The Effect of Cross Relaxation on the NMR Behaviour of Bovine White Matter

by

THORARIN ALBERT BJARNASON

B. Eng. (Engineering Physics) University of Saskatchewan, 2002

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF APPLIED SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

PHYSICS

THE UNIVERSITY OF BRITISH COLUMBIA

July 2005

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Abstract

In vitro experiments on 15 white matter samples from 5 bovine brains were performed on a $^1$H-NMR spectrometer at 24 and 37°C. A four pool model including intra/extracellular water, myelin water, non-myelin tissue, and myelin tissue was proposed to represent the NMR behaviour of bovine white matter. A cross relaxation correction was introduced to compensate for shifting of the measured data points and $T_2$ times over the duration of the CPMG measurement due to cross relaxation. This correction was found to be slight, providing evidence that myelin water fractions measured using a multi-echo technique are near physical values. At 24°C the cross relaxation times between myelin tissue and myelin water, myelin water and intra/extracellular water, and intra/extracellular water and non-myelin tissue were found to be approximately 227, 2064, and 402 ms, respectively. At 37°C these same cross relaxation times were 158, 1021, and 170 ms, respectively. The cross relaxation time between the two mobile pools at 24°C was found to be roughly two times bigger than the larger $T_1$ value of the measured bi-exponential $T_1$ times for white matter. However, at 37°C this cross relaxation time was of the same order as the measured mono-exponential $T_1$ value. Thus, one can conclude that cross relaxation is responsible for merging the two $T_1$ peaks at the higher temperature. The cross relaxation time was over an order of magnitude larger than the measured $T_2$ times and hence unable to merge the $T_2$ peaks together. The exchange rate between myelin water and myelin was found to be $11.8 s^{-1}$ at 37°C while the exchange rate between intra/extracellular water and non-myelin tissue was found to be $6.8 s^{-1}$. These exchange rates are of similar magnitude; indicating that the interaction between intra/extracellular water and non-myelin tissue cannot be ignored.
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<table>
<thead>
<tr>
<th>Symbol or Abbreviation</th>
<th>Meaning</th>
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<tbody>
<tr>
<td>$\gamma$</td>
<td>gyromagnetic ratio ($42.577 \text{MHz}/T$ for protons)</td>
</tr>
<tr>
<td>$\hbar$</td>
<td>Planck's constant ($1.0546 \times 10^{-34} \text{J} \cdot \text{s}$)</td>
</tr>
<tr>
<td>$\omega_0$</td>
<td>Larmor frequency ($128 \text{MHz}$ for protons at a magnetic field strength of $3T$)</td>
</tr>
<tr>
<td>$\mu_0$</td>
<td>permeability of free space ($4\pi \times 10^{-7} \text{N} / \text{A}$)</td>
</tr>
<tr>
<td>CPMG</td>
<td>Carr-Purcell-Meiboom-Gill</td>
</tr>
<tr>
<td>FID</td>
<td>free induction decay</td>
</tr>
<tr>
<td>IE</td>
<td>intra/extracellular</td>
</tr>
<tr>
<td>$M_2$</td>
<td>second moment</td>
</tr>
<tr>
<td>MF</td>
<td>mobile fraction</td>
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<tr>
<td>MR</td>
<td>magnetic resonance</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>MS</td>
<td>Multiple Sclerosis</td>
</tr>
<tr>
<td>MWF</td>
<td>myelin water fraction</td>
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<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NNLS</td>
<td>non-negative least squares</td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
</tr>
<tr>
<td>$T_1$</td>
<td>longitudinal, or spin-lattice, relaxation time</td>
</tr>
<tr>
<td>$T_2$</td>
<td>transverse, or spin-spin, relaxation time</td>
</tr>
<tr>
<td>TE</td>
<td>echo time</td>
</tr>
<tr>
<td>TR</td>
<td>repetition time</td>
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Acknowledgements

This manuscript exists because of the help and support of many people. As a token of my gratitude they get to see their names in print.

Alex M*Kay is a wonderful supervisor. His insight into the physics behind MRI is astounding, and his relaxed nature always keeps me grounded. Thank you Alex for your guidance and support and for taking a chance on a student with mediocre grades.

The MRI Research Group has provided a great working environment that is definitely unique. Our crowded space seemed less so with the open nature of our lab. Most importantly, the lab is nothing without the people involved. Corree Laule and Shannon Kolind are great to travel with and I thank you both for helping me feel comfortable in the lab when I began. Whenever I needed it, you both seemed able to spare time for me. Irene Vavasour and Charmaine Chia began the research that became my Masters project. Thank you both for your hard work. Burkhard Mädler and I have had many discussions about the physics of MRI, and I learned a lot from this. Thank you Boogie for our chats and for allowing me access to your library. Evan Minty and I also enjoyed talking shop, especially during our detours when intending to get coffee. Thank you all for being the MRI Research group, and thank you for constantly keeping me busy with tangential projects; I seem to focus best when my mind is somewhere else. Finally, I would like to acknowledge Ken Whittall; you left a bit of a legacy. Even from a distance I feel as though you have influenced my work and when I did have a chance to corner you with technical questions you were always prepared with an answer.

Linda Chandler. You helped me immensely. I seem to be a magnet for administrative hiccups, and you were always there to help me sort it out. Occasionally, when it was necessary, you picked up the phone and let them have it: for this I am grateful.

Tony Walters, thank you for keeping me in the green. You saved my hide on numerous occasions.

Thank you Qing San Xiang for being my second reader. I enjoyed the discussions we had and am always impressed with the way you explain complicated concepts so that when you are done, the concepts become simple.

This manuscript was written using OpenOffice.org software. I thank the OpenOffice.org community for the technical assistance they provided through the newsgroups. Without the support of the volunteers that make up this community, I would have been forced to use a much less desirable method to produce a thesis.

I thank the Multiple Sclerosis Society of Canada for their financial support of my research. I would also like to thank Ms Jackie Munroe for always being there when I had a question or a problem; you are very helpful.

Family. Without them we are nothing. Thank you everyone for your patience and support as I remain in school. Just knowing you are all there brings me peace. Thank you Amma and Merle; Poppa; Leon and Betty Ann; Sparky and Delta; Maja, Marlo and Sophia; Alicia and Geoff; Tyrell; Raj, Kitty, Neena and Seamus; Emma and Nia; Sunil, Carmen and Liam. Thank you all.

Most of all, I thank Tara times all the neutrinos in the universe. You are the rock I lean upon. I thank you for your endless support, patience, love and friendship. You are the best friend I could ever have. If I could, I would give you *all* the labradoodles.
For Afi
Chapter 1: Introduction

1 Introduction

Brain fascinates me. The complex arrangement of the nervous system is simply mind-boggling. For such a complicated and redundant system to be responsible for sentient beings is too much to comprehend. Unfortunately, even with redundancies, brain is susceptible to disease, damage, and deterioration. For humans to attempt to repair brain function is a monumental task; in fact I believe it is downright arrogant of humankind to expect to repair such a complicated system to a fully functional state. However, humankind has accomplished seemingly impossible tasks before; we can now circumnavigate the globe in a matter of hours. Such an ultimate success began with small ventures. I feel as though we are in the rowboat stage of circumnavigating the brain. The work I have conducted is equivalent to sanding out a ridge on the hull of the rowboat; the rowboat was already made and functional and I simply improved upon it slightly.

1.1 Myelin

The nervous system is complex and important; it is the means by which external stimuli is transmitted through a series of electrical and chemical processes to the most integral part of the nervous system, the brain, at which point the signals are interpreted and reactions are sent back down the neural pathways accordingly. This process is all accomplished using nerve cells, or neurons.

![Diagram of myelinated axons](image1.png)

Figure 1.1: A cartoon cross-section of myelinated axons. The myelin is characterized by the spiral enclosures of the axons. The regions of myelin water and intra/extracellular (IE) water are indicated. This cartoon is not drawn to scale.

The human brain consists of about $10^{11}$ neurons with roughly $10^{14}$ synaptic connections between them [1]. Brain neurons communicate with other neurons spread throughout the body and have the ability of transmitting nerve impulses from distances of fractions of a millimetre to several metres. Neurons receive signals from other neurons by bushy receptors called dendrites. An example of an exception to dentrites is the sensory neurons that generate a signal based on specialized receptors on the skin; other similar exceptions exist. After receiving a signal, the
dendrites convey the signal down the axon, which is a thin tube that can be up to one metre in length [2].

When a neuron is at rest a chemical balance is maintained. Negatively charged potassium ions are kept inside the axon at a ratio of 50:1 and positively charged sodium ions are kept outside at a ratio of 10:1, leaving the inside of the cell with an overall negative charge [3]. The result is the maintenance of an overall voltage potential much like a battery. The ionic charge is maintained over the distance of the axon by means of a membranous substance made of lipids and proteins called myelin, which acts as electronic insulation much like plastic does around conducting wire. The myelin sheath is stimulated at the nodes of Ranvier when a signal is received, permitting the ions to switch positions. This voltage reversal causes an electric impulse that travels along the membrane to the ends of the cell where it encounters spaces before the dendrites of the next neurons. These spaces are known as synapses [2].

Once the nerve impulse reaches the synapse, electrical transmission is no longer viable due to the gap existing between the neurons. Hence, the impulse is transferred to the next neuron by chemical means. The next neuron is then excited and the ions in it begin to switch places at the nodes of Ranvier. At the same time the first neuron, having conveyed the signal, returns to the resting state. [2]

For a brain to perform successfully, all these processes must occur in concert. Of interest in this manuscript is myelin. In the central nervous system myelin is made up of 70 to 85% lipid and 15 to 30% protein [4]. Unmyelinated axons conduct nerve impulses at a rate of less than 1 m/s, while myelinated axons of the same diameter convey signals roughly ten times faster with the largest myelinated axons propagating nerve impulses at a rate up to 100 m/s. Unmyelinated axons require about 100 times the thickness as myelinated axons to match the signal propagation speed of myelinated axons [5]. Myelin sheaths wrap themselves tightly around axons as shown in the cartoon of Figure 1.1 and are created by specialized glial cells called oligodendrocytes [6]. The myelin sheath is characterized by spiral layers of repeating units of membrane-cytoplasmic space-membrane-extracellular space having a thickness of about 150 to 160 Å [7].

1.2 Motivation

Because the myelin sheath speeds up signal propagation so rapidly it is an integral part of healthy neurons. Various diseases are capable of destroying myelin in brain, but Multiple Sclerosis (MS) will be given special emphasis in this manuscript. Statements made regarding quantifying myelin and the importance this has for MS is equally important for any biological process that decreases myelin content in brain.

1.2.1 Multiple Sclerosis

Multiple Sclerosis is an autoimmune disorder that destroys myelin in the central nervous system and affects approximately 50,000 Canadians [8] with the onset of disease generally diagnosed between the ages 20 and 40 with the ratio of occurrences for females:males varying from 2.1:1 to 3:1 [9]. Multiple Sclerosis is believed to be caused by both environmental and genetic factors, although the severity of the disease seems to be less correlated to either cause [10]. In normal brain myelin regenerates when the sheath is damaged or degenerated, however, MS seems to hinder the regeneration process. The location and severity of inflammation is different for every case and varies for each specific episode within an individual. The inflammation is believed to be caused by an autoimmune response that targets myelin antigens causing damage to myelin and in
some cases destruction of the axon. As a result, multiple areas of scar tissue, called lesions, are formed in the brain [11]. Lesions cause the slowing and blocking of nerve impulses along affected cells resulting with symptoms such as sensory loss, weakness, optic neuritis, bladder dysfunction, and cognitive impairment, just to name a few [12].

![Figure 1.2: Types of MS plotted as increasing disability over time. Relapsing-remitting, primary-progressive, secondary progressive, and progressive-relapsing MS are shown using panels RR, PP, SP, and PR, respectively.](image)

Four types of MS are currently diagnosed: relapsing-remitting, primary-progressive, secondary progressive, and progressive-relapsing. Relapsing-remitting MS and is characterized by a continual relapsing and remitting cycle of MS attacks with stable times between attacks. Relapse-remitting MS accounts for 85 to 90% of patients initially diagnosed with MS. An example graph of increased disability over time is presented in panel RR of Figure 1.2. Primary-progressive MS affects about 10% of the patient population. This form of the disease is characterized by increased disease severity over time with occasional plateaus and maybe temporary improvements. Primary-progressive MS lacks a remitting cycle and the patient's health continually deteriorates, as depicted in panel PP of Figure 1.2. Secondary progressive MS develops in about 50% of patients initially diagnosed with relapsing-remitting MS, although which cases will change form is unpredictable and could happen after any duration following the relapsing-remitting diagnosis. Secondary progressive MS is characterized by a patient initially diagnosed with relapsing-remitting MS who suddenly develops primary progressive MS symptoms. An example of increased disability over time for a patient with primary progressive MS represented by panel SP of Figure 1.2. Progressive-relapsing MS is characterized by a steady increase in disability accompanied by sudden spikes. After a short duration following the spike, the disability will relapse slightly, but not back to the level preceding the spike as shown in panel PR of Figure 1.2. Approximately 5 to 6% of patients have this form of MS. Multiple sclerosis is a serious disease with no known cure. Although one can live with MS for more than 35 years post diagnosis the disease is ultimately fatal. [12]
1.2.2 Imaging White Matter

The non-invasive nature of MRI has made it an invaluable tool for visualizing structural, biochemical, and functional changes in brain. Magnetic resonance imaging allows clinicians and researchers a view of the internal workings of brain; these people can now study disease progression in brain as opposed to only having access to escalating symptoms. Several techniques researchers commonly use to study MS are: $T_2$-weighted imaging, $T_1$-weighted imaging, proton density-weighted imaging, gadolinium-enhanced $T_1$-weighted imaging, diffusion-weighted imaging, magnetic resonance spectroscopy, functional magnetic resonance imaging, magnetization transfer imaging, and $T_2$-relaxation imaging [13]. All aforementioned techniques provide excellent qualitative visualization of MS in brain. Developing a quantitative technique is much more challenging, yet extremely useful for studying the progression of the disease. With proper quantification techniques various treatments can be developed and compared.

![Figure 1.3: An example of a $T_2$ distribution for white matter. The region between 10 and 50ms is assigned to myelin water, the region between 50 and 120ms is considered to result from the IE water compartments, and cerebrospinal fluid is assumed to consist of $T_2$ times greater than 1000ms.](image)

Many groups have adopted magnetization transfer techniques to study brain regions and MS lesions of diseased brain. This technique was developed by Wolff and Balaban based on Forsén and Hoffman's original work [14,15]. Magnetization transfer utilizes the fact that two distinct types of pools exist in brain, aqueous and non-aqueous, that communicate with each other through continuous magnetization exchange [14]. Magnetic resonance imaging is only capable of measuring signal from the aqueous pools. Magnetization transfer techniques consist of a preparation phase that disturbs magnetization vectors in the non-aqueous pool by means of an off resonance magnetization transfer pulse; the exchange process can be inferred by measuring changes in the mobile signal [15]. The current belief among magnetization transfer enthusiasts is that the myelin water/myelin interaction governs the effects seen using this technique [13]. One of the purposes of the work done in this manuscript was to investigate if the intra/extracellular (IE) water/non-myelin tissue interaction provides a comparable effect to the myelin water/myelin interaction in terms of cross relaxation.

Magnetic resonance imaging can be used to measure $T_2$ relaxation in brain. Such exponential relaxation curves can be fit using a distribution of $T_2$ times [16]. The compartmental nature of white matter leads to three often distinguishable $T_2$ components [17]: a short
component assigned to myelin water, an intermediate component associated with IE water, and a long component assigned to cerebrospinal fluid. An example of a $T_2$ distribution in white matter is shown in Figure 1.3. Similar distributions can be created for $T_1$ data. An intriguing observation is that in vitro samples at 24°C often exhibit bi-exponential decays characterized by two peaks in the $T_1$ distribution, but when the temperature is increased to 37°C the distribution indicates that the decay is mono-exponential with a $T_1$ time of roughly 1s. At both temperatures the $T_2$ distribution maintains a separation of the myelin water and IE water peaks. It has been assumed that cross relaxation is the likely cause of the merging of the $T_1$ peaks when the temperature is increased. For this behaviour to be true, the cross relaxation process must be slow enough to leave the $T_2$ distribution relatively unchanged, yet fast enough to merge the two peaks in the $T_1$ distribution at the higher temperature. One of the purposes of this manuscript was to confirm that the cross relaxation process is responsible for this merging behaviour. Similarly, cross relaxation should slightly shift the peaks towards each other in the $T_2$ distribution. A cross relaxation correction was developed in order to determine the extent that the peaks of the $T_2$ distribution were shifted in order to investigate if the measured values for $T_2$ and myelin water fraction are close to physical values.

Previously, investigators have used a two pool model of white matter to study cross relaxation between the aqueous and non-aqueous pools [18-20]. Using the results of the numerical solutions to the four pool model, the results of these previous studies were compared using the four pool model parameters to confirm that the two pool model is a simplified version of the four pool model.

1.3 Outline

Initially, general NMR theory will be reviewed in Chapter 2. The following chapter describes the experiments conducted and provides figures outlining the results of the experiments. The mathematics behind the two pool model are described in Chapter 4; the Zimmerman-Brittin model is introduced and a discussion of Edzes and Samulski's cross relaxation paper is presented. Chapter 5 begins with describing the four pool model of white matter. Following this description the numerical solution procedure is outlined and the applicable parameters are introduced. A description of the cross relaxation correction is developed with detailed maths provided in the Appendix. The results of the numerical solutions are presented in Chapter 6 by means of displaying the simulated fits as well as a summary of the resulting parameters. This chapter also contains a comparison of the four pool model parameters with known literature values. A comparison of the two pool model and the four pool model is also discussed. Finally, Chapter 7 closes the manuscript with concluding remarks, a description of current work, suggestions for future work, and advice for changes to the experiments conducted.
2 NMR Physics

Nuclear Magnetic Resonance (NMR) has been in the scientific spotlight in recent years. Nobel Prizes have been awarded to Magnetic Resonance Imaging (MRI) pioneers in the field of medicine and chemistry. In 2003 the Nobel Prize in Physiology or Medicine was awarded for the development of MRI technology; in 2002 the Nobel Prize in Chemistry was awarded for studying proteins using NMR; in 1991 the Nobel Prize in Chemistry was awarded for high resolution NMR spectroscopy. [21]

Isidor Rabi and colleagues developed the first NMR molecular beam experiments in 1938 [22-24]. These experiments involved sending a beam of molecules through a strong magnetic field, while two smaller, variable oscillating magnetic fields were placed orthogonal to the main field along the beam's path. These two orthogonal fields were swept through various frequencies, and as radio frequencies neared a special frequency, called the Larmor frequency, of the molecules in the beam, energy absorption was observed in the form of signal attenuation. This type of experiment allowed researchers to determine the gyromagnetic ratio of various atomic nuclei, and provided close agreement with conventional measures of the time such as band spectra and atomic beam measurements. This experimental method won Rabi the Nobel Prize for Physics in 1944.

A few years after Rabi's first beam experiments, NMR was observed in condensed matter. In 1945, Edward Purcell and colleagues reported on resonance absorption in the nuclear magnetic moment vectors in solids [25]. At the same time Felix Bloch and colleagues independently discovered this NMR effect and provided estimates of the spin-lattice relaxation time, or $T_1$, of water, and discussed how $T_1$ can differ for water in the solid or liquid phase [26,27]. It was from these experiments that Bloch formulated the famous Bloch equations [28]. Purcell and Bloch shared the Nobel Prize in Physics in 1952 for their efforts.

In 1950 Erwin Hahn first described the spin-echo [29,30]. Hahn initially considered these spin-echoes an annoying glitch, but this work has led some to consider Hahn's paper as a dividing point in NMR; there was NMR before the discovery of the spin-echo and NMR after the discovery [31]. The introduction of the spin-echo changed NMR from continuous-wave measurements, like the beam experiments of Rabi, to pulsed NMR that is commonplace today.

Richard Ernst introduced the Fourier transform for NMR spectroscopy in a paper he published in 1966 [32]. Eventually, these transforms were put to use in making images from NMR principles. For this contribution, Ernst was awarded the Nobel Prize for Chemistry in 1991.

In 1973 Paul Lauterbur generated the first image using NMR and a reconstruction method called backprojection; dubbing the technique used as zeugmatography, from the Greek ζευγματογραφία, meaning "that which is used for joining" [33]. Around the same time, Peter Mansfield and colleagues used a similar NMR technique to form images using a selective irradiative process [34]. For their efforts, Lauterbur and Mansfield were jointly awarded the Nobel Prize for Physiology or Medicine in 2003.

2.1 Larmor Frequency

Joseph Larmor was an Irish physicist and mathematician who lived from 1857-1942. Thus, he was a seasoned scientist during the exciting times when the field of physics was being revolutionized by the theories of quantum mechanics and relativity at the beginning of the
Chapter 2: NMR Physics

twentieth century. Larmor's formula, Larmor precession, and Larmor frequency all carry Larmor's name even though these discoveries were tangential to the majority of his research. Larmor precession is attributed to the splitting and polarization of spectral lines in a magnetic field. The rate of energy radiating from an accelerating electron was explained using Larmor's formula in terms of charge and acceleration, which breaks down for velocities close to the speed of light. Larmor postulated that electrons in a magnetic field orbit around some centre at a predictable frequency. This Larmor frequency is now considered a physical property of charged particles and the precession rate scales linearly with magnetic field by a proportionality constant known as the gyromagnetic ratio, which is different for each nucleus. The Larmor frequency is one of the most important concepts for magnetic resonance. Given the gyromagnetic ratio of proton nuclei as \( \frac{42.58 \text{MHz}}{T} \), and a magnetic field strength of \( 3T \), the Larmor frequency can be calculated to be \( 128 \text{MHz} \), which is in the radio frequency range. \[35\]

2.1.1 Quantum Mechanical Derivation of the Larmor Frequency

Two approaches can be taken to describe magnetic resonance: classical mechanical and quantum mechanical. This section provides an introduction to magnetic resonance theory using a quantum mechanical approach. First, a Hamiltonian, \( \mathcal{H} \), needs to be derived assuming one applies a static magnetic field, \( B \), to a sample with a magnetic moment vector, \( \mu \) [36]:

\[
\mathcal{H} = -\mu \cdot B
\] (2.1.1)

If one assumes the magnetic field has magnitude \( B_0 \) and is applied in the \( z \) direction, Equation 2.1.1 becomes:

\[
\mathcal{H} = -\gamma B_0 I_z
\]

where \( \gamma \) is an element dependent constant called the gyromagnetic ratio; \( \hbar \) is Planck's constant; and \( I_z \) are eigenvalues of the Hamiltonian and are also known as the spin angular momentum quantum numbers. The \( I_z \) values are in integer multiples of \( \gamma \hbar B_0 \). Thus, the allowable energies are:

\[
E = -\gamma \hbar B_0 m; \quad m = I_z, I_z - 1, ..., -I_z.
\]

For proton NMR, \( I_z = \frac{1}{2} \), so there are only two allowable energy states differing by \( \gamma \hbar B_0 \). This relationship, known as Zeeman energy splitting, is [36]:

\[
\hbar \omega = \Delta E = \gamma \hbar B_0.
\] (2.1.2)

One can simplify Equation 2.1.2 into the resonance equation:

\[
\omega_0 = \gamma B_0.
\] (2.1.3)

This rotational frequency is also known as the Larmor frequency. Of interest here is that Planck's constant does not appear in Equation 2.1.3, suggesting that the quantum mechanical approach provides results closely related to the classical picture [36]. In fact, the same result is derived in Section 2.1.2 using the classical approach.

2.1.2 Classical Mechanical Derivation of the Larmor Frequency

If one introduces a magnetic field vector, \( B \), to a system with a magnetic moment vector of \( \mu \), the change in total angular momentum of the system can be defined as [36]:

The angular momentum of the system can be related to the magnetic moment vector by a numerical factor known as the gyromagnetic ratio, \( \gamma \) [37]. Thus, one can rewrite Equation 2.1.4 as:

\[
\frac{d}{dt} J = \mu \times B .
\] (2.1.4)

One can explicitly define the magnetic moment vector as:

\[
\mu = \mu_x \hat{x} + \mu_y \hat{y} + \mu_z \hat{z}.
\]

A rotational operator, \( \Omega \), can be defined as operating on the vector directions as:

\[
\frac{d}{dt} (\hat{x} + \hat{y} + \hat{z}) = \Omega \times (\hat{x} + \hat{y} + \hat{z}).
\] (2.1.6)

Taking the left hand side of Equation 2.1.5 one can define the derivative as:

\[
\frac{d}{dt} \mu = \frac{d}{dt} \mu_x \hat{x} + \frac{d}{dt} \mu_y \hat{y} + \frac{d}{dt} \mu_z \hat{z} + \mu_x \frac{d}{dt} \hat{x} + \mu_y \frac{d}{dt} \hat{y} + \mu_z \frac{d}{dt} \hat{z}.
\] (2.1.7)

Thus, one can rewrite Equation 2.1.7 using \( \delta \) to indicate a change within the coordinate system while maintaining \( d \) to indicate a change of the vector direction:

\[
\frac{d}{dt} \mu = \left( \frac{\delta}{\delta t} + \frac{d}{dt} \right) (\mu_x \hat{x} + \mu_y \hat{y} + \mu_z \hat{z}),
\]

which can be rewritten using Equation 2.1.6 as:

\[
\frac{d}{dt} \mu = \frac{\delta \mu}{\delta t} + \Omega \times \mu .
\] (2.1.8)

Combining Equations 2.1.5 and 2.1.8 gives:

\[
\frac{\delta \mu}{\delta t} + \Omega \times \mu = \mu \times \gamma B ,
\]

and further manipulation results in:

\[
\frac{\delta \mu}{\delta t} = \mu \times (\gamma B + \Omega) .
\] (2.1.9)

Introducing an effective field \( B_{\text{eff}} \) such that \( B = B_{\text{eff}} - \Omega / \gamma \) and substituting it into Equation 2.1.9 yields:

\[
\frac{\delta \mu}{\delta t} = \mu \times (\gamma B_{\text{eff}}),
\] (2.1.10)

which represents the motion of \( \mu \) in a coordinate system rotating at \( \Omega \) [36]. Note the similarity of Equations 2.1.5 and 2.1.10 with this choice of effective field.

Now one can study the motion of \( \mu \) in a static magnetic field of \( B = B_0 \hat{z} \) and a rotational frame such that \( B_{\text{eff}} = 0 \) as:
\[ B_{\text{eff}} = 0 = \gamma B_0 \hat{z} + \frac{\Omega}{\gamma} \]

resulting with:

\[ \Omega = -\gamma B_0 \hat{z} = -\omega_0 \hat{z}. \]  

(2.1.11)

Equation 2.1.11 describes the angular frequency of \( \mathbf{\mu} \) in this static magnetic field. This rotational frequency is termed the \textit{Larmor frequency} and agrees perfectly in magnitude with the quantum derivation of Equation 2.1.3 in Section 2.1.1.

### 2.2 Longitudinal and Transverse Relaxation of Protons in a Magnetic Field

If protons, having spin angular momentum quantum number of \( \frac{1}{2} \), are placed in a large static magnetic field one would expect Zeeman energy splitting as described by Equation 2.1.2. Using Boltzmann statistics one can calculate the probability of a proton occupying one of the two Zeeman states at a given temperature \( T \) as [36]:

\[ P(E) = C e^{-\frac{\Delta E}{kT}} \]

where \( k \) is the Boltzmann constant and \( C \) is some algebraic constant. If one assumes \( P(E^{1/2}) \) to be the probability of the spin being in the higher Zeeman energy state of \( -\frac{1}{2} \), and \( P(E^{+1/2}) \) to be the probability of the spin being \( +\frac{1}{2} \), the ratio of the two probabilities can be written:

\[ \frac{P(E^{1/2})}{P(E^{+1/2})} = e^{\frac{\Delta E}{kT}} = e^{\gamma h \omega_0, kT} \]

If one assumes room temperature to be 300K, and the magnetic field strength to be 3T, then \( \Delta E/kT \approx 2.04 \times 10^{-5} \), resulting in a ratio of roughly 1.0000204. This ratio indicates that there is a net spin available for manipulating and measuring.

Once the small abundance of spins have been perturbed from the equilibrium state, interaction of water molecules with the local environment is responsible for the ultimate decay of signal in the case of the transverse relaxation time, or \( T_2 \), and the return of signal to equilibrium for the case of the longitudinal relaxation time, or \( T_1 \). Assuming water protons, the relaxation rates, which are the inverse of the relaxation times, can be described by [38]:

\[ \frac{1}{T_1} = \frac{3}{10} \left( \frac{\mu_0 \gamma^2 \hbar}{4 \pi r^3} \right)^2 \tau_c \left( \frac{1}{1 + \omega_0^2 \tau_c^2 + \frac{4}{1 + 4 \omega_0^2 \tau_c^2}} \right) \]

(2.2.1)

\[ \frac{1}{T_2} = \frac{3}{20} \left( \frac{\mu_0 \gamma^2 \hbar}{4 \pi r^3} \right)^2 \tau_c \left( 3 + \frac{5}{1 + \omega_0^2 \tau_c^2} + \frac{2}{1 + 4 \omega_0^2 \tau_c^2} \right) \]

(2.2.2)

where \( \mu_0 \) is the permeability of free space; \( r \) is the distance between spins and can be assumed to be constant; \( \gamma \) is the gyromagnetic ratio; \( \hbar \) is Planck's constant; \( \omega_0 \) is the Larmor frequency; and \( \tau_c \) is the correlation time, or time between collisions of molecules in motion. For short correlation times of \( \omega_0 \tau_c \ll 1 \), and thus fast motion, these rates are approximately equal:
The $M_2$ is defined as the second moment of the dipolar lineshape in the absence of motion. For bulk water, where the characteristic correlation time is of the order of $10^{12}$ s, $T_1 \approx T_2$ and ranges from 1 to 3 s. The $T_1$ value has a minimum when $\omega_0 \tau_c = 1$ and $T_2$ becomes less than $T_1$ as $\omega_0 \tau_c > 1$. For long correlation times of $\omega_0 \tau_c \gg 1$, and thus slow motion, $T_2 < T_1$.

In biological tissue $T_1$ is found to be less than that of bulk water, and generally $T_2$ is found to be less than, as opposed to equal to, $T_1$. Spin-lattice relaxation times are reduced from bulk water values because of local fluctuating magnetic fields due to fast rotational and translational motions of the spins [39]. Spin-spin relaxation times are reduced because of dipolar coupling interactions between nuclei [39]. A simple way of explaining why $T_1 \geq T_2$ follows. $T_1$ relaxation, following a radio frequency pulse that tips at least a portion of the magnetic moment vectors into the transverse plane, is the return of magnetic moment vectors to being parallel to the external magnetic field. While returning, the magnetic moment vectors must eventually be taken out of the transverse plane to align along the longitudinal axis. This process restores the longitudinal magnetization but destroys the transverse magnetization, placing an upper limit of $T_2 = T_1$. Additional processes such as: small inhomogeneities of the main magnetic field, magnetic moment vectors due to paramagnetic ions, and internuclear forces between adjacent spins also destroy the transverse magnetization, but do not affect the total energy of the spin system leaving the longitudinal component unchanged. Such processes affect $T_2$ and not $T_1$, leading to the inequality $T_2 < T_1$.

### 2.3 Bloch Equations

In 1946 Felix Bloch developed a set of phenomenological equations that describes the behaviour of the magnetic moment vectors of nuclei as they return to equilibrium in a constant magnetic field after being perturbed by a radio frequency gradient engaged at right angles to the main field [26-28]. This behaviour can be described as:

$$\frac{d}{dt} \mathbf{M}(t) = \gamma \mathbf{M}(t) \times \mathbf{B} - \frac{M_x(t)}{T_2} \mathbf{\hat{x}} - \frac{M_y(t)}{T_2} \mathbf{\hat{y}} - \frac{M_z - M_0}{T_1} \mathbf{\hat{z}}$$

(2.3.1)

where $\mathbf{M}(t)$ is the magnetic moment vector; $\gamma$ is gyromagnetic ratio; $\mathbf{B}$ is the constant magnetic field along the $\mathbf{\hat{z}}$ direction with intensity $B_0$; $T_2$ is the transverse relaxation time; $M_0$ is the magnetic moment vector at thermal equilibrium that aligns with $\mathbf{B}$ in the resting state; and $T_1$ is the longitudinal relaxation time. Equation 2.3.1 can be broken down into three equations along orthogonal axes:

$$\frac{d}{dt} M_x(t) = -\gamma M_y(t) B - \frac{M_x(t)}{T_2}$$

(2.3.2)

$$\frac{d}{dt} M_y(t) = -\gamma M_x(t) B - \frac{M_y(t)}{T_2}$$

(2.3.3)

$$\frac{d}{dt} M_z(t) = -\frac{M_z(t) - M_0}{T_1}$$

(2.3.4)

Substituting the Larmor relation, $\omega_0 = \gamma B_0$, the general solution of Equations 2.3.2-2.3.4 is:
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\[ M_x(t) = [M_x(0)\cos(\omega_0 t) + M_y(0)\sin(\omega_0 t)]e^{-i\omega T}; \]  
\[ M_y(t) = [-M_x(0)\sin(\omega_0 t) + M_y(0)\cos(\omega_0 t)]e^{-i\omega T}; \]  
\[ M_z(t) = M_0 + [M_z(0) - M_0]e^{-i\omega T}. \]  

If one assumes the special case where the initial magnetization is along the y-axis following a 90° excitation rf pulse, then \( M_x(0) = 0 \) and \( M_y(0) \equiv M_0^y \) giving:

\[ M_x(t) = M_0^y \sin(\omega_0 t)e^{-i\omega T}; \]  
\[ M_y(t) = M_0^y \cos(\omega_0 t)e^{-i\omega T}. \]  

One can define the transverse magnetization as \( M_{xy}(t) = M_x(t) - iM_y(t) \), allowing one to rewrite Equations 2.3.8 and 2.3.9 as a single expression:

\[ M_{xy}(t) = M_0^y e^{-i\omega T}e^{i\omega_0 t}. \]  

Figure 2.1: Time evolution of the magnetic moment vector using the Bloch equations. The static magnetic field is assumed to be constant along the z-axis. The panels on the left depict the magnetic moment vector along three orthogonal directions as a function of time. For the two transverse cases the signal oscillates about zero at the Larmor frequency. The image on the right is a 3-dimensional rendering of the magnetic moment vector over time.

The evolution of Equations 2.3.7 and 2.3.10 over time are depicted in Figure 2.1. The behaviour of the magnetic moment for each orthogonal direction is shown on the left, while the right side depicts how magnetic moment vector behaves in three-dimensional space.
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The oscillatory nature of this system, resulting from the presence of a strong static magnetic field, can be demodulated out of the transverse magnetization vector. Essentially, the demodulation process means that one can imagine the scenario depicted on the right side of Figure 2.1 using a reference frame that rotates at the same frequency, \( \omega_0 \), as the transverse magnetization vector. The result is a further simplified version of Equation 2.3.10:

\[
M_{xy}(t) = M_{0y} e^{-i\omega_0 t}.
\]  

(2.3.11)

2.4 Spin-Echoes

It is well known that a free induction decay (FID) curve will follow a 90°, or 90, rf pulse. In 1949 Hahn discovered that following the initial 90 pulse if one waited a time \( \tau \), and applied a second 90 pulse the result was an additional FID curve [29,30]. Hahn termed this process a spin-echo. The discovery of spin-echoes led to better comprehension of resonance phenomena. To understand why signal is recovered to produce a second FID, one needs to examine the other causes of signal decay in the transverse plane besides \( T_2 \). The decay time \( T_2 \) decay is characterized by the same factors as \( T_2 \), decay plus additional effects due to magnetic field inhomogeneity. \( T_2 \) can be described by the equation:

\[
\frac{1}{T_2^*} = \frac{1}{T_2} + \gamma \Delta B
\]  

(2.4.1)

where \( \gamma \) is the gyromagnetic ratio; and \( \Delta B \) is a measure of field inhomogeneity. Decay due to \( T_2 \) is not recoverable, however additional decay due to the inhomogeneity of the magnetic field is recoverable; a spin-echo is the result.

In 1954 Herman Carr and Edward Purcell elaborated on the spin-echo concept by using a 180 pulse following the initial 90 pulse [40]. In order to understand how this works it is best if one visualizes a reference frame with the \( z' \)-axis along the main magnetic field with the \( x' \)- and \( y' \)-axes spinning at the Larmor frequency, matching the rate of rotation of the transverse magnetization in the absence of magnetic field inhomogeneities. In this rotating frame there will be no precession about the \( z' \)-axis, but dephasing due to spin-spin interactions and magnetic field inhomogeneities will persist. With this reference frame in mind, one can denote the Carr-Purcell (CP) pulse sequence as:

\[
90_x, -\tau - 180_x, -\tau - \text{echo}.
\]

Assuming a strong magnetic field is along the \( z' \)-axis and the sample was initially at equilibrium, the 90\( _x \) pulse of this CP sequence results in a magnetic moment vector along the \( y' \)-axis. During the delay \( \tau \) the signal dephases due to spin-spin interactions and magnetic field inhomogeneities. After the delay of \( \tau \) a 180\( _x \) pulse is applied that flips the equatorial plane completely around the \( x' \)-axis. During the second, equal delay of \( \tau \) the magnetic moment vectors continue to dephase, but now instead of dephasing away from each other, they are dephasing back into each other towards the \( y' \)-axis; an echo results.

Carr and Purcell went a step further by repeating the echo creation \( N \) times using two techniques [40]. The first technique was defined as:

\[
90_x, -\tau - \left( 180_x, -\tau \right)_N - \Delta TR
\]
where $\Delta TR$ is the wait time before repeating the experiment. This CP sequence results in $N$ echoes with the last echo occurring at time $2N\tau$. This pulse sequence results in some of the echoes occurring during $180_\chi$ pulses. The second technique they investigated was:

$$90_\chi - \tau / 2 - (180_\chi - \tau)_{N} - \Delta TR.$$ 

This technique also results in $N$ echoes, with the final echo occurring at time $2N\tau$ and no echoes occurring during the $180_\chi$ pulses.

Unfortunately, the reproducibility of measurements taken using the CP method was low. In 1958 S Meiboom and D Gill suggested reasons why the experiment was not readily reproducible and proposed a modified version of the CP sequence to increase reproducibility, now known as the CPMG sequence [41]. Meiboom and Gill suggested that since the $90$ and $180$ pulses were along the same axis, if the $180$ pulses were not ideal a cumulative error would result because the successive pulses will rotate the magnetic moment vectors further and further out of the $x'y'$-plane. Meiboom and Gill suggested using a $90_\gamma$ pulse initially so that if the $180$ pulses were not exactly $180$, but were consistently the same value close to $180$, every second $180_\chi$ pulse will return any vectors to the $x'y'$-plane that were displaced out of the $x'y'$-plane by the original $180_\chi$ pulse. The CPMG pulse sequence was defined as:

$$90_\gamma - \tau - (180_\chi - 2\tau)_{N} - \Delta TR.$$ 

The resulting signal decay is shown in Figure 2.2. The oscillatory nature of the solid line is due to the measurements being taken in the lab frame as opposed to the rotating frame; the oscillations occur at the Larmor frequency. The overall signal decays exponentially before the first $\tau$ time
with an envelope that has a decay time of $T_2^*$. This decay is recovered due to the 180° pulse so that a non-recoverable decay envelope can be defined at even $\tau$ values. This longer decay has a decay constant of the sample's $T_2$ and the overall signal loss can be defined as:

$$S(2n\tau) = S(0)e^{-2n\tau/T_2}$$  \hspace{1cm} (2.4.2)$$

where $n$ is an integer placing the signal measurements midway between the 180° pulses.
3 Experiments

The data I used for my work were collected using experiments primarily conducted by Dr Irene Vavasour during her PhD studies on NMR in bovine brain [42]. Vavasour's study of bovine brain continued beyond her dissertation and some data were collected by a summer student, Charmaine Chia. The focus of my thesis was to determine a numerical solution to the four pool model of white matter using collected data from bovine brain. While I did not carry out the experiments listed in this section, knowledge of how the experiments were performed was of the utmost importance when determining the numerical solutions. This section of the thesis provides a background to the experiments conducted that were relevant to the numerical solutions.

NMR experiments were carried out on a modified Bruker SXP 4-100 2.1T NMR spectrometer operating at 90MHz. The data acquisition system included a locally built pulse programmer, a Rapid Systems digitizer, and an IBM compatible computer [43]. The temperature was maintained using a Bruker B-ST 100/700 temperature controller and an air flow device. The wait time before repeating the pulse sequence, ΔTR, for all experiments was 7s, and the integrity of samples was monitored by repeating the FID and CPMG sequence at the beginning and end of data acquisition [40,41]. Vavasour reported no significant changes over the course of the experiments [42].

3.1 Samples

Bovine brains were obtained within three hours of slaughter (GrandMaison beef farm, Langley, BC) and placed in a 1.0M phosphate buffered saline (Oxoid, ON) and/or (Sigma-Aldrich Canada, ON) cooled with ice or placed within a 4°C refrigerator. White matter tissue samples were cut from various regions of the brain and placed in a 10-mm outer diameter NMR tube. The samples were then placed in the NMR spectrometer and experiments were carried out at 24 and 37°C. White matter samples were taken from a total of five bovine brains, eleven samples were examined at 24°C and four samples at 37°C. Unless otherwise stated, the following experiments were carried out on all fifteen samples. One brain had cross relaxation experiments carried out on two samples at 24°C and three samples at 37°C.

3.2 Free Induction Decay

A modified FID experiment was used to separate the signals from the motionally restricted and mobile protons:

\[ 90° - \frac{\tau}{2} - (180° - \tau) - \Delta TR \]

where \(\tau=200\mu s\) with 100 signal averages. An example of the resulting decay is shown in Figure 3.1. The intensity measurements were taken along the \(y'\)-axis. The first 90° pulse laid the magnetization vectors along the \(y'\)-axis, where magnetization from the motionally restricted signal rapidly decays in a nonrecoverable fashion. The eight 180° pulses rephased signal dephased due to \(T_2^*\) decay in order for us to quantify the mobile signal decay.

The intensities from the top of the eight echoes were fit to a sum of two exponentials and extrapolated to \(t=0\) to obtain the initial signal, \(M(0)\). The FID signal for dipolar coupled protons

Portions of this chapter have been included in an article accepted for publication. Bjarnason TA, Vavasour IM, Chia CLL, MacKay AL. Characterization of the NMR behaviour of white matter in bovine brain. Magnetic Resonance in Medicine, In Production: July, 2005.
can be fit to a moment expansion equation given as [44]:

\[
S(t) = S(0) \left( 1 - \frac{M_2 t^2}{2!} + \frac{M_4 t^4}{4!} - \frac{M_6 t^6}{6!} \right) \tag{3.2.1}
\]

where $M_2$, $M_4$, and $M_6$ are the second, fourth, and sixth moments of the lineshape and $S(0)$ is the total signal at $t=0$. The initial portion of the decay curve, 17 to 42\,$\mu$s from the centre of the 90° pulse, was fit to Equation 3.2.1. The measured $M_2$ was an average over all protons in the sample and can be calculated using:

\[
M_2 = M_2^{mr} \left( \frac{S(0) - M(0)}{S(0)} \right) + M_2^{mb} \left( \frac{M(0)}{S(0)} \right) \tag{3.2.2}
\]

where $M_2^{mr}$ is the second moment of the motionally restricted protons and $M_2^{mb}$ is the second moment of the mobile protons. Assuming $M_2^{mb}=0$ one can calculate $M_2^{mr}$ using Equation 3.2.2. The mobile fraction, MF, of the sample was calculated as the ratio of $M(0)/S(0)$.

The average mobile fractions were determined, with standard deviation in brackets, to be 82.4 (3.3)% for 24°C and 81.3 (1.9)% for 37°C. These fractions were used in the numerical solutions to break down what portion of the NMR signal was due to mobile, ie water, or motionally restricted, ie tissue, protons.

### 3.3 Spin–Spin Relaxation

The mobile signal was further characterized using the following CPMG sequence:

\[
90_x - \frac{\tau}{2} - (180_x - \tau)_{4320} - \Delta \text{TR}
\]

where $\tau=100$, 200, or 400\,$\mu$s, with 200 signal averages. A description of this spin-echo acquisition is presented in Section 2.4. One data set was acquired for each of the three echo spacings. For the first 224 echoes every echo was sampled, and for the next 4096 echoes every eighth echo peak was sampled. Due to noisy initial data points, data acquisition started at 1\,ms.
Non-negative Least Squares Algorithm

If the decay curves were assumed to be multi-exponential in nature, the following general equation was used to describe the decay of the measured signal, \( y \), [16]:

\[
y_i = \sum_{j=1}^{M} s_j \exp\left(-t_i/T_{2j}\right), \quad i = 1, 2, \ldots, N
\]

(3.3.1)

where \( t_i \) are the measurement times; \( M = 160 \) and represents the number of values used in the \( T_2 \) partition, \( T_{2j} \) are logarithmically spaced \( T_2 \) times within the range of \( 1 \)ms to \( 10 \)s; \( N \) represents the total number of measured data points; and \( s_j \) are the relative amplitudes for each partitioned \( T_2 \) time. The non-negative least squares (NNLS) algorithm was used to minimize both \( \chi^2 \) and an energy constraint that smoothes the \( T_2 \) distribution, \( s(T_{2j}) \), providing better, consistent fits in the presence of noise [16,45]. The following expression was minimized:

\[
\chi^2 + \mu \sum_{j=1}^{M} s_j^2, \quad \mu \geq 0.
\]

(3.3.2)

The larger the \( \mu \) parameter the more the routine smooths the \( T_2 \) distribution at the cost of misfit, and for the case of \( \mu = 0 \) the \( \chi^2_{\min} \) fit would result [16]. Regularized smooth \( T_2 \) distributions were created by minimizing the expression shown in Equation 3.3.2 with the energy constraint of 

\[
1.01 \chi^2_{\min} \leq \chi^2 \leq 1.02 \chi^2_{\min}.
\]

Figure 3.2: Examples of \( T_2 \) distributions for CPMG data of a 24°C sample collected with echo spacings of 100, 200 and 400\( \mu \)s providing measurements out to 432, 736 and 1728ms, respectively. It is apparent that collecting out to times of the same order as the later peaks leads to a better resolved \( T_2 \) distribution.

An example of \( T_2 \) distributions for each of the three \( \tau \) times is shown in Figure 3.2. The 100, 200, and 400\( \mu \)s measured decay curves ended at times 432, 736, and 1728ms, respectively. As expected, collecting to longer times better characterized the later peaks in the \( T_2 \) distribution. The geometric mean of the \( T_2 \) distribution between times 1 and 50ms was defined as the myelin...
water $T_2$ time, or $T_2^{\text{pw}}$. The IE water $T_2$ time, or $T_2^{\text{ie}}$, was defined by the range 50 to 700 ms [46,47]. Myelin water fractions (MWFs) were calculated by dividing the area of the $T_2$ distribution from 1 to 50 ms by the area between 1 and 700 ms [17]. The results for each $\tau$ are reported in Table 3.1. Slight changes are observed in both the MWF and $T_2$ values. They are all within one standard deviation of the measurements so a trend in these values according to $\tau$ cannot be commented on.

<table>
<thead>
<tr>
<th>Temp</th>
<th>$T_2^{\text{pw}}$</th>
<th>$T_2^{\text{ie}}$</th>
<th>MWF</th>
<th>$T_2^{\text{pw}}$</th>
<th>$T_2^{\text{ie}}$</th>
<th>MWF</th>
<th>$T_2^{\text{pw}}$</th>
<th>$T_2^{\text{ie}}$</th>
<th>MWF</th>
</tr>
</thead>
<tbody>
<tr>
<td>24°C</td>
<td>9.5 (2.2)</td>
<td>104 (16)</td>
<td>16.0 (5.9)</td>
<td>15.3 (9.5)</td>
<td>120 (21)</td>
<td>14.3 (3.2)</td>
<td>13.8 (7.3)</td>
<td>106 (18)</td>
<td>10.9 (3.2)</td>
</tr>
<tr>
<td>37°C</td>
<td>8.2 (1.4)</td>
<td>78.1 (8.9)</td>
<td>17.0 (6.0)</td>
<td>13.3 (3.3)</td>
<td>91.1 (6.9)</td>
<td>14.1 (5.2)</td>
<td>11.7 (3.7)</td>
<td>80.9 (8.0)</td>
<td>11.8 (4.6)</td>
</tr>
</tbody>
</table>

Table 3.1: Measured $T_1$ values, in ms, for myelin water and IE water as well as the MWF, in %. The measured values are stated for the three $\tau$ times used in the CPMG experiment. Standard deviations are shown in brackets.

### 3.4 Spin-Lattice Relaxation

![Figure 3.3: A typical $T_1$ distribution showing mono-exponential behaviour at 37°C while exhibiting bi-exponential behaviour at 24°C.](image)

The $T_1$s were determined using a modified inversion recovery pulse sequence:

$$90_x-\Delta TR$$
$$180_x-\tau-90_x-\Delta TR$$

The first part of the sequence rotated the magnetization vector about the $x'$-axis onto the $y'$-axis. The signal was then measured along the $z'$-axis and was characterized by a signal exponentially growing towards the equilibrium value. The second part of the pulse sequence rotated the magnetization vector onto the $-z'$-axis, where it was allowed to grow towards the equilibrium value for a duration of $\tau$. After a time $\tau$, the magnetization vector was rotated onto the $y'$-axis and allowed to grow back to the equilibrium value. By subtracting the second signal from the first, a curve decaying to zero was defined using the various $\tau$ times. For a signal with mono-exponential relaxation, the resulting signal would decay as:

$$S(\tau) = 2S(0)e^{-\tau/T_1}.$$
The $\tau$ values followed a geometric progression with 18 values between 1 and 5000 ms. 100 signal averages were taken.

The resulting decay curve was fit using a regularized NNLS algorithm to obtain smooth $T_1$ distributions. The range of $T_1$ times included 160 points logarithmically spaced within the range 1 ms to 10 s and the expression shown in Equation 3.3.2 was minimized with the energy constraint of $1.01X_{min}^2 \leq X^2 \leq 1.02X_{min}^2$ to obtain smooth $T_1$ distributions. This pulse sequence was used for 6 samples at 24°C and 4 samples at 37°C. Examples of $T_1$ distributions at 24 and 37°C are shown in Figure 3.3. For samples at 24°C the distributions were found to be bi-exponential with average $T_1$ times of 343 (83) ms for myelin and 943 (10) ms for non-myelin. At 37°C the $T_1$ distribution was found to be 830 (52) ms. These $T_1$ times were used in the numerical solutions to the four pool model.

### 3.5 Cross Relaxation – Free Induction Decay

Cross relaxation between the motionally restricted and mobile protons as monitored by the motionally restricted protons was measured using the Goldman-Shen pulse sequence [48]:

$$
\begin{align*}
90_\varphi - \delta - 90_{-\varphi} - \tau_{cr} - 90_\varphi - \Delta TR \\
90_\varphi - \delta - 90_{-\varphi} - \tau_{cr} - 90_\varphi - \Delta TR
\end{align*}
$$

For the first part of the pulse sequence, the initial 90$\varphi$ pulse took the magnetization vector out of equilibrium and placed it along the $y'$-axis. The magnetization vectors were allowed to dephase for a set time of $\delta=400 \mu s$, which allowed for complete dephasing of the motionally restricted signal with minimal effect on the mobile signal. The 90$_{-\varphi}$ pulse returned the remaining mobile vectors to the $z'$-axis. During the time $\tau_{cr}$ signal was transferred to the motionally restricted pools through cross relaxation, thus concluding the preparation phase. The resulting signal along the $z'$-axis was measured as an FID following the last 90$\varphi$ pulse. The second part of the pulse sequence used a similar preparation phase differing with a sign change for the second 90 pulse. The result is the signal being placed on the $-z'$-axis. The first curve was subtracted from the second resulting in a curve that decayed to zero for long times due to $T_1$ relaxation, but not negating the $T_1$ relaxation process as initially intended. 200 signal averages were taken. This experiment consisted of a total of 14 $\tau_{cr}$ times, all following a geometric progression between 100 $\mu s$ and 200 ms, and was performed on two samples at 24°C and three samples at 37°C.

The data points for each $\tau_{cr}$ time were determined from $S(0)-M(0)$; these variables were defined in Section 3.2. The measured points for each data set were scaled by the largest point in the set in order to compensate for any scaling factor resulting from the acquisition. The data for each temperature were then averaged. The results of this experiment are represented as boxes in Figure 3.4a for 24°C and b for 37°C. The motionally restricted signal increased rapidly and peaked around 150 ms for both temperatures. Beyond this time the signal began to decrease and was expected to eventually reach zero due to the inversion recovery experiment used. Signal recovery in this experiment can be attributed to cross relaxation.

### 3.6 Cross Relaxation – CPMG

Cross relaxation between the motionally restricted and mobile protons as monitored by the mobile protons was measured using the following pulse sequence:
The preparation phase is the same as described in Section 3.5. During the \( \tau_{cr} \) time magnetization was exchanged between the two mobile pools and with the motionally restricted pools. Following the preparation phase the CPMG portion of this experiment was collected as discussed in Section 3.3. For each \( \tau_{cr} \) time, the NNLS amplitudes corresponding to the two water pools were evaluated. This experiment was performed on the same samples as the experiment described in Section 3.5. The measured points for each data set were scaled by the initial IE water data point in the set to compensate for any scaling resulting from the data acquisition. The data for each temperature were then averaged together.

**Figure 3.4:** Averaged cross relaxation measurements collected for the myelin water pool, the IE water pool, and the non-separable non-aqueous pools. The data collected at 24°C are shown in a while 37°C data are shown in b. The intensities are scaled relative to each other and the y-axis was split in two in order to better display the data.

The mobile signal was separated into myelin and IE water using the NNLS algorithm that
minimized Equation 3.3.2 with the energy constraint of $1.01 \lambda^2_{\text{min}} \leq \lambda^2 \leq 1.02 \lambda^2_{\text{min}}$. The results of this experiment are reported as the data points represented by circles and diamonds in Figure 3.4a for 24°C and b for 37°C. The mobile signal was consistently characterized by an initial decrease of signal. Over the duration of the experiment, the myelin water data was observed to recover slightly. One would suspect that the IE water would eventually recover, but longer $\tau_{cr}$ times would be needed to confirm this. The changes in the mobile pools is believed to be due to cross relaxation.
Chapter 4: Two Pool Model

4 Two Pool Model

When studying magnetization exchange, there are some cases in which a two pool model is sufficient to characterize the system like in collagen and wood studies [49,50]. This system is well understood and is analyzed in Section 4.2. A schematic representation of a generic two pool is shown in Figure 4.1. In this schematic there are two magnetization pools, \( m_1 \) and \( m_2 \), with exchange rates of \( k_{21} \) and \( k_{12} \) with directionality as indicated in the diagram. The \( T_1 \) relaxation times for the two pools are not necessarily the same.

![Figure 4.1: A schematic representation of a two pool model.](image)

4.1 Laplace Transform

A technique that can be used to solve the system of differential equations in Section 4.2 is the Laplace transform. This tool was developed by Pierre Simon Marquis de Laplace and Oliver Heavyside in the nineteenth century [51]. The Laplace transform looks like a truncated form of the Fourier transform that is so familiar to those in the MRI field. The aesthetic difference is in the limit of integration, where the lower limit of the Laplace transform is zero as opposed to negative infinity. The Laplace transform is defined as a function \( F(s) \) such that for a function \( f(t) \) that exists for all values of \( t \geq 0 \), the following integral exists:

\[
F(s) = \mathcal{L}(f) = \int_0^\infty e^{-st} f(t) dt
\]

(4.1.1)

where \( \mathcal{L} \) denotes the action of taking the Laplace transform. The inverse of this transform can be written as:

\[
f(t) = \mathcal{L}^{-1}(F).
\]

Tables are often used to move between \( s \) space and \( t \) space [52]. The Laplace transform changes complicated integrations into simple algebra.

The Laplace transform of a derivative has a unique quality such that if \( df(t)/dt \) exists for all \( t \geq 0 \) the transform is:

\[
\mathcal{L}\left( \frac{df}{dt} \right) = s \mathcal{L}(f) - f(0) = sF(s) - f(0).
\]

(4.1.2)

Equipped with this tool, differential equations with initial values can easily be solved avoiding nasty integrals; this technique lends itself well to problems involving exponentials. Several
Laplace transform pairs as well as their viable ranges are listed in Table 4.1 [52].

<table>
<thead>
<tr>
<th>$f(t)$</th>
<th>$\mathcal{L}<a href="s">f(t)</a>$</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$</td>
<td>$\frac{a}{s}$</td>
<td>$s&gt;0$</td>
</tr>
<tr>
<td>$at$</td>
<td>$\frac{a}{s^2}$</td>
<td>$s&gt;0$</td>
</tr>
<tr>
<td>$e^{at}$</td>
<td>$\frac{1}{s-a}$</td>
<td>$s&gt;a$</td>
</tr>
<tr>
<td>$\frac{d}{dt} f(t)$</td>
<td>$sF(s)-f(0)$</td>
<td>$s&gt;0$</td>
</tr>
</tbody>
</table>

Table 4.1: A brief overview of some Laplace transforms and their viable ranges.

A simple procedure can be used and is best illustrated in the form of an example as follows.

**Initial Value Problem Example**

Solve for 'y' given:

$$\frac{d}{dt} y + 7y = -2e^{-8t}, \quad y(0) = 8.$$  

Using tables, generate the subsidiary equation,

$$sY - y(0) + 7Y = \frac{-2}{s+8}.  $$

$$Y (s+7) = \frac{8s+62}{s+8}.  $$

The solution to the subsidiary equation is thus:

$$Y = \frac{6}{s+7} + \frac{2}{s+8}.  $$

From the latter expression, the inverse Laplace transform can easily be taken in order to obtain the solution to the given problem as

$$y = \mathcal{L}^{-1}\left(\frac{6}{s+7}\right) + \mathcal{L}^{-1}\left(\frac{2}{s+8}\right) = 6e^{-7t} + 2e^{-8t}.  $$

The Laplace transform method for solving differential equations greatly simplifies integration of this type into simple algebra. This technique will be applied in Section 4.2.

**4.2 Zimmerman-Brittin Model**

A schematic representation of the Zimmerman-Brittin model is shown in Figure 4.1 [53]. For this model, $k_{21}$ and $k_{12}$ are the spin exchange rates and the $T_1$ decay for the separate pools can be
Chapter 4: Two Pool Model

defined as $T_{1i}$ for the first pool and $T_{12}$ for the second pool. Cross relaxation between the two pools occurs when two protons of opposite phase are near each other. Zeeman spin coupling can occur between the two phases by simultaneous spin flips. Such coupling leads to coupling of spin-lattice as well as spin-spin relaxation [49]. Magnetization along the $z$ axis can be normalized as:

$$m_i(t) = \frac{-\left(m_{i\text{orig}}(t) - m_{i\text{orig}}(\infty)\right)}{2m_{i\text{orig}}(\infty)}$$

where the superscript orig stands for the original magnetization before normalizing, $m_{i\text{orig}}(\infty)$ is the equilibrium magnetization amount, and $i$ stands for either pool and can be further defined in the following Bloch equations where $m_1$ and $m_2$ are the magnetizations in each pool:

$$\frac{d}{dt} m_1 = -k_{12} m_1 - \frac{m_1}{T_{11}} + k_{21} m_2$$  \hspace{1cm} (4.2.1)$$

$$\frac{d}{dt} m_2 = -k_{21} m_2 - \frac{m_2}{T_{12}} + k_{12} m_1$$  \hspace{1cm} (4.2.2)$$

A quick inspection of these equations indicate that whatever magnetization is lost by one pool is picked up by the other; the system is closed.

These two equations can be rewritten as:

$$\frac{d}{dt} m_1 = a_1 m_1 + a_2 m_2$$

$$\frac{d}{dt} m_2 = b_1 m_2 + b_2 m_1$$

where

$$a_1 = -k_{12} - \frac{1}{T_{11}} \quad a_2 = k_{21}$$

$$b_1 = -k_{21} - \frac{1}{T_{12}} \quad b_2 = k_{12}$$

This system of differential equations can be solved using the Laplace transform method as described in Section 4.1. First, the subsidiary equations are determined:

$$sM_1 - m_1(0) = a_1 M_1 + a_2 M_2$$

$$sM_2 - m_2(0) = b_1 M_1 + b_2 M_2$$

where $M_1$ is the Laplace transform of $m_1$, $M_2$ is the Laplace transform of $m_2$, and $m_1(0)$ and $m_2(0)$ are the initial values of the system. For the Laplace transform, $s$ is the dependent variable in Laplace space much like $k$ is the dependent variable in Fourier space.

These equations can easily be manipulated to:

$$(s - a_1)M_1 - a_2 M_2 = m_1(0)$$

$$-b_2 M_1 + (s - b_1)M_2 = m_2(0)$$

Isolating $M_1$ and $M_2$ gives us the Laplace space solutions:
Chapter 4: Two Pool Model

\[ M_1 = \frac{m_1(0)s-b_1 m_1(0)+a_2 m_2(0)}{s^2-(a_1+b_1)s+a_1 b_1-a_2 b_2} \]  
(4.2.3)

\[ M_2 = \frac{m_2(0)s-a_1 m_2(0)+b_2 m_1(0)}{s^2-(a_1+b_1)s+a_1 b_1-a_2 b_2} \]  
(4.2.4)

Another substitution can be done at this point so that:

\[ \begin{align*}
M_1 &= \frac{m_1(0)s-b_1 m_1(0)+a_2 m_2(0)}{(s+A)(s+B)} \\
M_2 &= \frac{m_2(0)s-a_1 m_2(0)+b_2 m_1(0)}{(s+A)(s+B)}
\end{align*} \]

where \( A \) and \( B \) can be solved for using the quadratic equation on the denominator of Equations 4.2.3 and 4.2.4 such that:

\[ \begin{align*}
A &= \frac{-a_1-b_1-\sqrt{(a_1+b_1)^2-4(a_1 b_1-a_2 b_2)}}{2} \\
B &= \frac{-a_1-b_1+\sqrt{(a_1+b_1)^2-4(a_1 b_1-a_2 b_2)}}{2}
\end{align*} \]

Inverse Laplace transforms can easily be taken if the equations are further massaged into forms that can match Laplace transform tables like the one shown in Table 4.1. Thus, the solution to the subsidiary equations are:

\[ \begin{align*}
M_1 &= \frac{\alpha}{s+A} + \frac{\beta}{s+B} \\
M_2 &= \frac{\xi}{s+A} + \frac{\zeta}{s+B}
\end{align*} \]  
(4.2.5)

(4.2.6)

where the variables introduced here are strictly to make these equations appear more aesthetically pleasing. These variables are:

\[ \begin{align*}
\alpha &= \frac{-a_2 m_2(0)-b_1 m_1(0)-A m_1(0)}{B-A} \\
\beta &= \frac{-a_2 m_2(0)+b_1 m_1(0)+B m_1(0)}{B-A} \\
\xi &= \frac{b_2 m_1(0)-a_1 m_2(0)-A m_2(0)}{B-A} \\
\zeta &= \frac{-b_2 m_1(0)+a_1 m_2(0)+B m_2(0)}{B-A}
\end{align*} \]

The inverse Laplace transform can be determined using Table 4.1 to be:

\[ \begin{align*}
m_1(t) &= \alpha e^{-At} + \beta e^{-Bt} \\
m_2(t) &= \xi e^{-At} + \zeta e^{-Bt}
\end{align*} \]  
(4.2.7)

(4.2.8)

both being a sum of two exponentials.
For completeness, the original variables are back substituted as:

\[ 2A = R_{11} + k_{12} + R_{12} + k_{21} - \sqrt{(R_{11} - R_{12} + k_{12} - k_{21})^2 + 4k_{12}k_{21}} \]  \hspace{1cm} (4.2.9)

\[ 2B = R_{11} + k_{12} + R_{12} + k_{21} + \sqrt{(R_{11} - R_{12} + k_{12} - k_{21})^2 + 4k_{12}k_{21}} \]  \hspace{1cm} (4.2.10)

\[ \alpha = \frac{(R_{12} + k_{21} - A)m_1(0) + k_{21}m_2(0)}{B - A} \]  \hspace{1cm} (4.2.11)

\[ \beta = \frac{(-R_{12} - k_{21} + B)m_1(0) - k_{21}m_2(0)}{B - A} \]  \hspace{1cm} (4.2.12)

\[ \xi = \frac{k_{12}m_1(0) + (R_{11} + k_{12} - A)m_2(0)}{B - A} \]  \hspace{1cm} (4.2.13)

\[ \zeta = \frac{-k_{12}m_1(0) + (-R_{11} - k_{12} + B)m_2(0)}{B - A} \]  \hspace{1cm} (4.2.14)

where \( R_{1u} \) is the reciprocal of \( T_{1u} \).

The rate constants, \( k_r \), are related by a cross-relaxation constant, \( T_{cr} \) as \([50]\) :

\[ \frac{1}{T_{cr}} = P_1k_{12} = P_2k_{21} \]  \hspace{1cm} (4.2.15)

where \( P_1 \) and \( P_2 \) are the probabilities of the protons being in pool 1 or 2, respectively.

---

On Edzes and Samulski's Cross Relaxation Paper

In 1978 Hommo T. Edzes and Edward T. Samulski published a landmark paper entitled: *The measurement of cross-relaxation effects in the proton NMR spin-lattice relaxation of water in biological systems: hydrated collagen and muscle* \([49]\).* This paper characterizes how apparent relaxation times, \( 1/A \) and \( 1/B \), are measured in place of true relaxation times of \( T_1 \) or \( T_2 \), depending on the pulse sequence used. Upon studying this paper in detail, however, I noticed an inconsistency with their Bloch equations with what I believe to be correct. Their Bloch equations are effectively the same as I have shown in Section 4.2 except they have \( a_2 = k_{12} \) and \( b_1 = k_{21} \). If one examines the Bloch equations using these changes, the system does not appear to be closed. If one assumes that the \( T_1 \) relaxation is infinite, then the net change in the first pool would be \( k_{12}m_2 - k_{12}m_1 \) and the net change in the second pool would be \( k_{21}m_1 - k_{21}m_2 \). For the system to be closed the net gain (or loss) of the first pool must result in the same net loss (or gain) in the second pool. Put another way, some fraction, \( k_{12} \), of \( m_1 \) is taken away from the first pool and a different fraction, \( k_{21} \), is delivered to the second pool. If such a delivery was done by Canada Post, one would surely complain! On a serious note, if one carries on with the calculations as shown in Section 4.2 the same apparent relaxation rates, \( A \) and \( B \), result. This is because the variables in question are coupled together in the derivation of these relaxation rates and appear nowhere independently.

The discrepancy occurs in the apparent intensities of the relaxation rates one would measure in such a system. These amplitudes are derived as \( \alpha \), \( \beta \), \( \xi \), and \( \zeta \) in Section 4.2. Following the same procedure with these different variables yields:
Chapter 4: Two Pool Model

\[ \alpha_{ES} = \frac{(R_{12} + k_{21} - A) m_1(0) + k_{12} m_2(0)}{B - A} \]

\[ \beta_{ES} = \frac{(-R_{12} - k_{21} + B) m_1(0) - k_{12} m_2(0)}{B - A} \]

\[ \xi_{ES} = \frac{k_{21} m_1(0) + (R_{11} + k_{12} - A) m_2(0)}{B - A} \]

\[ \zeta_{ES} = \frac{-k_{21} m_1(0) + (-R_{11} - k_{12} + B) m_2(0)}{B - A} \]

In order to obtain the exact solutions published in their paper, one needs to replace \(-A\) in the numerator of \(\alpha_{ES}\) and \(\xi_{ES}\) with \(-A + B - B\) and manipulate. Likewise, one needs to change \(B\) in the numerator of \(\beta_{ES}\) and \(\zeta_{ES}\) with \(B - A + A\) and manipulate to get:

\[ \alpha_{ES} = \frac{-(R_{11} + B) m_1(0) + k_{12} (m_2(0) - m_1(0))}{B - A} \]

\[ \beta_{ES} = \frac{(R_{11} - A) m_1(0) - k_{12} (m_2(0) - m_1(0))}{B - A} \]

\[ \xi_{ES} = \frac{-(R_{12} - B) m_2(0) + k_{21} (m_1(0) - m_2(0))}{B - A} \]

\[ \zeta_{ES} = \frac{(R_{12} - B) m_2(0) - k_{21} (m_1(0) - m_2(0))}{B - A} \]

which agrees exactly with Edzes and Samulski's results.

If one compares \(\alpha\) and \(\beta\) derived these two different ways there is a difference of:

\[ \frac{(k_{21} - k_{12}) m_2(0)}{B - A} \]

Similarly, if one compares \(\xi\) and \(\zeta\) derived these two ways there is a difference of:

\[ \frac{(-k_{21} + k_{12}) m_1(0)}{B - A} \].
The four pool model of white matter is similar in concept to the generalized two pool model presented in Section 4. The experiments conducted resulted in three readily distinguishable pools: non-aqueous tissue, myelin water, and IE water, as shown in Figure 3.4. If one assumes that the non-aqueous tissue pool can be broken down into two currently non-divisible non-aqueous pools, each associated with one of the water pools, then a biologically viable model of this system is a four pool model [20,42,54]. The model is shown schematically in Figure 5.1 and comprises of non-aqueous protons in myelin (m) and non-myelin (nm), water protons in myelin (mw) and in the intra/extracellular spaces (ie). If one assumes that each proton reservoir achieves internal equilibrium of magnetization rapidly compared to the rates of magnetization exchange between the proton reservoirs, one can describe the time dependence of the magnetization using the Bloch equations [49,53]:

\[
\frac{d}{dt} M_m = -k_{12} M_m - \frac{M_m - M_m^{(\infty)}}{T_1^m} + k_{21} M_{mw}
\]

\[
\frac{d}{dt} M_{mw} = -k_{21} M_m - \frac{M_{mw} - M_{mw}^{(\infty)}}{T_1^{mw}} - k_{23} M_{mw} + k_{12} M_m + k_{32} M_{ie}
\]

\[
\frac{d}{dt} M_{ie} = -k_{32} M_{ie} - \frac{M_{ie} - M_{ie}^{(\infty)}}{T_1^{ie}} - k_{34} M_{ie} + k_{23} M_{mw} + k_{43} M_{nn}
\]

\[
\frac{d}{dt} M_{nn} = -k_{43} M_{nn} - \frac{M_{nn} - M_{nn}^{(\infty)}}{T_1^{nn}} + k_{34} M_{ie}
\]

where the $M$s and $M^{(\infty)}$s denote the time dependent and equilibrium magnetizations of the four proton pools; the $T_1$s denote the spin-lattice relaxation times; and the $k$s denote the exchange.

Portions of this chapter have been included in an article accepted for publication. Bjarnason TA, Vavasour IM, Chia CLL, MacKay AL. Characterization of the NMR behaviour of white matter in bovine brain. Magnetic Resonance in Medicine, In Production: July, 2005.
rates between pools with directionality as indicated in Figure 5.1.

This four pool model provides three exchange processes: myelin with myelin water, myelin and IE water, and IE water with non-myelin tissue. These three processes can be characterized by cross relaxation times [49]:

\[
\frac{1}{T_{cr}^{m}} = \frac{p^{m}}{p^{m} + p^{mw}} k_{12} = \frac{p^{mw}}{p^{m} + p^{mw}} k_{21}
\]

(5.5)

\[
\frac{1}{T_{cr}^{D}} = \frac{p^{mw}}{p^{mw} + p^{ie}} k_{23} = \frac{p^{ie}}{p^{mw} + p^{ie}} k_{32}
\]

(5.6)

\[
\frac{1}{T_{cr}^{ie}} = \frac{p^{ie}}{p^{ie} + p^{nn}} k_{34} = \frac{p^{nn}}{p^{ie} + p^{nn}} k_{43}
\]

(5.7)

where \( T_{cr}^{m} \) is the cross relaxation time between the myelin and myelin water pools; \( T_{cr}^{D} \) is the cross relaxation time between the two water pools due to diffusion; and \( T_{cr}^{ie} \) is the cross relaxation time between the IE water and the non-myelin proton pools. The \( P \)'s are the probabilities of a proton being in one of the four proton pools.

Alternately, the cross relaxation times can be determined from the reciprocal rates using:

\[
T_{cr}^{m} = \frac{1}{k_{12}} + \frac{1}{k_{21}} ; \quad T_{cr}^{D} = \frac{1}{k_{23}} + \frac{1}{k_{32}} ; \quad T_{cr}^{ie} = \frac{1}{k_{34}} + \frac{1}{k_{43}}
\]

(5.8)

### 5.1 Numerical Solution

I spent roughly a month attempting to integrate the system of Equations 5.1-5.4 in a similar fashion as presented in Section 4. This task was daunting. The final solution involves solving a quartic equation, which in turn requires solving a cubic. When attempting to solve a cubic for the general case, one ends up with three sets of solutions, two of which could contain imaginary answers. With further contemplation it appeared possible to generate this general solution even with these obstacles. However, it became evident that solutions of the form provided in Equations 4.2.9-4.2.14 would each encompass more than a page. Deriving meaning from such complex equations would not be as easy as leaving the original derivative equations alone.

Thus, to investigate how well the four pool model represents the data summarized in Section 3 a computer program was created using Matlab (The MathWorks, MA). The Bloch Equations 5.1-5.4 were adapted in an incremental fashion as:

\[
M_{m}(i+1) = \left( -k_{12} M_{m}(i) - \frac{M_{m}(i) - M_{m}(\infty)}{T_{1}} + k_{21} M_{wm}(i) \right) t + M_{m}(i)
\]

(5.1.1)

\[
M_{wm}(i+1) = \left( -k_{23} M_{wm}(i) - \frac{M_{wm}(i) - M_{wm}(\infty)}{T_{wm}} - k_{32} M_{m}(i) \right) t + M_{wm}(i)
\]

(5.1.2)
where \( t \) was the timestep set to 0.1 ms, which is reasonable since the cross relaxation times were found to be much longer, and \( i \) was cycled from 1 to 2000. The initial magnetizations were determined one of two ways for the mobile signal. The initial value determined using NNLS was used if it was the largest, otherwise, the initial value was set to the average of the initial value and the maximum value of the data shown in Figure 3.4. The measured initial non-aqueous value, as shown as squares in Figure 3.4, was split evenly into myelin and non-myelin non-aqueous values. The mobile equilibrium values were assumed to be the same as the initial values. The non-aqueous equilibrium values were estimated using the proton densities as determined in Equation 5.1.5. The \( T_i \) values were determined as described in Section 3.4. For numerical solution purposes it was assumed that \( T_{mx} = T_{w} \) and \( T_{wm} = T_{1} \).

It is important when performing the numerical solutions to work with the actual relative amount of protons present in the samples. The mobile fraction, \( MF \), was determined as described in Section 3.2 and the MWFs were determined as described in Section 3.3 and were modified during the numerical solution process using a cross relaxation correction as discussed in Section 5.1.1. The probability of a proton being found in the various pools was:

\[
P_{m} = P_{mw} \times MF; \\
P_{ie} = MF - P_{m}; \\
P_{m} = P_{nn} = 0.5(1 - MF)
\]

where \( P_{mw}, P_{ie}, P_{m}, \) and \( P_{nn} \) represent the probability of a proton being found in the myelin water, IE water, myelin tissue, or non-myelin tissue proton pools, respectively. The variable parameters of the numerical solutions were three \( T_{cr} \)s and a scalar parameter that was necessary because the two cross relaxation experiments used to collect the data were done at different times.

### 5.1.1 Cross Relaxation Correction

In the transverse plane, cross-relaxation occurs over the duration of the CPMG measurement. Thus, without a cross relaxation correction, one will artificially measure smaller values for cross relaxation times, \( T_2 \), and MWFs. In order to compensate, one needs to investigate the Bloch equations in the transverse plane. The behaviour of the magnetization in the mobile pools during the CPMG experiment can be described by:

\[
\frac{d}{dt} M_{m} = k_{32} M_{ie} - \frac{M_{nn}}{T_{2}} - k_{23} M_{nw}
\]

(5.1.6)

\[
\frac{d}{dt} M_{nw} = k_{23} M_{nn} - \frac{M_{nn}}{T_{2}} - k_{32} M_{iw}
\]

(5.1.7)

When one compares these two equations to Equations 5.1-5.4 one immediately notices that the
two equations defining the motionally restricted pools are absent and that the cross-terms with these pools are also gone. Since the decay mechanism of the motionally restricted pools cause the pools to decay faster than the CPMG experiment can characterize them, one need not include these pools in the equations presented here. Instead, one can assume a drain in mobile signal resulted from interactions with these motionally restricted pools. In fact, some wood experiments determined that the cross-relaxation between mobile and motionally restricted pools partially, and in some cases completely, determined the $T_2$ decay \[50\]. Thus, the terms $k_{21}$ and $k_{34}$ are assumed to be constituents of $1/T_2^{mw}$ and $1/T_2^{i\epsilon}$, respectively.

One can solve the system of Bloch Equations 5.1.6 and 5.1.7 to get \[49\], Section 4:

$$M_{nw}(t) = \alpha e^{-\beta t} + \beta e^{-\beta t}$$

$$M_{ie}(t) = \xi e^{-\beta t} + \zeta e^{-\beta t}$$

defining:

$$2A = R_2^mw + k_{32} + R_2^i\epsilon + k_{32} - \sqrt{(R_2^mw - R_2^i\epsilon + k_{32} - k_{32})^2 + 4k_{23}k_{32}}$$

$$2B = R_2^mw + k_{23} + R_2^i\epsilon + k_{32} + \sqrt{(R_2^mw - R_2^i\epsilon + k_{32} - k_{32})^2 + 4k_{23}k_{32}}$$

$$(\alpha + \xi) = \frac{(R_2^i\epsilon + k_{23} + k_{32} - A)M_{nw}(0) + (R_2^mw + k_{23} + k_{32} - A)M_{ie}(0)}{B - A}$$

$$(\beta + \zeta) = \frac{(-R_2^i\epsilon - k_{23} - k_{32} + B)M_{nw}(0) + (-R_2^mw - k_{23} - k_{32} + B)M_{ie}(0)}{B - A}$$

where $R_2$ is the reciprocal of $T_2$ and the $k_{ij}$s are defined in Equation 5.6. The values $(\alpha + \xi)$ and $(\beta + \zeta)$, and measured relaxation rates $A$ and $B$, vary for given intensities, relaxation rates, and cross relaxation time. In order to correct for the cross-relaxation effect, Equations 5.1.8-5.1.11 needed to be examined so that a cross relaxation correction could be done. This cross relaxation correction could be used to determine the initial intensities and relaxation rates prior to the CPMG measurement using the measured values and cross relaxation time.

Solving Equations 5.1.8-5.1.11 for the relaxation rates and initial intensities gives:

$$\frac{1}{T_2^{mw}} = R_2^mw = -k_{23} + \frac{1}{2}(A + B + Q)$$

$$\frac{1}{T_2^{i\epsilon}} = R_2^i\epsilon = -k_{32} + \frac{1}{2}(A + B - Q)$$

$$M_{nw}(0) = \frac{(\alpha + \xi)(2k_{32} - B + A + Q) + (\beta + \zeta)(2k_{23} + B - A + Q)}{2(k_{23} - k_{32} - Q)}$$

$$M_{ie}(0) = \frac{(\alpha + \xi)(2k_{23} - B - A - Q) + (\beta + \zeta)(2k_{32} + B - A - Q)}{2(k_{23} - k_{32} - Q)}$$

where $Q = \sqrt{B^2 - 2AB + A^2 - 4k_{23}k_{32}}$. A more in-depth presentation of the mathematics used to derive the Equations 5.1.12-5.1.15 are presented in the Appendix.

Recalling Equation 5.6, $k_{23}$ and $k_{32}$ depended on the cross relaxation time as well as the
relative proton percentages, $P^{mw}$ and $P^{se}$, which can be derived from the unknown values $M_{mw}(0)$ and $M_{se}(0)$. So, when doing the cross relaxation correction one needs to first estimate values for $M_{mw}(0)$ and $M_{se}(0)$ in order to determine $k_{23}$ and $k_{32}$ to use on the right hand side of Equations 5.1.14 and 5.1.15. Good starting values are $M_{mw}^{1}(0)=(\beta+\zeta)$ and $M_{se}^{1}(0)=(\alpha+\xi)$. 
6 Results and Discussion

The cross relaxation – FID experiment provided rapid reappearance of the motionally restricted signal initially and peaked around $\tau_{cr} = 150\, ms$ for both temperatures, as shown by the boxes in Figure 6.1. Beyond this time the signal decreased and was expected to eventually reach zero due to the inversion recovery experiment used. Both mobile pools, shown as diamonds and circles in Figure 6.1, decreased initially for small $\tau_{cr}$ times as the mobile pools replenished magnetization to the non-aqueous pools. The initial rate of change in the signal happened at a greater rate for all pools when at the higher temperature. These mobile pools were expected to eventually reach equilibrium for long $\tau_{cr}$ as $T_1$ relaxation allowed these pools to recover to steady state values.

The Zimmerman-Brittin model was used to characterize the $\tau_{cr}$s, and is ideal for $T_{cr}^{mw}$ because myelin and myelin water are closely associated in the bilayers [53]. The Brownstein-Tarr model [55] would have been a better choice to model the cross relaxation due to diffusion, $T_{cr}^D$, but would have introduced another level of complexity into the numerical solution process. Thus, $T_{cr}^D$ can be assumed to be a first order approximation of the diffusion process. The numerical solution agrees quite well with the measured data as shown in Figure 6.1. A summary of the parameters used in the numerical solutions are presented in Table 6.1. The $T_{cr}$ values were all smaller by roughly a factor of two at the higher temperature. The reciprocal rates scale with their corresponding $T_{cr}$ values as described by Equation 5.8. The estimates for the time constant due to diffusion, $T_{cr}^D$, were 2064\,ms for the 24°C case and 1021\,ms for the 37°C case. This decrease of exchange time as temperature increases is an expected result if one attributes the cross relaxation time between the two water pools to be governed by diffusion, which is a temperature driven process [56].

The free induction decay of the motionally restricted pool goes to zero rapidly due to the relatively large unaveraged dipolar interactions between adjacent protons. In white matter, magnetization is transferred from the aqueous pool to the non-aqueous pool by the cross relaxation process. When in the transverse plane, the non-aqueous pool's newly acquired magnetization is then quickly dephased and thus lost. As a result, this cross relaxation process places an upper limit on the $T_2$ for the two water pools, i.e,

$$T_2^{mw} \leq \frac{P^{mw}}{P^m + P^{mw}} T_{cr}^{mw} = \frac{1}{k_{21}}$$

$$T_2^{ie} \leq \frac{P^{ie}}{P^{ie} + P^{mw}} T_{cr}^{ie} = \frac{1}{k_{34}}$$

where $T_2^{mw}$ and $T_2^{ie}$ are the $T_2$ times for myelin and IE water, respectively.

Table 6.1 summarizes the results of the numerical solutions along with error estimates. These error estimates were performed by allowing the parameter to vary during a numerical solution, while keeping the other parameters constant, so that the change of the sum of squares was 5%. For the cross relaxation parameters the error estimates are indicated by a “+” change and a “−” change. The reciprocal rates result from the cross relaxation parameters and the error estimates are presented following the “±” symbol.

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Portions of this chapter have been included in an article accepted for publication. Bjarnason TA, Vavasour IM, Chia CLL, MacKay AL. Characterization of the NMR behaviour of white matter in bovine brain. Magnetic Resonance in Medicine, In Production: July, 2005.
Chapter 6: Results and Discussion

Figure 6.1: Averaged cross relaxation measurements collected for the myelin water pool, the IE water pool, and the non-separable non-aqueous pools. The corresponding fits used in the numerical solution are shown as lines with \( a \) representing the data and fit for the 24\(^\circ\)C case and \( b \) representing the 37\(^\circ\)C case. The intensities are scaled relative to each other and the y-axis was split in two in order to better display the data.

It was found that \( 1/k_{31} \) was 122±13ms for myelin water and \( 1/k_{34} \) was 349±90ms for IE water at 24\(^\circ\)C. For 37\(^\circ\)C these reciprocal rates were found to be 85±32 and 149±46ms, respectively. If cross relaxation were the only mechanism responsible for water \( T_2 \) relaxation, then the measured \( T_2 \)s would be equal to these reciprocal rates [50]. The measured values for the \( T_2 \) times were considerably lower with 13.8 (7.3)ms for \( T_2^{\text{myel}} \) and 106 (18)ms for \( T_2^{\text{IE}} \) at 24\(^\circ\)C and 11.7 (3.7) and 80.9 (8.0)ms at 37\(^\circ\)C, respectively. Thus, as expected, cross relaxation between tissue and the corresponding mobile pools is only one of the mechanisms responsible for \( T_2 \).
Chapter 6: Results and Discussion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>24 °C</th>
<th>37 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{\text{cr}}^p$</td>
<td>2064 ±356</td>
<td>1021 ±653</td>
</tr>
<tr>
<td>$T_{\text{cr}}^{\text{mw}}$</td>
<td>227 ±76</td>
<td>158 ±75</td>
</tr>
<tr>
<td>$T_{\text{cr}}^{\text{rel}}$</td>
<td>402 ±119</td>
<td>170 ±62</td>
</tr>
<tr>
<td>$1/k_{12}$</td>
<td>104 ±11</td>
<td>73 ±30</td>
</tr>
<tr>
<td>$1/k_{21}$</td>
<td>122 ±13</td>
<td>85 ±32</td>
</tr>
<tr>
<td>$1/k_{32}$</td>
<td>310 ±44</td>
<td>142 ±39</td>
</tr>
<tr>
<td>$1/k_{34}$</td>
<td>1753 ±276</td>
<td>879 ±321</td>
</tr>
<tr>
<td>$1/k_{43}$</td>
<td>349 ±90</td>
<td>149 ±46</td>
</tr>
</tbody>
</table>

Table 6.1: Numerical solution generated cross relaxation times, corresponding reciprocal exchange rates and a summary of $T_2$s used in the numerical solutions. Standard deviations are shown in brackets and error estimates follow the $+$ and $-$ signs. All units are in ms.

A cross relaxation correction was performed on the data during the numerical solution in order to generate the fits, as described in Section 5.1.1. To show how much this correction affects the $T_2$ values and MWFs, values from the CPMG experiment were taken for the $\tau=400\mu s$ case of Table 3.1. The results of this exercise are shown in Table 6.2. One can see that the correction is slight and within the error estimates and standard deviations. This result indicates that the measured values for MWFs and $T_2$s are close to physical values.

<table>
<thead>
<tr>
<th>Temp</th>
<th>$T_2^{\text{mw}}$ [ms]</th>
<th>$T_2^{\text{rel}}$ [ms]</th>
<th>MWF [%]</th>
<th>$T_2^{\text{mw}}$ [ms]</th>
<th>$T_2^{\text{rel}}$ [ms]</th>
<th>MWF [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>24°C</td>
<td>13.8 (7.3)</td>
<td>106 (18)</td>
<td>10.9 (3.2)</td>
<td>14.6 ±9.2</td>
<td>112 ±25</td>
<td>12.4 ±5.1</td>
</tr>
<tr>
<td>37°C</td>
<td>11.7 (3.7)</td>
<td>80.9 (8.0)</td>
<td>11.8 (4.6)</td>
<td>12.7 ±5.2</td>
<td>88 ±13</td>
<td>14.4 ±5.9</td>
</tr>
</tbody>
</table>

Table 6.2: Measured $T_2$ values and MWF values along with cross relaxation corrected values for both temperatures at which the experiments were carried out. Standard deviations are shown in brackets. Error estimates follow "±" and represent the maximum change resulting on the output value from the cross relaxation correction routine when the input parameters were varied by the standard deviation of the measured values.

6.1 Comparison With Literature

The following sections compare the results of the study done for this manuscript with other studies published in the literature. The first study was conducted by Irene M Vavasour, Kenneth P Whittall, David KB Li, Alex L MacKay and was entitled Different magnetization transfer effects exhibited by the short and long $T_2$ components of human brain [57]. In this study magnetization transfer experiments were conducted using different magnetization transfer delay...
times. Next, John G Sled and G Bruce Pike's study: *Quantitative imaging of magnetization transfer exchange and relaxation properties in vivo using MRI* was investigated [18]. Their magnetization transfer study was conducted on agar gels, uncooked beef, and *in vivo* human white, gray and normal appearing white matter as well as MS lesions. In their study some cross relaxation parameters were reported. Clare Morrison and R Mark Henkelman's study entitled *A model for magnetization transfer in tissues* [19] investigates the use of a two pool model to interpret magnetization transfer results of bovine white and gray matter, optic nerve, muscle, and liver as well as human blood and cerebral spinal fluid. They reported various cross relaxation parameters used for modeling their data. Finally, a similar four pool model to the one presented in this manuscript was published by Greg J Stanisz, A Kecojevic A, Micheal J Bronskill, R Mark Henkelman in the document entitled *Characterizing white matter with magnetization transfer and T₂* [20]. This study was performed on bovine optic nerve using similar theory and methods as presented in Henkelman et al's previous papers [19,58].

### 6.1.1 Vavasour *et al*

A previous study by Vavasour *et al* [57] investigated exchange between the myelin water pool and the IE water pool for human brain *in vivo*. In this previous study a $T₂$ measurement was conducted following various delay times after a magnetization transfer pulse. For delay times longer than about 200ms both pools were found to be in equilibrium. This result is in agreement with the current *in vitro* study where it was found that the reciprocal rate, $1/k_{35}$, at which myelin water loses signal to the IE water pool was 142ms at 37°C.

### 6.1.2 Sled and Pike

John Sled and Bruce Pike used a two pool model to characterize magnetization transfer exchange in frontal white matter of *in vivo* human subjects [18]. In their experiment the variable $k_f$ represented the rate that the mobile, or “free”, pool lost signal to the non-aqueous pool. Upon studying the differential equations they used the following relation comprised with nomenclature of this manuscript can be determined:

$$k_f M_f = k_{21} M_{mw} + k_{34} M_{ie}$$

where $M_f$ represents the mobile magnetic moment vectors and the variables on the right hand side of the equation are the same as defined in Section 5. Assuming the free pool is made up of both mobile pools:

$$M_f = M_{mw} + M_{ie},$$

one can derive an expression of Sled and Pike's variable using the nomenclature of this manuscript:

$$k_f = \frac{k_{21} M_{mw} + k_{34} M_{ie}}{M_{mw} + M_{ie}}.$$

Similarly, the rate at which signal from the non-aqueous, or “restricted”, pool loses signal to the mobile pool, $k_r$, can be determined:

$$k_r = \frac{k_{12} M_{m} + k_{43} M_{mn}}{M_{m} + M_{mn}}.$$  

(6.1.2)

Another variable reported in Sled and Pike's paper was the fraction of protons that are restricted.
In the nomenclature of this document, this variable can be defined as:

\[ F = \frac{1 - MF}{MF} \]  

(6.1.3)

where MF is defined in Section 5.1. Within Pike and Sled's paper, an additional method of calculating \( k_r \) was presented [18]:

\[ k_r = \frac{k_f}{F} \]  

(6.1.4)

<table>
<thead>
<tr>
<th>Current Study</th>
<th>Sled and Pike's Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 24^\circ C )</td>
<td>( 37^\circ C )</td>
</tr>
<tr>
<td>( k_f ) (s(^{-1}))</td>
<td>3.5 ± 1.3</td>
</tr>
<tr>
<td>( k_r ) (s(^{-1}))</td>
<td>14.3 ± 2.8</td>
</tr>
<tr>
<td>( F )</td>
<td>0.214 ± 0.049</td>
</tr>
</tbody>
</table>

Table 6.3: Determined values, including propagated errors, of the current study compared to Sled and Pike's results [18]. Sled and Pike's results were found using human frontal white matter in vivo. *Normal appearing white matter of a multiple sclerosis patient. Calculated using Equation 6.1.4.

A comparison of the values determined for this current study to Sled and Pike's results are shown in Table 6.3. For the \( 37^\circ C \) case the rate values agree within error while the fraction restricted, \( F \), does not. A close correlation is not expected, however, since the measurements were taken on two different mammals, one in vitro and the other in vivo. One would expect the related parameters to agree on an order of magnitude scale, which they do.

### 6.1.3 Morrison and Henkelman

Morrison and Henkelman also used a two pool model to characterize magnetization transfer in bovine white matter at room temperature [19]. The parameters of interest in their paper are \( R \) and \( R\cdot M_0^s / R_s \). In order to compare results, one needs to examine the Bloch equations they cited in a previous paper by the same group [58]. In Henkelman et al's nomenclature, \( R \) is the rate constant that characterizes the rate of transfer of magnetization between the mobile, or "liquid", and non-aqueous, or "semi-solid", pools.

Using the nomenclature defined by Equations 6.1.1 and 6.1.2 allows one to define a cross relaxation time parameter in a similar way to that presented in Section 5:

\[ \frac{1}{T_{cr}} = \frac{P_f}{P_f + P_s} k_f = \frac{P_s}{P_f + P_s} k_r \]

where \( P_f \) is the probability of a proton being found in the mobile pool and \( P_s \) is the probability of
a proton being found in the non-aqueous pool. Thus:

\[ T_{cr} = \frac{1}{k_r} + \frac{1}{k_r} \]  

(6.1.5)

One of Henkelman et al's parameters relates to the nomenclature developed in Equation 6.1.5 as:

\[ RM_0^B = k_f = \frac{P_r^p + P_r^s}{p_f} \cdot \frac{1}{T_{cr}} \]  

(6.1.6)

In Henkelman et al's paper, the concentration of protons in the semi-solid pool are represented by \( M_0^A \) and the liquid concentration was \( M_0^B \). The liquid concentration was normalized in Henkelman et al's work; this normalization can be described as:

\[ M_0^A = \frac{p_f}{P_f} ; \quad M_0^B = \frac{p_f}{P_f} \]

Substituting the above relation into Equation 6.1.6 provides:

\[ R = \frac{P_r^p + P_r^s}{P_r^p} \cdot \frac{1}{T_{cr}} \]

or

\[ R = \frac{P_r^p + P_r^s}{P_r^p} \cdot \frac{1}{T_{cr}} \]  

(6.1.7)

<table>
<thead>
<tr>
<th>Current Study</th>
<th>Morrison and Henkelman's Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R ) ( (s^{-1}) )</td>
<td>( 15.8 \pm 9.3 )</td>
</tr>
<tr>
<td>( k_f/R_A )</td>
<td>( 2.8 \pm 1.6 )</td>
</tr>
<tr>
<td>( k_f/R_A )</td>
<td>( 2.0 \pm 0.1 )</td>
</tr>
</tbody>
</table>

Table 6.4: Determined values, including propagated errors, of the current study at 24°C compared to Morrison and Henkelman's results [19]. Morrison and Henkelman's results were determined on bovine white matter at 20-22°C.

An additional parameter used in Morrison and Henkelman's work was \( R_A \) which was defined as \( 1/T_1 \) for the mobile pool [19]. In the current study the \( T_1 \) of white matter at 24°C was found to biexponential. Thus, in order to compare results with Morrison and Henkelman's work the following relation was used:

\[ R_A = \frac{MWF}{T_1^{me}} + \frac{(1-MWF)}{T_1^{se}} \]  

(6.1.8)

Using the values in Table 6.1 for 24°C \( R_A = 1.26 \pm 0.27s^{-1} \). In order to compare with another parameter set out by Morrison and Henkelman, the following simplification was made using Equation 6.1.6:

\[ \frac{RM_0^B}{R_A} = \frac{k_f}{R_A} \]  

(6.1.9)

A comparison of the values determined for this study to Morrison and Henkelman's
results are shown in Table 6.4. The parameters of Morrison and Henkelman's study agree within error to the values calculated for the current study. This finding provides evidence that the two pool model is indeed a simplification of the four pool model. One can calculate the parameters of a two pool model using the determined parameters of the four pool model. This process cannot be reversed, however as one can see from Equations 6.1.1 and 6.1.2.

6.1.4 Stanisz et al

Using a magnetization transfer experiment combined with a $T_2$ experiment, Stanisz et al [20] used a similar four pool model to characterize the exchange process of bovine optic nerve at 20°C. Their analysis and theory was closely related to Morrison and Henkelman's work described in Section 6.1.3.

<table>
<thead>
<tr>
<th>Current Study</th>
<th>Stanisz et al's Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{21}(s^{-1})$</td>
<td>8.2 ± 0.9</td>
</tr>
<tr>
<td>$k_{34}(s^{-1})$</td>
<td>2.9 ± 0.7</td>
</tr>
<tr>
<td>$T_{cr}^{D}$</td>
<td>2064 ± 1285</td>
</tr>
<tr>
<td>$k_{21}T_{1}^{nw}$</td>
<td>2.81 ± 0.98</td>
</tr>
<tr>
<td>$k_{34}T_{1}^{ie}$</td>
<td>2.70 ± 0.90</td>
</tr>
</tbody>
</table>

Table 6.5: Determined values, along with propagated errors, of the current study at 24°C compared to Stanisz et al's results [20]. Stanisz et al's results were determined for bovine optic nerve at about 20°C.

The parameters of interest in comparing with Stanisz et al's work are summarized in Table 6.5. Based on the values of Stanisz et al, pool B represents myelin water and the associated tissue while pool A represents IE water and non-myelin tissue. Their superscripts S and L represent the non-aqueous, or "semi-solid", tissue and aqueous, or "liquid", pools. Examining the Bloch equations of Stanisz et al it becomes immediately obvious that:

$$R_{B}M_{B}^{S}=k_{21}$$, \quad $$R_{A}M_{A}^{S}=k_{34}$$.

(6.1.10)

Utilizing Equation 6.1.5 the following relation can be derived:

$$T_{cr}^{D} = \frac{1}{k_{AB}} + \frac{1}{k_{BA}}$$

(6.1.11)

where [20]

$$k_{BA} = k_{AB} \frac{M_{A}^{S}}{M_{B}^{S}}$$.

The $M$'s are the relative proton populations of the two liquid pools. The final relations are reminiscent of Equation 6.1.3 and are:

$$\frac{R_{B}M_{B}^{S}}{R_{B}^{L}} = k_{21}T_{1}^{nw}$$, \quad $$\frac{R_{A}M_{A}^{S}}{R_{A}^{L}} = k_{34}T_{1}^{ie}$$.

(6.1.12)

Major differences are seen between the compared parameters in Table 6.5. Of interest are the pseudo-first-order rate constants between the liquid and tissue pools. Stanisz et al found that
the exchange rate between myelin water and myelin was much larger than the exchange rate between IE water and the non-myelin tissue pool. The results from the current study found that these two pseudo-first-order rate constants were of the same order; this finding does not support the theory that the exchange between myelin and myelin water is the dominating exchange mechanism for all white matter regions. Discrepancies in these values may be due to the different myelin contents between white matter (~13%) and optic nerve (~32%), the different temperatures at which the experiments were performed, the fact that Stanisz et al's study used a different acquisition method that did not measure a non-aqueous signal directly, both experiments were conducted at slightly different magnetic field strength, and the values reported in this manuscript were determined using a cross relaxation correction.

6.1.5 Summary
The main conclusion from Stanisz et al's work was that the IE water/non-myelin tissue interaction was found to be much smaller than the myelin water/myelin tissue interaction, thus they concluded that the magnetization transfer effect was mostly governed by the myelin water/myelin tissue interaction [20]. The results from the current study indicate that both interactions are of the same order, thus the IE water/non-myelin tissue interaction cannot be ignored when looking at the magnetization transfer effect. The results from the current study were compared to studies that used a two pool model to describe magnetization transfer [18,19]. The Bloch equations from these studies were compared with the Bloch equations of the current study in order to derive relationships between the parameters of the separate studies; they agreed well with each other.
Chapter 7: Conclusion

7 Conclusion

7.1 Concluding Remarks

The NMR signal from bovine white matter, following the dephasing of the non-aqueous signal, was represented using four proton pools: myelin water, intra/extracellular (IE) water, myelin, and non-myelin tissue. A large body of literature has been published using a two pool model to represent white matter in magnetic transfer experiments on the basis that the exchange of magnetization between the aqueous pools and the non-aqueous pools is governed by the interaction between myelin water and myelin. The purpose of this work was to investigate whether or not this assumption was valid, to investigate how well a four pool model can represent the MR signal collected from white matter, and to confirm that a two pool model is a simplified version of the four pool model. The four pools used were a simple way of representing the complexity of white matter and the agreement the numerical solutions provide with the experimental data was excellent. Similar experiments on bovine white matter using the two pool model provided parameters that could be calculated from the variables determined using a four pool model; thus confirming that the two pool model of white matter is a simplification of the four pool model. The numerical solutions to the four pool indicate that the exchange rate between IE water and its non-aqueous pool was of the same order as the exchange rate between myelin water and myelin; the interaction between IE water and its non-aqueous pool cannot be ignored.

The cross relaxation time between myelin water and IE water at 24°C was found to be twice as long as the measured $T_1$s of these two pools, allowing the measured decay to be bi-exponential since the signal of each of the two pools does not have enough time to mix via cross relaxation over the duration of the measurement. At 37°C the cross relaxation time was of the same order as the measured mono-exponential $T_1$ value. This observation indicated that the increased cross relaxation rate between the mobile pools caused mono-exponential $T_1$ measurements at 37°C. The increased cross relaxation rate is presumably a result of the expected increase of water diffusion between 24 and 37°C; in pure water the self diffusion rate has been observed to increase roughly 40% over a similar temperature range [56]. At both temperatures the cross relaxation time between myelin water and IE water was significantly longer than the measured $T_2$s, allowing for consistent measurements of multi-exponential decays.

Introducing a cross relaxation correction to compensate for cross relaxation effects over the duration of the CPMG measurement caused slight shifts in the measured $T_1$ and myelin water fraction values with a slightly larger shift occurring for the higher temperature. The minor shifts in measured values provide evidence that measured myelin water fractions and $T_2$ values are near physical values.

7.2 Current and Future Work

If one were to repeat the experiments performed for this thesis, it would be useful to collect data out to longer $T_1$ times. Such experiments would confirm that the mobile pools will equilibrate for long $T_1$ due to the $T_2$ process. Also, the cross relaxation – FID experiment could be modified so that the signal would not decay to zero for long $T_2$. This type of cross relaxation – FID experiment was used in an attempt to negate $T_1$ effects. After further consideration it was discovered that the experiment used did not negate the $T_1$ effect, but instead made the numerical solution determination more difficult.

I am currently involved in analyzing some in vivo human data that utilized a
magnetization transfer preparation pulse to null the non-aqueous signal. After waiting various delay times, CPMG measurements were taken and $T_2$ analysis provided quantification of the myelin water and IE water pools as they lost signal to the non-aqueous pools while communicating to each other and equilibrating through the $T_1$ process.

![Figure 7.1: Examples of the $T_2$ distributions for delay times of 0, 18, 51, 84, and 118 ms shown in panels a, b, c, d, and e, respectively. The y-axes are of arbitrary intensities. The $T_2$s used in the partition were 20, 80, 120, and 2000 ms.](image)

The data was collected on a 1.5-T GE Signa clinical MR scanner using a preparation phase consisting of a 19 ms sinc magnetization transfer pulse followed by various delay times per acquisition. The $T_2$ acquisition had the CPMG sequence parameters: TR = 3.8 s, TE = 10 ms for the first 32 echoes and 50 ms for the remaining 16 echoes, field of view of 22 cm, matrix size of 64x64, and 2 averages. The experiments were performed on normal volunteers: 21 volunteers were examined with the delay times of 0, 18, 51, 84, and 118 ms; 19 volunteers were examined with delay times of 0, 84, 118, 218, and 318 ms; and 18 volunteers were examined with delay times of 0, 318, 468, 618, and 768 ms. Delay times of 0 ms indicate that no MT preparation phase was used. The scanner was not retuned between scans in order to maintain constant image scaling for each volunteer.

Regions of interest were drawn on five white matter structures for each volunteer: the genu of the corpus callosum, splenium of the corpus callosum, posterior internal capsules, major forceps, and minor forceps. Within each ROI the pixel intensities were averaged for each
time point. The scans for each delay time were registered to the initial scan to compensate for any volunteer motion between scans. The curves were then fit using NNLS [16] with a $T_2$ partition made up of four components: 20, 80, 120, and 2000ms [57]. These components were chosen to match the $T_2$ times measured in previous studies of normal human brain [17,59,60]. The four components were used in the partition instead of using an arbitrary number of $T_2$ components in the partition because of the variability found in the intensities and positions of the short $T_2$ components due to the low signal to noise ratio resulting from only taking two signal averages. An example of the $T_2$ distributions for the delay times ranging from 0 to 118ms is presented in Figure 7.1. One can see the changes in the fit as the delay time was increased.

Figure 7.2: Two examples of MWF and IE water fraction as a function of delay time following a magnetization transfer preparation pulse. The panel $a$ represents data collected from the genu of the corpus callosum while panel $b$ was collected from the splenium of the corpus callosum. Both data sets were collected on normal volunteers.

For each brain ROI the $T_2$ distribution was averaged together and graphs of myelin water fraction and IE water fraction were made as a function of delay time. The fractions were
determined for each scan as a ratio of the variable of interest, myelin water or IE water, with the
total water signal from the zero-delay case, with the sum of all the components being
representative of total water signal. This scaling was done in order to remove any scaling the
scanner could introduce when collecting data for different volunteers. Example graphs of the
genu and splenium of the corpus callosum are shown in Figure 7.2. Both the curves are
characterized by an initial drop in signal as the mobile pools exchange magnetization with the
nulled motionally restricted pools. The genu shows rapid recovery of MWF and the beginning of
the recovery of the IE water fraction around the delay time of 500ms. The splenium is even more
interesting; the signal from booth pools was originally lost as they exchanged magnetization with
their motionally restricted pools. The MWF signal begins recovering around the 20ms mark and
then begins to decrease again at about 200ms as the IE water signal begins to recover. Between
roughly 100 and 350ms the myelin water signal is concave down and the IE water signal is
concave up. From 350 to 800ms the myelin water signal is concave up and the IE water signal is
concave down. This switching of concavity is indicative that these two variables are closely
related for the splenium. If one considers this concavity phenomenon in the corpus callosum data
one can see the phenomenon there as well, just not to the same extent.

The future work of this in vivo project will be to attempt to model the data using the four
pool model and the procedure applied by this thesis work on in vitro bovine brain. In the near
future, we will be looking into using a three T2 model consisting of T2 times of 20, 80, and
2000ms. When one begins to model the data one will have to recall that, unlike the in vitro study,
the motionally restricted signal was not accessible. Thus, one will need to model the signal
assuming the motionally restricted pool is present and interacting with the mobile pools. The
numerical solutions will require solving for four parameters: $T^{2}_{cr}$, $T^{mw}_{cr}$, $T^{ie}_{cr}$, and mobile
fraction (MF). If one can use an estimate of the MF, the fit will only require three parameters.
One will also have to remember to include a cross relaxation correction for the CPMG
measurement. Ideally, one could also incorporate a fitting routine.

If one were to repeat this in vivo study the addition of water bottles into each scan would
be useful, so one could scale each signal by an external standard, allowing one to plot relative
intensities instead of MWF and IE water fractions.
8 Bibliography


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Chapter 8: Bibliography


Chapter 9: Appendix

9 Appendix

Determining the cross relaxation correction was algebraically intense. I utilized some mathematics software to aid in the derivation. The following section outlines the derivation in detail.

9.1 Doing the Zimmerman-Brittin Backwards

The following is Maple [Maplesoft, Waterloo, ON] code that was used to determine the actual intensities and decay rates given the measured values.

> restart:

> In the Zimmerman-Brittin model, one derives what the measured values for relaxation and intensity would be if one is given the initial intensities, relaxation rates and cross relaxation relaxation rate. This maple program inverts this process and determines the actual initial intensities and relaxation rates given the cross relaxation rate and measured signal intensities and relaxation rates.

> The following are declarations of the measured intensities. It is assumed that we measure AlphaXi = $\alpha + \xi$, and BetaZeta = $\beta + \zeta$.

> alpha := (a[2]*m[20] - b[1]*m[10] - A*m[10])/(B - A);

\[ \alpha = \frac{a_2 m_{20} - b_1 m_{10} - Am_{10}}{B - A} \]

> beta := (-a[2]*m[20] + b[1]*m[10] + B*m[10])/(B - A);

\[ \beta = \frac{-a_2 m_{20} + b_1 m_{10} + Bm_{10}}{B - A} \]

> xi := (b[2]*m[10] - a[1]*m[20] - A*m[20])/(B - A);

\[ \xi = \frac{b_2 m_{10} - a_1 m_{20} - Am_{20}}{B - A} \]

> zeta := (-b[2]*m[10] + a[1]*m[20] + B*m[20])/(B - A);

\[ \zeta = \frac{-b_2 m_{10} + a_1 m_{20} + Bm_{20}}{B - A} \]

> The following values A and B correspond to the measured relaxation rates, where A corresponds to the smaller rate.


\[ eq1 = A = \frac{-a_1 - b_1 - \sqrt{a_1^2 + 2a_1b_1 - b_1^2 + 4b_2a_2}}{2} \]


\[ eq2 = B = \frac{-a_1 - b_1 + \sqrt{a_1^2 + 2a_1b_1 - b_1^2 + 4b_2a_2}}{2} \]
eq2 := B = \frac{-1}{2}a_1 - \frac{1}{2}b_1 + \frac{1}{2}\sqrt{a_1^2 + 2a_1b_1 + b_1^2 + 4b_2a_2} \\

> At this point the variables are substituted in.
> a_1 := -R_{mw} - k_{12}
> a_2 := k_{21}
> b_1 := -R_{ie} - k_{21}
> b_2 := k_{12}

> Solve for actual decay rates.
> SolveRates := solve( {eq1, eq2}, {R[mw], R[ie]} );
>
> SolveRates := \{ R_{re} = \text{RootOf}(\_Z^2 + (2k_{21} - B - A)\_Z - k_{21}A + k_{21}^2 - B k_{21} + BA),
> R_{mw} = A - k_{12} - \text{RootOf}(\_Z^2 + (2k_{21} - B - A)\_Z - k_{21}A + k_{21}^2 - B k_{21} + BA) \cdot k_{21} + B) \}

> allvalues( SolveRates );
>
> The myelin water relaxation rate should be the larger of the two, since it decays faster.
> R_{mw} = \frac{1}{2}A - k_{12} + \frac{1}{2}B + \frac{1}{2}\sqrt{B^2 - 2BA + A^2 - 4k_{12}k_{21}}

> R[ie] := 1/2*A + 1/2*B - 1/2*(B^2 - 2*B*A + A^2 - 4*k[12]*k[21])^(1/2) - k[21];
> R_{re} = -k_{21} + \frac{1}{2}B + \frac{1}{2}A - \frac{1}{2}\sqrt{B^2 - 2BA + A^2 - 4k_{12}k_{21}}

> Now, solve for the actual intensities.
> eq3 := AlphaXi = alpha + xi;
eq3 := \(\frac{k_{13} m_{10} \cdot \left(-\frac{1}{2} - B \cdot \frac{1}{2} \cdot \sqrt{B^2 - 2BA + A^2 - 4k_{13} k_{21}}\right)}{B - A}\) m_{10} \cdot A m_{10} \\

\[eq3 := \frac{k_{13} m_{10} \cdot \left(-\frac{1}{2} - B \cdot \frac{1}{2} \cdot \sqrt{B^2 - 2BA + A^2 - 4k_{13} k_{21}}\right)}{B - A}\] \\

\[eq4 := \frac{\text{BetaZeta} = \beta + \zeta;}{B - A}\] \\

\[eq4 := \frac{\text{BetaZeta} = \beta + \zeta;}{B - A}\] \\

\[\text{SolveIntensities} := \text{solve}\left(\{eq3, eq4\}, \{m[10], m[20]\}\right);\] \\

\[\text{SolveIntensities} := \text{solve}\left(\{eq3, eq4\}, \{m[10], m[20]\}\right);\] \\

\[m_{10} = \frac{1}{2 \left(k_{21} + k_{13} \cdot \sqrt{B^2 - 2BA + A^2 - 4k_{13} k_{21}}\right)} \left(\text{BetaZeta} B \cdot \text{AlphaS} + 2 k_{21} \text{AlphaS} + \text{AlphaS} A + 2 \text{BetaZeta} k_{21}\right)\] \\

\[m_{20} = \frac{1}{2 \left(k_{21} + k_{13} \cdot \sqrt{B^2 - 2BA + A^2 - 4k_{13} k_{21}}\right)} \left(\text{BetaZeta} B \cdot \text{AlphaS} - \sqrt{B^2 - 2BA + A^2 - 4k_{13} k_{21}} + \text{AlphaS} A\right)\]