for the water permeability of the membrane in the filtration process. On the other hand, the acetyl groups of cellulose esters are hydrophobic sites which serve as cross-links to restrict the swelling about the hydroxyl groups so that, to a first approximation, only water and not hydrated ions can permeate the polymer matrix [34]. In practice however, water molecules cannot diffuse through the membrane over a short time scale, unless a pressure gradient exists across the membrane. In this case, the flux J through the ultrafiltration membrane is directly proportional to the applied transmembrane pressure P [35]:

$$J = K \cdot \Delta P, \quad (2.56)$$

where the constant K depends on the membrane properties. Thus, if no pressure is applied, there is no net flux through the membrane.

Hydration layer

The fact that cellulose esters contain hydrophilic groups suggests that there is a strong polar interaction between H_2O molecules and the filter membranes. This interaction results in a dynamically oriented structure of water called the *hydration layer* between the free water and the cellulose ester molecules. This hydration layer exhibits properties that are different from those of free water. Some characteristic properties of hydration water that are important from the point of view of NMR measurements are [36]:

- restricted translational and rotational motions of the water molecules due to macromolecular-water interactions;
- partial orientation of the water molecules with macromolecular chains;
- anisotropic diffusion along the hydration layer.

According to Bottomley [36], water bound to various different macromolecules typically undergoes exchange diffusion with the free pool of water with a correlation

time of about $10\ \mu\text{s}$, which may be identified as the residence time of water molecules in the bound state. The thickness of the layer extends over 1 to 3 molecular dimensions. Also, the T_2 relaxation time is about 10 times shorter for the hydration layer than for the free water.

Chapter 3 Materials and Methods

All experiments were conducted on the Siemens SP-4000 1.5 Tesla MR imager of the Ottawa Hospital, which is equipped with a gradient system capable of producing magnetic field gradient pulses up to 10 mT/m. In order to maximize the signal-to-noise ratio (SNR), the 30 cm head coil of the imager was used as a *rf* transmitter and receiver for all procedures. The NMR sequences were developed with the PARGEN® sequence editor and operated via the NUMARIS® software. As mentioned previously, two types of sequences were used in this work: the PGSE sequences were employed for short diffusion times between 50.6 ms and 93.3 ms, while the PGSTE sequences were used for longer diffusion times going from 160 ms to 1020 ms. More details about each sequence are given in the next section.

3.1. Description of the NMR Sequences

PGSE sequences

The timing of a PGSE sequence is depicted in Fig. 3.1. In chronological order, each component of the sequence has the following purpose: firstly, the 90° *rf* pulse is combined with the G_{+z} gradient in order to define a slice, perpendicular to the z -direction, within which the spins are labelled and the bulk magnetization is turned into the xy -plane. The G_{-z} component compensates for the T_2^* dephasing that occurs during the application of the G_{+z} gradient. At the same time, a compensation gradient is also applied along the y -direction to compensate for the dephasing that occurs later in the sequence due to the readout gradient.

Afterwards, the first *diffusion sensitizing gradient* (DSG) pulse, G_{D1} , is introduced to dephase the spins along the y -direction. This DSG is followed by the application of a 180° pulse during which the G_{+x} gradient is applied to select a

Figure 3.1: A schematic representation of the PGSE sequence used for diffusion measurements. The gradients G_{+x} and G_{+z} applied during the application of the rf pulses define a column along the readout direction (y -direction). In this case, the diffusion-sensitization gradients are applied in the y -direction, $TE = 111$ ms, and $T_d = 50.6$ ms.

Figure 3.2: Positioning of the column of interest (COI) via two slice selection processes.

second slice perpendicular to the x -direction. The intersection of this slice and the one obtained with the 90° pulse defines the *column of interest* (COI) within which the diffusion is measured, as shown in Fig. 3.2. The COI has a cross-section of $10 \text{ mm} \times 10 \text{ mm}$ and its axis is along the y -direction.

Following the application of the 180° pulse, the phase accumulated by the spins during the application of G_{D1} is recovered partially or completely by the application of the second DSG, G_{D2} . At the time of the spin-echo, TE , the readout on the column is performed. The details of this process will be given later.

Finally, crusher gradients are placed after the data acquisition interval in order to quench any coherent signal still remaining. After these gradients have been applied, no more rf or gradient pulses are applied so that the spin system can return to thermal equilibrium before the sequence is repeated. The *repetition time*, defined as the time from one acquisition to the next, was set to 1500 ms for all sequences.

Figure 3.3: PGSTE sequence used for diffusion measurements. For the sequence shown in this figure, $TE = 59$ ms and $T_d = 160$ ms.

PGSTE sequences

The timing of PGSTE sequences, shown in Fig. 3.3, is similar to the timing of the PGSE sequences, except that i) the 180° pulse is replaced by two 90° pulses and ii) the column of interest is now defined by the intersection of the slices selected using the three gradients G_{x1} , G_{z2} and G_{z3} , which are T_2^* -compensated by the application of G_{x2} and G_{z1} . As mentioned in Section 2.2, the purpose of the second 90° pulse is to store the magnetization in the longitudinal direction, parallel to the main magnetic field (in the z -direction), for a time interval τ_2 . During this interval, crusher gradients are applied in the three Cartesian directions to quench any FID produced by the second 90° pulse or small spin-echoes that could be engendered by imperfect pulses. The third 90° pulse is used to return the magnetization to the transverse plane before the application of G_{D2} .

Figure 3.4: a) Real and imaginary parts of the signal induced at the receiver coil as a function of time and b) the same signal as a function of frequency obtained by performing a FFT on the real and imaginary components and calculating the absolute value. The data shown were obtained from [19].

Readout process

The readout measurements of the NMR signal are performed in *quadrature*, i.e. the signal is actually detected by two different coils rotated 90° with respect to each other in the transverse plane. This means that the current induced in first coil is a quarter of a cycle ahead of the current in the second coil. These two currents are known as the *real* and *imaginary* components of the signal. Examples of these components are shown in Fig. 3.4a.

During the readout process, the current induced in the receiver coils - the echo - is amplified, filtered and the carrier (i.e. resonant) frequency is electronically subtracted from the signal. The real and imaginary components of the signal are then sent to the analog-to-digital converter (ADC) which generates an array of 1024 complex values. Afterwards, a fast Fourier transform (FFT) of this array converts the time-dependent NMR data to frequency-dependent data. These data provide a spatial localization of the signal because the readout gradient, G_{READ} , causes a linear variation of the Larmor frequency as a function of position along the COI. Accordingly, the absolute value of the Fourier-transformed NMR signal gives a *signal profile* with the form of Fig. 3.4b, which can be interpreted as the

Figure 3.5: A schematic diagram of a hemodialysis filter (model CA-110, Baxter inc., Chicago, IL) which was used as a phantom. Inlets *A* and *D* allow a fluid to flow inside the fibres, while inlets *B* and *C* are linked to the volume outside the fibres.

position-dependent magnetization, M , along the COI.

3.2. Description of the Phantoms

In some areas of the white matter, the axonal system can be thought of as a bundle of long parallel fibres of circular cross section. Hemodialysis filters (CA-110, Baxter inc., Chicago, IL) were used as phantoms to represent this system in our experiments [37]. These filters contain hundreds of long parallel hollow fibres. Each fibre has an internal diameter of $200\ \mu\text{m}$ [38] and the fibre membranes, made of cellulose acetate, have a thickness of $10\ \mu\text{m}$. The diameter and the membrane thickness of the fibres have been verified using optical microscopy.

As shown in Fig. 3.5, the fibres of the filter are imbedded in a cylindrical polycarbonate case in such a way that two liquids can flow: one inside and one outside of the fibres¹. Accordingly, the intraluminal and/or extraluminal volume(s) of the filter can be filled, depending on the experiment. Plastic deflectors are placed under inlets *B* and *C* in order to distribute evenly any liquid flowing into the extraluminal space.

¹ When used for hemodialysis, the blood flows inside the fibres and the dialysing fluid flows in the opposite direction outside the fibres.

The permeability of the fibres to water is zero when no transmembrane pressure is applied. This has been verified by leaving the fibres filled with water for several days, without observing any transmission of water into the extraluminal compartment. This also agrees with the technical specifications given by Baxter [38] according to which the fibres are non-permeable in the absence of a pressure gradient.

3.3. Experimental Procedures

Measurement protocol for static fluid

To fill the phantom with water, a reservoir and a siphon hose were used. At first, the reservoir was filled with tap water at room temperature. Then, the siphon was connected to inlet A or B, depending on whether the intra- or extraluminal volume was to be filled. The flow of the siphon was maintained for approximately 5 minutes in order to make sure that all air was flushed out of the volume to be filled.

When the inside of the fibres had to be filled, the filter was immersed in a container of water immediately after the siphon was stopped to prevent air from re-entering the fibres. Then, the extraluminal compartment was filled (if necessary) and inlets B and C were closed tightly using plastic caps. In the case where *only* the extraluminal volume had to be filled, all inlets were closed using plastic caps after the filling of the extraluminal space, but the filter was not immersed in water to make sure that water could not enter the inside of the fibres by inlets A or D.

The temperature of the water in the container was measured immediately before the phantom was placed in the head coil of the imager, parallel to the main magnetic field $\mathbf{B}_0$. Then, an MR sequence called a *localizer* was run in order to obtain the exact position of the phantom in the magnet. This sequence, based on the sequence SE_15B130 of Siemens, rapidly produces a set of 3 T_1 -weighted images in the sagittal (yz), coronal (xz) and transverse (xy) planes. The purpose of these images is twofold: firstly, they allow us to verify the alignment of the filter with the direction of $\mathbf{B}_0$ and decide on the position of the COI within which the diffusion is to be measured. Secondly, the cross-sectional image of the filter in the transverse plane is used to calculate the *intraluminal volume fraction*, f , i.e. the fraction of

Sequence name	Type	G_{READ} (mT/m)	ADC (μs)	G_D dir.	TE (ms)	τ_2 (ms)	δ (ms)	Δ (ms)	T_d (ms)
YAP	PGSE	3.0 $\hat{y}$	8192	$\hat{y}$	111	-	36	62.6	50.6
YHF	PGSE	3.0 $\hat{y}$	8192	$\hat{z}$	111	-	36	62.6	50.6
YAPL	PGSE	3.0 $\hat{y}$	8192	$\hat{y}$	159	-	40	106.6	93.3
YHFL	PGSE	3.0 $\hat{y}$	8192	$\hat{z}$	159	-	40	106.6	93.3
STYAP15	PGSTE	1.0 $\hat{y}$	16382	$\hat{y}$	59	140, 240, 355, 490, 640, 800, 1000	15	τ_2+25	τ_2+20
STYHF15	PGSTE	1.0 $\hat{y}$	16384	$\hat{z}$	59	140, 240, 355, 490, 640, 800, 1000	15	τ_2+25	τ_2+20

Table 3.1: Sequences used for D_a vs T_d measurements and their respective parameters.

the space taken by the volume inside the fibres over the total volume available for fluid inside and outside the fibres. To calculate f , a particular function of the NUMARIS® software called MEAN was used. This function allows us to measure the average signal in a particular region of an image. Thus, by measuring the mean signal in the circular region of the cross-sectional image containing the fibres when only the intraluminal volume is filled with water, and by repeating this procedure when both intraluminal and extraluminal spaces are filled, it is possible to obtain f from the ratio of the two measurements (considering that only a negligible amount of water is present in the matrix of the fibre walls).

The D_a vs T_d measurements were made with the COI placed perpendicular to the filter, in the *anterior-posterior* (AP) direction (i.e. vertically). The DSG pulses were applied along two directions: the *head-feet* (HF) direction (parallel to the fibres and the bore of the magnet) and the AP direction (perpendicular to fibres and the bore of the magnet). For each sequence, 10 DSG strengths (0.5, 1.5, 2.5, ..., 9.5 mT/m) were applied to obtain a well-defined diffusion decay. A total of 20 acquisitions were performed each time a sequence was used, the signal being averaged directly on the complex echo data.

Figure 3.6: Experimental set-up for phantom experiments with a slow flow in the extraluminal compartment.

In this experiment, the value of D_a was measured for 9 values of T_d ranging from 50.6 ms to 1020 ms. Table 3.1 contains the parameters of all the sequences that were utilized. The time required to run all the sequences, considering 10 DSG strengths and 20 acquisitions per sequence, was approximately 2.5 hours.

After all experiments, the phantoms were emptied by blowing air into the compartments and stored in a dry place. However, because the phantom fibres are hydrophilic and because the access to the extraluminal compartment is difficult, it is impossible to flush out completely all the water from the extraluminal compartment after an experiment. To ensure that the extraluminal space was completely dry, a new filter was utilized for each experiment.

Measurement protocol in the presence of forced flow

Some experiments were performed with water flowing in the extraluminal compartment. This flow was forced by a diaphragm pump (LMI Milton Roy LE-95T, Acton, MA) placed outside the magnet room. As shown in Fig. 3.6, water was directed into the magnet bore via plastic tubing connected to inlet B of the phantom, while a second piece of tubing carried the water back from outlet C of the phantom to

Sequence name	Type	G_{READ} (mT/m)	ADC (μ s)	G_D dir.	TE (ms)	τ_2 (ms)	δ (ms)	Δ (ms)	T_d (ms)
DIFFOPT	PGSE	$3.0 \hat{j}$	8192	$\hat{j}$	139	–	57	69.6	50.6
DIFFOPTHF	PGSE	$3.0 \hat{j}$	8192	$\hat{z}$	139	–	57	69.6	50.6
DIFFOPT300	PGSTE	$1.0 \hat{j}$	16382	$\hat{j}$	59	300	15	325	320
DIFFOPTHF300	PGSTE	$1.0 \hat{j}$	16384	$\hat{z}$	59	300	15	325	320

Table 3.2: Sequences used to study the shape of diffusion decays and their respective parameters.

the water reservoir. The frequency of the pump was set to 1 Hz, which is a reasonable approximation of the human heart rate. The stroke level of the pump was then adjusted to obtain a flow of about 90 ml/min, the minimum flow offered by the pump at this frequency. It has been observed that the pressure exerted by the pump in this configuration was too low to make the phantom fibres permeable. Flow measurements were done by determining the time needed to fill a 25 ml graduated cylinder with water flowing back from the phantom.

The purpose of these experiments was to study the shape of the diffusion decays in presence of a very slow flow. To achieve this task, the measurements were done using the same kinds of sequences as mentioned previously, except that a large number of b -values were used to improve the definition of the diffusion decays. At $T_d = 50.6$ ms, 32 b -values were considered, while at $T_d = 320$ ms, 26 b -values were used. The details of the sequences are given in Table 3.2.

Measurement protocol for white matter

The shape of the diffusion decay at $T_d = 50.6$ ms has also been studied *in vivo* in the white matter of 4 normal and healthy volunteers. Subjects were placed supine, head first into the magnet. A set of T_1 -weighted images in the coronal and transverse planes were obtained using the sequence SE_15B130 with $TE = 15$ ms and $TR = 513$ ms. Two examples of these scout images are shown in Fig. 3.7.

Using these images, the position of the COI was determined using the DISTANCE function of the NUMARIS® console, which allowed us to evaluate the position of a point on a MR image with a precision of 0.1 mm. Then, the coordinates of

Figure 3.7: Positioning of the COI in the white matter. a) Sagittal view and b) transverse view with the predominant orientation of the axonal fibres.

the COI were entered manually into the sequence file using the PARGEN® editor. The COI was positioned superolateral to the ventricle of the left hemisphere, in a white matter region named the *corona radiata*. In this region of the brain, the orientation of the nerve fibres is known [39, 40] to be predominantly in the *right-left* (RL) direction in the central region of the brain, while in the anterior and posterior cerebral regions, the preponderant orientation of the fibres is along the AP direction. The COI placement and the dominant orientation of the axonal fibres are depicted in Fig. 3.7b.

These measurements were done using the DIFFOPT sequence. This sequence was run for 32 different b -values with 20 acquisitions per point. In this particular case, the signal averaging was performed on the signal profiles instead of the echo data. For every volunteer, the DSC pulses were applied in the AP direction. Each volunteer stayed for approximately 40 minutes in the imager, including the preparation time.

3.4. Data Analysis Programs

Before proceeding to the data analysis, all the spectroscopy files were converted from the NUMARIS format to the ASCII format via a short FORTRAN program called NUM2ASCII such that each file contains one signal profile in the form of Fig. 3.4b. Using another FORTRAN program, the b -values corresponding to each signal profile were calculated from Eq. 2.49.

The analysis of experimental data was done using home-made software called DPRO99, written in IDL (Research Systems, Boulder, CO). This software allows the user to read every signal profile related to a particular experiment and visualize them all at once via a graphical interface. The pre-calculated b -values corresponding to each signal profile can be entered manually into DPRO99 or loaded from a data file. Moreover, the baseline can be removed from each signal profile before the data analysis.

Once a b -value is assigned to each profile, the user can proceed to the calculation of D_a . The value of D_a is obtained by performing an exponential fit to the values

Figure 3.8: Determination of D_a . In a) are presented the signal profiles obtained using 10 different b -values. A cut through these profiles, for example at $y = 0$ mm, gives the diffusion decay shown in b). An exponential fit of this decay allows the determination of D_a . If several diffusion decays are fitted, e.g. between -60 mm and 60 mm, then a more accurate value of D_a can be calculated.

of the signal profiles as a function of b for a given position. For example, Fig. 3.8a shows the signal profiles obtained when running the PGSE sequence YAP for a head coil phantom - a spherical container filled with water and generally used for calibration purposes - for 10 different b -values. A cut at $y = 0$ mm through these profiles gives a diffusion decay which decreases exponentially, as shown in Fig. 3.8b. In this particular case, the slope of the semi-log plot has been evaluated as $(2.06 \pm 0.10) \times 10^{-5} \text{ cm}^2/\text{s}$, where the error corresponds to the standard deviation of the slope. From Eq. 2.48 it can be seen that the slope of such a curve is equal to D_a . In order to get a more accurate value of D_a , the weighted mean of all the values of D_a fitted in the range $-60 \text{ mm} \leq y \leq 60 \text{ mm}$ was calculated, giving a final result for D_a of $(2.073 \pm 0.011) \times 10^{-5} \text{ cm}^2/\text{s}$. In the present work, the measured values of D_a will refer to the mean of the values calculated for the region of the signal profiles where the profiles are roughly constant.

Fitting method

The fitting routine of DPRO99 is based on a Marquardt least-squares minimization algorithm [41], the standard of non-linear least-squares routines [42]. The Marquardt method performs a least squares fit to any non-linear function of an arbitrary number of parameters. Given trial values for the parameters, this method proceeds iteratively to converge on the parameter values that minimize χ^2 . The same trial values were used for all fits, although the Marquardt algorithm is reasonably insensitive to the starting values of the parameters [43].

Two types of fit were performed in this study. The main one is an exponential fit used to find the value of D_a from the diffusion decays. In this case, the experimental data were fitted to the equation

$$\frac{M}{M_0} = \exp(-bD_a). \quad (3.1)$$

in order to extract D_a . However, in some situations, the diffusion decay deviated from the exponential shape. When it presented a biexponential shape, the diffusion decay was fitted to

$$\frac{M}{M_0} = f_{\text{slow}} \exp(-bD_a^{\text{slow}}) + (1 - f_{\text{slow}}) \exp(-bD_a^{\text{fast}}). \quad (3.2)$$

where D_a^{slow} is the slowly diffusing component of the diffusion decay, D_a^{fast} is the fast diffusing component and f_{slow} is the fraction of the signal due to D_a^{slow} . The diffusion decay could have been simply fitted to Eq. 3.2 using the Marquardt method. However, to make sure that the ranges of b -values related to D_a^{slow} and D_a^{fast} were relatively the same for all decays, the fit was performed in two steps, as suggested by Bevington [43]. Firstly, the best estimate of D_a^{slow} was calculated by performing a monoexponential fit (Eq. 3.1) in the region of high b -values. On a semi-log plot, this fit corresponded to the slope of the best estimate of a straight line in the region of high b -values. Moreover, the intercept of this fit with the y -axis gave the value of f_{slow} . Secondly, in order to calculate D_a^{fast} , the contribution of D_a^{slow} was subtracted from the diffusion decay and a second monoexponential fit was performed on the corrected decay.

Figure 3.9: a) Tissue model of the white matter and b) the 2D-geometry used in MC simulations.

All fits were checked for self-consistency by making sure that D_a^{fast} contributed less than about 1% to the data that was used for the calculation of D_a^{slow} .

3.5. Monte-Carlo Model

Consider a white matter model in which the axons are represented by infinitely long cylinders of radius R , closely packed and parallel to each other, as shown in Fig. 3.9a. In the two-dimensional MC model that was implemented, each axon was represented by a circular cell placed on a periodic triangular lattice of lattice constant $2R_m$. The free diffusion coefficients in the intracellular and extracellular spaces were respectively D_I and D_E , while the spin-spin relaxation constants in each compartment were $(T_2)_I$ and $(T_2)_E$.

The MC model is based on a numerical model developed by Szafer *et al.* [44]. Essentially, the simulations proceed as follows:

1. At $t = 0$, a number N of spins are distributed randomly in the intracellular and/or extracellular environment(s). Each spin is described by a complex number m_j for which the real and imaginary parts represent the components on the magnetization in the transverse plane. After the application of the 90° pulse, all spins are in phase with each other and $m_j(0) = 1.0$. However, immediately after the application of the first DSG, the phase of each spin

depends on its initial position and also on the value of $\gamma G \delta$, such that $m_j(0^+) = m_j(0^-) \exp(i\gamma G \delta x_j)$.

2. Once the initial phases and positions of the spins have been determined, the program simulates the Brownian motion of particles for a period of time T_d during which the spins experience a series of small steps of duration dt . Each step consists of x and y components with random lengths following a gaussian probability distribution. This distribution has a standard deviation equal to $\sqrt{2D_I dt}$ for the intracellular displacements and $\sqrt{2D_E dt}$ for the extracellular displacements. Furthermore, at each time interval dt , the magnetization of each spin decays according to $m_j(t + dt) = m_j(t) \exp(-dt/T_2)$.
3. Our implementation of this model allows us to make the cells permeable. When a particle is incident on the membrane of permeability P , it has a probability p of being transmitted through the membrane. Otherwise, it is specularly reflected. The probability of transmission is given by the formula [44]

$$p = \frac{2P}{\langle v \rangle} \quad (3.3)$$

where $\langle v \rangle$ is the average velocity of the particles in the medium. When a particle goes through the barrier, the length of the diffusing step is rescaled to take into account the difference between D_I and D_E . For example, if a particle goes from a position inside the cell to a position (x_f, y_f) in the extracellular space by crossing the wall at (x_w, y_w) , then the final position is rescaled according to the following expressions:

$$\begin{cases} x_f \rightarrow x_w + \sqrt{\frac{D_E}{D_I}} (x_f - x_w) \\ y_f \rightarrow y_w + \sqrt{\frac{D_E}{D_I}} (y_f - y_w) \end{cases} \quad (3.4)$$

The T_2 attenuation of m_j also gets rescaled by considering the amount of time spent by the particle in each compartment. For instance, if during the interval dt the spin stays for a time dt_I in the intracellular space and $(dt - dt_I)$ in the extracellular space, then m_j evolves according to

$$m_j(t + dt) = m_j(t) \exp[-dt_I / (T_2)_I] \exp[-(dt - dt_I) / (T_2)_E]. \quad (3.5)$$

4. Finally, after a time T_d , the dephasing effect of the second DSG is applied to all spins such that $m_j(T_d^+) \rightarrow m_j(T_d^-) \exp[iqx_j(T_d)]$. By adding the contribution of each individual magnetization, the total echo signal can be obtained:

$$M(\gamma G \delta, T_d) = \left| \sum_{j=1}^N m_j(T_d) \right|. \quad (3.6)$$

At this point, the value of D_a may be calculated in two different ways: from the mean squared displacement of the particles (Eq. 2.7) or equivalently, from the diffusion decay of the total magnetization (Eq. 2.37). In this work, the first approach was used when only $D_a(T_d)$ was needed. $M(\gamma G \delta, T_d)$ was utilized to study the shape of the diffusion decay for different conditions of the cellular environment, or when there was T_2 inhomogeneities between the intra- and extracellular environments.

Delayed reflection

It is possible in our model to delay the reflection of a particle on a barrier, i.e. that each time a spin encounters a wall, it ‘sticks’ there for a predetermined amount of time before being specularly reflected. This allowed us to evaluate how the residency time of a molecule in the hydration layer or in a pore of the membrane affects the shape of the diffusion decay and the value of D_a .

Planar geometry

The model presented previously allowed the study of diffusion in the direction perpendicular to the fibre orientation. In order to simulate the diffusion of molecules in the direction parallel to the fibres, a second two-dimensional planar model, shown in Fig. 3.10, was used. In this model, the particles diffused between two, infinitely long planes separated by a distance L . This situation corresponds to the diffusion of molecules *inside* a cylindrical fibre if the distance L between the planes is set equal to the average distance between opposite sides of a fibre along the direction perpendicular to it. In other words, L is not equal to the diameter d of the fibre, but to the mean distance that separates two points of its circular cross-section along a given direction, $\pi d/4$.

Figure 3.10: The planar MC model used to calculate D_a for water diffusing within a cylindrical fibre. The planes are separated by a distance $L = \pi d/4$, where d is the diameter of the fibre.

Chapter 4 Results

In the present chapter, the calibration results obtained in measuring the ‘free’ diffusion coefficient of water, D , for different values of T_d are first described. The measurements of D_a as a function of T_d for restricted diffusion of water inside the phantom are also presented. Then, the shape of some diffusion decays that were obtained from *in vivo* measurements is shown and the results of a phantom study in which a slow flow was forced into the extraluminal compartment is given. Finally, different plots that demonstrate the validity of our MC simulations and give interesting information on the T_d -dependence of D_a in real white matter tissues are presented. All these results will be analyzed in detail in the next chapter.

4.1. Calibration Results

Before proceeding to the phantom experiments, all the sequences were tested by measuring the diffusion coefficient of water, D , in the absence of spatial restriction. Each sequence has been run for a phantom consisting of a plastic container filled with tap water. The water temperature was measured before and after the experiment, with a value of $(21.0 \pm 0.5)^\circ\text{C}$. By interpolating the data from Mills [45] who measured the free diffusion coefficient of water in the range $1\text{--}45^\circ\text{C}$, the value of D at this temperature is $(2.077 \pm 0.025) \times 10^{-5} \text{ cm}^2/\text{s}$.

Fig. 4.1 shows the results obtained for each sequence. The solid points and open points refer to diffusion sensitizations in the HF direction and AP direction respectively. The first two points for the AP and HF sensitizations were acquired using PGSE sequences, while all the other points were obtained using PGSTE sequences. The solid line represents the expected value of D and the dashed lines the error range for this value due to the uncertainty on the temperature. Finally, the error

Figure 4.1: Measurement of the coefficient of diffusion of water, D , using all the sequences. The solid line represents the value of D and the dashed lines are the error bars on this value due to the uncertainty on the water temperature.

bars on the experimental values are the standard deviations of D_a calculated from the exponential fits on the signal profiles.

4.2. Restricted Diffusion in the Phantom

The results of the different experiments that were performed on the phantom are now presented. The measured values of D_a are given for the case where the diffusion of water is restricted inside and/or outside the phantom fibres, for T_d varying between 50 ms and 1020 ms. All measurements were performed for diffusion sensitization parallel and perpendicular to the fibre orientation.

Restricted diffusion of water in the intraluminal volume

Fig. 4.2 shows the measured value of D_a as a function of $\sqrt{T_d}$ in the situation where only the inside of the fibres is filled with water. The solid points represent the values of D_a when the diffusion is measured along the HF' direction, i.e. along the direction parallel to the fibres (D_{a+}). The open points represent the values of D_a for diffusion sensitization in the AP direction, which is perpendicular to the fibre orientation (D_{a-}). Again, the first two points of each curve were acquired using PGSE sequences, while all the other points were obtained using PGSTE sequences. The dashed line illustrates the value of D which is $(2.15 \pm 0.01) \times 10^{-5} \text{ cm}^2/\text{s}$ for water at $(22.4 \pm 0.1)^\circ\text{C}$, by interpolation of Mills's data [45]. Here, D can be interpreted as the expected value of D_{a+} in the ideal case where all the fibres are perfectly aligned with the DSG's and specularly reflect all the molecules colliding with their inner walls.

On the other hand, the solid line depicts the value of D_{a-} as a function of $\sqrt{T_d}$ calculated from the theoretical equation of Söderman. Considering a cylinder with a diameter of $200 \mu\text{m}$, the diffusion decay $M(b)$ was obtained from Eq. 2.54 and the values of $D_{a- \text{Sod}}$ were calculated from Eq. 2.55. The initial slope of the diffusion decay was calculated from only two points: $M(0) = 1.0$ and the value of M obtained from Söderman's equation for a very small value of b , i.e. 50 s/mm^2 .

Finally, the square points represent the values obtained from a MC simulation.

Figure 4.2: D_{a+} and D_{a-} as a function of $\sqrt{T_d}$ for water diffusing inside the phantom hollow fibres. The dashed line represents the ‘free’ diffusion coefficient of water and the solid line the value of D_{a-} as a function of $\sqrt{T_d}$ calculated from Söderman’s equation. Simulation results for D_{a-} are depicted by the square points and were obtained using $R = 100 \text{ } \mu\text{m}$, $N = 30000$, $dt = 15 \text{ } \mu\text{s}$, and $D_I = D = 2.15 \times 10^{-5} \text{ cm}^2/\text{s}$.

The simulation parameters used are $R = 100 \mu\text{m}$, $N = 30000$ particles, $dt = 15 \mu\text{s}$ and $D_I = 2.15 \times 10^{-5} \text{ cm}^2/\text{s}$. The walls were considered non-permeable in the simulation, reflecting specularly and instantaneously all the molecules colliding with them.

Restricted diffusion of water in the extraluminal volume

The values of D_a as a function of $\sqrt{T_d}$ for water only in the volume outside the fibres are shown in Fig. 4.3. Again, the solid points and the open points illustrate respectively the values of D_a for DSC's parallel ($D_{a\parallel}$) and perpendicular ($D_{a\perp}$) to the fibre orientation. The dashed line represents the value of D for water at $(22.5 \pm 0.1)^\circ\text{C}$, which from [45] is evaluated at $(2.16 \pm 0.01) \times 10^{-5} \text{ cm}^2/\text{s}$.

A Monte-Carlo simulation was run to find what the value of $D_{a\perp}$ as a function of $\sqrt{T_d}$ should be in the situation where the fibres are as shown in Fig. 3.9. In this simulation, it was considered that the intraluminal volume fraction, f , of the total volume was 0.363. To calculate this number, we measured the ratio of the signal intensities of two filter cross-sectional images: one acquired when only the intraluminal compartment is filled and the other when both compartments are filled. This ratio was 0.30 ± 0.01 . But for the simulation, the volume taken by the fibre membrane itself in the intraluminal volume fraction must also be considered. That is why f was multiplied by the factor $\frac{\pi(R+t_m)^2}{\pi R^2}$, where t_m is the thickness of the membrane. Considering that the phantom fibres have an inner radius of $100 \mu\text{m}$ and a wall thickness of $10 \mu\text{m}$, the measured value of f was increased by 21% for the present simulation. Accordingly, R was set to $110 \mu\text{m}$ and the value of R_m was set to $173.87 \mu\text{m}$ such that

$$f = \frac{\pi R^2}{6R_m^2 \tan(\pi/6)} = 0.363. \quad (4.1)$$

where the numerator represents the area of a fibre circular cross-section, and the denominator the area of the hexagonal unit cell. A total of $N = 30000$ particles were followed and dt was set to $15 \mu\text{s}$ in the simulation.

Figure 4.3: D_{a+} and D_{a-} as a function of $\sqrt{T_d}$ for water outside the hollow fibres of the phantom. The dashed line indicates the value of D . The MC results were obtained using $N = 30000$, $R = 110 \text{ } \mu\text{m}$, $dt = 15 \text{ } \mu\text{s}$, $f = 0.363$ and $D_E = D = 2.15 \times 10^{-5} \text{ cm}^2/\text{s}$.

Figure 4.4: D_a and $D_{a\perp}$ as a function of $\sqrt{T_d}$ for water both inside and outside the hollow fibres of the phantom. The dashed line indicates the value of D . The MC results were obtained using $N = 30000$, $R = 105 \mu\text{m}$, $dt = 15 \mu\text{s}$, $f = 0.300$, and $D_I = D_E = D = 2.15 \times 10^{-5} \text{ cm}^2/\text{s}$.

Restricted diffusion of water in both intraluminal and extraluminal volumes

Fig. 4.4 depicts the results obtained for D_a and $D_{a\perp}$ for the case where both intra- and extraluminal compartments were filled with water. The temperature of the water during this experiment was $(22.4 \pm 0.1)^\circ\text{C}$ and the corresponding value of D is $(2.15 \pm 0.01) \times 10^{-5} \text{ cm}^2/\text{s}$ [45].

In the MC model, the fibre walls are considered infinitely thin. For this reason, R was set to $105 \mu\text{m}$ in the simulation of the present experimental configuration. The other MC parameters were set to $R_m = 182.56 \mu\text{m}$ such that $f = 0.300$, $N = 30000$ and $dt = 15 \mu\text{s}$.

4.3. *In Vivo* Study

Before the other results that have been obtained on the phantom are presented, the results of some measurements that were performed *in vivo* in the white matter of 4 human volunteers will be presented. The purpose of these measurements is to demonstrate the anisotropy of D_a in the white matter and, especially, show that the shape of the diffusion decay is not monoexponential in all regions.

In Fig. 4.5a are presented the signal profiles that were obtained on a human volunteer for 32 b -values, using a sequence (DIFFOPT) with diffusion sensitization along the AP direction. By performing an exponential fit on these profiles at different positions, it is possible to calculate the different values of D_a as a function of position. The result of such a calculation is presented in Fig. 4.5b. It appears that D_a is not constant along the COI. This was expected since the orientation of the axonal fibres is not the same everywhere in the region under study (as shown in Fig. 3.7b, the predominant fibre orientation is in the RL direction in the central region, and in the AP direction for the anterior and posterior regions).

The main interest in doing these *in vivo* experiments was to study the shape of the diffusion decays obtained in cerebral white matter. In Fig. 4.6, two diffusion decays observed respectively in the central and posterior regions of the COI are presented. These decays were extracted from the signal profiles shown in Fig. 4.5a at positions #1 and #2 respectively. On those decays, a straight line corresponding to a monoexponential fit performed in the region where $b \geq 250 \text{ s/mm}^2$ was traced. Diffusion decays obtained in the same regions of the brain are also presented for 3 other volunteers in Figs. 4.7, 4.8 and 4.9.

Figure 4.5: a) Signal profiles acquired *in vivo* in the white matter of a human volunteer, using 32 b -values. b) Values of D_a as a function of position resulting from exponential fits on the signal profiles.

Figure 4.6: Diffusion decays obtained at positions #1 and #2 on the signal profiles of volunteer #1 shown in the previous figure.

Figure 4.7: Diffusion decays obtained in the central and posterior regions of the COI for volunteer #2.

Figure 4.8: Diffusion decays obtained in the central and posterior regions of the COI for volunteer #3.

Figure 4.9: Diffusion decays obtained in the central and posterior regions of the COI for volunteer #4.

4.4. Effects of a Forced Flow in the Phantom Extraluminal Volume

The aim of this experiment was to simulate a hypothetical extracellular fluid motion that would be caused by the hydrostatic pressure exerted on cerebral tissues by the blood pulsations inside the brain vasculature. Actually, the specific objective of this experiment was to investigate the effect of a slow flow on the shape of the diffusion decays and assess what kind of information it is possible to extract from those decays. To achieve this task, several b -values were used in order to produce well-defined diffusion decays, as in the *in vivo* study. First of all, the diffusion decays were obtained for water simply diffusing in the phantom, i.e. when no flow was forced. Then the experiment in which flow was involved for two distinct experimental configurations was performed: in the first one, water was flowing in the extraluminal compartment of the phantom while nothing was inside the fibres. In the second configuration, the water was flowing outside the fibres, but the lumens were filled with water before the experiment. For each configuration, the diffusion decays were measured at both $T_d = 50.6$ ms and $T_d = 320$ ms.

Results for $T_d = 50.6$ ms

The first results that are presented are some examples of the diffusion decays obtained in the absence of a flow. In Fig. 4.10, the diffusion decays corresponding to each experimental configuration are shown, for both parallel and perpendicular DSG orientations. All the diffusion decays have a monoexponential shape.

However, when a pulsed flow is forced into the extraluminal compartment of the phantom, the shape of the diffusion decays changes radically. Figures 4.11 and 4.12 depict some examples of the diffusion decays obtained in this situation. For both parallel and perpendicular orientations of the DSG's, whether the intraluminal volume contains water or not, the diffusion decays deviate from the exponential behaviour observed when no flow is involved.

Consequently, the values of $D_{a||}$ and $D_{a\perp}$ cannot be simply calculated from monoexponential fits as before. It was assumed that the decays were biexponential and biexponential fits were performed on the signal profiles acquired from 2 different

Figure 4.10: Examples of the monoexponential diffusion decays obtained for DSG directions perpendicular and parallel to the fibre orientations, in the absence of flow in the diffusion phantom. In (a,b) only the extraluminal compartments is filled with water while in (c,d) both intra- and extraluminal compartments contain water.

Figure 4.11: Examples of the biexponential diffusion decays obtained for $T_d = 50.6$ ms in the presence of flow in the extraluminal compartment of the diffusion phantom, when the intraluminal volume is empty. In a) DSG's are applied perpendicular to the fibre orientation, while in b) the DSG's are applied parallel to the fibres.

Figure 4.12: Examples of the biexponential diffusion decays obtained in the presence of flow in the extraluminal compartment of the diffusion phantom for $T_d = 50.6$ ms, when the intraluminal compartment is filled with water. In a) DSG's are applied perpendicular to the fibre orientation, and in b) the DSG's are applied parallel to the fibres.

Configuration of experiment #1 ($T_d = 50.6$ ms)	Mean results from the biexponential fits ($D_a \times 10^{-5}$ cm ² /s)	
	DSG's perpendicular to fibres	DSG's parallel to fibres
Air inside the fibres	$D_{a_{\perp}}^{\text{slow}} = 1.61 \pm 0.07$	$D_a^{\text{slow}} = 2.09 \pm 0.09$
	$D_{a_{\perp}}^{\text{fast}} = 5.1 \pm 0.9$	$D_a^{\text{fast}} = 7.4 \pm 0.7$
	$f_{\text{slow}} = 0.70 \pm 0.05$	$f_{\text{slow}} = 0.78 \pm 0.06$
Water inside the fibres	$D_{a_{\perp}}^{\text{slow}} = 1.85 \pm 0.07$	$D_a^{\text{slow}} = 1.97 \pm 0.05$
	$D_{a_{\perp}}^{\text{fast}} = 5.7 \pm 1.5$	$D_a^{\text{fast}} = 7.6 \pm 1.0$
	$f_{\text{slow}} = 0.82 \pm 0.06$	$f_{\text{slow}} = 0.79 \pm 0.03$

Configuration of experiment #2 ($T_d = 50.6$ ms)	Mean results from the biexponential fits ($D_a \times 10^{-5}$ cm ² /s)	
	DSG's perpendicular to fibres	DSG's parallel to fibres
Air inside the fibres	$D_{a_{\perp}}^{\text{slow}} = 1.64 \pm 0.13$	$D_a^{\text{slow}} = 2.10 \pm 0.07$
	$D_{a_{\perp}}^{\text{fast}} = 4.6 \pm 0.8$	$D_a^{\text{fast}} = 8.7 \pm 2.5$
	$f_{\text{slow}} = 0.82 \pm 0.05$	$f_{\text{slow}} = 0.89 \pm 0.03$
Water inside the fibres	$D_{a_{\perp}}^{\text{slow}} = 1.85 \pm 0.08$	$D_a^{\text{slow}} = 1.93 \pm 0.05$
	$D_{a_{\perp}}^{\text{fast}} = 6.1 \pm 3.5$	$D_a^{\text{fast}} = 7.5 \pm 2.9$
	$f_{\text{slow}} = 0.90 \pm 0.03$	$f_{\text{slow}} = 0.90 \pm 0.04$

Table 4.1: Results calculated from biexponential fits performed on the diffusion decays obtained in the presence of a flow in the extracellular compartment ($T_d = 50.6$ ms).

experiments. Tables 4.1a and 4.1b show the mean results that were obtained by fitting the signal profiles at several positions. The fits allowed us to extract, from the profiles related to each experimental configurations, a slow diffusing component, D_a^{slow} , and a fast diffusing component, D_a^{fast} . The fraction of the decay attributed to D_a^{slow} was designated as f_{slow} . Note also that the water temperature in both experiments was 21.0 °C and thus the diffusion coefficient of water is about 2.08×10^{-5} cm²/s [45].

Results for $T_d = 320$ ms

The same measurements that were performed for $T_d = 50$ ms have also been done for $T_d = 320$ ms. Again, the shape of the diffusion decays was affected by the flow and the decays deviate from the exponential behaviour, as shown in Figs. 4.13 and 4.14.

Configuration of experiment #1 ($T_d = 320$ ms)	Results from an biexponential fit ($D_a \times 10^{-5}$ cm ² /s)	
	DSG's perpendicular to fibres	DSG's parallel to fibres
Air inside the fibres	$D_{a_{\perp}}^{\text{slow}} = 1.77 \pm 0.16$ $D_{a_{\perp}}^{\text{fast}} = 11 \pm 3$ $f_{\text{slow}} = 0.71 \pm 0.07$	$D_{a_{\parallel}}^{\text{slow}} = 1.69 \pm 0.14$ $D_{a_{\parallel}}^{\text{fast}} = 12 \pm 3$ $f_{\text{slow}} = 0.81 \pm 0.06$
Water inside the fibres	$D_{a_{\perp}}^{\text{slow}} = 1.87 \pm 0.06$ $D_{a_{\perp}}^{\text{fast}} = 12 \pm 4$ $f_{\text{slow}} = 0.90 \pm 0.06$	$D_{a_{\parallel}}^{\text{slow}} = 1.61 \pm 0.09$ $D_{a_{\parallel}}^{\text{fast}} = 8 \pm 3$ $f_{\text{slow}} = 0.88 \pm 0.04$

Configuration of experiment #2 ($T_d = 320$ ms)	Results from an biexponential fit ($D_a \times 10^{-5}$ cm ² /s)	
	DSG's perpendicular to fibres	DSG's parallel to fibres
Air inside the fibres	$D_{a_{\perp}}^{\text{slow}} = 1.99 \pm 0.12$ $D_{a_{\perp}}^{\text{fast}} = 7.0 \pm 2.0$ $f_{\text{slow}} = 0.88 \pm 0.04$	$D_{a_{\parallel}}^{\text{slow}} = 1.52 \pm 0.15$ $D_{a_{\parallel}}^{\text{fast}} = 6.0 \pm 1.6$ $f_{\text{slow}} = 0.82 \pm 0.08$
Water inside the fibres	$D_{a_{\perp}}^{\text{slow}} = 2.01 \pm 0.11$ $D_{a_{\perp}}^{\text{fast}} = 9 \pm 3$ $f_{\text{slow}} = 0.88 \pm 0.04$	$D_{a_{\parallel}}^{\text{slow}} = 1.70 \pm 0.08$ $D_{a_{\parallel}}^{\text{fast}} = 5.0 \pm 1.0$ $f_{\text{slow}} = 0.88 \pm 0.05$

Table 4.2: Results calculated from biexponential fits performed on the diffusion decays obtained in the presence of a flow in the extracellular compartment ($T_d = 320$ ms).

The results of the biexponential fits for the different experimental configurations are presented in Table 4.2. These results will be discussed in detail in the next chapter.

4.5. MC Simulations of Water Diffusion in White Matter

The MC model developed to analyze phantom results was also used to describe diffusion processes in real tissues. Different simulations were performed in order to better understand the effect of physical and geometrical properties of the axonal system on the measurable values of D_a . With these simulations, the validity of the MC codes could be checked by comparing the results with results of the literature.

To demonstrate the effect of the different cellular properties on D_a , a tissue model very similar to the one used by Szafer *et al.* [44] was considered. In their MC model, the authors used a *square* cell geometry with the relevant parameters

Figure 4.13: Examples of the biexponential diffusion decays obtained in the presence of flow in the extraluminal compartment of the diffusion phantom for $T_d = 320$ ms, when the intraluminal volume is empty. In a) DSG's are applied perpendicular to the fibre orientation, and in b) the DSG's are applied parallel to the fibres.

Figure 4.14: Examples of the biexponential diffusion decays obtained in the presence of flow in the extraluminal compartment of the diffusion phantom for $T_d = 320$ ms, when the intraluminal compartment is filled with water. In a) DSG's are applied perpendicular to the fibre orientation, and in b) the DSG's are applied parallel to the fibres.

(axon size, cellular volume fraction, D_I , D_E) adjusted to what is typically found in white matter. For the purpose of comparison, we considered exactly the same values for the intra- and extracellular diffusion coefficients, D_I and D_E , that were used by Szafer *et al.* Although the model is based on a circular cell geometry, the same cellular area ($7.3 \mu\text{m}^2$) and the same cellular volume fraction ($f = 0.8$) were also utilized.

Figure 4.15a shows the values of D_{a-} calculated by Szafer *et al.* for a set of axonal systems with different membrane permeabilities, assuming $(T_2)_I = (T_2)_E$. The corresponding values obtained from our MC model are shown in Fig. 4.15b. In these simulations, a total of $N = 10000$ particles were followed and D_{a-} was calculated from the mean squared displacement of the molecules since the relaxation effects are not an issue when T_2 is homogeneous.

However, when a difference exists between intra- and extracellular T_2 relaxation rates, it is necessary to calculate D_a from the initial slope of the diffusion decay. For example, Figs. 4.15c and 4.15d correspond to the same systems considered in Figs. 4.15a and 4.15b, except that now different relaxation times, $(T_2)_I = 70$ ms and $(T_2)_E = 200$ ms, have been assigned to each compartment. Again, the diffusion of $N = 10000$ particles was simulated.

Figure 4.15: MC calculation ($N = 10000$ particles) of $D_{a_m}(T_d)$ for different membrane permeability values, P . The results in (a,c) are those of Szafer considering cells with square cross-sections, while our results for cells with circular cross-sections are presented in (b,d). For all plots, the cellular area is $7.3 \mu\text{m}^2$, $f = 0.8$, $dt = 5 \mu\text{s}$, $D_E = 2.5 \times 10^{-5} \text{ cm}^2/\text{s}$ and $D_I = 1.0 \times 10^{-5} \text{ cm}^2/\text{s}$. The plots in (a,b) were produced assuming $(T_2)_I = (T_2)_E$, while in (c,d) $(T_2)_I = 70 \text{ ms}$ and $(T_2)_E = 200 \text{ ms}$.

Chapter 5 Analysis and Discussion

5.1. Calibration Results

The results obtained from the measurements of the diffusion coefficient D , shown in Fig. 4.1, demonstrate that the sequences used in this research are working properly. These results are in agreement with the expected value of D for water at 21.0 °C. However, the experimental values of D measured along the HF direction are lower than the values of D measured along the AP direction for 7 of the 9 values of T_d . A possible reason for this phenomenon is the calculation of the b -values which does not consider any anisotropic background gradients that could exist in the magnet. It could also be associated with a small temperature shift (≤ 0.5 °C) between the two sets of measurements. But overall, the experimental results are accurate enough that the measurements of D_a in the other experiments can be trusted.

5.2. Measurements of D_a as a Function of T_d in the Phantom

Results of D_a and $D_{a\perp}$ for water diffusing in the intraluminal and/or the extraluminal compartment(s) of the phantom are depicted in Figs. 4.2 to 4.4. The first observation that can be made about these three graphs is the anisotropy of D_a in the phantom. For every configuration, the values of $D_{a\parallel}$ are clearly larger than the values of $D_{a\perp}$. This was expected since the movement of the water molecules in the direction perpendicular to the fibres is hindered by the fibre membranes and their mean square displacement is reduced. Moreover, the values of $D_{a\parallel}$ are roughly constant for the three experiments, while the values of $D_{a\perp}$ decrease as a function of $\sqrt{T_d}$. This is explained by the fact that, in the direction perpendicular to the fibres, the relative number of molecules hitting the membranes increases with the diffu-

sion time. Thus, as T_d gets longer, the displacement distribution of the molecules deviates more and more from its behaviour in a freely diffusing medium.

However, the experimental values of D_{a+} and D_{a-} are not exactly the results that were expected *a priori*. For instance, in Fig. 4.2, the values of D_a are systematically lower than the diffusion coefficient of water. For fibres that are perfectly aligned along the DSG direction and that have specularly reflecting membranes, the value of D_a should, in theory, be constant as a function of T_d and be equal to D . On the other hand, the values of D_{a-} are also systematically lower than the values of $D_{a- \text{solid}}$ calculated from Söderman's equation for fibres with a diameter of 200 μm . The same behaviour is observed in Figs. 4.3 and 4.4: the diffusion coefficient of water is higher than the measured values of D_a and the values of D_{a-} are lower than the values calculated from MC simulations.

How can this difference between experimental results and analytical (or simulated) results be explained? If there was a deviation from the expected results only for D_a , then the alignment of the fibres with the DSG orientation could be questioned. However, the results of D_{a-} are also lower than the values calculated from Söderman's equation and from MC simulations.

A possible cause for obtaining values of D_a lower than predicted could be the presence of molecular segments protruding from the fibre walls. Remember that the fibre membrane is made of long cellulose acetate chains. Due to the hydroxyl groups, these chains are planar and bonded with each other rather tightly. Thus, it is not likely that the cellulose acetate membrane has molecular segments sticking out from the surface. Moreover, in order to have a significant effect, there would have to be a lot of these segments and they would have to protrude well out from the surface. But an inspection of fibres using optical microscopy showed that the inner and outer boundaries of the cylindrical membranes were very well defined on an image with a magnification of 40X.

Looking at Fig. 4.2, it appears that the difference between experimental and calculated values of D_a increases slightly with T_d . This phenomenon could happen if the water molecules 'stick' to the surface for a time τ every time they collide with

the walls. In such a case, the mean square displacement of the molecules for a given diffusion time T_d would be reduced and accordingly so would D_a . Two possibilities have been thought of to explain why molecules would not diffuse normally in the vicinity of the membrane. The first one is the hydration layer that is caused by the hydrophilicity of the cellulose acetate and within which the molecular diffusion is not completely free due to polar interaction of molecules with the walls. The second possible explanation is related to the porosity of the fibre membrane. In producing the $D_{a, \text{SMD}}$ curve in Fig. 4.2 and the $D_{a, \text{MC}}$ curves in Figs. 4.3 and 4.4, it was assumed that the fibre membranes are perfectly flat surfaces that do not interact with the molecules and that the latter are immediately reflected specularly when they collide with the membrane. But the fibre walls have many pores which are hydrophilic and are often a few microns deep. Accordingly, a molecule incident on a pore in the fibre wall is not likely to simply rebound from the membrane but will probably spend some time inside the pore. Note however that the average pore size is much smaller than the membrane thickness so that a molecule entering a pore is not likely to move all the way through the membrane, except if a pressure gradient is applied.

To verify how these phenomena could affect the calculated values of D_a , different simulations were performed in which the molecules, when they encounter a barrier, stay for a certain amount of time on the barrier before being reflected.

MC simulations with delayed reflections

The first simulations that have been run were performed to understand how delayed reflections affect the value of $D_{a, \text{MC}}$ for water molecules inside a fibre. As a numerical model for this system, the diffusion of a particle placed between two parallel planes was simulated. The distance between the planes was set to $157 \mu\text{m}$ and simulations were performed for *delay times*, τ , equal to 0, 500, 1000, 2000 and $3000 \mu\text{s}$. Results are shown in Fig. 5.1. As we can see, the values of $D_{a, \text{MC}}$ are roughly the same as the coefficient of diffusion of water when $\tau = 0 \mu\text{s}$. However, as τ increases, the value $D_{a, \text{MC}}$ deviates more and more from D as T_d gets longer because the time spent

Figure 5.1: Calculated values from MC simulations of D_a as a function of $\sqrt{T_d}$ for water diffusing between two planes separated by 157 μm . Each time a molecule encounters a plane, it ‘sticks’ there for a period $\tau = 0, 500, 1000, 2000$ or $3000 \mu\text{s}$. The parameters used for the simulations are $D = 2.15 \times 10^{-5} \text{ cm}^2/\text{s}$, $N = 30000$ and $dt = 15 \mu\text{s}$.

Figure 5.2: Calculated values from MC simulations of D_{a-} as a function of $\sqrt{T_d}$ for water diffusing inside a cylinder with $R = 100 \text{ } \mu\text{m}$. When a molecule encounters the cylinder boundaries, its reflection is delayed for a period $\tau = 0, 500, 1000, 2000$ or $3000 \text{ } \mu\text{s}$. Simulation parameters were set to $N = 30000$, $D_l = 2.15 \times 10^{-5} \text{ cm}^2/\text{s}$, and $dt = 15 \text{ } \mu\text{s}$.

by the molecules when they are sticking to the walls reduces their mean square displacement, and thus D_a .

MC simulations have also been performed to evaluate the effect of delayed reflections on the values of $D_{a-}(T_d)$ for molecules diffusing within a fibre. The results are depicted in Fig. 5.2. Again, the calculated values of D_{a-} deviate from $D_{a- \text{solid}}$ with increasing T_d .

A comparison of the simulated results in Figs. 5.1 and 5.2 with the experimental results of Fig. 4.2 suggests that the sticking time of the molecules on the membrane would be between $1000 \text{ } \mu\text{s}$ and $2000 \text{ } \mu\text{s}$. This is much longer than typical molecular residency times in the bound state of a hydration layer ($\approx 10 \text{ } \mu\text{s}$). Thus, the effect

of the hydration layer alone is not enough to explain the results obtained. However, the effect of the pores could cause a delay of 1000 μs or 2000 μs in the reflection of a molecule. Indeed, consider water with a coefficient of diffusion $D = 2 \times 10^{-9} \text{ m}^2/\text{s}$. During 1000 μs , the root-mean-square displacement, $\sqrt{\langle x^2 \rangle}$, along a given direction will be from Eq. 2.7,

$$\begin{aligned}\sqrt{\langle x^2 \rangle} &= \sqrt{2T_d D} \\ &= 2 \mu\text{m}.\end{aligned}$$

Considering that the average pore size in cellulose acetate membrane ranges between 0.1 μm and 1 μm , it is reasonable to think that a water molecule can stay up to 2000 μs inside a pore when it encounters the membrane. Also, the hydration layer on the walls of each pore may tend to increase the residency time of molecules within them. The water molecules that enter these hydrophilic pores will likely interact with the cellulose acetate several times before leaving the pore.

Effect of delayed reflections on diffusion decays

In the scenario where molecules are not immediately reflected when colliding with the membrane, only the molecules that are close enough to the walls at time zero that they can reach the membrane during T_d will have a reduced D_a . For water diffusing inside a cylindrical fibre of radius R , the fraction of molecules able to collide with the walls will grow until $\sqrt{\langle r^2 \rangle} = R$. In other words, for short diffusion times, there will exist two groups of spins: one diffusing at D with no chance to interact with the walls and another also diffusing at D but having a reduced D_a because of interactions with the walls. Such a difference should be observable as 2 components in the diffusion decay. The larger diffusion coefficient, D_a^{fast} , will correspond to the spins not interacting with the walls. This component will initially be 100%, but will reduce with T_d to 0%.

The question is whether or not it is possible to extract these two components from the diffusion decays obtained experimentally. MC simulations have been run in order to study the effect of delayed reflections on the shape of the diffusion

Figure 5.3: Simulated diffusion decays for water diffusing inside a cylindrical fibre during 50 ms with $\tau = 0 \mu\text{s}$ and $\tau = 2000 \mu\text{s}$. The other parameters used for the simulation are $N = 10000$, $dt = 15 \mu\text{s}$, $R = 100 \mu\text{m}$ and $D = 2.22 \times 10^{-5} \text{ cm}^2/\text{s}$.

decay for diffusion perpendicular to the fibres. In Fig. 5.3, the simulated decays at $T_d = 50 \text{ ms}$ are shown for $\tau = 0 \mu\text{s}$ and $\tau = 2000 \mu\text{s}$ in the b -value range that has been studied experimentally. The slope of the decay is steeper when $\tau = 0 \mu\text{s}$ because D_a is larger in the absence of sticking. Furthermore, the diffusion decay deviates slightly from being exponential when $\tau = 2000 \mu\text{s}$. However, this deviation is subtle and it would be impossible to distinguish it on the diffusion decays that were obtained experimentally because the data are too noisy for high b -values. A SNR high enough to see such a deviation on experimental data would require several hours of acquisition with the imager that was utilized.

In summary, the results for $D_{a\parallel}$ and $D_{a\perp}$ as a function of T_d are in reasonable agreement with the results calculated from Söderman's expression and MC simu-

lations. Better agreement is obtained when the effect of the pores is taken into consideration. The residency time that has been evaluated ($\tau \approx 1000$ to $2000 \mu s$) is consistent with the average pore size in the fibre membranes and should not have caused a notable change in the shape of the diffusion decays.

5.3. Comparison with White Matter Axons

One difficulty with comparing our phantom model with white matter tissues is that the phantom fibres and the axonal fibres have quite different diameters: $200 \mu m$ for the phantom fibres and about $5 \mu m$ for the axons. For a convenient comparison of axonal systems with different sizes, the results were plotted in terms of the *relative* apparent diffusion coefficient, D_a/D , as a function of the unitless *reduced time*, T . In a study of Tanner [27] about diffusion in a system partitioned by infinite planes, the reduced time has been defined as

$$T \equiv \frac{T_d D}{a^2}, \quad (5.1)$$

where D is the coefficient of diffusion of the fluid and a is the separation between the planes. In the present case, a will be considered as the *diameter* of the fibres.

In Fig. 5.4, the values of D_a/D calculated from Söderman's equation are plotted as a function of T for different fibre radii ranging between $2.5 \mu m$ and $100 \mu m$. These values correspond to the calculated values of D_a/D for $50 \text{ ms} \leq T_d \leq 1000 \text{ ms}$. They are all lying in different regions of a same curve and there is no overlapping of the values for $R = 2.5 \mu m$ with the values for $R = 100 \mu m$. The results for $R = 100 \mu m$ are in the upper region of the curve describing the 'free' diffusion. In this region, the fraction f_C of molecules that can collide with the membrane during a time interval T_d is low. Actually, if we refer to Fig. 5.5, then this fraction corresponds to the shaded area near the membrane boundaries and can be written as:

$$f_C = \frac{\pi (R^2 - r^2)}{\pi R^2}, \quad (5.2)$$

where R is the radius of the fibre and r determines the region within which the molecules are too far from the walls to reach them. In other words, only the particles

Figure 5.4: Relative apparent diffusion coefficient, D_a/D , as a function of reduced time for water diffusing within non-permeable fibres with different diameters. Each curve was calculated from Söderman's equation for $50 \text{ ms} \leq T_d \leq 1000 \text{ ms}$, using $b = 500 \text{ s/mm}^2$ and $D = 3.0 \times 10^{-5} \text{ cm}^2/\text{s}$.

Figure 5.5: The average fraction of the molecules able to reach the boundaries in a time T_d is $f_C = \frac{2R\sqrt{\langle x^2 \rangle} - \langle x^2 \rangle}{R^2}$.

whose $\sqrt{\langle x^2 \rangle} \leq R - r$ have, on average, the possibility to hit the walls. Hence,

$$\begin{aligned}
 f_C &= \frac{R^2 - \left(R - \sqrt{\langle x^2 \rangle}\right)^2}{R^2} \\
 &= \frac{2R\sqrt{\langle x^2 \rangle} - \langle x^2 \rangle}{R^2} \\
 &= \begin{cases} \frac{2(R\sqrt{2DT_d} - DT_d)}{R^2} & (\sqrt{2DT_d} \leq R) \\ 1 & (\sqrt{2DT_d} \geq R) \end{cases} \quad (5.3)
 \end{aligned}$$

In Fig. 5.6, f_C is plotted as a function of $\sqrt{T_d}$ for water inside fibres with $R = 2.5, 10, 50$ and $100 \mu\text{m}$. Considering that the observation time is limited to about 1 s experimentally, we see that molecules within an axon ($R = 2.5 \mu\text{m}$) do not need so much time to reach the boundaries and thus can experience several collisions with the cellular membrane. On the other hand, the value of f_C after 1 s for the phantom fibres ($R = 100 \mu\text{m}$) is less than 100% and the effect of restriction for water inside the phantom fibres is much less than for the intracellular fluid inside the axons. Thus, in this particular case of water diffusing *inside* a fibre, the restriction effects in axons fibres are considerably more important than those observed in phantom fibres, considering that the range of diffusion times studied experimentally is 50–1000 ms.

Nevertheless, if we consider a system of fibres with permeable membranes – a more realistic description of white matter axons – then the situation is different. MC simulations were performed for axonal systems with fibres of different sizes and permeabilities. In these simulations, D was set to $3.0 \times 10^{-5} \text{ cm}^2/\text{s}$, the approximate diffusion coefficient for water at body temperature, inside and outside the fibres. The calculated values of D_a/D for $50 \text{ ms} \leq T_d \leq 1000 \text{ ms}$, assuming a cellular volume fraction of 0.8, are plotted in Fig. 5.7.

As we can see, for non-permeable fibres and short T 's, the effect of changing the fibre size is about the same as observed in Fig. 5.4. For longer T 's however, D_a/D becomes constant since the extracellular diffusion of molecules is not completely restricted in space, but only tortuous. When the fibres are permeable, this constant value is higher because the intracellular particles now have a certain probability of

Figure 5.6: f_C as a function of $\sqrt{T_d}$ for water inside fibres with $R = 2.5, 10, 50$ and $100 \text{ } \mu\text{m}$.

Figure 5.7: D_a/D as a function of reduced time for different systems of permeable fibres. All the values of D_a were obtained for $50 \text{ ms} \leq T_d \leq 1000 \text{ ms}$. 6 axonal sizes and 3 membrane permeabilities ($P = 0 \text{ cm/s}$ (white), 0.001 cm/s (grey) and 0.01 cm/s (black)) were considered. The MC parameters used are $N = 30000$, $D_I = D_E = D = 3.0 \times 10^{-5} \text{ cm}^2/\text{s}$ and $f = 0.8$.

leaving the cells and traveling longer distances. If the permeability is high enough (e.g. > 0.01 cm/s), the diffusion regimes are almost always constant whatever the fibre radius is. In this situation, only a proportionality factor differentiates the diffusion processes within two fibre systems of different sizes. Accordingly, the phantom would be a better representation of the axonal system if its fibres were permeable. Different uses of such a phantom will be discussed in Section 6.4.2.

5.4. *In Vivo* Study

The calculated values of D_a as a function of position in the white matter were presented in Fig. 4.5b. In this plot, D_a is lower in the central region of the COI than in the anterior and posterior regions. This is explained by the fact that the DSG's of the NMR sequence were applied in the AP direction, i.e. parallel to the axons in the anterior and posterior regions, but perpendicular to the nerve fibres in the central region.

To evaluate D_a along the COI, exponential fits were performed on the diffusion decays. However, a closer look at those decays revealed that they are not always monoexponential. The diffusion decays presented in Fig. 4.6, although they are noisy, clearly demonstrate a deviation from exponential behaviour. The diffusion decays obtained on the second volunteer (Fig. 4.7) are more ambiguous: in both central and posterior regions, the shape of the decay seems to be almost monoexponential. Finally, the diffusion decays acquired on volunteers #3 and #4 (Figs. 4.8 and 4.9) also present deviations from the exponential shape, but the deviation observed for volunteer #3 is less important than those observed for volunteers #1 and #4.

Although only 8 diffusion decays are presented in this work, hundreds of decays were studied. It was observed that the shape of the diffusion decay presents important variations from one region of the white matter to another, and also from one volunteer to another. It was attempted to quantify the slowly diffusing component and the fast diffusing component by performing a biexponential fit on each decay. This exercise has not been successful because of the poor SNR of the decays, their spatial variation and the difference between the results obtained from subject to

subject. Nevertheless, there is no doubt that the magnetization decay in the white matter has more than one component. This is consistent with several recent studies [17, 18, 19].

Different explanations have been proposed in the literature to explain the additional components observed in the diffusion decays. Gates and Cameron [46, 19] suggested that the fast diffusing component could be the result of extracellular fluid motion due to the hydrostatic pressure exerted on the cerebral tissues when blood pulses through the brain vasculature. It has already been observed that this mechanism causes a pulsatile motion of the CSF in the nearby ventricles [47] and hence could produce an oscillatory movement of the fluid between the axons. To verify how such a flow affects the shape of the diffusion decays, different phantom experiments were performed in the presence of a pulsed interstitial flow.

5.5. Measurements of D_a in the Presence of Extraluminal Flow

Analysis of measurements at $T_d = 50.6$ ms

The results of the biexponential fits performed on signal profiles were presented in Table 4.1. As demonstrated before, the extraluminal flow causes the diffusion decays to deviate from the exponential behaviour. Accordingly, the fast diffusing component, D_a^{fast} , must be attributed to the flow and the slowly diffusing component, D_a^{slow} , to the real diffusion such as observed when no flow is present.

Indeed, the values of D_a^{slow} are in good agreement with the values of D_a presented in Section 4.2 for restricted diffusion in the different compartments of the phantom. For example, in Fig. 4.3, the value of $D_{a||}$ obtained for diffusion in the extraluminal compartment was $(2.05 \pm 0.05) \times 10^{-5} \text{ cm}^2/\text{s}$, while the values of $D_{a||}^{\text{slow}}$ extracted from the biexponential fits for air-filled fibres were $(2.09 \pm 0.09) \times 10^{-5} \text{ cm}^2/\text{s}$ and $(2.10 \pm 0.07) \times 10^{-5} \text{ cm}^2/\text{s}$ for the two experiments performed. For perpendicular diffusion sensitization (and air-filled fibres), the values of $D_{a\perp}^{\text{slow}}$ are slightly lower than the value of $D_{a\perp}$ obtained when no flow is present: $(1.93 \pm 0.11) \times 10^{-5} \text{ cm}^2/\text{s}$. When the fibres are filled with water, the agreement is better; the value of $D_{a\perp}^{\text{slow}}$ ($1.85 \times 10^{-5} \text{ cm}^2/\text{s}$) is almost the same as it was in the absence of flow (1.88×10^{-5}

cm²/s in Fig. 4.4), and the values of $D_u^{\text{slow}} (\sim (1.95 \pm 0.05) \times 10^{-5} \text{ cm}^2/\text{s})$ are consistent with the result obtained without the flow $((2.05 \pm 0.03) \times 10^{-5} \text{ cm}^2/\text{s})$. Thus, it is possible to distinguish accurately the diffusional component from the flow component in the diffusion decays.

The fraction of the decays attributed to the slowly diffusing component, f_{slow} , is 70% or higher for all measurements, even those for water only in the extraluminal volume. Remember that the flow is pulsed with a frequency of about 1 Hz. In consequence, for each cycle of the pump, the water spends a part of the time flowing in the filter and the remaining part staying relatively still. But even during the period when water is flowing, all the molecules do not move with the same velocity. Indeed, a fluid flowing along a solid always experiences some friction at the interface. This so-called *shear stress* causes the water near the fibre walls to be more or less stationary [48]. Moreover, the *boundary layer* near the wall will cause drag for the next layer of fluid such that the friction effect will be gradually communicated to layers which are deeper still. Consequently, the velocity profile of the flowing water between the fibres is definitely not constant as a function of space or as a function of time.

From the data we have, it is difficult to quantify the relative effects of these factors on the value of f_{slow} . However, it is possible to evaluate how f_{slow} should change depending on whether the intraluminal volume is filled or not. For instance, consider f_{slow} to be 70% when there is no water inside the fibres. Since the intraluminal volume fraction, f , is 0.3 for the phantom used and 100% of the water inside the fibres is *only* diffusing, the value of f_{slow} when the fibres are filled should be

$$\begin{aligned} f_{\text{slow}}^{\text{water inside}} &= 100\% \cdot f + f_{\text{slow}}^{\text{air inside}} \cdot (1 - f) \\ &= 30\% + 70\%(1 - 0.3) \\ &= 79\%. \end{aligned} \tag{5.4}$$

Thus, when f_{slow} is initially 70%, it should increase by 9% when adding water in the intraluminal volume. For f_{slow} initially at 89%, the increase should be approximately 3%. This is in good agreement with the measurements.

Note also that a flow component is detected in the direction perpendicular to the fibres. This component may be caused by some vortices or turbulence in the extracellular volume. The unidirectionality of the flow is also compromised by the fact that the water entering in the filter is perpendicular to the fibres before it meets the deflector. The deflector allows the flow to be more uniformly distributed in the filter but it does not guaranteed that the flow will be perfectly parallel to the fibres. Nevertheless, the measured values of D_a^{fast} are very consistent with each others for both DSG orientations. Furthermore, the results for D_a^{fast} are higher than the measured values of D_a^{fast} for the same experimental configuration as expected.

Analysis of measurements at $T_d = 320$ ms

Overall, the results of the biexponential fits presented in Table 4.2 are in agreement with those obtained for $T_d = 50.6$ ms. The results are however less accurate than those for $T_d = 50.6$ ms since only 26 b -values were used at $T_d = 320$ ms. Moreover, the SNR of the diffusion decays was reduced because a PGSTE sequence was used, and hence half of the magnetization was lost. Also, since the COI is aligned perpendicular to the direction of flow, a significant amount of ‘labelled’ spins leaves the column during the interval T_d .

Actually, a flow of 90 ml/min was measured at the outlet of the pumping line. Considering that the cross-section of the filter is 8.3 cm², it was calculated that the flow corresponds to a bulk motion of the water in the extracellular volume of about 0.3 cm/s. The COI is 1 cm thick, and thus for a bulk displacement of the water of 0.3 cm/s, about 10% of the spins will leave the COI between the beginning of the sequence and the readout. It was attempted to do some measurements for diffusion times longer than 320 ms but the quality of the diffusion decays was really too low to provide any useful information.

Thus, the effect of a pulsed flow on the diffusion decays was unambiguously observed in different experimental configurations and for two diffusion times. The slowly diffusing components extracted from the decays, D_a^{slow} , were in good agreement with the previous measurements of Section 4.2. Finally, even if the results of

f_{slow} are not completely understood, their variation from one experimental configuration to another is reasonable.

5.6. MC Simulations of Water Diffusion in White Matter

In Fig. 4.7, the MC results obtained using a circular cell geometry are presented along with the results published by Szafer *et al.* [44] for a square cell geometry. In the situation where $(T_2)_I = (T_2)_E$, the results obtained (Fig. 4.15b) are very similar to the results of Szafer *et al.* (Fig. 4.15a), particularly for low permeabilities. When the permeability of the cells is increased, the values of D_a for circular cells are slightly higher than for square cells. This can be explained by the fact that for a single diffusion step of a particle inside a square cell, the particle can only be reflected one or two times (e.g. if the particle is in a corner of the square). However, a particle inside a circular cell can be reflected more than two times if the particle is incident on the membrane with a very small angle. Each time a particle encounters the wall, it has a given probability of being transmitted through the membrane. Consequently, the probability for a particle inside a circular cell of being transmitted outside the cell (where $D_E \gtrsim D_I$) is more important than for a particle inside an equivalent square cell, and thus the value of D_{a-} is higher.

When $(T_2)_I \neq (T_2)_E$, simulation results (Fig. 4.15d) that are consistent with those of Szafer *et al.* (Fig. 4.15c) were also obtained. But in this case, the shape of the different curves of $D_{a-}(T_d)$ is more complex. For highly permeable membranes, the molecules can transfer almost freely between the intra- and extracellular environments, so the diffusion decay of the magnetization after a time T_d is essentially the same for all molecules. Accordingly, D_{a-} is almost unaffected by the T_2 inhomogeneity of the system. However, for poorly permeable membranes, the diffusion decay of the magnetization in the intracellular environment may be very different from the one in the extracellular space. For instance, in the simulations (Fig. 4.15d), a large fraction of the intracellular magnetization is lost as T_d gets longer because of the short T_2 relaxation time. Consequently, the value of D_{a-} increases because it becomes more representative of diffusion in the extracellular environment.

Hence, the agreement between the MC simulations and those of Szafer *et al.* is very good considering that systems with different cell and lattice geometries were studied. It appears from these results that the value of D_{a-} for longer diffusion times could be useful for determining the permeability of the axonal membranes, but that the differences between $(T_2)_I$ and $(T_2)_E$ may bias the measurement of D_{a-} when T_d gets too long.

Chapter 6 Conclusions and Future Directions

6.1. Restricted Diffusion Inside the Phantom

The diffusion time dependence of D_a in the range $50 \text{ ms} \leq T_d \leq 1020 \text{ ms}$ was clearly established for water diffusing inside the phantom. It was shown unambiguously that the diffusion occurring inside and/or outside the phantom fibres is anisotropic. The measured values of D_a were not constant as a function T_d as was expected *a priori* and the results of D_{a+} also deviated from the MC calculations. Nevertheless, it was demonstrated that these behaviours are likely caused by the porous nature of the phantom membranes. The reflection of molecules by the membrane is not instantaneous and this causes D_a to decrease for longer T_d 's. However, the effect of this molecular trapping on the shape of the diffusion decays is too low to be detected, as demonstrated by the simulations.

It appears from our data that even for a well-defined system such as the phantom that was utilized, which is much simpler than the axonal system *in vivo*, the interpretation of D_a can remain a complicated task. But a phantom study such as the one presented in this work is necessary because it makes possible the calibration of NMR sequences before they are used for *in vivo* tissues. It also provides useful information that can be used to refine the MC models. For example, the molecule-membrane interactions were at first neglected in the simulations. It is likely that this kind of interaction is also important in the white matter. For instance, it is known that some proteins of the myelin sheath are hydrophobic [49] and thus polar interactions should be included in the simulations.

6.2. Effect of a Slow Flow in the Extraluminal Volume

It was clearly demonstrated that a slow, pulsed flow in the extraluminal volume modifies significantly the shape of the diffusion decays measured in the phantom. It was also shown that two distinct components can be extracted from these decays: one attributed to the flow and the other to the real diffusion movement of the particles. The component attributed to diffusion was properly isolated since its values were relatively accurate and in good agreement with the results obtained in the absence of flow.

In most tissues of the human body, perfusion and/or other types of fluid movements happen and can contaminate the measured values of D_a if care is not taken to avoid this. By preventing this type of contamination, the isolation and the suppression of the extra flow component in the diffusion decays could improve the quality of data measured in different clinical applications of diffusion MRI.

6.3. *In Vivo* Measurements

The *in vivo* study presented in this thesis is not exhaustive. The shape of the diffusion decays was described rather qualitatively because of their poor SNR and the difference in the results from one volunteer to another. Obtaining a better SNR would have required us to do more acquisitions and leave the subjects in the imager for several hours, what was unfortunately not possible. However, it was demonstrated that the diffusion decays in white matter deviate from the exponential behaviour and thus that an accurate calculation of D_a cannot be done from a 2-point attenuation curve.

The imager at the Ottawa Hospital uses 15 years old technology. It will be replaced soon by a state-of-the-art imager capable of producing magnetic field gradients with amplitude at least 3 times stronger than those that were utilized. Considering that the b -values are proportional to the square of the gradient amplitude, the range of reachable b -values will be about one order of magnitude larger. Stronger gradients will also allow us to reduce the echo time, TE , and hence get a better SNR.

Measurements of D_a for diffusion times at least 10 times shorter will also be possible with such a gradient system. Furthermore, phase-array coils and better amplifiers will increase significantly the SNR. The switching rate of these new gradients will also be more rapid so that the use of ultrafast imaging techniques, such as *echo-planar imaging* (EPI), will be possible. These techniques would allow us to do the phase encoding required to produce diffusion-weighted images, without increasing the acquisition time.

6.4. Future Directions

The MC calculations presented in this work clearly show that the T_2 inhomogeneities in the axonal system and the permeability of cellular membranes have a major effect on the value of D_a . We conclude this work by explaining how the present phantom study could be extended to study experimentally the effect of these two parameters.

Inhomogeneous T_2 relaxation

In the white matter, the higher protein content and viscosity in the intracellular environment causes $(T_2)_I$ to be shorter than $(T_2)_E$ [44, 50, 51]. For long diffusion times, this T_2 inhomogeneity can considerably increase the values of D_a measured *in vivo*, as demonstrated in the simulation results of Fig. 4.15d.

In the phantom experiments we performed, we did not use different liquids in the intra- and extraluminal volumes in order to prevent any effect of T_2 inhomogeneities on D_a . But it would be interesting to perform phantom experiments that more closely resemble the real white matter environment. It is known that the average T_1 and T_2 relaxation times in white matter axons are about 760 ms and 80 ms respectively [51]. It is also known that the properties of the cerebrospinal fluid (CSF) found in the extracellular environment are almost the same as water so that $T_1 \gtrsim 4$ s and $T_2 \gtrsim 2$ s. Recently, Tofts *et al.* [52] identified and characterized different liquids whose coefficient of diffusion covers the range of apparent diffusion coefficients measured in the brain. For each of these liquids, they also measured T_1 and T_2 . Their results are shown in Table 6.1.

Liquid	$D (\times 10^{-5} \text{ cm}^2/\text{s})$ at 22°C	T_1 (ms)	T_2 (ms)
cyclohexane C_6H_{12}	1.401	2328	1329
cycloheptane C_7H_{14}	0.942	2156	1598
cyclooctane C_8H_{16}	0.525	1608	1234
<i>n</i> -octane C_8H_{18}	2.250	1932	193
<i>n</i> -nonane C_9H_{20}	1.719	1748	140
<i>n</i> -decane $\text{C}_{10}\text{H}_{22}$	1.327	1526	145
<i>n</i> -undecane $\text{C}_{11}\text{H}_{24}$	1.060	1331	204
<i>n</i> -dodecane $\text{C}_{12}\text{H}_{26}$	0.827	1160	163
<i>n</i> -tridecane $\text{C}_{13}\text{H}_{28}$	0.667	999	173
<i>n</i> -tetradecane $\text{C}_{14}\text{H}_{30}$	0.533	869	198
<i>n</i> -pentadecane $\text{C}_{15}\text{H}_{32}$	0.430	751	178

Table 6.1: Values of D , T_1 and T_2 for different liquids, as measured by Tofts *et al.*

We think that such liquids could be used in a phantom study to get a better model of the white matter tissue. A good configuration could be, for instance, to fill the extraluminal compartment of the phantom with water and the intraluminal compartment with *n*-dodecane. We have run MC simulations to find out what the experimental results would look like for such an experiment. Results are depicted in Fig. 6.1. The curve is very different from what we measured using only water in Fig. 4.4. Firstly, D_a increases as a function of T_d because as the observation time increases, the fraction of the signal coming from water also increases until D_a reaches the free diffusion coefficient of water. The same phenomenon is observed for D_{a-} , except that at some point ($T_d^{\frac{1}{2}} \approx 0.7 \text{ s}^{\frac{1}{2}}$), the value of D_{a-} does not saturate but begins to decrease because of the spatial restriction.

In this simulation, the interactions between the molecules and the membrane were not considered. They may vary from one type of molecule to another, but this would need to be assessed experimentally.

Phantom measurements with permeable fibres

One problem with the phantom experiment we have done is that no exchange occurs between the intra- and extraluminal compartments, while in the white matter the cellular membranes of the axons are permeable. It has been shown [53] that, for

Figure 6.1: MC simulation of the diffusion inside a phantom of two fluids with different T_2 relaxation times and diffusion coefficients. In this case, the fibres were filled with *n*-dodecane and the extraluminal volume was filled with water. The parameters used to obtain $D_{a_{\perp}}$ are $N = 30000$, $R = 105 \mu\text{m}$, $f = 0.30$, $D_I = 0.83 \times 10^{-5} \text{ cm}^2/\text{s}$, $D_E = 2.25 \times 10^{-5} \text{ cm}^2/\text{s}$, $(T_2)_I = 163 \text{ ms}$ and $(T_2)_E = 2500 \text{ ms}$. The values for $D_{a_{\parallel}}$ were obtained using cell dimensions 10000 times larger in order to minimize the restriction effects.

relatively simple membrane systems *in vitro*, the permeability, P , of the membranes can be determined from the values of D_{a-} using long diffusion times. However, it is not clear if this approach can be extended easily to complex systems such as the human brain *in vivo*, the values of D_{a-} being strongly affected by T_2 inhomogeneities, flow, and perfusion as T_d increases.

A phantom study could be very useful in the development of an experimental protocol for the measurement of P . The same kind of phantom we used could be utilized in this study, but the fibre membranes should be permeable and well characterized. If such a study could lead to the measurement of P *in vivo*, then it would likely have important clinical ramifications. For instance, permeability-weighted MR images could be generated in order to visualize regions of normal and abnormal permeability in tissues. In white matter diseases such as multiple sclerosis, it might be possible to distinguish areas where the myelin sheath and/or the axonal membrane have been compromised. Permeability imaging could also be used in the diagnosis of ischemic injuries. Indeed, it has been shown that D_a in cerebral tissues drops significantly in the minutes following a stroke [54, 55]. This phenomenon could be attributed to a reduction in the water transport in the membranes of injured cells and thus a reduction of P . Accordingly, the P -weighted images could be very useful in the detection of areas of the brain affected by a stroke. But this procedure would not be restricted to brain only. It could potentially be used to detect abnormalities in cell membrane permeability in other tissues of the human body as well.

In brief, considerable work remains to be done in the field of diffusion MRI. Phantom studies will be a part of this work since it is often necessary to test new methods on systems with a degree of complexity lower than real tissues.

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