

EXPLORING EXCHANGE AND TRANSPORT DYNAMICS IN COMPLEX SYSTEMS
THROUGH NUCLEAR MAGNETIC RESONANCE

by

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DEDICATION

To Mom, Ronni, Dad, Mindy, Luke, Nadine, B and Eleanor, I wouldn't be here without the constant support and love from you all. To Grandpa and Grandma, I was my own woman and that was the best advice to give me. To Eleanor, I hope this shows you the sky is the limit.

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ABSTRACT

Nuclear magnetic resonance (NMR) is uniquely qualified for non-invasive studies of systems providing insights into macro-, meso-, and microscale structures. NMR relaxation and diffusion methods are applied to characterize transport and magnetization exchange dynamics in various complex systems. These techniques are highly sensitive to molecular mobility restrictions which correlate to the ability to monitor thermodynamic phase transitions and changes in molecular environment.

NMR diffusion and relaxation measurements are applied to characterize the effect of xylose on transport within zeolite beads. The ability for NMR to explore the transport phenomenon on multiple length and time scales is exploited to characterize how the introduction of xylose effects the transport structure of the bead.

Eigenvector simulations of magnetization evolution within a coupled pore system during multidimensional NMR measurements, T1-T2 relaxation correlation experiments, allowed for insights into complex diffusion and exchange occurring within multiple systems. Additionally, multidimensional relaxation NMR measurements, in the form of varying echo-time spin-spin relaxation dispersion $T2(\tau)$ and spin-spin relaxation exchange T2-T2 experiments, are demonstrated to successfully characterize thermodynamic structural rearrangements of two natural straight-chained hydrocarbons and a natural wax. Temperature dependent magnetization exchange was found in both the longitudinal and transverse magnetization. The results indicate the ability of NMR relaxometry to detect magnetization exchange without mass or molecular exchange, also known as spin diffusion, including in the transverse magnetization. Spatial domain extent can be inferred from the exchange timescale and an estimate of the spin diffusion coefficient.

NMR relaxometry methods were extended to glycerol behenate, a common pharmaceutical component. Glycerol behenate was decomposed into its three base components to explore how polymorphic structure and exchange depend on temperature within each pure lipid through $T2(\tau)$ and T2-T2 NMR relaxation experiments. These methods allowed for in-situ monitoring of thermodynamic dependent exchange across domains in addition to decoupling of transverse and longitudinal exchange. The results allow for calculation of exchange length scales across the micro- and mesoscales within the lipids. Ultimately, multidimensional NMR relaxometry is successfully demonstrated to be an effective technique for characterizing and monitoring structural changes in lipids across various phase transition temperatures and time and length scales.

CHAPTER ONE

INTRODUCTION

Soft-matter systems are ubiquitous throughout everyday life and crucial to modern medicine. From Chapstick® to ice-cream, soft-condensed matter is the basis [1] for many modern-day amenities. Understanding and designing techniques to explore these systems is essential to developing creamier ice-cream and a smoother lip balm. More importantly, pharmaceutical companies rely on soft-condensed matter in the form of lipids, macromolecular mixtures, to hold active ingredients in many pills and tablets. [2] This lipid matrix is one means to control for drug release rates. Choosing the incorrect lipid can cause incorrect temporal release rates [2] which could have destructive effects on the body. Because of this, fast and effective techniques are in need as quality assurance for these lipids.

Currently, dissolution techniques, paired with methods such as Raman spectroscopy and X-ray spectroscopy, are used to explore the lipid matrix along with release rates within the pharmaceutical industry. [2-4] While these methods give great information on the macroscale, such as temporal information of water retention and expulsion from the dissolution techniques [5] and microscale information of lipid structure from the spectroscopy [6], these are complementary techniques and must be paired to have a robust view of the system over the hierarchy of length and time scales which interact to control use. In response to this shortcoming, the work in this thesis employs nuclear magnetic resonance (NMR) as the primary experimental technique to explore multiple soft-condensed matter systems due to its ability to concurrently obtain

structural information on the macro, meso, and microscale. [7, 8] NMR is uniquely qualified to non-invasively probe systems through the same physics as MRI machines in the hospital. Through this technique, information about the structure of local molecular environments, inter- and intra-molecular exchange, and diffusion dynamics, which impact pharmacological function, can be obtained without destroying the system. Thus, information, analogous to spectroscopy techniques, are obtained in addition to new insights into system dynamics. [9] This allows for long term monitoring through the ability to retest systems multiple times which allow for dissolution probing to occur.

Throughout the development of NMR [10, 11], two modalities, solid-state NMR and liquid-state NMR, have generated different hardware for application to solid material and liquid solution. Communication between these two sub-techniques is limited due to different NMR physics in each phase. A broad and diverse sector of soft-condensed matter has been explored through these methodologies, for example in polymers where the solid-state techniques are applied to rigid crystalline polymer systems [12, 13] and liquid-state techniques applied to polymer solutions. [14] In this work, semi-solid systems, which undergo solid-solid crystalline to rotator phase transitions systems, are examined with NMR relaxometry and diffusion techniques, typically used in liquids, extending, and expanding the reach and potential application of this method.

Additionally, NMR can use high magnetic fields (upwards to 7 T) or low magnetic fields (around 0.35 T). High-field NMR is used primarily in this thesis with a 250MHz superconducting magnet (5.9 T). The high magnetic field strength coupled with the high magnetic field gradient is well suited for systems with low signal. Soft-

condensed matter, due to the semi-solid structure, is characterized by low signal and strong dipolar coupling which causes line broadening. [7, 8, 15]

In this thesis, NMR is used to (1) characterize biomolecular transport in zeolite catalytic beads [16], (2) characterize through a simulation of a coupled two pore system of various geometries [17], (3) explore phase transitions and exchange dynamics in lipids of various complexities (eicosane, octacosane, and beeswax), and (4) study glycerol behenate in its base components to explore the dynamics of each component and their interaction with each other. ^1H proton NMR excitation and detection is utilized in all the projects.

Outline

This thesis begins with an introduction to the basic NMR theory such as spin angular momentum, the underlying quantum mechanical formalism, and the definitions of relaxation in Chapter 2. In Chapter 3, more advanced topics are covered, expanding on the basic theory of Chapter 2 to cover experimental and analytical techniques utilized in the following chapters. These topics need to be understood to fully understand the scope of the projects discussed in this dissertation. Examples of these subjects are diffusion and exchange measurements and how NMR is uniquely qualified to explore systems in which mesoscale domains of many microscale-molecular structures impact macroscale behavior. Multi-dimensional NMR relaxation correlation pulse sequences are also discussed. The novel scientific work reported comes after these introductory chapters, where four different systems are examined.

The impact of transport of biomolecules in zeolite catalytic beads is examined in Chapter 4. Catalytic beads are used to increase yield in chemical reactions and novel new applications to generating furans from sugars is of current interest. [18] While macroscopic information such as percent yield is known, the microscale physics of how and why this happens is often limited. In this chapter, commercial zeolite beads are examined to determine how interactions with xylose create a large yield in making furfuran. [18] In this work, the diffusion dynamics are explored before and after the introduction of xylose into the water solution which the beads reside in, in order to measure changes in molecular transport. The work presented in this chapter is published in *Molecules*. [16]

In Chapter 5, experimental results in which a non-physical T_1 - T_2 relaxation correlation signal rise in systems due to coupling of diffusive eigenmodes in two domains are explored. [19] The modified NMR signal for analysis is expected to decrease monotonically in time as the system evolves back to equilibrium. The observation of an increase in signal is scientifically interesting from a modelling perspective [19] and physically understood in a limited fashion until this work. In Chapter 5, the NMR signal is simulated through a coupled-pore model system to explore the impact of exchange between pores. [17, 19] Using the Bloch-Torrey [20] equations to evolve the magnetization, a simulated signal is obtained for a given physical coupled pore system. A large parameter space of surface relaxivity and diffusive exchange is explored, and two base cases are used to explain the physicality of how the signal characterized the exchange and diffusion governing the system. Four systems are examined with this

simulation in mind to explain the physical basis of the signal behavior, a microporous bead pack, model hydrates during formation, and beeswax undergoing a solid-solid phase transition. The manuscript in this work was published in *Journal of Magnetic Resonance*. [17]

Chapter 6 extends the exploration of beeswax from Chapter 5 and includes research on solid-solid phase transitions in lipids of increasing complexities. Solid-solid phase transitions are shown to alter molecular dynamics which impact dipolar coupling, providing more structural detail than current methods used in the field. Difficult to detect solid-solid phase transitions are measured through NMR where differential scanning calorimetry, the current method, often shows only limited indications of these transitions. Additionally, exchange dynamics from spin diffusion, some of the first detections using transverse magnetization through variable echo time $T_2(\tau)$ dispersion [21] and T_2 - T_2 final magnetization exchange [22] technique, are shown for octacosane and beeswax. This manuscript is submitted to *Physical Chemistry Chemical Physics*.

Chapter 7 extends the techniques from Chapter 6 to glycerol behenate, a commonly used lipid in pharmaceuticals. This lipid is composed of, mono-, di-, and triglycerides of approximately 18/52/28 percent by weight. Glycerol behenate is decomposed into its base mono-, di-, triglyceride components to be examined for second order phase transitions and exchange, then a mixture of each component is systematically made to explore the interactions between each lipid domain. This work enhances the knowledge of how the lipid interacts separately from the active ingredients within

pharmaceutical drugs with the goal of understanding spatial phase separation due to composition. [6, 23]

Finally, in Chapter 8 conclusions are drawn from all the work presented in this thesis. This dissertation provides concrete examples of how the usage of NMR can yield structural and dynamical information on not only the microscale of individual molecules but also the mesoscale where structures on the order of nanometers are present. This chapter further discusses future avenues to explore, including the potential for extending these techniques to industry for quality control and research and development.

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CHAPTER TWO

INTRODUCTION TO NUCLEAR MAGNETIC RESONANCE

Most of the introductory work in this thesis will be referencing the work by Sir Paul Callaghan [1] and Anatole Abragam [2]. Without these two giants in the field nuclear, magnetic resonance would not be where it is today.

Quantum Mechanics of NMR

Nuclear magnetic resonance (NMR) measurements are dependent on the interaction of the quantum mechanical property each nuclei have, called spin, and an externally applied magnetic field. The macroscopic properties investigated through NMR are possible due to materials consisting of statistically large ensembles of these spins, however, due to this, it is fundamental to understand the quantum mechanical nature making up these interactions.

Spin Angular Momentum

Spin angular momentum is an intrinsic quantum mechanical property in each nuclei which has no classical analog. The spin state of a nuclei is defined by the quantum number I , which is either an integer or half-integer and describes the nuclei in its stable ground state. [1, 2] I is composed of a basis set of discrete values of the azimuthal quantum number, m , where m ranges from $-I, -I + 1, \dots, I - 1, I$, and characterizes all possible energy states of that system. In the example of a proton, $I = 1/2$, this means

there are two possible states, $m = \pm 1/2$, referred to as ‘spin down’ and ‘spin up’ respectively.

Interpretations of the molecular environment of NMR measurements stem from measuring an observable quantity which depicts the energy state of the system. In order to measure the system through NMR techniques, the quantum state must be altered. Mathematically, this is represented in the eigenvalue equation

$$A|\alpha\rangle = a|\alpha\rangle, \quad (2.1)$$

where A is the operator or observable of interest, $|\alpha\rangle$ is the basis state of A , and a is the eigenvalue or observed value of A . For a concrete example, $I_z|m\rangle = m|m\rangle$, can be interpreted as m is the observed value of I_z , the angular momentum.

In quantum states, the possible state is often described as a superposition of states. With this, we are faced with the Heisenberg uncertainty principle which states we cannot simultaneously know the momentum and position of the nuclei at the same time. The most we can obtain is the probability of each. In the spin-1/2 system with definitive I_z , the observable measurement would be either ‘spin-up’ or ‘spin down.’ However, if the system is a superposition of multiple states given by $|\Psi\rangle = \sum a_m|m\rangle$, the measurement taken would result in the expectation value, or mathematically

$$\langle\Psi|I_z|\Psi\rangle = \sum |a_m|^2 m, \quad (2.2)$$

where $|a_m|^2$ is the probability of measuring $|\Psi\rangle$ within that quantum state. Until this measurement is taken, the system will stay as a superposition of all the possible states.

Since this thesis is concerned with the dynamics of systems, it is useful to understand this within the context of the Hamiltonian, or total energy operator $\mathcal{H}$. These dynamics are governed by the Schrödinger equation.

$$i\hbar \frac{\partial}{\partial t} |\Psi(t)\rangle = \mathcal{H} |\Psi(t)\rangle \quad (2.3)$$

The Hamiltonian includes both kinetic and potential energy of the system while $|\Psi(t)\rangle$ is what is evolving in time. $\hbar$ is Plank's constant over 2π . With a system which has a stationary Hamiltonian in time, the Schrodinger equation becomes

$$|\Psi(t)\rangle = U(t) |\Psi(0)\rangle \quad (2.4)$$

Where $U(t)$ is the evolution operator and is defined as $U(t) = \exp(-\frac{i\mathcal{H}t}{\hbar})$.

Zeeman Effect and Spin Magnetism

If an external magnetic field, $\mathbf{B}_0$, is applied to the spins, then the Zeeman interaction occurs. Without an applied field, nuclei are oriented randomly in space, however, after a field is applied, the nuclei will orient themselves parallel or anti-parallel to the field with slight preference for the parallel alignment. The spins will split their energy level states. The lower energy state is parallel with the magnetic field and is the 'spin up' state, $|+1/2\rangle$, while the higher energy state is anti-parallel and is called the 'spin down' state, $|-1/2\rangle$.

Atomic nuclei have a magnetic dipole, $\boldsymbol{\mu}$, which is proportional to the angular momentum by a constant denoted as the gyromagnetic ratio, γ . With the interaction of a magnetic field, the interaction energy is characterized both classically and quantum mechanically as $-\boldsymbol{\mu} \cdot \mathbf{B}_0$. If this field is oriented along the z-axis, and the spin is spin-1/2,

then the Hamiltonian can be written as [2] $\mathcal{H} = -\gamma B_0 I_z$. In this situation, the energy levels are split with an energy separation of $\Delta E = \gamma B_0 \hbar$ shown in Figure 2.1.

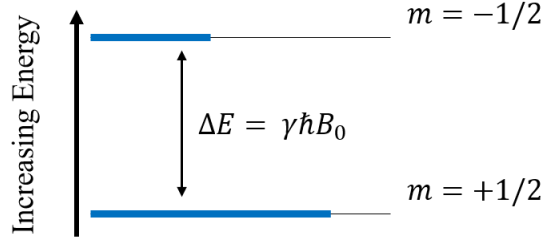


Figure 2.1: The energy levels of a spin $\frac{1}{2}$ bearing molecule with the application of a magnetic field are shown above. This splitting of the spin-up and spin-down states is called the Zeeman effect.

The gyromagnetic ratio is nuclei dependent. Hydrogen, ^1H , has a particularly large value γ of compared to other nuclei, $\gamma = 2.675 \times 10^8 \text{ rad}/(\text{T} \cdot \text{s})$ in addition to being abundant within materials. This is why ^1H is used heavily in NMR detection and is the nuclei detection used in this thesis.

Within a magnetic field, all states will precess around at the Larmor frequency, ω_0 , defined as [1, 2]

$$\omega_0 = \gamma B_0 \quad (2.5)$$

Not only is this the frequency at which the precession around the applied field occurs, but it is also the frequency of flipping between energy states, primarily in this case, ‘spin up’ and ‘spin down.’ This transition between energy states is mitigated through energy absorption or emission and is the crux of NMR signal detection.

Given this transition between energy states being detected through NMR methods, the population of spins in each state is important. The quantum properties can be characterized through classical statistics over the system ensemble. The ensemble

average of the expectation value [1, 2], $\overline{\langle \Psi | I_z | \Psi \rangle} = \frac{1}{2} \left[\overline{\left| a_{+\frac{1}{2}} \right|^2} - \overline{\left| a_{-\frac{1}{2}} \right|^2} \right]$, is the difference between the energy state populations. These populations within these states are given by the Boltzmann distribution from classical statistical mechanics and is given by a probability [1, 2]

$$P_m = \frac{\exp\left(-\frac{\gamma B_0 m \hbar}{k_B T}\right)}{\sum_m \exp\left(\frac{\gamma B_0 m \hbar}{k_B T}\right)}, \quad (2.6)$$

where $\Delta E = \gamma B_0 m \hbar$ is the energy of each state, k_B is the Boltzmann constant, and T is the absolute temperature of the system. There is a slight polarization, I_z , aligned with the applied static magnetic field due to the slight preference of spins toward the lower energy state of ‘spin up.’ However, it is not possible to measure this polarization directly. The energy difference between the energy states is significantly smaller than $k_B T$ in addition to having no phase coherence between the spins.

Measurement of the x-component, I_x , yields phase coherence information between the +1/2 and -1/2 states. [1, 2]

$$\overline{\langle \Psi | I_x | \Psi \rangle} = \frac{1}{2} \left[\overline{a_{+1/2}^* a_{-1/2}} + \overline{a_{+1/2} a_{-1/2}^*} \right] \quad (2.7)$$

This indicates how the relative phase is important and not absolute phase between spins. Not only is the polarization important, but the phase is as well. In order to simply explore both of these, the spin density matrix ρ is introduced. [1, 2]

$$Tr[A\rho] = \overline{\langle \Psi | A | \Psi \rangle} = \overline{\langle A \rangle} = \sum_{\Psi} \rho_{\Psi} \langle \Psi | A | \Psi \rangle \quad (2.8)$$

where ρ evolves according to the Schrödinger equation, is Hermitian, and directly provides the ensemble averaged expectation value for a measurement A . For systems of spin $\frac{1}{2}$ and in exploring the observable I , ρ becomes the following [1, 2]

$$\rho = \begin{bmatrix} \frac{1}{2} + \overline{\langle I_z \rangle} & \overline{\langle I_x \rangle} - i\overline{\langle I_y \rangle} \\ \overline{\langle I_x \rangle} + i\overline{\langle I_y \rangle} & \frac{1}{2} - \overline{\langle I_z \rangle} \end{bmatrix} \quad (2.9)$$

Here is where the macroscopic NMR measurement coalesces with the quantum theory.

The spin density matrix implies all states of independent spin $\frac{1}{2}$ nuclei may be characterized through the three components of the vector $(\overline{\langle I_x \rangle} \mathbf{i} + \overline{\langle I_y \rangle} \mathbf{j} + \overline{\langle I_z \rangle} \mathbf{k})$. Which in macroscopic terms is equivalent to describing the net magnetization vector in the laboratory reference frame. [1, 2]

$$\mathbf{M} = N\gamma\hbar(\overline{\langle I_x \rangle} \mathbf{i} + \overline{\langle I_y \rangle} \mathbf{j} + \overline{\langle I_z \rangle} \mathbf{k}) \quad (2.10)$$

Since we usually define B_0 to be along the z-axis, we can directly detect and measure I_x and I_y through NMR where I_x is the real component and I_y is the imaginary component, and N is the number of spins per unit volume.

Excitation and Signal Detection

It is advantageous to adopt a semi-classical view of NMR to understand the macroscopic properties which are detected and measured through the spin magnetization vector $\mathbf{M}$. In this view, the angular momentum vector is $\mathbf{M}/\gamma$. The rate of change of the angular momentum is simply related to the torque on the system as [1]

$$\frac{d\mathbf{M}}{dt} = \gamma \mathbf{M} \times \mathbf{B} \quad (2.11)$$

where $\mathbf{B}$ is the magnetic field. When the system is on resonance, the oscillation of the $\mathbf{B}_1$ field and $\mathbf{M}$ occur at the Larmor frequency, $\omega_0 = \gamma B_0$. The radio frequency (rf) field is defined as [1]

$$\mathbf{B}_1(t) = B_1 \cos(\omega_1 t) \mathbf{i} - B_1 \sin(\omega_1 t) \mathbf{j} \quad (2.12)$$

Using $\mathbf{B}_1$ as the magnetic field applied in Equation (2.11) in addition to the static $B_0 \mathbf{k}$ field, the cross product produces the following:

$$\begin{aligned} \frac{dM_x}{dt} &= \gamma [M_y B_0 - M_z B_1 \sin(\omega_1 t)] \\ \frac{dM_y}{dt} &= \gamma [M_z B_1 \cos(\omega_1 t) - M_x B_0] \\ \frac{dM_z}{dt} &= \gamma [M_z B_1 \sin(\omega_1 t) - M_y B_1 \cos(\omega_1 t)] \end{aligned} \quad (2.13)$$

Assuming the initial condition of [1] $\mathbf{M}(t) = M_0 \mathbf{k}$

$$\begin{aligned} M_x &= M_0 \sin(\omega_0 t) \sin(\omega_1 t) \\ M_y &= M_0 \cos(\omega_0 t) \sin(\omega_1 t) \\ M_z &= M_0 \cos(\omega_1 t) \end{aligned} \quad (2.14)$$

where $\omega_1 = \gamma B_1$. This solution describes motion in the laboratory reference frame. $\mathbf{M}$ is in “free-precession” and precesses around $\mathbf{B}_1$ with frequency ω_1 for the duration of the rf pulse and $\mathbf{B}_0$ with frequency ω_0 shown in Figure 2.2.

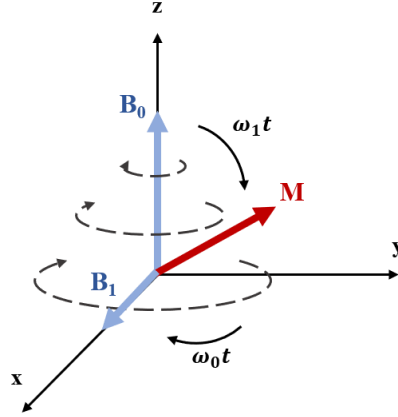


Figure 2.2: Applying a rotating magnetic field, B_1 , forces the magnetization down into the transverse plane. The magnetization will precess with the ω_1 frequency in time.

The angle of rotation of $\mathbf{M}$ around $\mathbf{B}_1$ is equal to $\omega_1 t$. The magnetization vector, $\mathbf{M}$, can be reoriented into the transverse plane through changing the duration of the rf pulse and the strength of $\mathbf{B}_1$. This reorientation into the transverse plane is denoted a 90° or $\frac{\pi}{2}$ pulse. Similarly, an rf pulse of $2t$ at the same $\mathbf{B}_1$ strength, or a doubling of the $\mathbf{B}_1$ strength, will result in the magnetization along the $-z$ -axis noted as a 180° or π pulse.

Signal is detected as the oscillating $\mathbf{M}$ decays back to equilibrium through an induced current in the rf coil. The free precession is detected through Faraday induction and is outputted as an oscillating voltage. The signal measured from the coil consists of a real and imaginary portion oscillating in quadrature resulting in the measurement of the phase and magnitude of the signal.

Reference Frames

For ease of describing spin dynamics further, it is useful to use a rotating reference frame around the static field $\mathbf{B}_0$ with frequency ω . The Zeeman Hamiltonian for the static $\mathbf{B}_0$ field is given by $\mathcal{H}_{Zeeman} = -\gamma B_0 I_z$. Since the frame is rotating at some

frequency, ω , this must be accounted for within the Hamiltonian. This correction is given by $\mathcal{H}_{rot} = \omega I_z$. The corrected Hamiltonian results in [1]

$$\mathcal{H}_{Zeeman-rot} = -\gamma(B_0 - \omega/\gamma)I_z \quad (2.15)$$

To tip the magnetization into the transverse plane, an oscillatory field, $\mathbf{B}_1$, is applied. The Hamiltonian becomes [1]

$$\mathcal{H}_{rot} = -\gamma(B_0 - \omega/\gamma)I_z - \gamma B_1 I_x \quad (2.16)$$

If the system is “on-resonance,” $\omega = \omega_0$, the apparent longitudinal field disappears leaving only the transverse field. The I_x operator is a linear combination of the raising and lowering operators, meaning for the case of a spin-1/2 nuclei, the spins flip between ‘spin-up’ and ‘spin-down’ over time at a rate of $\omega_1 = \gamma B_1$.

The equations describing the evolution of $\mathbf{M}$ with this reference frame reduces to the following [1]

$$M_x = 0$$

$$M_y = M_0 \sin(\omega_1 t) \quad (2.17)$$

$$M_z = M_0 \cos(\omega_1 t)$$

Assuming the applied $\mathbf{B}_1$ -field is oriented in the x-direction as shown in Figure 2.3.

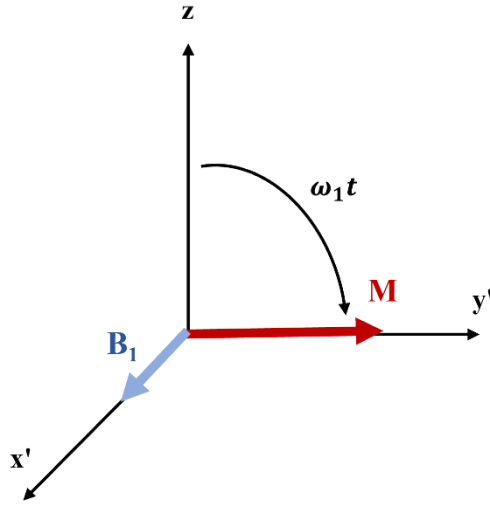


Figure 2.3: In the rotating reference frame, a rf pulse appears to simply push the magnetization into the transverse plane without rotation. This shift into the rotating frame simplifies the evolution of magnetization.

Free Induction Decay

The most basic NMR measurement is a single 90° pulse followed by signal acquisition in the time domain shown in Figure 2.4. The signal acquired is an exponentially decaying sinusoid and is called the “Free Induction Decay (FID).” It has an offset frequency, $\Delta\omega$, and is characterized as [1]

$$S(t) = S_0 \exp(i\phi) \exp(i\Delta\omega t) \exp\left(-\frac{t}{T_2^*}\right) \quad (2.18)$$

Where S_0 is the signal directly after the excitation and T_2^* is the spin-spin relaxation in the presence of $\mathbf{B}_0$ inhomogeneities. The exact mechanisms behind the signal decay will be discussed later.

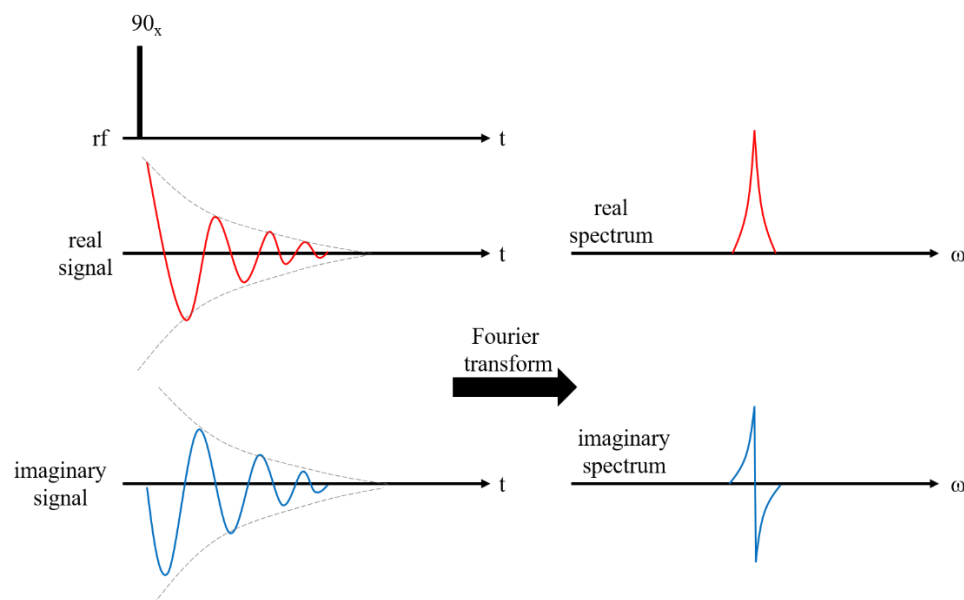


Figure 2.4: The real and imaginary signal from a 90°_x rf pulse can be decoupled through applying a Fourier transform. In the frequency space, the real spectrum will appear as an absorption line while the imaginary spectrum will appear as a dispersion distribution.

Being a complex signal in the time domain, moving to the frequency domain through a Fourier transformation allows for a simpler interpretation. [1]

$$F\{S(t)\} = s(\omega) = \int_{-\infty}^{\infty} S(t) \exp(i\omega t) dt \quad (2.19)$$

The real and imaginary portions of the signal are transformed into the absorption and dispersion section. The real part of the frequency domain signal is Lorentzian in nature for a liquid with a cyclic frequency of $\Delta\omega/2\pi$ and with a full-width-half-maximum (FWHM) of $1/\pi T_2^*$. The imaginary part of the signal has a greater FWHM than the real portion.

For solids, the correlation time between dipolar coupling is much shorter than liquids (1-100 μ s). This results in a Gaussian shape of the absorption spectrum.

Relaxation

After an rf pulse excites the spins into a higher energy state, the nuclei will relax back to equilibrium aligning with the $\mathbf{B}_0$ field, usually along the z-axis, through a relaxation mechanism called spin-lattice relaxation, or T_1 relaxation. This is an exchange of energy from the excited spin to the thermal reservoir, or lattice, in order to reestablish the Boltzmann distribution of spin states. Simply put, this relaxation is due to the spins returning to the lowest energy state. For a liquid at room temperature, this occurs on the order of 0.1-10s. Mathematically, this relaxation is represented as [1]

$$\frac{dM_z}{dt} = \frac{-(M_z - M_0)}{T_1} \quad (2.20)$$

with a solution [1]

$$M_z(t) = M_z(0) + M_0 \left(1 - \exp\left(-\frac{t}{T_1}\right) \right) \quad (2.21)$$

The relaxation of the spins, once they are in the transverse plane, is called spin-spin relaxation or T_2 relaxation. Spin-spin interactions dictate how long the signal in the transverse plane will last. This is the process in which the nuclei come into thermal equilibrium among themselves and is due to a loss of phase from decoherence or dephasing from molecular interactions. [1] Since these interactions are dependent on the structure and interactions of the spins, the range of possible times range from microseconds in ordered crystalline structures to seconds in disordered liquids.

Mathematically this relaxation is [1]

$$\frac{dM_{x,y}}{dt} = -\frac{M_{x,y}}{T_2} \quad (2.22)$$

with solution [1]

$$M_{x,y}(t) = M_{x,y}(0) \exp\left(-\frac{t}{T_2}\right) \quad (2.23)$$

Combining Equations (2.11), (2.21) and (2.23) the Bloch equations are formed. [1]

$$\begin{aligned} \frac{dM_x}{dt} &= \gamma M_y \left(B_0 - \frac{\omega}{\gamma} \right) - \frac{M_x}{T_2} \\ \frac{dM_y}{dt} &= \gamma B_1 - \gamma M_x \left(B_0 - \frac{\omega}{\gamma} \right) - \frac{M_y}{T_2} \\ \frac{dM_z}{dt} &= -\gamma M_y B_1 - \frac{(M_z - M_0)}{T_1} \end{aligned} \quad (2.24)$$

These were first presented by Felix Bloch in 1946. [3] The relaxation of spins can be described through the solution to these coupled differential equations.

A separate type of relaxation is T_2^* relaxation. This is decay stemming from the inhomogeneity of the $\mathbf{B}_0$ field. The non-uniform nature of the field causes a range of precession frequencies for the spins resulting in signal decay. This phenomenon may be reversed through time reversal with a spin-echo which will be discussed later in this chapter.

Spectral Density Functions

With spin-1/2 nuclei, the spin relaxation is dominated by the dipolar coupling described by the dipolar Hamiltonian, $\mathcal{H}_D$, which is a first-order perturbation to the Zeeman field. Since both T_1 and T_2 lead to stochastic fluctuations, it is important to understand how these effect the relaxation phenomenon observed through NMR techniques. In a relaxation experiment, molecular tumbling will cause fluctuations in dipolar interactions between nuclei. The relaxation times from this experiment are

sensitive to the Fourier transform of these fluctuations, or the spectrum of the auto-correlation function called the spectral density function, $J(\omega)$. The spectral density function describes how the interaction of the magnetic fields from each spin depend on time and the frequency of precession. For a spin-1/2 system in a static magnetic field, $\mathbf{B}_0$, there are three possible quantum transitions; zero-quantum, single-quantum, and double-quantum transition.

For a coupled spin state to stay at the same energy level, or to have a zero-quantum transition, the probability of transitioning is given by [1, 2]

$$W_0 = \frac{1}{10} \left(\frac{\mu_0 \hbar \gamma^2}{4\pi r_{ij}^3} \right)^2 J^0(\omega) \quad (2.25)$$

where r_{ij} is the radial distance between spins. For one of the spins to transition into a higher energy state, the single-quantum transition probability, it is given by [1, 2]

$$W_0 = \frac{3}{20} \left(\frac{\mu_0 \hbar \gamma^2}{4\pi r_{ij}^3} \right)^2 J^1(\omega) \quad (2.26)$$

and for the probability of both spins exciting to a new energy state, the double-quantum transition probability is given by [1, 2]

$$W_0 = \frac{3}{5} \left(\frac{\mu_0 \hbar \gamma^2}{4\pi r_{ij}^3} \right)^2 J^2(\omega) \quad (2.27)$$

Using (2.25) - (2.27), the spectral density functions for all possible transitions are [1, 2]

$$\begin{aligned} J^{(0)}(\omega) &= \frac{24}{15r_{ij}^6} \frac{\tau_c}{1 + \omega^2 \tau_c^2} \\ J^{(1)}(\omega) &= \frac{4}{15r_{ij}^6} \frac{\tau_c}{1 + \omega^2 \tau_c^2} \end{aligned} \quad (2.28)$$

$$J^{(2)}(\omega) = \frac{16}{15r_{ij}^6} \frac{\tau_c}{1 + \omega^2 \tau_c^2}$$

where τ_c is the rotational correlation time, the time it takes for the molecules to rotate by 1 radian.

Using Equation (2.28), T_1 and T_2 can be characterized in terms of $J(\omega)$. The spin-lattice relaxation in terms of the Lamour frequency is [1, 2]

$$\frac{1}{T_1} = \left(\frac{\mu_0}{4\pi}\right)^2 \gamma^4 \hbar^2 \frac{3}{2} I(I+1) [J^{(1)}(\omega_0) + J^{(2)}(2\omega_0)] \quad (2.29)$$

Notice T_1 relies on the single- and double-quantum transitions. This is due to the relaxation and transitions stemming from spin-lattice interactions. There must be a change in the total ensemble for relaxation to occur, hence the characterization by these transitions.

Spin-spin relaxation in terms of the Lamour frequency is given by[1, 2]

$$\frac{1}{T_2} = \left(\frac{\mu_0}{4\pi}\right)^2 \gamma^4 \hbar^2 \frac{3}{2} I(I+1) \left[\frac{1}{4} J^{(0)}(0) + \frac{5}{2} J^{(1)}(\omega_0) + \frac{1}{4} J^{(2)}(2\omega_0) \right] \quad (2.30)$$

Here there is a dependence on all three quantum transitions, zero-, single-, and double-quantum transitions. The addition of $J^{(0)}(0)$ dependence is due to the fact that T_2 relaxation does not need to take energy from the environment to occur. The relaxation can occur when the spins stay within the same energy state and exchange energy and implies there is an addition exchange mechanism other than Zeeman interaction contributing to T_2 relaxation. This addition term causes T_2 to relax at a rate equal to or faster than T_1 . The two relaxations are equal at short correlation times, as the correlation times increase, the zero-quantum term increases causing T_2 to increase while T_1 decreases down to a minimum shown in Figure 2.5.

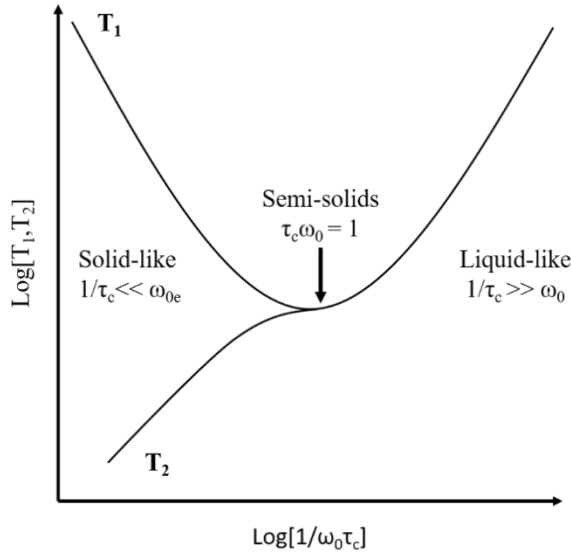


Figure 2.5: T_1 and T_2 as a function of correlation time, τ_c , is shown above. At short τ_c , in solids, the T_1 and T_2 of the sample are different with $T_1 > T_2$. At long correlation times, in liquids, T_1 and T_2 are approximately equal.

Why T_1 has a minimum while T_2 undergoes monotonic increase with τ_c can be explained through a quantum view as well. Since T_1 undergoes transitions through energy exchange with the local environment, at the minimum point in Figure 2.6 the energy input is equal to the energy needed to undergo a transition. A quanta of energy needed to transition is met more often when the system is on resonance.

Basic Pulse Sequences

NMR measurements hinge on unique combinations of rf pulses in combination with applied magnetic field gradients. Through parameters such as rf pulse timing, signal detection, and gradients, the detection of various observables can occur. The majority of complex pulse sequences are derived from four simple rf pulse sequences which will be

discussed in this section. These sequences are inversion recovery, the Hahn echo[4], the Carr-Purcell Meiboom-Gill (CPMG) pulse sequence [5, 6], and the stimulated spin-echo [7].

The pulse sequence information is presented in a pulse sequence diagram where timing is read from left to right. The rf pulse timing and what type of pulse is applied is usually shown in the top row with the applied magnetic field gradients below. Signal acquisition can be shown on either of the rows or on a separate line. Each sequence is cycled through N times to achieve the desired signal-to-noise (SNR). This also helps with phase cycling which is a method of cancelling out coherent noise and artifacts through varying the relative phase for each repetition.

Inversion Recovery

The inversion recovery sequence measures the T_1 relaxation of a sample shown in Figure 2.5. The spins are disturbed from equilibrium through applying a 180° rf pulse, inverting $\mathbf{M}$ to the -z-axis alignment. Due to T_1 relaxation, $\mathbf{M}$ will decay in magnitude over time. After some time τ , a 90° pulse draws $\mathbf{M}$ up to the transverse plane where signal detection occurs. Repeating the pulse sequence for various values of τ produces a curve defined through the equation

$$M_z(\tau) = M_0(1 - 2 \exp\left(-\frac{\tau}{T_1}\right)) \quad (2.31)$$

The cross over where the magnetization goes from $-\mathbf{M}$ to $+\mathbf{M}$ occurs at $\tau = 0.6931T_1$.

From this T_1 values can be extracted.

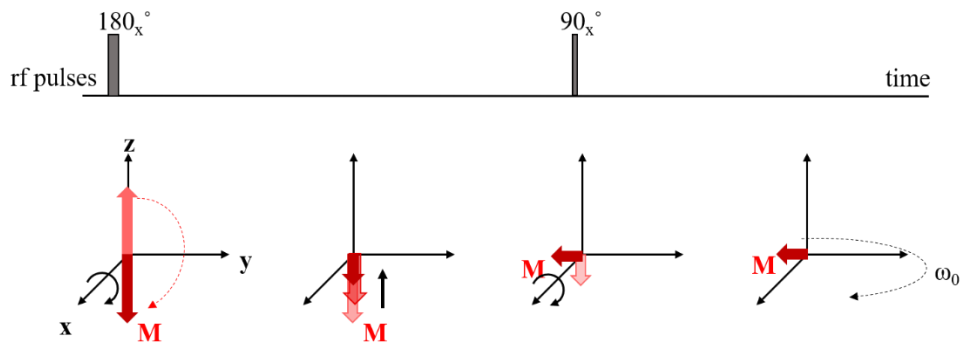


Figure 2.6: The inversion recovery pulse sequence begins with inverting the magnetization with a 180° pulse, after which, the magnetization will decay up toward positive z . After some time, a 90° pulse is applied, putting the magnetization into the transverse plane for detection.

Hahn Echo

Inhomogeneous $\mathbf{B}_0$ fields are unavoidable given NMR hardware limitations and sample preparation leading to dephasing of the magnetic moment of spins. The difference in field strength and orientation within the sample will lead to nuclei to precess at different frequencies in relation to the Larmor frequency causing dephasing in the transverse plane. This is called T_2^* relaxation. Fortunately, in 1950 Erwin Hahn developed an rf pulse sequence to rephase this decoherence paving the way for T_2 relaxation measurement methods. [4] If after the 90° pulse a 180° rf pulse is applied after a time τ , the dephasing will re-phase at the same rate of the initial dephasing and will produce an echo at 2τ , defined as the echo time τ_E . Since the spins are in the transverse plane, the magnetization will experience T_2 relaxation during τ but inhomogeneous effects will be refocused. Because of this, the echo amplitude will be less than the initial echo. This sequence is shown in Figure 2.7.

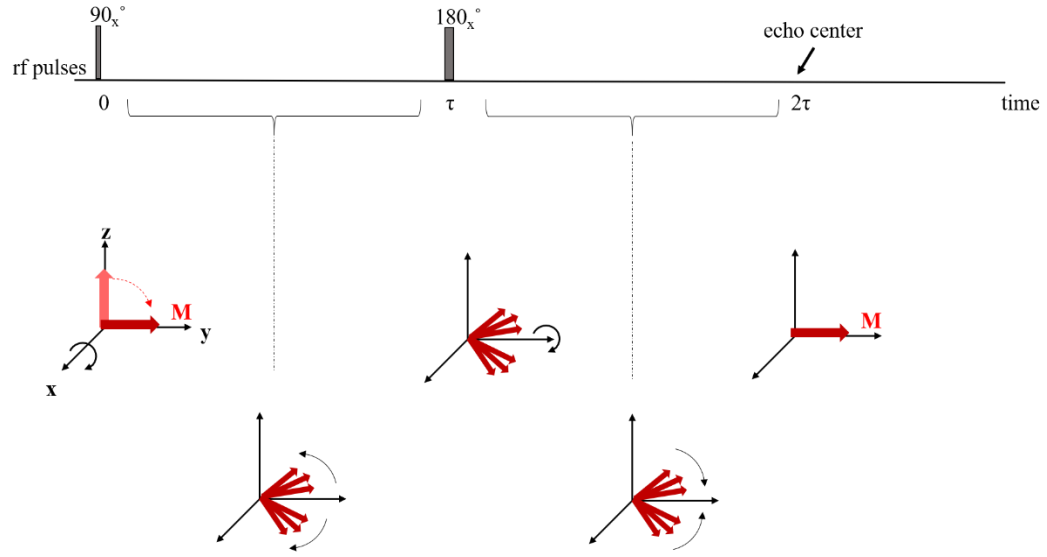


Figure 2.7: The Hahn echo, the heart of the CPMG train, is shown. It begins with a 90° pulse to put the magnetization into the transverse plane. Then the spins dephase until the 180° pulse is applied, reversing the dephasing, until τ time has passed. At this point the magnetization coalesce into an echo, centered at 2τ with a decay departed on T_2 only.

CPMG Echo Train

Four years after the discovery of the spin echo, in 1954, Carr and Purcell discovered by applying successive 180° rf pulses every 2τ , consecutive phase refocusing occurs [5, 6] shown in Figure 2.8. As discussed, each successive echo will have a decrease in amplitude due to T_2 relaxation, because of this, T_2 relaxation times can be obtained through this type of pulse sequence. The decay is fit by

$$M_{x,y} = M_0 \exp\left(-\frac{t}{T_2}\right) \quad (2.32)$$

Following this discovery, Meiboom and Gill modified this technique in 1958 through the use of quadrature 180_y° rf pulses which compensates for small turn-angle errors. Combining these to create the CPMG pulse sequence, direct measurement of T_2 relaxation times can occur with one experiment (Figure 2.8).

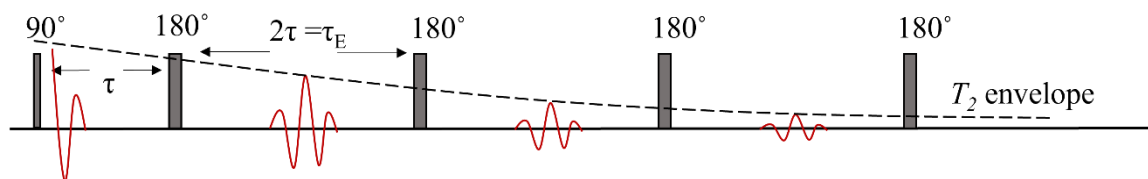


Figure 2.8: The Carl-Purcell-Meiboom-Gill (CPMG) pulse sequence, used primarily to explore T_2 relaxation is shown above. The sequence begins with a Hahn echo, then successive 180° pulses are applied until the signal has decayed due to dipolar coupling dephasing.

Stimulated Echo

In many materials, such as solids, the T_2 relaxation time is significantly shorter than the T_1 relaxation time. In these systems, it can be advantageous to store the magnetization coherence away from the influence of T_2 effects to explore other dynamics, such as translational motion of molecules. This is the core idea behind the stimulated echo. Through applying a 90_x rf pulse after some τ time after an initial 90_x rf pulse, the remaining magnetization will be stored along the $-z$ -axis where transverse relaxation does not occur and only longitudinal relaxation impacts the spins (Figure 2.9). After some desired time τ' , a third 90_x is applied, putting the magnetization back into the transverse plane where information about how the molecules have changed during the storage time can be examined. Once the magnetization is back in the transverse plane, the coherence will re-phase and produce an echo at $2\tau + \tau'$ similar to the Hahn echo described before.

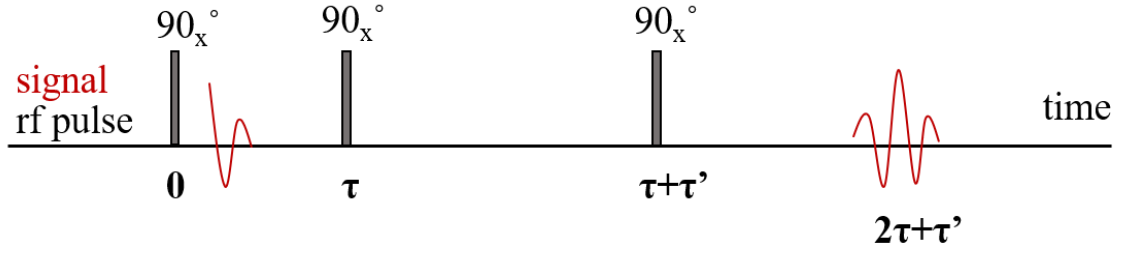


Figure 2.9: A stimulated echo pulse sequence is shown. The magnetization undergoes successive 90° pulses at $t = 0, \tau$, and $\tau + \tau'$ ending with an echo at $2\tau + \tau'$. This pulse sequence is used when the T_2 relaxation is much, much shorter than T_1 relaxation.

Gradients in NMR

Inhomogeneity in magnetic fields within a material is often unavoidable with NMR and causes broadening in the frequency domain of the spectrum. However, this broadening can be used to an advantage through deliberately applying a linearly varying magnetic field called a magnetic field gradient, $\mathbf{G}$. Through this application, the spins will precess around the field at different frequencies depending on spatial location. There will be a linearly spatial dependence which is the basis for magnetic resonance imaging (MRI) and NMR observations of spin motion. The crux of the theory comes from the Larmor equation $\omega_0 = \gamma B_0$. With the linearly applied gradient, the applied magnetic field becomes

$$B(\mathbf{r}) = B_0 + \mathbf{G} \cdot \mathbf{r} \quad (2.33)$$

Where $\mathbf{r}$ is the spatial coordinate, and this is visually shown in Figure 2.10. We will assume for this thesis that $\mathbf{B}_0$ is much greater than $\mathbf{G}$. It is apparent the frequency will have spatial dependence due to the applied gradient.

$$\omega(\mathbf{r}) = \gamma B_0 + \gamma \mathbf{G} \cdot \mathbf{r} \quad (2.34)$$

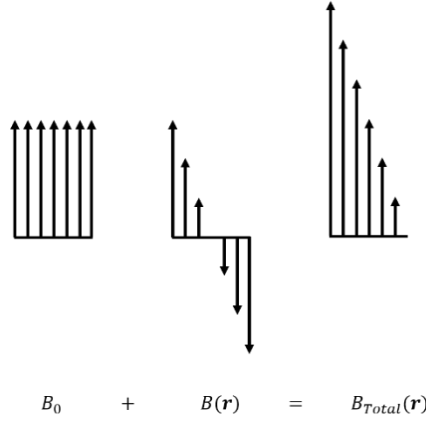


Figure 2.10: The application of a gradient to a static magnetic field imparts spatial information to the magnetic field.

k-Space and Magnetic Resonance Imaging

The evolution of a signal amplitude of a spin ensemble from an applied magnetic field gradient is [8]

$$S(t) = \iiint \rho(\mathbf{r}) \exp(i\gamma \mathbf{G} \cdot \mathbf{r}t) d\mathbf{r} \quad (2.35)$$

where $\rho(\mathbf{r})$ is the spin density matrix, $\mathbf{G}(\mathbf{r})$ is the gradient vector dependent on spatial location $\mathbf{r}$, and the gradient pulse duration t . Defining a new variable, $\mathbf{k}$

$$\mathbf{k} = \frac{\gamma t \mathbf{G}}{2\pi} \quad (2.36)$$

where $\mathbf{k}$ is the reciprocal space vector, and substituting it into Equation (2.35), a Fourier relationship between the normalized signal, $S_N(\mathbf{k})$, and the spin density, $\rho(\mathbf{r})$ is found.

$$S_N(\mathbf{k}) = \int \rho(\mathbf{r}) \exp(-i\mathbf{k} \cdot \mathbf{r}) d\mathbf{r} \quad (2.37)$$

$$\rho(\mathbf{r}) = \frac{1}{2\pi} \int S_N(\mathbf{k}) \exp(i\mathbf{k} \cdot \mathbf{r}) d\mathbf{r} \quad (2.38)$$

What this means is the Fourier transform of the signal, or spin density, can be viewed as a MR image of the material with spatially encoded information. This is the fundamental basis behind MRIs.

Similarly, the spin system signal can be expressed through the phase.

$$S = S_0 \exp(i\varphi) \quad (2.39)$$

where S_0 is the maximum initial signal and φ is the total accumulated phase shift at location $\mathbf{r}$ after applied gradient duration time t defined as

$$\varphi = \gamma \mathbf{G} \cdot \mathbf{r} t \quad (2.40)$$

While the gradient is applied, the spin phase evolves in time, essentially winding up in what is called the ‘magnetization helix’ which is shown in Figure 2.11. After a 90° rf pulse, the spins are in the transverse plane all with identical phases. As time progress with the introduction of a gradient, each spin will experience a difference magnitude of the magnetic field causing the ‘winding’ of the magnetization helix. The overall evolution of the helical phase pattern has a wavelength of $\lambda = \frac{2\pi}{\gamma G t}$ which can be seen to decrease in time.

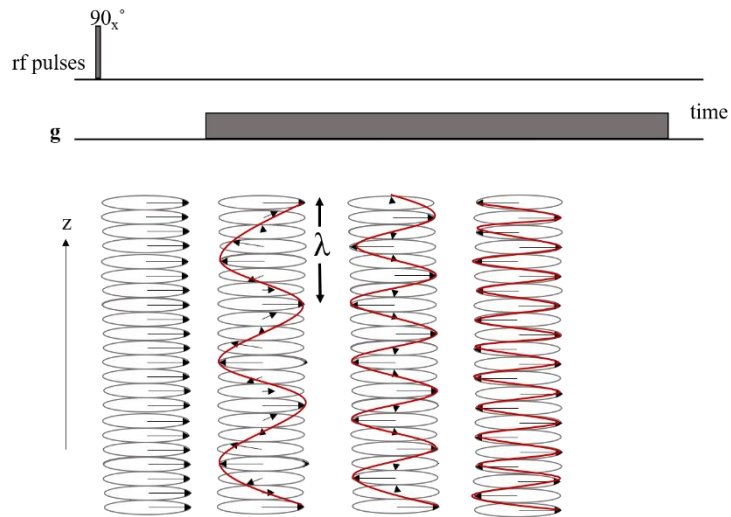


Figure 2.11: The application of a steady gradient after a 90° pulse winds up the magnetization helix. The longer the gradient is applied, the tighter the helix is with wavelength, λ , decreasing as time increases.

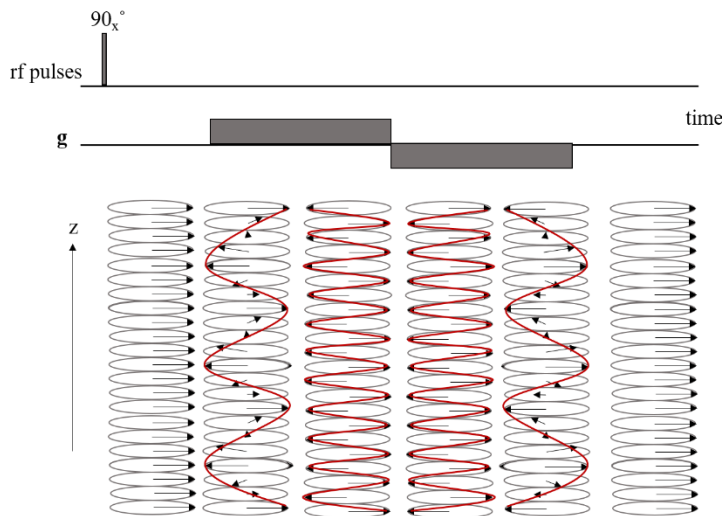


Figure 2.12: The magnetization helix can be unwound through applying an equal in amplitude and duration but opposite in sign gradient lobe. This reverses the winding of the spins and will result in an echo called a gradient echo.

This helix can be reversed though applying an addition gradient of equal but opposite sign producing a gradient echo (Figure 2.12) or through applying a 180° pulse followed

by a gradient of equal length and sign producing a spin echo (Figure 2.13). Taking a gradient echo, the sign change causes the spins to reverse phase and come together creating a perfect echo after a time τ , which is the time of the initial gradient duration. Similarly, for the spin echo, the application of the 180° rf pulse inverts the phase helix causing the subsequent gradient pulse to have the effect of unwinding the helix as well. Note the perfect echoes achieved are only possible if there is no motion causing perturbations to the phase. The effect of spin motion will be explored in later sections.

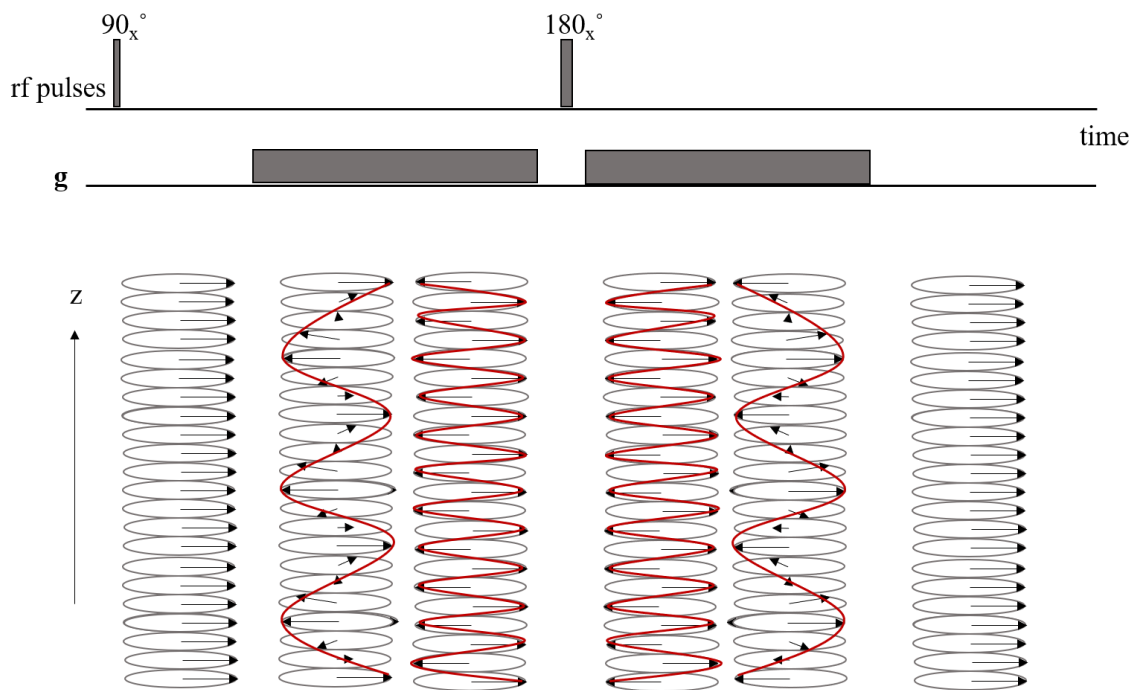


Figure 2.13: Similar to the gradient echo, a spin echo sequence reverses the winding of the magnetization helix resulting in an echo. However, this is done through an application of a 180° pulse, bisecting a gradient into two separate lobes with equal amplitude and duration. A 180° pulse effectively flips the sign of a gradient lobe.

k-Space Encoding

In order to obtain a well resolved MR image, a large amount or efficient amount of k -space must be traversed. This may be done through two methods, stepping through gradient values G , or through stepping through time t . The first is known as phase encoding and the latter is known as frequency encoding.

Starting with phase encoding (Figure 2.14), a systematic method of stepping through gradient amplitude values, called G_{phase} , with a fixed time interval is used. For one gradient step, one k -space value is obtained. While this is less efficient than frequency encoding discussed below, the FID is available for spectral resolution and there are no inhomogeneous broadening which could be associated with relaxation occurring.

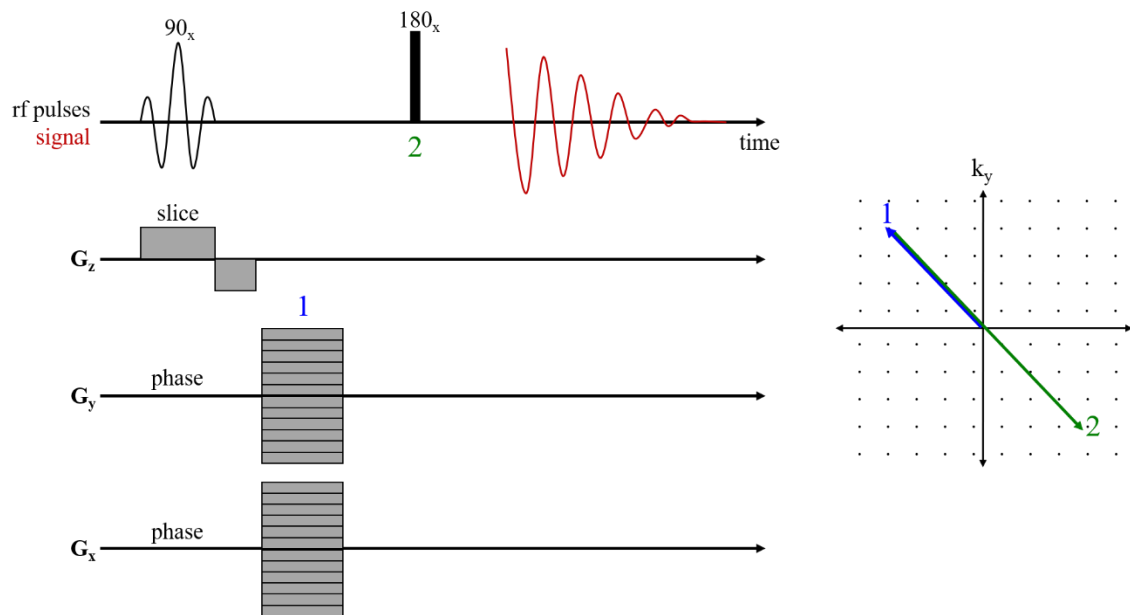


Figure 2.14: A 2D-imaging pulse sequence with only phase encoding is shown with a respective k -space path. The exciting of the spins in a specific xy plane is done with the rf and slice gradients. The phase gradients in x and y traverse the k space, winding the magnetization helix up in (1), then the 180° pulse inverts this (2). The echo is then obtained.

Moving onto frequency encoding (Figure 2.15), the signal is sampled under a constant gradient denoted as G_{read} . In this method, an entire line of k -space is obtained instead of a single point as in phase encoding methods. While appearing to be more efficient, there are possibilities for artifacts to occur.

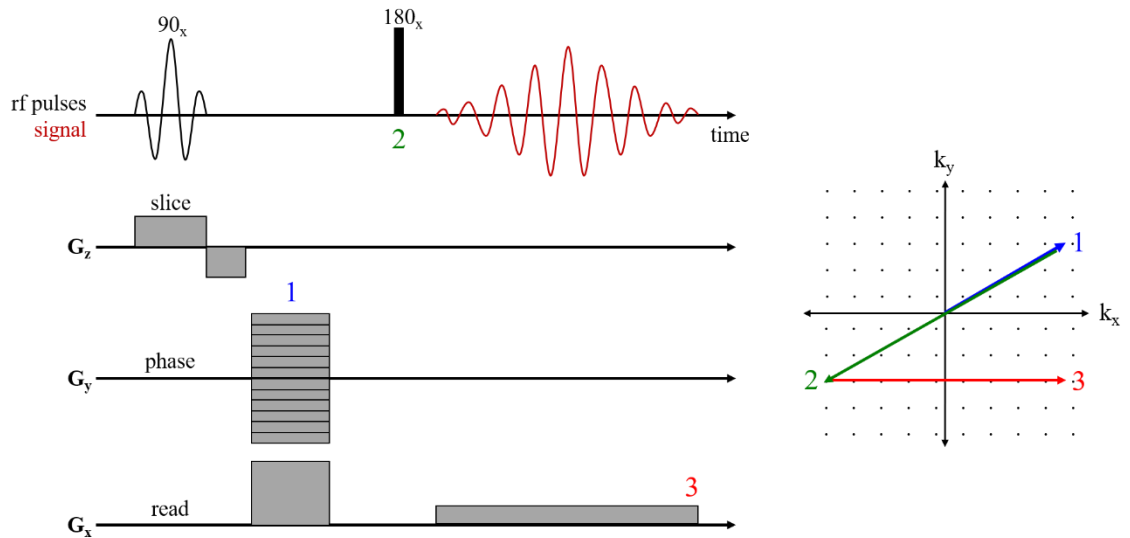


Figure 2.15: A different type of 2D-imaging is shown above. Similar slice selection is used (1), and the inversion of the magnetization helix is also used (2). The difference comes with the echo acquired during the read gradient (3) allowing an entire line in k -space to be sampled (red). This allows a more efficient sampling of k -space.

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CHAPTER THREE

ADVANCED NMR TOPICS

This chapter relies on work from Sir Paul Callaghan [1] and Anatole Abragam[2].

Molecular Self-Diffusion and Propagators

In 1855 Adolf Fick [3] envisioned the process of diffusion as the evolution of particles in a concentration gradient. This yielded Fick's second law, or the diffusion equation,

$$\frac{\partial n}{\partial t} = D \nabla^2 n \quad (3.1)$$

which describes the process known as mutual diffusion.

Self-diffusion was first explained by Albert Einstein with the driving force being thermal fluctuations. Based on this idea, the Stokes-Einstein relationship was derived [4, 5].

$$D = \frac{k_B T}{6\pi\eta R} \quad (3.2)$$

where the denominator is the Stokes drag for a spherical particle with effective radius R moving through a medium of viscosity η . Using this relationship in combination with Fick's law, a description of Brownian motion is that of a stochastic and probabilistic process. Because of this, Brownian motion and diffusion processes can be described as the probability the particle will travel a specific distance during a specific time, also called a propagator. Since NMR deals with statistical ensembles of spins, an average propagator is useful to implement when discussing displacement [1].

$$\bar{P}(\mathbf{R}, t) = \int \rho(\mathbf{r}, 0) P(\mathbf{r}|\mathbf{r} + \mathbf{R}, t) d\mathbf{r} \quad (3.3)$$

where $\rho(\mathbf{r}, 0)$ is the initial probability density of the particle at location $\mathbf{r}$ at time 0, and $P(\mathbf{r}|\mathbf{r} + \mathbf{R}, t)$ is the conditional probability of moving to position $\mathbf{r} + \mathbf{R}$ at time t . This means $\bar{P}(\mathbf{R}, t)$ is the average probability of the particle moving from position $\mathbf{r}$, at time 0 and moving a distance $\mathbf{R}$ over time t .

This propagator obeys Fick's law and from that, the solution for Brownian motion and self-diffusion is a Gaussian which evolves over time shown in Figure 3.1.

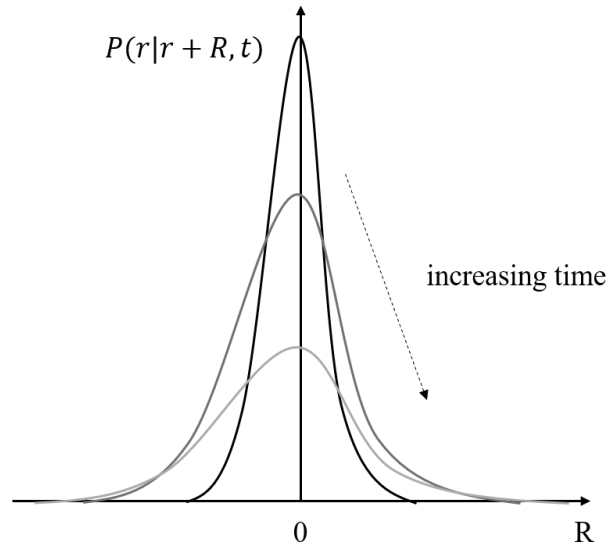


Figure 3.1: The Gaussian propagator, $P(\mathbf{r}|\mathbf{r} + \mathbf{R}, t)$, over time decreases in amplitude and spreads out further in space.

The Gaussian nature leads to the Einstein relations:

$$\langle (x' - x)^2 \rangle = 2Dt \quad (3.4)$$

$$\langle (\mathbf{r}' - \mathbf{r})^2 \rangle = 6Dt \quad (3.5).$$

In this thesis, the hardware used restricts the mean square displacement obtained to 1D, or as given in Equation (3.4).

Normalized Echo Signal

Measuring molecular motion with NMR techniques is the basic process of tagging spins at an initial time, encoding positions through applying spatially varying gradients, then observing the diffusion and flow over a fixed time period. If there is motion or translational fluctuations, the spins will accumulate a phase shift during the measurement causing a decay in the echo amplitude over time. The echoes are obtained through time varying gradients, $\mathbf{g}(t)$, which are used to encode for motion as well. For one echo, the total signal is given by[1]

$$M_+(t) = M_+(0) \langle \exp(i\gamma \int_0^t \mathbf{g}^*(t') \cdot \bar{\mathbf{r}}_j(t') dt') \rangle \quad (3.6)$$

If the position vector is Taylor expanded, for (3.7) to be non-zero, meaning for an echo to be formed, the zeroth moment must be zero[1].

$$M_0 = \int_0^t \mathbf{g}^*(t') dt' = 0 \quad (3.7)$$

With translational motion or any type of diffusion the echoes will decay with time, because of this, it is useful to introduce the idea of a normalized echo signal. The signal is normalized by the signal with no gradient applied, or the initial echo in the measurement shown as[1]

$$E(t) = \frac{M_+(t)|_{\mathbf{g}^*(t') \neq 0}}{M_+(t)|_{\mathbf{g}^*(t') = 0}} \quad (3.8)$$

where $\mathbf{g}^*(t')$ is the effective gradient applied. Using the normalized echo signal, $E(t)$, normalizes out any relaxation effects occurring during the measurement leaving only the effects of the effective gradient. Additionally, this normalizes the echo signal allowing for comparison between samples.

Moments of the Gradient

Residual phase is accumulated in systems where spins experience coherent motion. For a spin j , travelling along a path $\mathbf{r}_j(t')$ with an applied effective gradient $\mathbf{g}^*$, this phase is given by [1]

$$\varphi_j(t) = \gamma \int_0^t \mathbf{g}^*(t') \cdot \mathbf{r}_j(t') dt' \quad (3.9)$$

Physical insights can be made by expanding $\mathbf{r}_j(t')$ with a Taylor series

$$\mathbf{r}_j(t) = \mathbf{r}_0 + \frac{d\mathbf{r}_j}{dt} + \frac{1}{2!} \frac{d^2\mathbf{r}_j}{dt^2} + \frac{1}{3!} \frac{d^3\mathbf{r}_j}{dt^3} + \dots \quad (3.10)$$

where the first derivative characterizes velocity, the second is acceleration, and the third jerk as expected. The phase then can be expressed in terms of the moments as shown below [1].

$$\varphi_j(t) = \gamma \left[\mathbf{r}_0 \int_0^t \mathbf{g}^*(t') dt' + \mathbf{v}_0 \int_0^t \mathbf{g}^*(t') t' dt' + \frac{\mathbf{a}_0}{2} \int_0^t \mathbf{g}^*(t') t'^2 dt' + \dots \right] \quad (3.11)$$

The integrals in (3.11) are the zeroth, first, second and third moment respectively and the generalized moment is defined as $m_n = \frac{1}{n!} \int_0^t \mathbf{g}^*(t') t'^n dt'$. For an echo to occur, the zeroth moment must be equal to zero and thus does not contribute to the accumulated

phase or signal, this is called the echo condition. The second and third moment encode for the velocity and acceleration of the system respectively, [1]

$$m_1 = v_0 \int_0^t \mathbf{g}^*(t') t' dt' = g \Delta \delta \quad (3.12)$$

$$m_2 = \frac{a_0}{2} \int_0^t \mathbf{g}^*(t') t'^2 dt' = g \Delta \delta^2 \quad (3.13)$$

where δ is the duration of the gradient waveform and Δ is the time between gradients.

The echo amplitude may be written in terms of moments as [1]:

$$E(t) = \exp [i\gamma \mathbf{r}_0 m_0 + i\gamma v_0 m_1 + i\gamma a_0 m_2 + \dots] \quad (3.14)$$

Position, velocity and acceleration can be measured directly through ensuring all other moments go to zero by choosing specific time dependence of the effective gradient, $\mathbf{g}^*(t')$. [1]

Stejskal-Tanner Experiment and q -Space

Prior to 1965, constant and steady gradients were always present during NMR measurements complicating the observation of slow diffusion due to the spreading of frequencies. A breakthrough occurred with the work of Stejskal and Tanner in measuring self-diffusion through a new pulse sequence. [6] The new pulse sequence implemented rectangular shaped gradients at finite times and was named the pulse gradient spin-echo (PGSE). Due to the variable nature of T_2 relaxation times, there are two common pulse sequences used for diffusion encoding in attempts to capture all signal prior to relaxing; these are named pulse gradient spin-echo (PGSE from here on) and pulse gradient stimulated-echo (PGStE from here on). These are both shown in Figure 3.2. Each pulse

sequence uses a combination of rf pulses, gradient pulses of duration length δ and amplitude g , and an observation time of Δ in which spins are allowed to diffuse between the application of each gradient. In addition to the development of the pulse sequence, Stejskal and Tanner noticed if the narrow pulse approximation ($\delta \ll \Delta$) was met, then propagator formalism could be implemented to describe the transport occurring. [7] The narrow pulse approximation can be rephrased to be the assumption that the gradients applied are short enough that motion during the application is negligible. Both the PGSE and PGStE are equivalent in imparting motion caused phases, but PGStE preserves the signal from T_2 relaxation during the time between the second and third rf pulses.

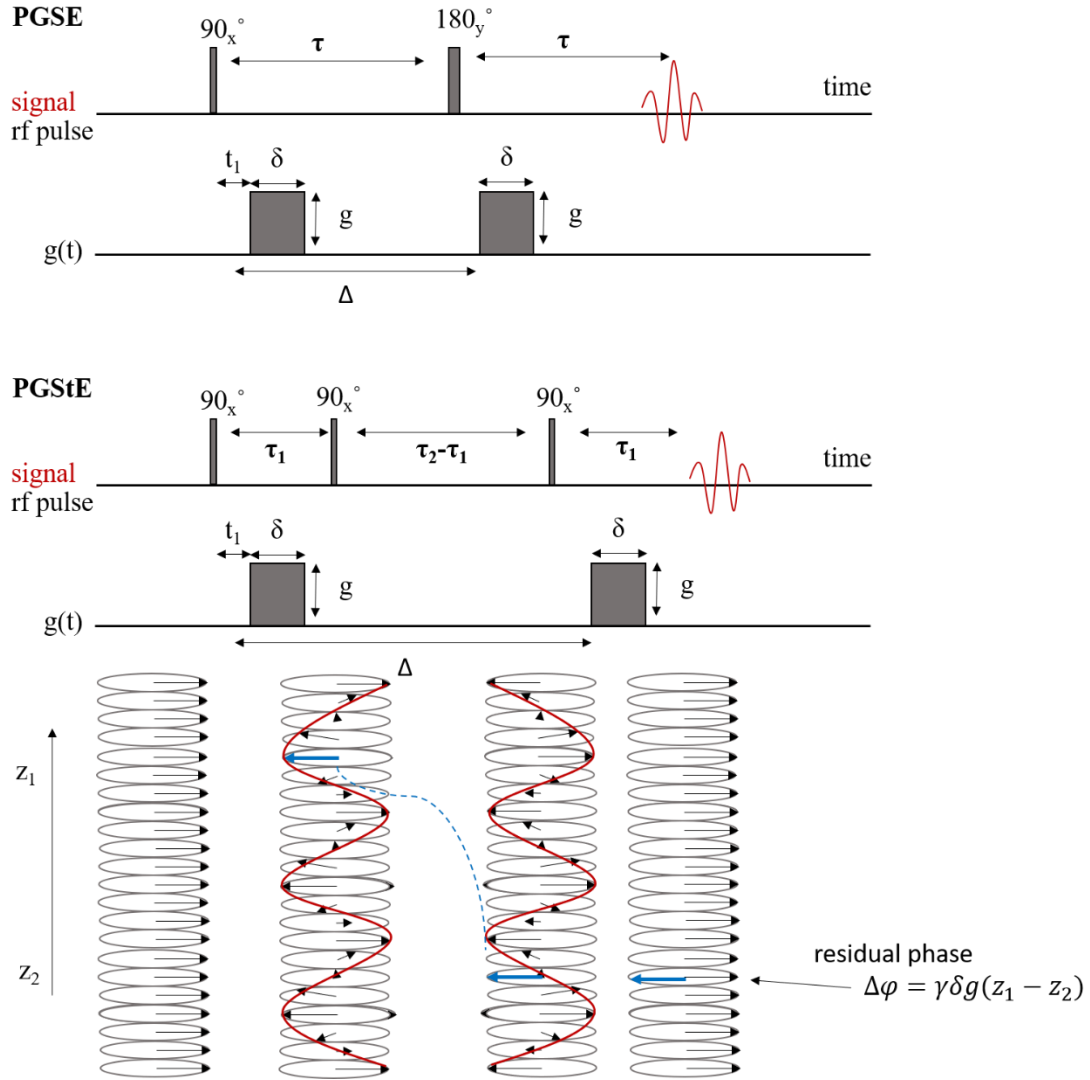


Figure 3.2: A PGSE (top) and PGStE (bottom) pulse sequence are shown above. The key difference between a PGSE and PGStE sequence is a 180° pulse in the PGSE is replaced with two 90° pulses in the PGStE. This is implemented when the sample has short relaxation times (*i.e.*: porous media and polymers). The winding of the magnetization helix is the same for each of these pulse sequences. Spatial displacement is measured in terms of net phase accumulation.

The application of the first gradient winds up the magnetization helix and imparts a phase of $\gamma\delta\mathbf{g} \cdot \mathbf{r}$ on a spin at position $\mathbf{r}$. After the final gradient, the helix is unwound, and the net phase of a given spin is $\Delta\phi = \gamma\delta\mathbf{g} \cdot (\mathbf{r}' - \mathbf{r})$. Note, if the spin does not

move between the two gradients (during duration Δ), $\mathbf{r}' = \mathbf{r}$, then the residual net phase is zero and the echo train will have no attenuation. In a PGStE experiment, the magnetization is stored along the z axis between the two gradients to remove relaxation due to dipolar coupling, or T_2 relaxation. Because of this, a PGStE experiment is utilized in systems when $T_1 > T_2$. It should be mentioned, T_1 relaxation is occurring during the storage time between gradients and Δ should not approach or exceed the T_1 . If motion is occurring during the experiment, then the echo attenuation will follow the well-known Stejskal-Tanner relation [6]

$$E(g) = \exp[-\gamma^2 g^2 \delta^2 D(\Delta - \delta/3)] \quad (3.15)$$

where D is the diffusion coefficient of the molecules in the system and is found from the echo attenuation of the signal, typically with respect to g . This decay comes from incoherent phase shifts throughout the sample. The pulse sequence combination of rf pulses and gradient applications rephases the spins themselves. The gradient amplitude, g , is varied in magnitude, with other parameters constant during the experiment, and is used to remove relaxation effects from the signal decay. The diffusion time Δ is varied to explore the displacement time dependence of diffusion within the system. This is often simplified through introducing a new variable $b = \gamma^2 g^2 \delta^2 (\Delta - \delta/3)$. Plotting the natural log of the signal amplitude against this variable b , the slope is D . After extracting the diffusion coefficient, information such as the mean-squared-displacement of the spin yield insight into the structure of the system through the Einstein relation $\langle z^2 \rangle = 2D\Delta$ [5] assuming it is in the absence of coherent flow.

Another insight Stejskal had [6] was using the propagator formalism in explaining probability densities of general motion in a sample. Using this, the signal from an ensemble of spins would be given by [6]

$$E(\mathbf{g}, \Delta) = \int \rho(\mathbf{r}) \int P(\mathbf{r}|\mathbf{r}', \Delta) \exp(i\gamma \mathbf{g} \cdot [\mathbf{r} - \mathbf{r}']) d\mathbf{r}' d\mathbf{r} \quad (3.16)$$

Where $\rho(\mathbf{r})P(\mathbf{r}|\mathbf{r}', \Delta)$ is treated as the probability of a spin to begin at $\mathbf{r}$ and move to $\mathbf{r}'$ after time Δ . Equation (3.3) can be rewritten in terms of a reciprocal space vector to displacement defined as:

$$\mathbf{q} = \frac{\gamma \delta \mathbf{g}}{2\pi} \quad (3.17)$$

With this, Eq (3.3) can be rewritten in terms of $\mathbf{q}$ space

$$\bar{P}(\mathbf{R}, t) = \int \rho(\mathbf{r}) P(\mathbf{r}|\mathbf{r} + \mathbf{R}, \Delta) d\mathbf{r} \quad (3.18)$$

$$E(\mathbf{q}, t) = \int \bar{P}(\mathbf{R}, t) \exp(i2\pi \mathbf{q} \cdot \mathbf{R}) d\mathbf{R} \quad (3.19)$$

Note, during q-space ‘imaging’ the distribution of spin displacements is measured, while during a k-space imaging experiment the spin density distribution is measured.

Bloch-Torrey Theory for Diffusion and Flow

In 1956, Torrey modified the Bloch equations to describe spin phase evolution and provided a more general approach. [8] An additional term was added such that the flow and self-diffusion was described in terms of magnetization transport. Essentially, magnetization is treated as a fluid in a convection diffusion conservation equation creating the Bloch-Torrey equation [8]

$$\frac{\partial M_+}{\partial t} = -i\gamma \mathbf{r} \cdot \mathbf{g} * (\mathbf{t}) M_+ - \frac{M_+}{T_2} + D \nabla^2 M_+ - (\mathbf{v} \cdot \nabla) M_+ \quad (3.20)$$

where $M_+ = M_x + iM_y$ and Eq (3.20) assumes a scalar diffusion coefficient. The solution to the differential equation above is [8]

$$M_+(\mathbf{r}, t) = A(t) \exp[-i\gamma \mathbf{r} \cdot \int_0^t \mathbf{g} * (t') dt'] \exp(-t/T_2) \quad (3.20)$$

where $A(t)$ is a phase factor defined by the spreading occurring in the spin bearing molecules from flow and diffusion, the first exponential term arises from the magnetic field gradient causing a helical phase distribution centering the echo at 1, and $\exp(-t/T_2)$ describes the spin-spin T_2 relaxation. With the echo condition of the first moment being zero, $A(t)$ is equal to the normalized echo attenuation $E(t)$. [8]

$$E(t) = \exp \left[-D\gamma^2 \int_0^t \left(\int_0^{t'} \mathbf{g}^*(t'') dt'' \right)^2 dt' \right] \exp[i\gamma \mathbf{v} \cdot \int_0^t \int_0^{t'} \mathbf{g}^*(t'') dt'' dt'] \quad (3.21).$$

This equation is only valid if the echo is evaluated at the echo center. If evaluated at the echo center, isotropic diffusion is assumed. If not, then another time-dependent term, like the structure factor, is included. This term arises from gradients dephasing due to spin location and it is necessary to average over all $\mathbf{r}$.

Restricted Diffusion

Up until this point, we have assumed uniform diffusion throughout our experiments. The diffusion coefficient in stationary bulk systems returned from the PGSE experiment is the self-diffusion coefficient for the fluid or sample, D_0 . However, rich information can be obtained from systems with restricted environments due to the correlation between the microstructure of the system and the diffusion coefficient. The

NMR PGSE experiment signal is uniquely qualified to measure this primarily due to the non-invasive manner in which this technique can probe the molecular displacement of the spins. Through the displacement, diffusion coefficients are calculated. In using the PGSE pulse sequence with this type of confined geometry, the measured value is the effective diffusion coefficient, D_{eff} , and is a function of the observation time, Δ . Implementing the well-known Einstein relationship, the mean-squared-displacement can be found through (3.22)

$$D_{eff} = \frac{\langle (\mathbf{r}(t) - \mathbf{r}(0))^2 \rangle}{6t} \quad (3.22)$$

where in this case t is the displacement observation time Δ of the experiment. With restricted environments, the observation time is extremely important in understanding the sampling of the system. In Figure 3.3, three situations are shown. The first is bulk fluid where the spin carrying molecule is able to move about randomly in Brownian motion without any restriction. This is seen as a straight line in the mean-squared-displacement plots. If instead the molecule is in a closed, isolated pore, at short observation times, the molecule will diffuse at the rate of self-diffusion, however, at a long enough time, the molecule will encounter the pore walls. As $t \rightarrow \infty$, the molecule will experience enough collisions with the walls such that $D(t) \rightarrow 0$. With interconnected pores, the initial bulk-like self-diffusion is seen as well. However, unlike the isolated pore, at long enough time the molecule may travel to other pores meaning $D(t) \rightarrow D_{eff}(\infty)$. [1]

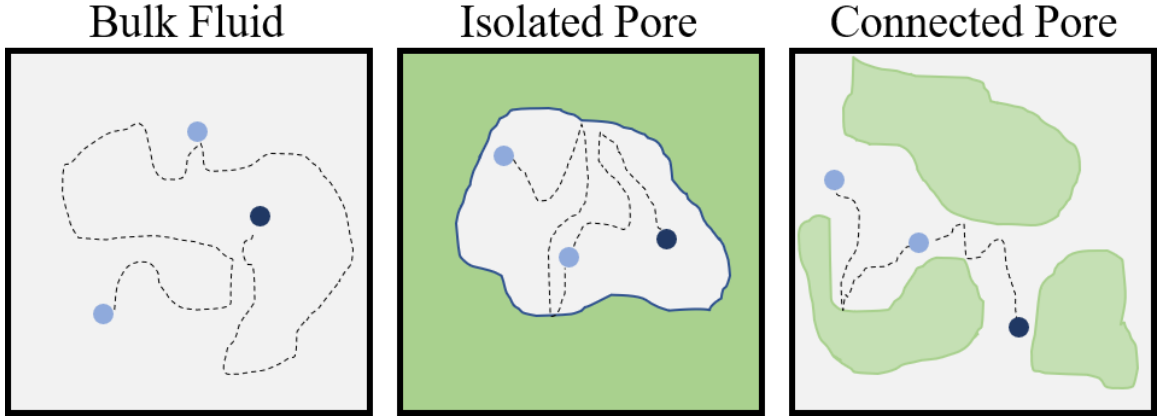


Figure 3.3: Bulk fluid, an isolated pore, and a connected pore network are shown to describe the difference in spin dynamics. In a bulk fluid, the spin undergoes self-diffusion through Brownian motion. In an isolated and connected pore, the spin at short times experiences only bulk fluid motion, however, at longer times the spin in the isolated pore will appear to have no net motion. The connected pore system allows the spin to sample other pores but with restricted motion.

In looking at the limits of the connected pores, at short times the molecule samples only the pore in which it is currently in. Information about the surface to volume ratio can be obtained using this short time limit approach [1]

$$\frac{D_{eff}(t)}{D_0} = 1 - \frac{(D_0 t)^{1/2} S}{V} \quad (3.23)$$

Similarly, in the long time limit, information about the connectivity, or tortuosity (α), is obtained [1]

$$\lim_{t \rightarrow \infty} \frac{D_{eff}(t)}{D_0} = \frac{D_{eff}(\infty)}{D_0} = \frac{1}{\alpha} \quad (3.24)$$

Note, in the inversion of the tortuosity a larger ratio indicates a higher tortuosity. A large tortuosity indicates a low connectivity of the system.

Spin Relaxation in Inhomogeneous Porous Media

With restricted diffusion environments, often each pore or confined geometry may have separate relaxation time scales. The relaxation times must be kept in mind while using the PGSE sequence when choosing a Δ value in order to exclude any relaxation weighting effects. Simply, Δ must be short enough such that the spin bearing molecules do not relax enough to disrupt the diffusion data collected. There are two methods for describing the interplay between relaxation and diffusion: the exchange model, in which a relaxation value is assigned to each physical region and diffusion has the effect of transferring the molecules to each region; and the surface relaxation model, where the bulk relaxation effects are decoupled from the interactions of the molecules near the walls experiencing restricted diffusion. In this section the first method, the exchange model, will be discussed.

In the exchange model with multi-site relaxation, the relaxation timescale of a region i is represented as $T_{(i)}$ which describes both T_2 and T_1 relaxation. In each region, the spin occupancy is P_i , where naturally $\sum P_i = 1$. Exchange is described as spin bearing molecules “hopping” between sites of different relaxation values. The timescales of interest come from considering the characteristic ‘exchange time,’ τ_E , in relation to the relaxation time $T_{(i)}$. For slow exchange, $T_{(i)} \ll \tau_E$, the magnetization relaxation decay is described as multi-exponential [1]

$$M(t)/M(0) = \sum P_i \exp(-t/T_{(i)}) \quad (3.25)$$

whereas in the fast exchange regime, $T_{(i)} \gg \tau_E$, the magnetization decay is given by a single exponential decay

$$\frac{1}{T} = \sum P_i \exp(-t/T_{(i)}) \quad (3.26)$$

$$M(t)/M(0) = \exp(-\frac{t}{T}) \quad (3.27)$$

In fast exchange, the spins will effectively experience all regions of the system and average out to an effective relaxation time. Unfortunately, in the intermediate regimes, neither of these models can explain the non-exponential decays which occur.

Brownstein-Tarr Theory

This problem of multi-site exchange in an inhomogeneous porous sample was explored in detail by Brownstein and Tarr[9] who used the approach of Bloch and Torrey [8] in treating magnetization as a fluid which diffuses and obeys Fick's Law. This is the second method of exploring exchange in a multi-site system. The differential equation governing this model is

$$D\nabla^2 M(\mathbf{r}, t) = \frac{\partial M(\mathbf{r}, t)}{\partial t} \quad (3.28)$$

where $M(\mathbf{r}, t)$ is the probability of finding magnetization at location $\mathbf{r}$ and time t such that the molecule has not relaxed. $M(\mathbf{r}, t)$ can be due to both spin-spin and spin-lattice relaxation. As the spins interact with the walls, the spins will relax, making the walls a magnetization sink in the system and enhancing the relaxation time in the restricted environment. The Brownstein- Tarr solution to (3.28) is a normal mode solution given by

$$M(r, t) = M(0) \sum_{n=0}^{\infty} I_n \exp(-t/T_n) \quad (3.29)$$

where I_n is the relative contribution of the normal mode with the eigenvalue of T_n and is defined as $I_n = a_n \int dr u_n$, a_n is the pore length scale and u_n is the eigenfunction solution.

T_n and u_n are the eigenvalue and eigenfunction solution to the Helmholtz equation

$$u_n/T_n + D\nabla^2 u_n = 0 \quad (3.30)$$

with a boundary condition of

$$D\hat{n} \cdot \nabla u_n(\mathbf{r}) + \bar{\rho} u_n(\mathbf{r})|_S = 0 \quad (3.31)$$

where $\bar{\rho}$ is the surface relaxivity which describes the average surface relaxation sink strength. For all geometries, I_n and T_n are dependent on $\frac{\bar{\rho}a}{D}$. [9]

Like the first method, there are limiting cases to explore, fast diffusion, intermediate diffusion, and slow diffusion regimes all of which are determined by the ratio of $\frac{\bar{\rho}a}{D}$. In the fast diffusion regime, $\frac{\bar{\rho}a}{D} \ll 1$, the magnetization decay is characterized by a single exponential decay and is dominated by the slowest relaxing mode $T_0^{-1} \sim \bar{\rho} S/V$. In this case, the magnetization diffusion rate is much greater than the relaxation rate occurring at the walls represented by the surface relaxivity term. For the slow diffusion regime, $\frac{\bar{\rho}a}{D} \gg 1$, T_n is essentially independent of the surface relaxivity since the relaxation occurring at the walls dominates. This is often called “diffusion-limited” exchange. [1, 9] A multi-exponential relaxation is seen, characterized by the slowest mode as well as higher order modes. The slowest mode is given by $T_0^{-1} \sim a/\bar{\rho}$. The higher order modes, T_n^{-1} , are independent of $\bar{\rho}$ and are of the order of $a^2/n^2\pi^2 D$, these higher order modes are observed in the diffusion limited regime. In using this relationship, an estimate of pore sizes in the system is obtained. As in most science, the

intermediate regime is difficult to determine but is known to have multi-exponential behavior as well.

In a porous system, the measured relaxation rates are a combination of the bulk fluid, T_B , the spins interacting with the surface, $T_{1,2,S} = \rho_{1,2}S/V$, and for spin-spin relaxation, the relaxation due to local inhomogeneous magnetic fields, T_D . The last can be ignored if the CPMG pulse sequence is implemented with short enough echo spacing to refocus the phase shifts due to diffusion. [1] The relaxation rates are given by

$$\frac{1}{T_1} = \frac{1}{T_{1,B}} + \frac{1}{T_{1,S}} \quad (3.32)$$

$$\frac{1}{T_2} = \frac{1}{T_{2,B}} + \frac{1}{T_{2,S}} + \frac{1}{T_{2,D}} \quad (3.33).$$

In the fast diffusion regime, the bulk term can be ignored leaving

$$\frac{1}{T_{1,2}} = \rho_{1,2}S/V \quad (3.34).$$

Multidimensional Relaxation NMR

A distribution of pores or phases in a system result in a multi-exponential relaxation decay. This will result in a distribution of relaxation rates which can be determined through using an inverse Laplace transformation as the analysis technique. The ILT is essential in analyzing single and multidimensional experimental data.

Inverse Laplace Transform (ILT)

The inverse Laplace transform (ILT) is given by

$$f(R) = \mathcal{L}^{-1}\{S(t)\} \quad (3.35)$$

$$S(t) = \mathcal{L}\{f(R)\} = \int_0^\infty f(R) \exp(-Rt) dR \quad (3.36)$$

Here $f(R)$ is the probability of finding the decay rate R , and $S(t)$, is the time domain signal acquired in the NMR experiment. The relaxation rate distribution resides in $f(R)$, which is where the ILT comes in to extract it. Unfortunately, the ILT is ill-defined, asymmetric and not easily implemented, but, an analytic expression does exist [1]

$$f(R) = \mathcal{L}^{-1}\{S(t)\} = \frac{1}{2\pi i} \int_{\gamma-i\infty}^{\gamma+i\infty} S(t) \exp(Rt) dt \quad (3.37).$$

This is a contour integral in the complex plane. The variable γ in this case is the vertical contour to the right of a singularity. This integral is ill-posed due to the potential for an increasingly large exponent caused by noise in $S(t)$ resulting in divergence. Experimental limitations of NMR hardware make this noise unavoidable. A practical approach to this problem was proposed by Stephen Provencher in 1982. [10] Provencher suggested using a regularized non-negative least squared method with the ILT to account for noise. Since the signal is digitized in experiments, the signal first is written in discrete form

$$S(t_i) = \sum_{j=1}^M \exp(-t_i R_j) f(R_j) + \epsilon_i \quad (3.38)$$

where i represents the discrete time sampling, and j represents the number of relaxation rate domains in $f(R)$. The other term, ϵ_i , correlates to the error from noise in the measurement. The equation can be optimized through minimizing this term. Additionally, this method requires prior knowledge that $f(R)$ is positive, and the application of parsimony, meaning the simplest solution is expected to be the correct physical situation. Provencher implemented this last requirement through a method known as Tikhonov

regularization. [10] This is the method in which the goal is to minimize the value of the following

$$V(\alpha) = \|\underline{K}f - \underline{S}\|^2 + \alpha^2 \|\underline{\Gamma}f\|^2 = \text{minimum} \quad (3.39)$$

where Γ is an operator which favors smoothness with minimum number of peaks in the relaxation distribution, K is the kernel, S is the measured signal from the experiment, f is the relaxation rate distribution and the least squares solution, and α is the regularization parameter. The last term of (3.39) is [10]

$$\|\underline{\Gamma}f\|^2 = \int_{R_{min}}^{R_{max}} [f''(R)]^2 dR \quad (3.40)$$

The solution vector f has a corresponding value for each element R_j through discretization. This results in the following [10]

$$\begin{aligned} V(\alpha) &= \sum_i^N \sum_j^M (S_i - \exp(-t_i R_j) f_j)^2 + \alpha^2 \sum_j^M (2f_j - f_{j+1} - f_{j-1})^2 \\ &= \text{minimum} \quad (3.41) \end{aligned}$$

The first term in the summation is the residual, X^2 , and is minimized through selecting an α value which minimizes the second term, the error term. The value is selected through parsimony by minimizing the value. A large α value will fit small features of the distribution and implies a high level of confidence in the fine structures. However, if the chosen value is too large, ‘pearling’ will occur which the noise is over fitted, and more detail is shown than exists in the original data.

T_1 - T_2 Relaxation Correlation Experiments

The T_1 - T_2 pulse sequence allows both the spin-lattice, T_1 , and spin-spin, T_2 , relaxation to be probed in one NMR experiment (Figure 3.4 [1]). This experiment starts off by first encoding for T_1 for a given inversion time between the initial 180 and 90 pulses, τ_1 , after which the remaining signal is fed into a CPMG sequence to measure T_2 decay with echo spacing of $\tau_E = 2\tau_2$. The echo maxima are located at $t_n = n\tau_E$ where n is the echo number. This sequence is looped through N times cycling through N separate inversion times.

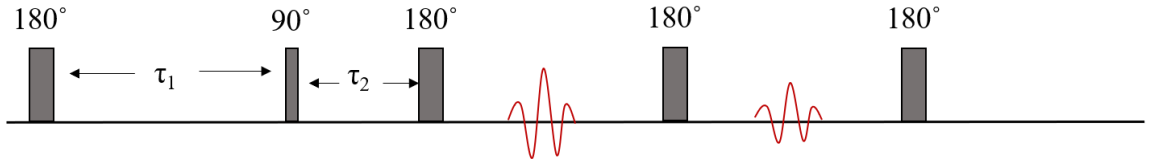


Figure 3.4: A T_1 - T_2 pulse sequence is shown. The experiment begins with an inversion recovery pulse to encode for T_1 relaxation. After time τ_1 , the remaining signal is fed into a CPMG train to encode for T_2 relaxation. This is repeated N times with different τ_1 times.

The normalized signal from this measurement is given by [1]

$$S(t_1, t_2) = \iint (1 - 2\exp(-R_1 t_1)) \exp(-R_2 t_2) f(R_1, R_2) dR_1 dR_2 \quad (3.42)$$

Where the relaxation rates, R_1 and R_2 , are the inverse of the respective relaxation times, and $f(R_1, R_2)$ is the relaxation spectrum density. To use the ILT as an analysis method, the experimental signal must be discretized. This is done through discretizing the independent encoding times τ_1 and τ_2 into t_{ji} where $j = 1$ or 2 .

$$S(t_{1i}, t_{2j}) = \sum_{k=1}^{M_1} \sum_{l=1}^{M_2} (1 - 2\exp(-R_{1k}t_{1i})) \exp(-R_{2l}t_{2j}) f(R_{1k}, R_{2l}) + \epsilon_{i,j} \quad (3.43)$$

Through the ILT, the relaxation distribution map $f(R_{1k}, R_{2l})$ can be obtained. A schematic indicating the expectation of these 2D distributions is shown in Figure 3.5.

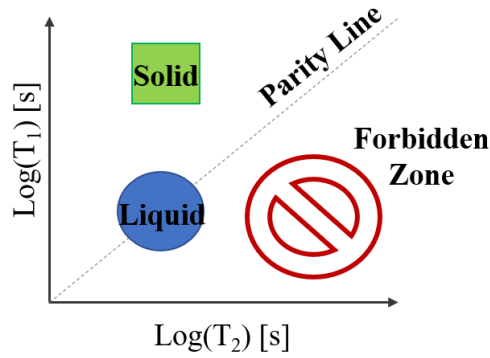


Figure 3.5: After an analysis with the ILT, a 2D distribution is obtained and a schematic of what can be expected is shown above. Due to the physical nature of relaxation, there is a nonphysical portion of the plot noted as the forbidden zone. In the physically relevant portions, information about the restriction in the system is found.

The diagonal dashed line through the plot is called the ‘parity line,’ which represents when $T_1 = T_2$. All populations which occur on the parity line are characterized as a liquid, more specifically as a non-viscous bulk liquid. Populations to the left the parity line are characterized as solids or highly viscous fluids. Recalling back to Chapter 2, $T_1 > T_2$ is the physical limitations of these parameters, thus populations which appear below the parity line are nonphysical and in the ‘forbidden’ region. However, sometimes in experimental analysis populations appear in this zone. This is an artifact due to an unnatural increase in the acquired signal at small inversion times shown in the raw time domain data (Figure 3.6).

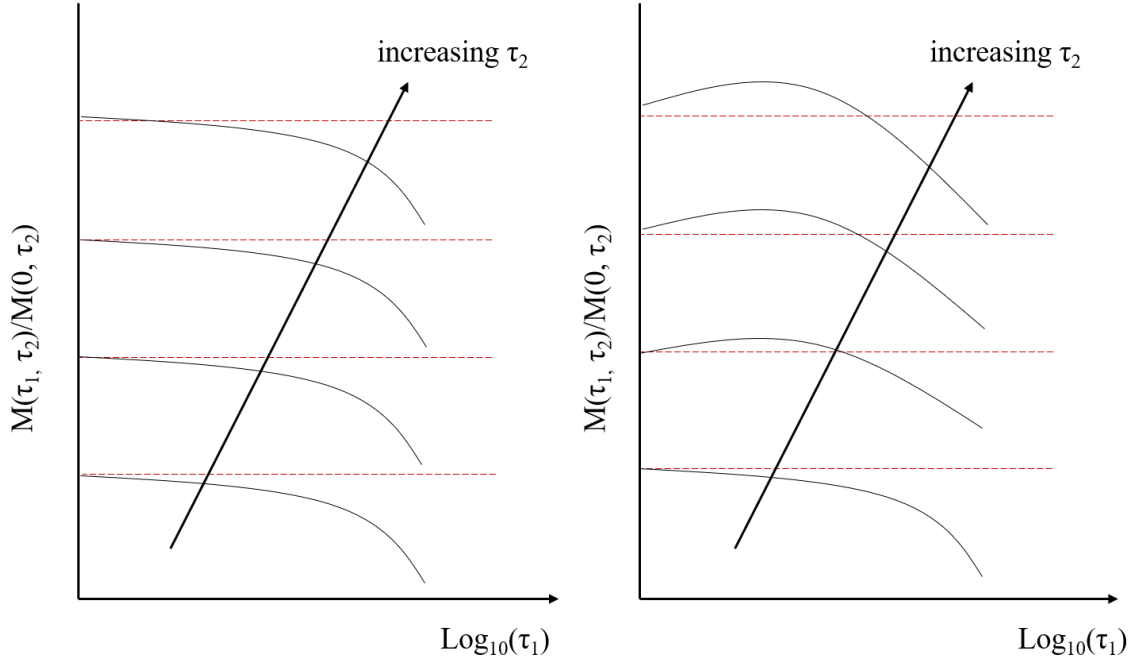


Figure 3.6: The time domain signal of the T_1 - T_2 experiment prior to the ILT is shown. It is expected that as the inversion time increases, and the echo number increases there is a monotonic decrease in signal due to relaxation (left). If there is a nonphysical peak in the forbidden region, there is always an increase in the signal at short inversion times and longer echo values (right). This phenomenon will be explored in more detail in Chapter 4.

Figure 3.6 shows a schematic of modified time domain data from the T_1 - T_2 experiment. The signal is modified in this manner to be fed into the ILT for analysis. What is expected is shown on the left, as we increase the inversion time, the magnetization monotonically decays. However, under certain conditions and certain systems an anomalous rise in signal is observed for short τ_1 times. This increase becomes larger at longer τ_2 times. The cause of the signal increase is due to complex diffusion and exchange dynamics in an interconnected structured. [11, 12] This will be discussed further in Chapter 5.

T_2 - T_2 Relaxation Exchange Experiments

The T_2 - T_2 relaxation exchange pulse sequence is implemented to explore exchange dynamics in a system (Figure 3.7 [1]). This experiment encodes for T_2 relaxation twice, separated by a mixing time, τ_m , where the magnetization is stored in the longitudinal domain. During this time, spins are allowed to migrate and exchange between domains. During the first encoding, the number of echoes is varied to observe both long and short relaxation times.

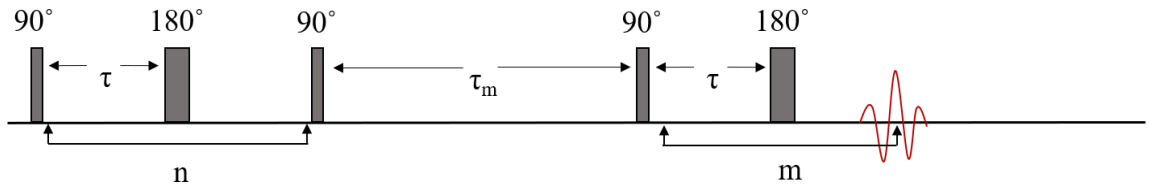


Figure 3.7: A T_2 - T_2 exchange experiment is shown above. T_2 relaxation is first encoded during the first CPMG train, the magnetization is then stored in the longitudinal plane while the spins are allowed to exchange for τ_m time. After which the spins are encoded for the second time with another CPMG train.

This experiment is implemented to gain insight into timescales of exchange occurring during the mixing time. After analysis through the ILT, the correlation map may look like Figure 3.8 shown below.

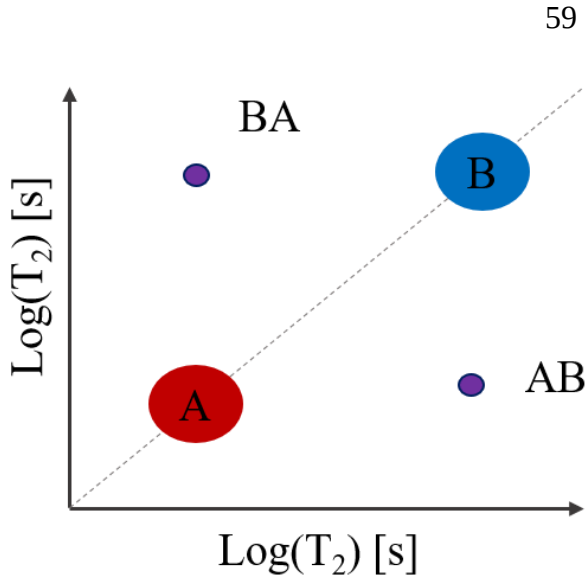


Figure 3.8: A T_2 - T_2 correlation exchange map is shown. The first and second encoding of relaxation is on the y and x axis respectively. Exchange is shown as off diagonal peaks indicating spins migrated from population A into population B or vice versa.

Any population residing on the dotted (diagonal) line indicates $T_{2(1)} = T_{2(2)}$ and no exchange occurred during the mixing time. However, populations which appear to the left or the right of the dotted line signify exchange occurred between populations during the mixing time. It is important to note the first T_2 encoding period, where the magnetization begins, is shown on the y-axis and the second T_2 encoding period, where the magnetization ends after the mixing time, is shown on the x-axis.

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CHAPTER FOUR

IMPACT OF XYLOSE ON DYNAMICS OF WATER DIFFUSION IN MESOPOROUS
ZEOLITES MEASURED BY NMR

Contribution of Authors and Co-Authors

Manuscript in Chapter 4

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Contributions: Designed and executed NMR experiments. Analyzed and interpreted data. Assisted in writing manuscript and provided comments and feedback.

Co-Author: Joelle E. Romo

Contributions: Conceptualized project. Created and provided catalytic beads. Helped with data interpretation. Provided feedback and comments on manuscript.

Co-Author: Stephanie G. Wettstein

Contributions: Conceptualized project. Helped with data interpretation. Provided feedback and comments on manuscript.

Co-Author: Joseph D. Seymour

Contributions: Assisted in design and implementation of NMR experiments.

Helped with data interpretation. Assisted in writing of manuscript. Provided feedback and comments on manuscript.

Manuscript Information Page

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IMPACT OF XYLOSE ON DYNAMICS OF WATER DIFFUSION IN MESOPOROUS ZEOLITES MEASURED BY NMR

Abstract

Zeolites are known to be effective catalysts in biomass converting processes. Understanding the mesoporous structure and dynamics within it during such reactions is important in effectively utilizing them. Nuclear magnetic resonance (NMR) T_2 relaxation and diffusion measurements, using a high-power radio frequency probe, are shown to characterize the dynamics of water in mesoporous commercially made 5A zeolite beads before and after the introduction of xylose. Xylose is the starting point in the dehydration into furfural. The results indicate xylose slightly enhances rotational mobility while it decreases translational motion through altering the permeability, K , throughout the porous structure. The measurements show xylose inhibits pure water from relocating into larger pores within the zeolite beads where it eventually is expelled from the bead itself.

Introduction

Pulsed gradient spin echo (PGSE), or pulsed field gradient (PFG) nuclear magnetic resonance (NMR) is a preeminent method for characterization of transport and structure in porous media systems. [1,2] Despite the fact that application to nanoporous systems is challenging, due to the small structural length dimensions which generate complex rotational and translational molecular dynamics over a hierarchy of scales, significant characterization of systems such as zeolites has been attained. [3–6] The application of zeolites in catalytic conversion of biomass to fuel and chemical products is

an area of growing application [7], and recent research has shown that zeolite beads have the potential to catalyze sugar to furan dehydration reactions [8]. Studies of water molecular dynamics in zeolites in the presence of biomolecules by NMR have been limited to solid state NMR spectroscopy, PGSE NMR using a single displacement observation time [9], and PGSE NMR to study water in zeolites [10,11] while solid state NMR has also been used to study solvents in zeolites [12]. Here displacement time-dependent PGSE NMR was applied to study the impact of xylose on water dynamics in zeolites for heterogeneous catalysis of sugars to furans. [8,13]

Zeolites are known for their chemical and thermal stability, versatility [14], and have been widely used in biomass conversion reactions [15–18] including xylose dehydration to furfural. For example, research by Gao et al. found xylose dehydration reactions with ZSM-5 resulted in furfural yields of 51.5% in an aqueous system. [17] Other researchers have looked at the use of powdered silicoaluminophosphates (SAPOs), a class of small-pore zeolites, in various solvent systems to maximize furfural production from xylose, achieving moderate yields. [15] In order to improve catalyst recovery, Romo et al. used dual-layered zeolite beads (versus powdered zeolites) to convert xylose to furfural and achieved yields of up to 45%, indicating zeolite beads have the potential for sugar upgrading. [8] Although microporous zeolites beads are promising for sugar dehydration reactions, the zeolite pore size can create a diffusion limited system. This is particularly true for substrates such as sugars, which have large kinetic diameters.

Commercially available Linde Type A (LTA) zeolite beads consist of $\sim 3\ \mu\text{m}$ crystallites made up of $\sim 5\text{\AA}$ molecular cages, which are compressed with binder into 3

mm beads that are 86% microporous. [8] Molecular transport in zeolite systems of this type of structure have been modeled as porous media with periodic permeable inclusions [6,19,20]. This results in an effective $1/D_{eff} = 1/D_o + 1/Kl$ where D_o is the molecular diffusion within a pore structure and K is the permeability, reflecting transport resistance between pore structures separated by length scale l . [19,20] In the zeolite system studied here, D_o is the diffusion within the zeolite crystal and K the permeability at the zeolite crystal grain interfaces within the 3 mm bead and D_{eff} the NMR measured diffusion.

Diffusion measurements by NMR are obtained by application of magnetic field gradient pulses which attenuate the voltage signal due to magnetization dephasing caused by random diffusive motion. The measured signal normalized by the signal with no gradient is given by $E(g, \Delta) = \frac{S(g, \Delta)}{S(0, \Delta)} = \exp \left[-\gamma^2 g^2 \delta^2 D \left(\Delta - \frac{\delta}{3} \right) \right]$, where γ is the gyromagnetic ratio, g is the gradient amplitude, δ is the gradient duration, and Δ is the gradient separation, which is the time the nuclei are allowed to displace. PGSE NMR thus measures the time dependent effective diffusivity $D(\Delta)$, which characterizes the length scale of the restricted diffusion dynamics of a fluid in a pore at short times, as. [2,21,22]

$$D(\Delta) = D_o \left[1 - \frac{4}{9\sqrt{\pi}} \frac{S}{V} (D_o \Delta)^{1/2} \right] \quad (4.1)$$

Here D_o is the free liquid diffusion, D the PGSE NMR displacement time for the spins, and $S/V = 3/R$ the surface to volume ratio of a spherical pore of characteristic length scale radius R . While the normalized form of the measured signal, by $E(g, \Delta)$, factors out T_1 spin-lattice and T_2 spin-spin magnetization relaxation effects, the measured signal is weighted by the T_2 relaxation if T_2 times are present which are less than the

PGSE echo time, as the signal from those spins are fully relaxed before being encoded for diffusive motion. T_2 relaxation is due to dipolar coupling of the NMR active spins, ^1H protons in the experiments conducted in this work, and interaction with solid surfaces in porous media. Longer T_2 relaxation times occur when the dipolar coupling is averaged out by rotational diffusion, as in liquids, and shorter when rotational mobility is restricted.

Results

T_2 relaxation measurement of water xylose solution in bulk has a large peak at 1167.1 ms due to water and a small peak at 126 ms from the xylose. T_2 relaxation of pure water and of the 20% wt. xylose solution in the 5A bead indicate two primary populations of relaxation behavior as shown in Figure 4.1. The fast relaxing, short T_2 relaxation populations are at 0.619 ms for water and 0.752 ms for the xylose solution. These sub millisecond relaxation times demonstrate the significant restriction of rotational mobility and interactions of primarily water and the zeolite surfaces (kinetic diameter 2.7 Å) [23] in the zeolite micropores, since the xylose (kinetic diameter 6.8 Å) [24] is too large to be within those pores. The presence of the xylose generates an increase in the T_2 relaxation time. The slower relaxing, longer T_2 populations in the zeolite beads at 8.300 ms for pure water and 9.100 ms for the xylose solution are due to the molecules in the inter-crystalline mesopores of the bead. In this more mobile population, the presence of the xylose induces a slight increase in the rotational mobility of the protons in the system. In the pure water in zeolite system there is a small peak at 806 ms which is associated with water in macro pores or leaking to the bead surface, while this population is suppressed in

the xylose solution system. Due to only 20% of the solution being xylose, the signal is primarily from water. Spectral resolution is not possible in the zeolite beads due to the signal broadening caused by the restrictions of the solution within the zeolite, and the T_2 relaxation distributions show little signal changes after the introduction of xylose. Therefore, the NMR signal obtained will be primarily attributed to water within the system.

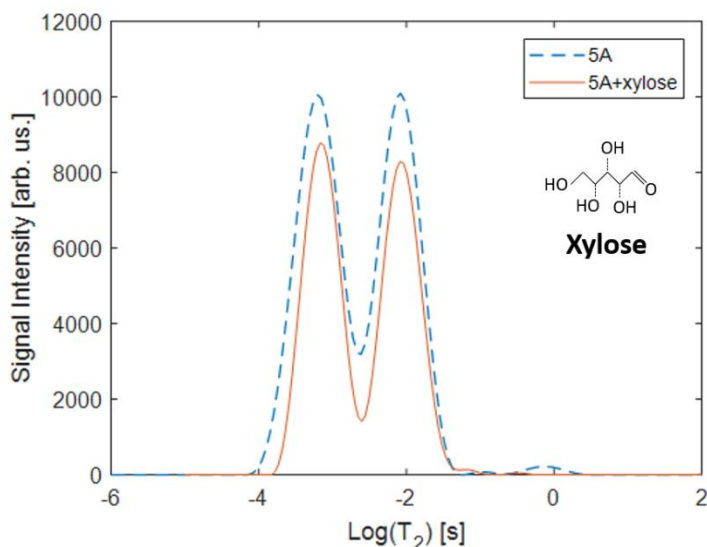


Figure 4.1. T_2 distributions comparing the core zeolite Grace 5A beads before and after the addition of 20% wt. xylose to the water permeating through the system. In the 5A beads with water there are two large populations at $T_2 = 6.193 \times 10^{-4}$ s and 8.300×10^{-3} s and a small population at longer $T_2 = 0.806$ s. The presence of xylose eliminates the longer relaxation component and slightly shifts the two dominant populations to slightly longer relaxation times $T_2 = 7.518 \times 10^{-4}$ s and 9.100×10^{-3} s. The 20% wt. xylose in water solution has a large relaxation population at $T_2 = 1.167$ s and a small population at 1.260×10^{-1} s representing the biopolymer and water relaxation times (not shown). The chemical structure of xylose is shown in the upper right.

The displacement time dependent pulse gradient stimulated echo (PGStE) NMR signal attenuation data as a function of increasing pulsed gradient strength is shown in [Figure](#)

4.2. The data are plotted in a standard Stejskal–Tanner plot format in which the slope of the curve indicates the diffusion coefficient. [2] The data exhibits biexponential behavior with a fast and slow diffusing component. It is important to note that the ^1H proton signal measured comes only from the longer T_2 relaxation population since the sub millisecond relaxing populations are filtered out by the 4.32 ms echo time of the stimulated echo experiment. The slow diffusing component increases in quantity as a percentage of the total signal as the displacement observation time is increased.

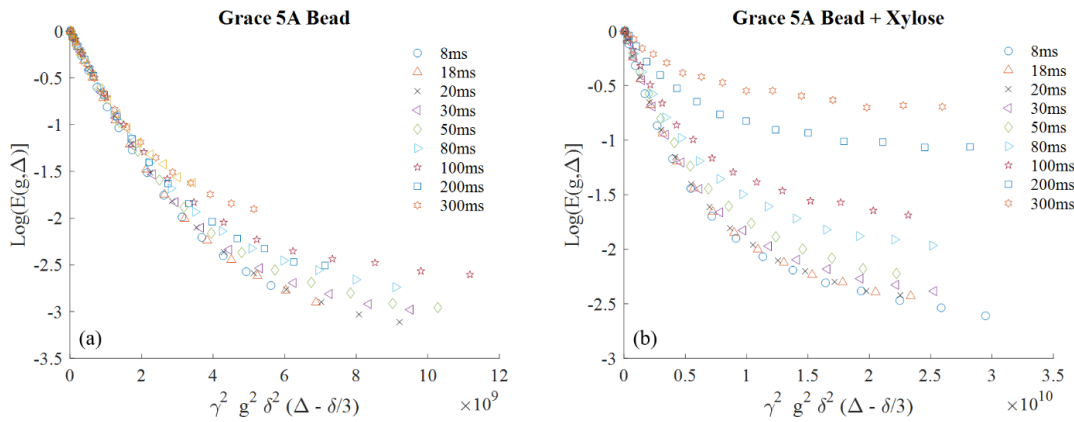


Figure 4.2. Stejskal–Tanner plots of Grace 5A beads (a) and Grace 5A beads with xylose (b) are shown. With increasing displacement observation time Δ from 8 to 300 ms, the attenuation of signal decreases for the pure water and xylose solution saturated beads. The gradient duration δ was 1 ms with a maximum gradient ranging from 0.500 to 1.9021 T/m in order to sample out to a similar point in the gradient domain. The data are well fit by a biexponential model with a fast and slow diffusion component $p_f \exp[-D_f x] + p_s \exp[-D_s x]$

This can be seen by fitting a biexponential model $p_f \exp[-D_f x] + p_s \exp[-D_s x]$ to the data and determining the population in the fast and slow decay regions, Figure 4.3. In the pure water system, there is an initial increase in the amount of fast diffusion component, and commensurate decrease in the slow diffusion population,

which is associated with the water moving into larger pore regions of the beads during the 20 min of each initial short displacement time Δ experiment. After the $\Delta = 50$ ms experiment, the signal proportion in the slow component increases. This can be attributed to a transient redistribution of the pure water into larger pore spaces followed by a loss of the water signal in the large pores due to dephasing of the signal or drainage from the large pores out of the sensitive region of the rf coil over the hours long experimental time for all the displacement times Δ . While any long-time scale transient redistribution of the water prohibits determining exchange between the fast and slow diffusion populations it does not negatively impact the assessment of the length scales associated with the diffusion dynamics. Of interest is the impact of the xylose in solution on the distribution of ^1H proton signal in the fast and slow diffusion populations, in that it generates a more significant loss of fast diffusion signal. The xylose maintains more liquid within the slow diffusion population than the pure water and inhibits the initial redistribution of the water into the larger pores during displacement times $\Delta < 50$ ms. experiments.

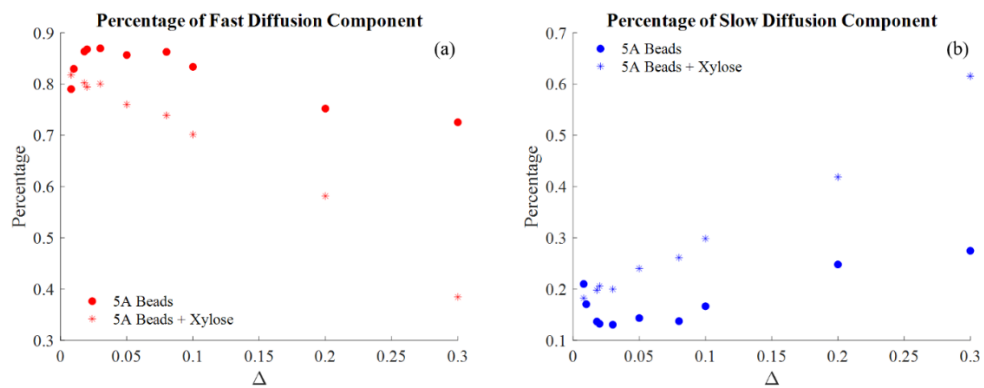


Figure 4.3. The population percentage of the fast (a) and slow (b) diffusion populations with varying Δ . The signal is weighted toward the slower diffusing regions before and after the addition of the xylose with increasing Δ .

The primary results of the PGStE measurement are the $D(\Delta)$ data for the fast and slow diffusion populations shown in [Figure 4.4](#). The data are plotted against $\Delta^{1/2}$ so that the short displacement time data provide S/V and the long displacement time data provide the tortuosity. [\[21,22\]](#) The beads with pure water show an increase in diffusion coefficient for both the fast and slow component for displacement times $\Delta > 80$ ms. This is consistent with a possible draining of the beads over the total experimental run and the loss of the fast diffusion population and precludes determination of the tortuosity, however further studies are required to determine the origin of this effect. The decrease in the fast diffusion coefficient with pure water to a value less than 1/2 that of free water indicates a diffusion length scale of the order of $l = (2D\Delta)^{1/2} = 9.49\mu m$ at $\Delta = 50$ ms, representative of multiple mesopore transport. The xylose solution is much more restricted in the largest pores and has a reduction in diffusion to less than 1/5 the free water value. The slow diffusion data for the pure water in the bead displays the classic $\Delta^{1/2}$ decay predicted by theory at short times. [\[21,22\]](#) Determination of the length scale from Equation (4.1) gives $R \sim 3.87 \mu m$ consistent with the inter-crystal mesopore length. The reduction of the slow diffusion coefficients in the xylose solution beads relative to the beads in pure water indicates a decrease in the permeability K at the zeolite crystal grain surfaces.

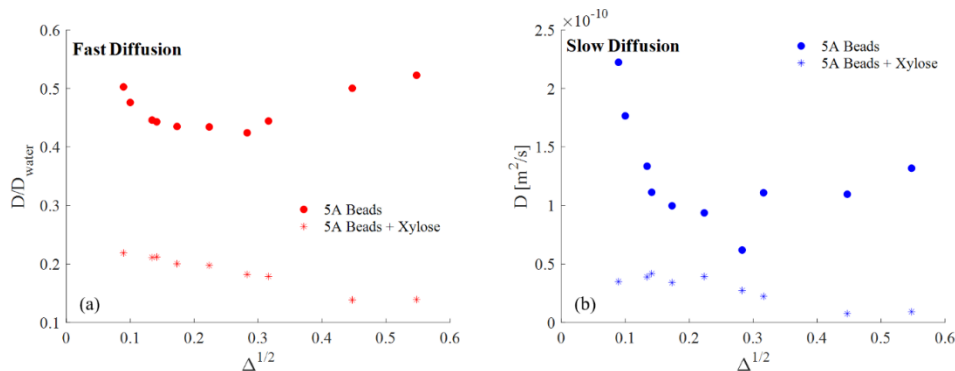


Figure 4.4. Diffusion coefficients calculated from the biexponential fits to the Stejskal–Tanner plots at each Δ , plotted against $\Delta^{1/2}$ for the zeolite beads with the pure water and xylose solution for the fast (a) and slow (b) diffusion component. The fast diffusion is plotted normalized by the free water diffusion coefficient $2.0 \times 10^{-9} \text{ m}^2/\text{s}$. The presence of the xylose significantly decreases both the fast and slow diffusion coefficients in the beads. The increase in diffusion of pure water in the zeolite at longer Δ indicates some nonstationary water redistribution.

Discussion

The NMR data presented indicate the presence of a biomolecule such as xylose alters the rotational mobility of water in zeolite crystal beads slightly, while generating larger changes in translation mobility. The xylose solution alters the interplay of fluid distribution between fast and slow diffusion populations. The increase in the percentage of fluid undergoing slow diffusion in the xylose solution relative to the pure water implies that the fluid in the largest pores is more prone to drain from the beads. Any seepage from the larger pores means caution must be used in interpreting changes in the fast diffusion coefficient with displacement observation time Δ . The slow diffusion component is associated with the zeolite crystal length scale. The slow diffusion coefficients measured for the pure water and the xylose solution indicate the xylose decreases the permeability at the zeolite crystal interfaces. The xylose also makes the

slow diffusion behavior less time dependent over displacement timescales from $\Delta = 8$ to 100 ms than in pure water, consistent with a decrease in permeability restricting water translational diffusion even on shorter timescales. This demonstrates that xylose dehydration reactions in zeolite bead catalysts can be impacted by diffusion limitations, even with mesoporosity.

Materials and Methods

Commercially obtained Zeolite 5A beads (W. R. Grace & Co., Columbia, MD, USA) are composed of 4.2 Å molecular cages grown to ~ 3 µm crystallites indicated by manufacturer's data and then compressed with binder into 3 mm beads. The exact crystal size within the bead is not measured, but there is crystallinity as seen by the XRD reported in our previous manuscript [8]. The beads were first saturated with water by placing 0.3 g of beads into a 15 mL pressure tube. Approximately 4 g water and a stir bar were added to the tube and then sealed with a Teflon cap with front-seal Kalrez o-ring. The pressure tube was then placed in an oil bath set at 443 K for 10 min with a stir rate of 600 rpm. The beads were then filtered from the liquid, gently blotted with a paper towel, and then placed in the NMR tube. For the beads containing xylose, a similar method was used, but the water was replaced with a 20% wt. xylose solution.

After the heat treatment period the catalytic beads were placed in a 5 mm glass NMR tube were returned to room temperature and loaded into the NMR probe in the magnet. Diffusion and T_2 relaxation experiments were performed in a Bruker 250 MHz superconducting magnet using a custom built high-power ^1H rf probe and 5 mm rf coil (Bruker, Karlsruhe). Sample temperature was controlled through the Bruker BTU system

with N₂ gas flow and kept at 292 K throughout all experiments. A standard Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence [1,2] was used for T_2 measurements with an echo time of $\tau_E = 192 \mu\text{s}$, 10,000 echoes, a dwell time of $4 \mu\text{s}$, and $7.4 \mu\text{s}$ 180° rf pulses at power of 100 W. Each measurement had 64 averages. The diffusion measurements were acquired using a pulsed gradient stimulated echo (PGStE) pulse sequence [1,2] with $\delta = 1$ ms, with maximum gradients ranging from 1.9021 T/m to 0.5003 T/m dependent on the displacement time Δ value which spanned 8–300 ms. The stimulated echo pulse sequence with an echo time of $\tau_E = 4.32$ ms was averaged 64 times.

The T_2 relaxation experimental data was processed through a Fredholm integral, also commonly described as an inverse Laplace transform method. The diffusion data were not analyzed through this method due to having only 16 echoes. This analysis technique works optimally with more echoes. The diffusion data were fit directly from the Stejskal–Tanner plots using a bi-exponential fitting process to obtain the diffusion coefficients.

Conclusions

NMR can provide data on the impact of biomolecules on the translational and rotational dynamics of water in zeolite beads. The significant decrease in diffusion due to decreased permeability at the zeolite crystal interfaces could impact the reaction dynamics and the catalyst performance.

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CHAPTER FIVE

TRANSPORT MODEL FOR INTERPRETING T_1 - T_2 MEASUREMENTS IN COMPLEX
SYSTEMSContribution of Authors and Co-Authors

Manuscript in Chapter 5

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Contributions: Assisted in design and implementation of NMR simulation and experiments. Helped with data interpretation. Assisted in writing of manuscript. Provided feedback and comments on manuscript.

Manuscript Information Page

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TRANSPORT MODEL FOR INTERPRETING T_1 - T_2 MEASUREMENTS IN COMPLEX SYSTEMS

Abstract

A 1D two region coupled pore model with discrete pore coupling is developed to elucidate the eigenmode interactions in regions with different surface relaxivity. Numerical solution of the model and simulation of the correlation experiment for varying surface relaxivity, pore connectivity and pore size ratio indicate the role of negative eigenmodes and overlap of T_1 and T_2 eigenmodes in generating a time domain signal increase with inversion recovery time, t_1 . The eigenmodes and eigenfunctions are considered in detail providing connection between the mathematical model and the diffusion dynamics and spin physics of the system. Physical systems, *i.e.* a microporous glass bead pack, a cyclopentane/water hydrate former, and beeswax, showing experimentally measured T_1 - T_2 time domain signal rise are considered within the limitations of the model.

5.1: Introduction

Transverse (spin-spin) and longitudinal (spin-lattice) relaxation have long been known to depend on pore size and surface proportions in porous media [1] and material properties such as molecular density and viscosity [2]. Restrictions in rotational and translational diffusion due to the physicochemical environment of the spin-bearing molecules generate variations in relaxation process that lead to important effects in observed relaxation behavior. Multi-dimensional relaxation methods provide the means

to study the coupling of T_1 and T_2 relaxation mechanisms, or modes, through T_1 - T_2 correlation experiments and the interplay of T_2 - T_2 correlations over a T_1 storage time [3-5]. A complicating aspect of such measurements is the inversion of the time-domain data from an experiment by a regularized Fredholm integral method *i.e.* inverse Laplace transform (ILT) [6], or other nonlinear inversion method. The presence of negative eigenmodes in the spectral form of the data and the implications of these types of modes for inversion stability in such spectra has been broadly discussed [7-10] .

Analysis of T_1 - T_2 correlation and T_2 - T_2 exchange experiments by direct use of the time-domain voltage signals provides a route to avoiding the artifacts due to data inversion [8, 9, 11]. The interpretation of exchange phenomena in the time domain of T_2 - T_2 experiments indicate signal curvature, a *cosh* modulation, due to exchange dynamics between two T_2 populations [11]. In T_1 - T_2 experiments, Song *et al.* [8] have demonstrated an increase in signal amplitude with inversion recovery time, t_1 for different echo times $t_2 = n\tau$ due to coupling of pore spin dynamics in a two-pore porous media and discussed the effect in terms of negative eigenmodes in the T_1 - T_2 spectra. In this paper, the eigenfunction solution framework of Song, *et al.* [12] is further developed to explore a coupled pore system and understand anomalous observed voltage signal increases in T_1 - T_2 experiments by time-domain analysis. It is found that negative eigenvalue modes are a necessary, but not sufficient condition, for the signal increase.

The motivation for the development in this paper is two-fold. First, the model, which explicitly couples the pores through magnetization conservation and exchange [13, 14], provides enhanced understanding of the T_1 - T_2 experiment for the characterization of

classical multiscale porous media [15]. Second, the model and its solution can provide routes to interpretation of molecular dynamics in reacting and soft matter systems in which such signal increases have been observed. Specifically, in addition to the first observations of signal rises in the T_1 - T_2 signal [7], signal-rise results have been observed in a model hydrate forming system [16], a polymer-solvent system undergoing a glass or gel transition [17] and waxes undergoing solid-solid rotator phase transitions [18]. In order to develop a solid basis for interpretation in this wide range of systems, the model is analyzed over a range of parameters related to pore- or population-size ratio, pore connectivity or exchange between pores, and the relaxation modes specific to each pore type. The analysis allows identification of regions of parameter space for which the increase in signal with experimental inversion recovery time t_1 is predicted, demonstrating that the coupling of the T_1 - and T_2 -relaxation modes in individual pores can be probed by the T_1 - T_2 experiment. The relaxation modes are coupled in terms of whether they are in the fast or slow diffusion limit [19].

In Section 5.2, we present the development of a coupled, two-region model for T_1 - T_2 signal prediction by extending the framework for the single-region model presented by Song, *et al* [12] through the use of an exchange boundary condition at the pore-pore interface [13, 14]. The model parameter space is then explored in Section 5.3 to demonstrate the range of behaviors that might be observed in a T_1 - T_2 experiment, including the signal rise behavior noted above. In Section 5.4, the model is then considered in the context of measurements in three different two-phase/two-region

systems. Section 5.5 synthesizes the results and insights from application of the model to provide some useful guidelines for using the T_1 - T_2 sequence in complex settings.

5.2: Model Development

5.2.1: Settings and Assumptions

The model to be developed here addresses the generic problem of the dynamics of diffusion and relaxation in multi-phase, multi-region systems using the ideas first proposed in an NMR context by Brownstein and Tarr [19, 20]. By the term *phase*, we mean a portion of a system with distinctly different transport properties (flow, diffusion and reaction, etc.) or NMR properties (relaxation, magnetization, etc.). These distinct properties are homogeneous in character, *i.e.* they occur within the volume of the phase, and so appear as part of the differential balance expressions for the model. For example, a phase might be composed of a porous media (a mixture of fluid and solid materials) and be characterized, through coarse-graining (averaging, smoothing), by an effective diffusion coefficient and effective NMR longitudinal and transverse relaxation times that would appear in the Bloch-Torrey equations [21]. The model of Belton and Hills [14] is an example of two-phase model in which the relaxation and transport properties change across interfaces in a system made up of plant cells or heterogeneous gels.

The term *region*, will identify a portion of the system with distinct length-scale features but not distinct transport or relaxation properties that would characterize a phase. That is, relaxation and transport properties do not change across regions as they would for phases. The two-pore model presented by Zielinski, *et al.* [15] is an example of a two-region system composed of regions with different length scales (a small pore coupled to a

large pore) but with the diffusion and relaxation properties that are the same in each of the pores. The random-walk simulations of Song, *et al.* [8] for diffusion and relaxation in porous glass particles is also a two-region model in that it explicitly assumes constant diffusion and relaxation parameters throughout all pore regions.

This study will develop a two-region model; specifically a two-pore model, based on the ideas put forth in Zielinski, *et al.* [15] and incorporating the boundary condition suggested by Belton and Hills [13, 14] for inter-region exchange and relaxation processes. Figure 5.1 presents the features of single- and two-pore models that we might consider. The single-pore model (Fig. 5.1a) consists of a pore with a characteristic length scale, L . In the interior of the pore, spins diffuse with diffusion coefficient D , and relax with relaxation rates $R_1 = 1/T_1$ and $R_2 = 1/T_2$. The pore surface, A_s , is impermeable to the spins, but the spins can undergo surface relaxation with surface relaxivity rate coefficient ρ_1 for longitudinal magnetization and ρ_2 for transverse magnetization. Computations for this system will follow the developments of Song, *et al.* [12] and simplify modeling efforts by using one spatial dimension (Fig. 5.1c).

Features of a two-pore model are shown in Figure 5.1b. This system is composed of pores with two distinct pore sizes, L_A , taken as the larger pore and L_B , the smaller pore. The pores are primarily enclosed by surfaces that have the same type of relaxation characteristics as the single-pore model. The pores can have different values for the surface relaxation rate constants, ρ_i , ($i = 1, 2$) but have the same *homogeneous* relaxation rate constants, $R_i = 1/T_i$ and diffusion coefficients. The pores can be connected

(coupled) over the joint areas denoted A_i . The nature of the dynamics on these interfacial surfaces is key to the distinctive character of the two-pore model [13, 14].

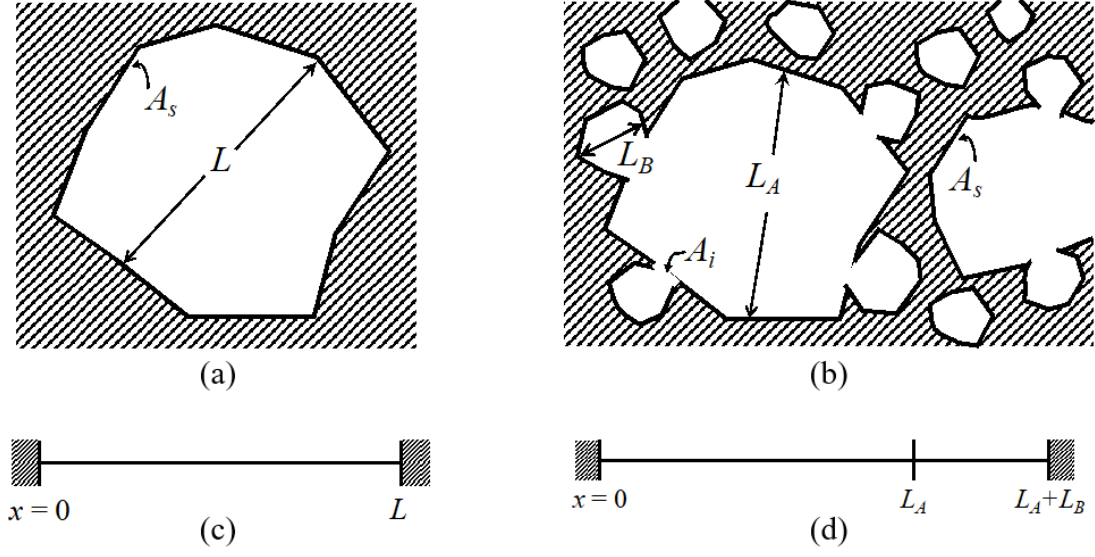


Figure 5.1: Settings and geometries for a single-pore (a), (c) and two-pore (b), (d) models.

As was done in the single-pore case, computations for the two-pore model are simplified by using the one-dimensional geometric representation shown in Figure 5.1d. In the reduction from the two- and three-dimensional reality of the pore setting to a one-dimensional computational setting, detail is lost. Hence, the specific property parameters (used in the one-dimensional version of the two-pore model) should be considered as estimates of intrinsic values.

5.2.2: The Pulse Sequence

The aim for developing the model is to simulate the time-domain signal from an NMR pulse sequence. The T_1 - T_2 pulse sequence is summarized concisely as $[\square - t_1 - \square/2 - t_e - (\square - t_e - acq - t_e)_n]$ [3, 8]. The duration of the inversion-recovery interval, t_1 , following the initial π -pulse is stepped through a series m of logarithmically spaced values and encodes the longitudinal-relaxation characteristics of the material into the signal. At the end of a t_1 -interval, a $\frac{\pi}{2}$ -pulse generates a time-domain signal from the sample. This signal is acquired as a Carr-Purcell-Meiboom-Gill (CPMG) echo train using a π -pulse separation time of $2t_e$ and consisting of N data points acquired at equal spaced times $t_{2,n} = 2nt_e, n = 1, 2, \dots, N$. This portion of the sequence encodes the transverse-relaxation characteristics of the material into the signal. The net result of the experiment is a two-dimensional array of voltage signals covering the full extent of the t_1 and t_2 values, $s_{m,n} = s(t_{1,m}, t_{2,n})$.

5.2.3: Governing Equations

The goal now is to develop a model that predicts the signal for the experiment. We assume the pulses in the sequence are hard pulses whose time duration is short compared to all other processes of interest in the system. The RF pulses thus set the initial conditions for simulating the evolution of the spin system during the inter-pulse intervals. A predictive model of the system evolution between the pulses is based on the formalism proposed by Song, *et al.* [12] and on the use of the Bloch-Torrey equations [22] to describe the dynamics of diffusion and relaxation processes. The magnetic field within the pores is assumed to be uniform so that dephasing due to diffusion in a non-

uniform field caused by susceptibility differences in the sample can be ignored. The Bloch-Torrey (BT) equations for a phase during the inter-pulse intervals then take the form,

$$\frac{\partial M_T}{\partial t} = D \nabla^2 M_T - R_2 M_T \quad (5.1)$$

for the transverse magnetization and

$$\frac{\partial M_z}{\partial t} = D \nabla^2 M_z - R_1 (M_z - M_{eq}) \quad (5.2)$$

for the longitudinal magnetization. If we define the generic phase magnetization $m_i = M_{T,i}$ for the transverse magnetization in phase i or $m_i = M_{zi} - M_{eq,i}$ for the longitudinal magnetization for phase i , Eqs. (5.1) and (5.2) become

$$\frac{\partial m_i}{\partial t} = D_i \nabla^2 m_i - R_{ij} m_i \quad i = A, B \quad j = 1, 2 \quad (5.3)$$

Eq. (5.3) encompasses both the Bloch-Torrey equations, which explores the effect of diffusion on evolving magnetization [22], and the Bloch-McConnell equations, which explores the effect of reaction and chemical exchange and relaxation on evolving magnetization [23]. For the Bloch-McConnell equations, $\frac{\partial m_i}{\partial t} = (-R_{i,1} - k_{i,1})(m_i - m_i^0) + k_{j,1}(m_{j,1} - m_{j,1}^0)$, where i and j are the pore regions, diffusion is neglected to focus on reaction exchange mechanisms [23] indicated by $k_{i,1}$. This method has been used to robustly quantify exchange occurring in various systems [5, 24-26]. However, in this work, we focus on the diffusion equation parameters and assume a homogenous and diagonalized bulk relaxation.

This two-phase formulation provides direction for a more detailed model but the focus of the present study is to develop a two-pore, *i.e.*, a two *region*, model. The regions share common values of the diffusivity ($D_i \rightarrow D$) and relaxation rates ($R_{ij} \rightarrow R_j$). These simplifications permit the elimination of the homogeneous relaxation term from Eq. (5.3) by using the transformation $m_i = u_i e^{-R_j t}$ to obtain

$$\frac{\partial u_i}{\partial t} = D \nabla^2 u_i \quad i = A, B \quad (5.4)$$

From this result, we note two important items. First, the diffusion equations in Eq. (5.5) take the same mathematical form for both longitudinal and transverse components and so a common solution process for simulating the pulse sequence will be effective. Second, the homogeneous relaxation processes will not play any role in the interpretation of the final results as they have been “factored out” of the solution.

As shown in Figure 5.1, the surface area of the pores in a two-pore system can be divided into separate categories each requiring potentially different boundary conditions (B.C.’s) to characterize them: areas over which there is relaxation of the magnetization at the fluid-solid surfaces (A_s) and areas across which the pores are connected (A_i). At the fluid-solid interfaces, the rate of surface relaxation is matched by the flux of magnetization to those surfaces

$$\mathbf{n} \cdot D \nabla u_i|_{A_s} = -\rho_{ij} u_i|_{A_s} \quad i = A, B \quad j = 1, 2 \quad (5.5)$$

where ρ_{ij} is the surface relaxation rate constant for relaxation time mode j (longitudinal relaxation time mode $j = 1$, transverse relaxation time mode $j = 2$), at a fluid-solid surface in pore region i . At the pore-pore boundaries, two B.C.’s are needed since both u_A and u_B

are present. Assuming these interfaces do not accumulate magnetization leads to the condition

$$\mathbf{n} \cdot D \nabla u_A|_{A_i} = \mathbf{n} \cdot D \nabla u_B|_{A_i} \quad (5.6)$$

Though retained in the expression, the diffusion coefficient plays no role in this boundary condition. The second B.C.'s at the pore-pore interfaces expresses the mechanism of inter-pore transport as

$$\mathbf{n} \cdot D \nabla u_A|_{A_i} = k_c (u_B - u_A)|_{A_i} \quad (5.7)$$

where k_c is a interface conductance, *i.e.*, $1/k_c$ is a interface resistance, which controls exchange of magnetization between regions, analogous to the membrane permeability used by Belton and Hills [13, 14].

In the limit as $k_c \rightarrow \infty$, the pore-pore interface offers no resistance to the exchange of magnetization and Eq. (5.7) reduces to an expression of the continuity of magnetization $u_B|_{A_i} = u_A|_{A_i}$ on those interfaces. In the limit as $k_c \rightarrow 0$, the boundary is impermeable and Eq. (5.7) reduces to separate no-flux conditions, one for each region (*i.e.*, $\mathbf{n} \cdot \nabla u_A|_{A_i} = 0$ and $\mathbf{n} \cdot \nabla u_B|_{A_i} = 0$). Depending on the exact nature of the interface, this no-flux condition might not be an appropriate limiting behavior. For example, Zielinski, *et al.* [15] added a relaxation term to the inter-pore boundary condition in the development of their model. As a result, when $k_c \rightarrow 0$ in their model, these portions of the pore surfaces would still act as relaxation sinks for magnetization in the pores with the potential for a different rate of relaxation than the fluid-solid surfaces of the pores. To allow for flexibility in handling these limiting behaviors and provide a

physically consistent model with respect to the connection between pores, we follow Zielinski, *et al.* [15] and add a surface-relaxation term to B.C.'s at the pore interfaces (Eq. (5.7)). Hence, at the surfaces A_i , we will use the conditions

$$\mathbf{n} \cdot D \nabla u_A|_{A_i} - \rho_{c,Aj} u_A|_{A_i} = \mathbf{n} \cdot D \nabla u_B|_{A_i} - \rho_{c,Bj} u_B|_{A_i} \quad (5.8a)$$

$$\mathbf{n} \cdot D \nabla u_A|_{A_i} - \rho_{c,Aj} u_A|_{A_i} = k_c (u_B - u_A)|_{A_i} \quad (5.8b)$$

where $\rho_{c,ij}$, $i = A, B$, and $j = 1, 2$ are relaxation rate constants specific to interfacial areas between pores.

5.2.4: Scaling and Solution

The one-dimensional Cartesian version of the diffusion equation with the geometric parameters given in Figure 5.1d will be the basis of the solutions we present. L_A is used as a length scale for the model, $t_D = L_A^2/D$ is used as the time scale, and M_{eq} is used to scale magnetizations. The key dimensionless parameters in the model are then the region-size ratio ($\beta = L_B/L_A$), the surface-relaxation Damköhler numbers of the second kind [27] ($\delta_{i,j} = \rho_{i,j} L_A/D$), the interface-relaxation Damköhler numbers ($\delta_{c,ij} = \rho_{c,ij} L_A/D$), and the scaled inter-pore transfer coefficient ($\kappa_c = k_c L_A/D$).

There are 6 parameters in the model and that number can be reduced by connecting the interface-relaxation to the corresponding surface-relaxation through

$$\delta_{c,ij} = \frac{\delta_{i,j}}{1 + \kappa_c} \quad (5.9)$$

This relation provides useful limiting physical behaviors. As $\kappa_c \rightarrow 0$, $\delta_{c,ij} \rightarrow \delta_{i,j}$ so that when the pores are not connected, all pore surfaces are surfaces with the same relaxation characteristics. As $\kappa_c \rightarrow \infty$, $\delta_{c,ij} \rightarrow 0$ so that when the regions are fully connected, there

is neither interface resistance nor interface relaxation at these boundaries. Hence, the boundary conditions serve to enforce the continuity of magnetization and magnetization flux at the interface between the regions and the system becomes a single-pore system with length $(1 + \beta)$. Clearly other relations between $\delta_{i,j}$ and $\delta_{c,ij}$ could be proposed but the expression above is simple, reduces the number of parameters in the model, and provides useful limiting behavior for our model of a two-pore system.

The full model for diffusion and relaxation during an inter-pulse interval is summarized in Appendix A. Here, we provide the essential features of the solution needed for the discussion to come. The solution for the scaled magnetization components has the form

$$u(z, \tau) = u_A(z, \tau) + u_B(z, \tau) = \sum_{k=1}^{\infty} a_k e^{-\lambda_k \tau} \phi_k(z) \quad (5.10)$$

The key computational problem involves finding the eigensystem of the problem, $\{\lambda_k, \phi_k(z)\}$, consisting of the eigenvalues (λ_k) and their associated eigenfunctions ($\phi_k(z)$).

The eigenvalues will lead to the observed/effective relaxation rates due to the interaction of diffusion and surface relaxation within a pore characterized by the Damköhler numbers $\delta_{i,j}$, and inter-pore exchange of magnetization, characterized by the inter-pore exchange coefficient, κ_c . Each portion of the T_1 - T_2 pulse sequence will have its own eigensystem and so, we will refer to the T_1 eigensystem for the dynamics during the inversion-recovery portion and the T_2 eigensystem for the dynamics of the spin-echo portions.

5.2.5: Pulse-Sequence Simulation

Using the solutions and eigensystems outlined in Appendix A, the signal from the T_1 - T_2 sequence can be predicted following the framework provided by Song, *et al.* [8]. Appendix B provides details of the development and here we summarize the key portions of that simulation. During the inversion-recovery interval, the magnetization evolves according to

$$u(z, \tau) = \sum_m a_m^{(1)} e^{-\lambda_m^{(1)} \tau} \phi_m^{(1)}(z) \quad 0 < \tau < \tau_1 \quad (5.11)$$

where $\tau_1 = t_1/t_D$ is the scaled duration of the T_1 evolution interval. With the state of the system at the end of the τ_1 interval, $u(z, \tau_1)$, providing initial conditions, the system evolves during the spin-echo interval according to

$$u(z, \tau) = \sum_n a_n^{(2)} e^{-\lambda_n^{(2)} (\tau - \tau_1)} \phi_n^{(2)}(z) \quad (\tau > \tau_1) \quad (5.12)$$

The signal for the experiment is acquired during this interval and, using $h(z)$ to represent the coil response function[28], the final expression for the signal is (Appendix B, Eq. (B. 7)),

$$\begin{aligned} S(\tau_1, \tau_2) &= \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} p_m^{(1)} G_{mn} h_n^{(2)} e^{-r_m^{(1)} \tau_1} e^{-r_n^{(2)} \tau_2} \\ &= \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} P_{mn} e^{-r_m^{(1)} \tau_1} e^{-r_n^{(2)} \tau_2} \end{aligned} \quad (5.13)$$

where $r_k^{(i)} = r_i + \lambda_k^{(i)}$ are the observed relaxation rates. This form of the signal clearly shows that an ILT will recover the amplitudes P_{mn} of the two-dimensional relaxation spectrum of the system.

The amplitudes P_{mn} in Eq. (5.13) are composed of three factors. The first $p_m^{(1)}$ is the projection of the initial magnetization onto the T_1 eigensystem while the third factor $h_n^{(2)}$ is proportional to the projection of the coil response function [28] onto the T_2 eigensystem. The second factor $G_{mn} = \frac{\langle \phi_m^{(1)}, \phi_n^{(2)} \rangle}{\langle \phi_n^{(2)}, \phi_n^{(2)} \rangle}$, has been termed the “overlap integral” by Song, *et al.* [7]. It represents the mixing of the two eigensystems, *i.e.* G_{mn} measures the projection of the m^{th} T_1 eigenfunction onto the n^{th} T_2 eigenfunction. As has been discussed extensively in the literature [7, 8, 10, 29], the P_{mn} coefficients in Eq. (5.13) are not necessarily all positive, a fact that presents challenges for current ILT numerical routines.

The T_1 - T_2 time domain signal rise with τ_1 is most clearly observed by scaling the signal in Eq. (5.13) by the initial value of the magnetization and plotting the reconstituted signal $S(\tau_1, \tau_2)/S(0, \tau_2)$ as a function of τ_1 for different values of τ_2 [8]. This scaling process removes primary relaxation effects due to homogeneous T_1 and T_2 processes and allows us to focus on differential effects of the inter-pore interactions. While the normalization signal $S(0, \tau_2)$ is easily obtained from Eq. (5.13), experimentally this quantity must be estimated from the shortest values of τ_1 available in a measurement.

5.3: Model Features and Predictions

In this section, we explore the parameter space of the model to gain insights into the physics of the system and identify regions where the anomalous signal rise most commonly occurs. Detailed analyses of the eigenfunctions, 2D ILT spectra and time domain signals are provided for two cases: one where the signal rise does occur and one

where the rise does not occur. Our aim is to use these base cases to provide physical insights into what information the T_1 - T_2 experiment can provide with respect to the molecular dynamics in systems where the rise effect has been observed.

5.3.2: Model Phase Space

There are six dimensionless parameters to work with: four Damköhler numbers, $\delta_{i,1}, \delta_{i,2}, i = A, B$, the inter-pore exchange parameter, κ_c , and pore-size ratio, β . The solution space is potentially quite large and so our approach to understanding the physics of the model is to first explore relaxation-contrast effects and then investigate the effects of exchange and pore-size ratio. Note that the use of the interface/pore-surface relaxation relation (Eq. (5.9)) eliminates the the need to provide explicit values of $\delta_{ci,j}$.

The Damköhler numbers, $\delta_{i,j}$, represent the ratio of the rate of surface relaxation to the rate of diffusion in pore i for relaxation mode T_j . When $\delta_{i,j} \ll 1$, pore i is referred to as being in the fast diffusion regime[19]. In the fast-diffusion limit, diffusion acts to maintain an essentially uniform level of magnetization throughout the pore and so the first eigenmode dominates the solution expression (Eq. (5.10)) for a pore. In this regime, the system response is essentially mono-exponential. When $\delta_{i,j} \gg 10$, the pore is referred to as being in the slow diffusion regime[19]. In the slow-diffusion limit, significant gradients develop in the pores and the interaction of diffusion and relaxation is more complicated. The net result is that the effects of higher order eigenmodes come in to produce a multi-exponential system response [19].

Each of the pores ($i = A, B$) will have two relaxation modes at its surfaces, corresponding to longitudinal relaxation characterized by $\delta_{i,1}$ and transverse relaxation

characterized by $\delta_{i,2}$. To manage the complexity of possible relaxation combinations amongst pores, we use a two-element labelling system to characterize the diffusion state of a pore. The first element of the label will refer to the T_1 relaxation mode ($\delta_{i,1}$) and the second label the T_2 relaxation mode ($\delta_{i,2}$) since a pore could be in a fast or slow regime for each of the relaxation modes. For example, we will identify a pore as being fast/slow when $\delta_{i,1} \ll 1$ and $\delta_{i,2} \gg 10$. Similar considerations apply to labeling a pore as fast/fast or slow/slow. The slow/fast mode combination would require $\rho_{i,1} > \rho_{i,2}$ for a pore, a condition which violates the premise that NMR longitudinal relaxation rates should be less than or equal to transverse rates.

The result of this consideration is that each pore can be in one of three conditions (fast/fast, fast/slow, and slow/slow) so there are nine possible ways to combine the labels for two pores. Exploring these combinations, it was found that the condition where the A pore is in the fast/slow regime and the B pore is in the slow/slow regime produced the most significant rise effects. In contrast, the combination of the same modes for both pores, i.e. slow/slow regime for pore A combined with the same the slow/slow or fast/fast regime for pore B did not show substantial (or any) rise effects.

Figure 5.2 summarizes the range of behaviors that were found for this combination of regimes for the A and B pores. The specific parameter values used in the computations are provided in the figure caption. From the figure, we observe the following:

- As $\delta_{A,1}$ increases (Fig. 5.2a) from 10^{-3} to a maximum of 10^3 , equivalent to $\delta_{A,2}$ (all other parameters fixed), the A pore moves from having mixed T_1/T_2 modes of fast/slow to being a pore with slow/slow mode. The transition is largely complete

once $\delta_{A,1} > 10$ and is not present when $\delta_{A,1} > \delta_{B,1}$. The reduction in the signal rise suggests that strong contrast in pore relaxation mode physics is an important factor leading to signal rise.

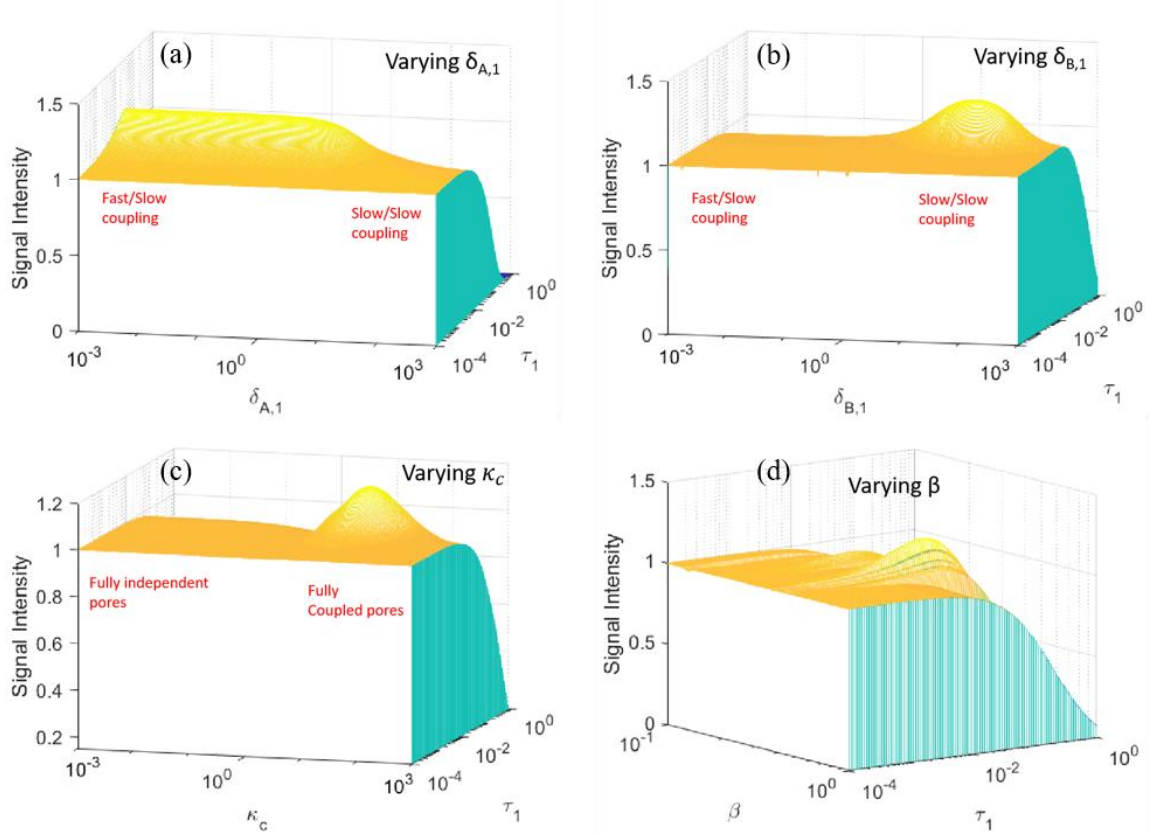


Figure 5.2: Parameter phase spaces for $\delta_{A,1}$ (a), $\delta_{B,1}$ (b), κ_c (c) and β (d) are shown. The total time domain signal at $\tau_2 = 2$ is shown. Unless varied, all parameters were kept constant as $\delta_{A,1} = 0.001$, $\delta_{A,2} = 1000$, $\delta_{B,1} = 100$, $\delta_{B,2} = 1000$, $\beta = 0.33$ and $\kappa_c = 10$. Substantial signal increase appears when the A pore is in fast/slow regime (a) and the B pore is in the slow/slow regime (b). (c) The exchange rate must be intermediate between being too restricted ($\kappa_c \ll 1$, separate pores) and too connected ($\kappa_c \gg 1$, single pore) for significant increases in signals. The geometry of the system, governed by β (d), shows multiple pore ratios for which the increase is seen with no increases occurring at small ($\beta < 0.1$) or large ($\beta > 1$) values.

- As $\delta_{B,1}$ decreases (Fig. 5.2b) from 10^3 (all other parameters fixed), the B pore moves from being a slow/slow pore to a fast/slow pore. For the transition, there is only a relatively small range of values of $\delta_{B,1}$ that lead to a rise in the signal. Further increases in $\delta_{B,1}$ (ending at $\delta_{A,1}$) make the B pore similar to the A pore and that combination of conditions does not lead to signal increase. This result supports the observation noted for Fig. 5.2a that a strong inter-pore relaxation contrast is needed for a rise to occur.
- Varying the parameter κ_c (Fig. 5.2c) over a range from 10^{-3} to 10^3 shows that for small values the system consists of uncoupled or separate pores while at large values the system consists of an effective single pore made up of the two fully coupled pores. The effect of inter-pore exchange on signal rise shown here makes it clear that a rise occurs for intermediate values of κ_c . At the extremes, inter-pore exchange removes any relaxation contrast between the pores which is not conducive to significant signal rises.
- The parameter β (Fig. 5.2d) should range over $0 < \beta < 1$ characterizing the change from a system with one pore ($\beta = 0$) where no signal rise is expected to a two-pore system of equal-sized pores ($\beta = 1$). The transition is not as smooth as those shown in Figures 5.2a and 5.2b. We do not presently have a clear reason for this lack of smoothness and further analysis is required to resolve this issue. However, it is clear that signal increases occur for $0.25 < \beta < 0.5$ for the given parameter set. Given the physical relevancy of the model [9, 11, 21, 28] the value $\beta = 0.33$ is chosen for detailed analysis going forward.

The primary insights, then, from Figure 5.2 are that the conditions that favor substantial signal rise include a strong contrast in the cross-pore longitudinal relaxation parameters ($\delta_{A,1} \gg \delta_{B,1}$) and an intermediate value of the inter-pore exchange rate, κ_c . Viewing the scaled exchange rate as a ratio of characteristic times or rates, $\kappa_c = \frac{k_c L_A}{D} = \frac{L_A^2/D}{L_A/k_c} = \frac{t_D}{t_{ex}} = \frac{r_{ex}}{r_D}$, it is clear that the exchange rate should be fast (an order of magnitude factor of 10, appears to be the case here) compared to the diffusion rate for the system to ensure that the relaxation-contrast between the pores is able to generate a signal rise. However, too high of an exchange rate reduces the relaxivity contrast between pores and dampens or eliminates any potential for a signal rise.

5.3.3: Eigensystems, Spectra, and Time-Domain Signal Features

With a general idea of the conditions which lead to a rise in the time domain signal, we now look into the mechanisms for signal rise by comparing the details of two base cases considered in Fig. 5.2 (the slow/slow (*A*), slow/slow (*B*) and fast/slow (*A*), slow/slow (*B*)). The first case to consider is when both pores are in the slow diffusion regime (i.e., $\delta_{A,1}, \delta_{A,2}, \delta_{B,1}$, and $\delta_{B,2} > 10$). Figure 5.3 summarizes the computational results for this slow/slow setting in the *A* and *B* pores.

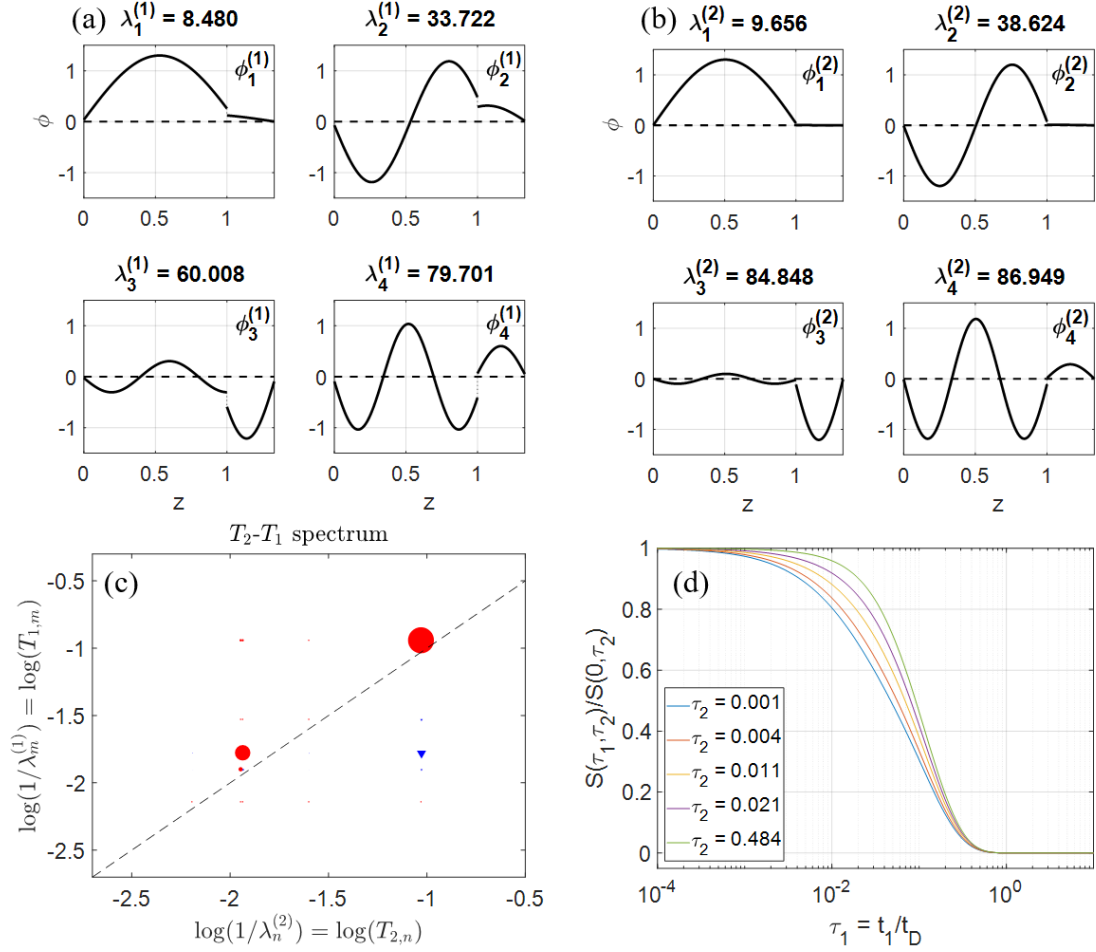


Figure 5.3: Results for eigensystem and signals using a base case with coupling between identical slow diffusion modes in two pores for the parameter set $\beta = 0.33$, $\delta_{A,1} = 100$, $\delta_{A,2} = 1000$, $\delta_{B,1} = 100$, $\delta_{B,2} = 1000$, and $\kappa_c = 10$. First four (a) longitudinal eigenfunctions, (b) transverse eigenfunctions and (c) the computed spectrum (P_{mn} values). In (c) blue triangles indicate negative P_{mn} values and red circles indicate positive values. The size of a marker indicates the magnitude of the value. The computed signal for the system is shown in (d). No anomalous rise in time domain signal is observed for this combination of relaxation modes.

The first four eigenvalue/eigenfunction pairs are shown in Figure 5.3a and demonstrate the discontinuous nature of the eigenfunctions at the interface due to a finite rate of transfer of magnetization there. Higher-order eigenfunctions oscillate with increasing frequency as expected from the nature of the eigenvalue problem.

Additionally, the first eigenfunction, as the last one to decay to zero in the solution (based on Eq. (5.10)), is a useful indicator of the magnetization profile in the system. Due to the large values of the Damköhler numbers, the magnetization at the pore ends are nearly zero and there are significant gradients in each of the pores as a result.

Figure 5.3b summarizes the same information for the T_2 eigensystem. There is substantial similarity to the T_1 eigensystem (Figure 5.3a) because of the similar Damköhler numbers in this case. Figure 5.3c provides the predicted relaxation-time spectrum. Note that there are minor negative modes as well as modes below the $T_1 = T_2$ diagonal of the spectrum. Finally, Figure 5.3d shows the reconstituted time domain signal. There is no signal rise in this case due to the positioning of the dominant relaxation modes above and along the $T_1 = T_2$ diagonal.

The example in Figure 5.3 provides a fuller understanding of the elements of the solution to the model. Recall from Eq. (5.13) that the signal is a weighted sum of exponential terms. The weights are the mode amplitudes, P_{mn} , which comprise three key factors ($p_m^{(1)}$, G_{mn} , and $h_n^{(2)}$) whose values are summarized in Table 5.1 for $m = n = 4$ terms in the two eigensystems.

Table 5.1: Values of the elements of the first four mode amplitudes in Figure 5.3c. Red denotes a negative value

m	$p_m^{(1)}$	G_{mn}				$h_n^{(2)}$	P_{mn}			
		n					n			
		1	2	3	4		1	2	3	4
1	0.99	1.03	0.06	0.02	0.04	0.63	0.64	0.00	0.01	0.01
2	0.08	0.08	1.00	0.09	0.09	0.00	0.01	0.00	0.01	0.01
3	0.98	0.10	0.39	1.08	0.27	0.21	0.06	0.00	0.22	0.06
4	0.15	0.03	0.06	0.12	0.89	0.17	0.01	0.00	0.01	0.02

The values of $p_m^{(1)}$ are the generalized Fourier expansion coefficients in the expansion of the initial magnetization in terms of the T_1 eigensystem. From the eigenfunctions in Figure 5.3a and the values of $p_m^{(1)}$ in Table 5.1, we see that the value of $p_3^{(1)}$ is important in the reconstruction of the initial condition due to the way that the third eigenfunction provides signal in the B pore relative to the A pore compared to first eigenfunction.

The matrix of the eigenfunction overlap integral G_{mn} values is strongly diagonal, reflecting the similarities of the relaxation aspects of the two eigensystems. Finally, the values of $h_n^{(2)}$ are proportional to the generalized Fourier expansion coefficients in the expansion of the coil response function in terms of the T_2 eigensystem. The specific values of $h_n^{(2)}$ in this case arise from the logic that lead to the $p_m^{(1)}$ values: modes with some spatially symmetric character are weighted most heavily in the synthesis of the (constant, symmetric) coil-response function, $h(z)$.

Figure 5.4 repeats the elements of Figure 5.3 for the case where the A pore is in the fast/slow mode and the B pore is in the slow/slow mode. The T_1 eigensystem (Figure 5.4a) has changed from the previous case (Figure 5.3a) while the T_2 eigensystem is unchanged. The reduction in the Damköhler number $\delta_{A,1}$ in the A pore has led to a smaller first eigenvalue and a magnetization profile (using the first eigenfunction as a guide) with gentler gradients but less strong symmetry, a result that will have important consequences in the discussion below.

The time domain signal for this case (Fig 5.4d) shows the rise behavior with a magnitude that increases with τ_2 . The spectrum (Fig 5.4c) is similar to that of the prior case (Figure 5.3c) but there are important differences. The negative P_{31} mode amplitude (below the diagonal) is much stronger compared to the dominant P_{11} mode in this case. Table 5.2, below, summarizes the numerical details using the first four T_1 and T_2 eigenfunctions following the structure of Table 5.1.

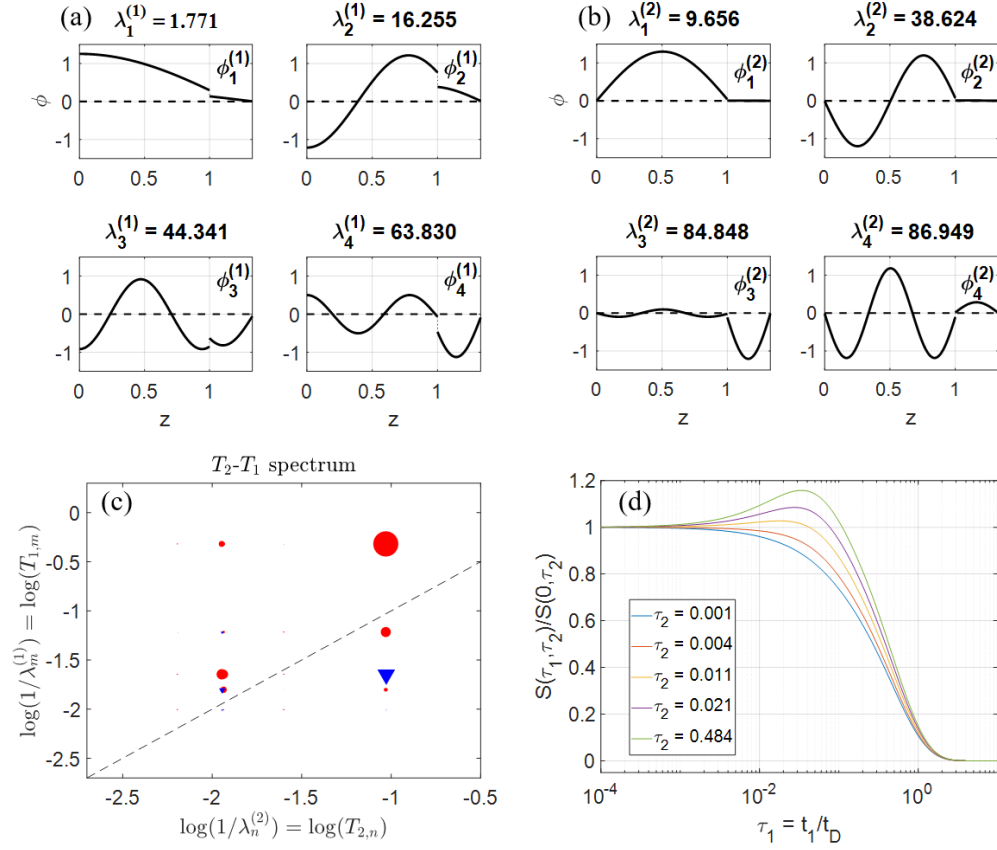


Figure 5.4: Results for eigensystem and signals using a base case with coupling between fast and slow diffusion modes in two pores for the parameter set $\beta = 0.33$, $\delta_{A,1} = 0.001$, $\delta_{A,2} = 1000$, $\delta_{B,1} = 100$, $\delta_{B,2} = 1000$, and $\kappa_c = 10$. First four (a) longitudinal eigenfunctions, (b) transverse eigenfunctions and (c) the computed spectrum (P_{mn} values). In (c) blue triangles indicate negative P_{mn} values and red circles indicate positive values. The size of a marker indicates the magnitude of the value. The computed signal for the system is shown in (d). An anomalous rise in time domain signal is observed for this combination of relaxation modes.

Table 5.2: Values of the elements of the first four mode amplitudes in Figure 5.4c. Red denotes a negative value.

m, n	$p_m^{(1)}$	G_{mn}				$h_n^{(2)}$	P_{mn}			
		n					n			
		1	2	3	4		1	2	3	4
1	1.02	0.92	0.23	0.03	0.23	0.62	0.58	0.00	0.01	0.04
2	0.37	0.41	0.88	0.08	0.11	0.00	0.22	0.00	0.07	0.12
3	0.45	0.79	0.44	0.79	1.70	0.20	0.22	0.00	0.07	0.13
4	0.55	0.04	0.24	0.31	0.35	0.16	0.01	0.00	0.03	0.03

With the change in the spatial characteristics of the first T_1 eigenfunction in pore A (compare Fig. 5.3a and Fig. 5.4a), more of the $p_m^{(1)}$ terms are needed to synthesize the (constant) initial condition. Importantly, though reduced in magnitude relative to the first, the value of $p_3^{(1)}$ remains negative and that negative value ultimately translates to the negative eigenmode amplitude at P_{31} . The overlap value associated with this negative-going mode, G_{31} , has increased substantially over the value for the slow/slow (A) slow/slow (B) case (Table 5.1) due to the decrease in similarity between the two eigensystems. The net result is a stronger negative mode amplitude for P_{31} .

The contrasts between the results in Figures 5.3 and 5.4 amplify the insights into the cause of a rise in the signal that were found in Figure 5.2. Specifically, the key requirements for a signal rise are first a strong contrast in the relaxation characteristics between the two pores (compare the first eigenfunctions in Figures 5.3 and 5.4) and

second an inter-pore exchange rate that is larger than the diffusion rate ($\kappa_c > 1$) and intermediate to the pore T_1 relaxation rates ($\delta_{A1} < \kappa_c < \delta_{B1}$).

5.3.4: Time Domain Signal Increase

Observations of anomalous time domain signal increase results in T_1 - T_2 measurements of Thrane, *et al.*, Williamson, *et al.*, and Nelson, *et al.* are additional motivation for development of the exchange model. As shown in Fig. 5.4, the two-region model predicts such a rise in the T_1 - T_2 signal, thus providing a tool for investigating the physics that underlie the effects.

The spectra in Figures 5.3c and 5.4c suggest that the interaction of the dominant mode, P_{11} , with the primary negative mode, P_{31} , is key to the development of an increase in the time domain signal amplitude. Because the P_{31} mode means that the short-lived third T_1 mode is coupled to the longest-lived first T_2 mode, a stronger negative value for the P_{31} mode means that the signal as a function of τ_1 will involve a component that decays early in a T_1 sense yet lasts longer in a T_2 sense. Such a setting could lead to a signal rise [9].

A demonstration of how this type of coupling can lead to the observed rise behavior is shown in Figure 5.5. Figure 5.5a presents the signal components from the case shown in Figure 5.3 (no rise observed) while Figure 5b does the same for the signal in Figure 5.4 (rise observed). The time evolution of six significant modes from those figures all take the form

$$f_m(\tau_1; \tau_{2n}) = P_{mn} e^{-\lambda_m^{(1)} \tau_1} e^{-\lambda_n^{(2)} \tau_{2n}} \quad (5.14)$$

for a given set of τ_{2n} values.

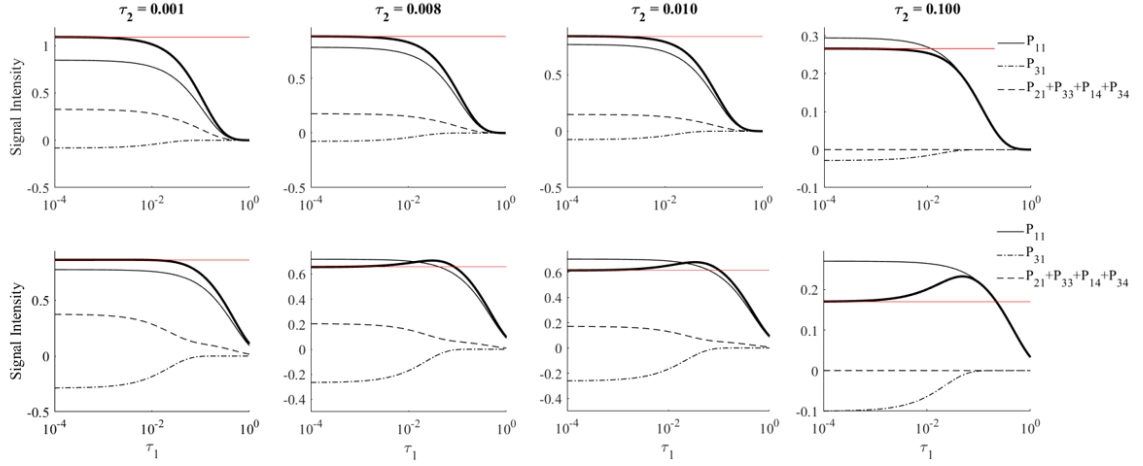


Figure 5.5: Demonstration of the contribution of the P_{mn} components to the time domain signal increase for a T_1 - T_2 experiment. Lines show the evolution of the signals according to Eq. (5.14) from the six dominant modes identified from Figures 5.3c and 5.4(c) along with their sum (heavy black line) with the four smallest positive modes combined for clarity. The thin (red) solid line locates the value of the sum as $\tau_2 \rightarrow 0$ and helps to identify the rise in the signal with increasing τ_1 . (a) Shows the slow/slow mode coupling case of Fig. 5.3 and (b) the fast/slow mode coupling of Fig. 5.4 for increasing τ_2 or echo number.

At short values of τ_2 , there is little differential T_2 weighting of the modes, and all modes contribute in proportion to their P_{mn} value to the observed signal. In Figure 5.5, the relative strength of the P_{11} mode, along with the smaller positive modes (P_{33} , P_{34} , P_{13} , and P_{14}) add together to balance out any effect of the negative P_{31} mode might have on the signal. However, by $\tau_2 = 0.1$, most of the smaller, higher-eigenvalue modes have decayed to zero leaving only the P_{11} and P_{31} modes, modes that are associated with the longest T_2 mode. If the magnitude of the P_{31} is much smaller than the dominant P_{11} mode (as it is in Figure 5.3), the increase in the signal due to the decay of this negative model is not of importance and as the total signal drops to zero without a rise in the τ_1

dimension (Figure 5.5a). When P_{31} is larger (Figure 5.4), however, the decay of the mode makes a noticeable addition to the signal *i.e.*, a signal rise is observed.

From this discussion, though negative mode amplitudes are necessary for generating a rise in the T_1 - T_2 time-domain signal, it is clear that they are not sufficient for the rise to appear. For example, negative modes are part of the system shown in Figure 5.3, but the magnitudes of those modes are not large enough to cause the offsets shown in Figure 5.4. In such cases, the T_1 - T_2 signal is spread out in the τ_1 domain as a function of the particular τ_2 value. Also, if the largest negative mode has a decay rate that is closer to the dominant positive mode decay rate, the rise in the signal could actually appear as a spread in the T_1 - T_2 signal decay as τ_1 increases.

5.4: Experimental Observations

The increase in the time domain signal for T_1 - T_2 experiments was first reported by Song *et al.* [8] for a microporous glass bead system. Diffusive coupling between the internal microscale pores within the beads and macroscale pores due to bead packing was shown to generate an increase in the reconditioned time domain signal [8]. An increase in the T_1 - T_2 time domain signal was also observed for a polymer/solvent (HPMCAS/acetone) system in a weak gel state above the glass transition temperature [17] and in the formation of hydrates of agglomerates of hydrate cages [16]. Here data for the microporous glass bead system, the cyclopentane/water hydrate system and beeswax undergoing a solid-solid rotator phase transition [18] are presented. The data is discussed in the context of using the exchange model to demonstrate the insight into measurements

of complex systems that is possible. The model as investigated here is most directly applicable only to the microporous beads. Extension to include bulk relaxation heterogeneity is necessary to further quantify the dynamics in the other systems.

5.4.1: Microporous Bead Pack

The data of Song *et al.* [8] was reproduced using the same 150 μm microporous beads (CPG2000A, Millipore) in a water-saturated bead pack. A 2MHz Rock Core Analyzer (Magritek Ltd, Wellington NZ) was used to acquire T_1 - T_2 correlation data. The T_1 domain was sampled with 32 logarithmically spaced values of τ_1 from 1 to 1000 ms and a CPMG readout train with a $\tau = 800 \mu\text{s}$ pulse spacing and 1500 echoes. The data is shown in Fig. 5.6 and clearly indicates a signal rise in the t_1 domain with increases in t_2 . The results here are also in accord with those of Song, *et al.* with signal maxima occurring around $t_1 \sim 0.01$ s and the onset of the growth of the rise in the signal appearing at about 320 ms.

In addition to particle size, Song, *et al.* [8] provide material characteristics that can provide estimates of the parameters for the two-pore model presented here. The total porosity of the system was reported as $\epsilon = 0.8$. The pore volume of the system was equally apportioned between the interior and exterior of the beads. Using the reported external porosity of $\epsilon_e = 0.4$, the interior porosity of the particles is calculated to be $\epsilon_i = 0.67$. The internal length scale of the beads was reported as $l_i = 0.3\mu\text{m}$.

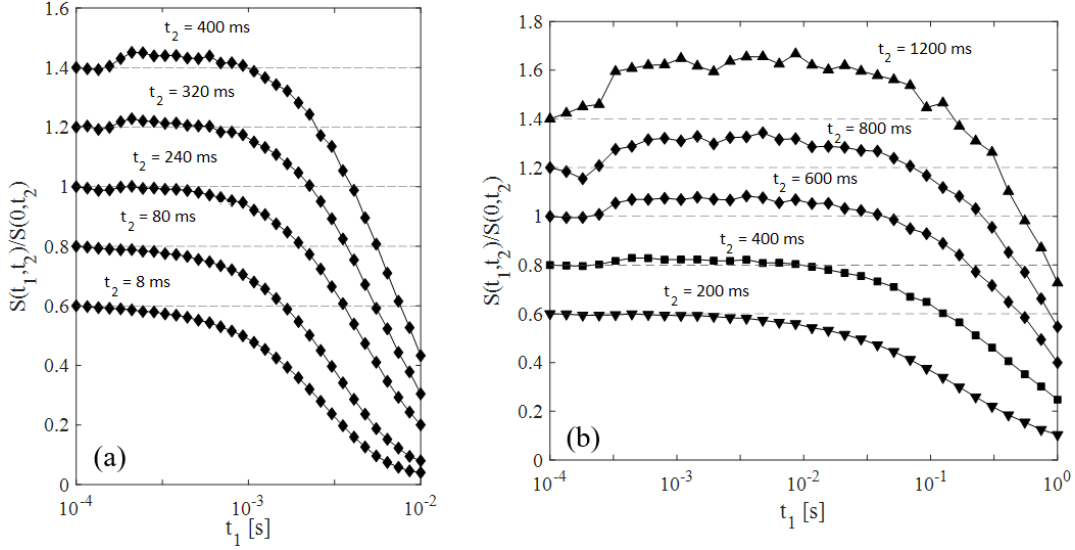


Figure 5.6: T_1 - T_2 time domain signal for 150 μm microporous bead pack in water at 2 MHz. The signal rise is detected at 320 ms and grows through the end of the CPMG train.

Our initial analysis will assume pore A is represented by the water external to the beads. To establish a length scale, L_A , we use a unit cell of width l_e that contains a layer of thickness l_p representing the water-saturated bead. Hence, for the interval $l_p < x < l_e$, there is only water. From geometric considerations and the equal apportioning of water between the regions in the system, we have that $\frac{\epsilon_p V_p}{V_e - V_p} = \frac{\epsilon_p l_p}{l_e - l_p} = 1$ which implies $l_e = (1 + \epsilon_p)l_p = (5/3)l_p$. Hence, with l_p approximately the particle radius, the length-scale for the region A is given as $L_A = l_e - l_p = \epsilon_p l_p = (2/3)l_p \approx 50 \mu\text{m}$. From this length scale and the diffusion coefficient of water ($D = 2.5 \times 10^3 \mu\text{m}^2/\text{sec}$), the time scale for the system is estimated to be $t_D = L_A^2/D \sim 1$ sec. A similar computation using spheres rather than flat layers to represent the phases leads to $t_D \sim 0.16$ sec and we infer a reasonable estimate of the diffusion time to be in the range of these values. . Measurements by Song,

et al. of surface relaxation rates (at 2 MHz) suggest that ρ_1 and ρ_2 are on the order of 1 and 10 $\mu\text{m/s}$, respectively, leading to the guideline that $\frac{\delta_{A,2}}{\delta_{A,1}} = \frac{\rho_1}{\rho_2} \sim 10$. From the scaling information so far, the initial estimates of the Damköhler numbers are $\delta_{A,1} \sim 0.01$ and $\delta_{A,2} \sim 0.1$ indicating a fast longitudinal diffusion mode and a fast to intermediate transverse diffusion mode.

Identifying the B pore with the micropores in the beads leads to the finding that $L_B \sim 0.31 \mu\text{m}$, and so $\beta \sim 10^{-2}$. As was made clear from the discussion of Figure 5.2d, we would not expect a substantial rise to occur for this pore-size ratio. Thus, our approach, will be to find a value of β that produces a rise and use that value to indicate an effective size of the B pore relative to the A pore. With regard to Damköhler numbers for the B pore, Song, *et al.* [8] suggest that the internal relaxation rates for the internal bead micropores will be much higher than the external rates due to the small micropore length scale. We anticipate that both homogeneous relaxation in pore B along with $\delta_{B,1}$ and $\delta_{B,2}$ is greater than the values in the A pore to provide the slow/slow conditions needed to generate the rise behavior. This argument suggests a second *phase* as we have defined for this model rather than two simple regions. More on both of these issues will be provided in the discussion to follow.

Based on these considerations, a range of model parameter combinations was explored in an effort to capture the trends in the data shown in Fig. 5.6. The results are summarized in Figure 5.7. To be clear, the comparison here is not an optimized fit of the model to the data. Rather, the parameters were chosen (see the caption for Figure 5.7 for

specifics) to be consistent with the data, the parameters provided by Song, *et al.*, and with the criteria we have developed for what conditions do and do not lead to predictions of a rise in the reconstituted signal for the experiment.

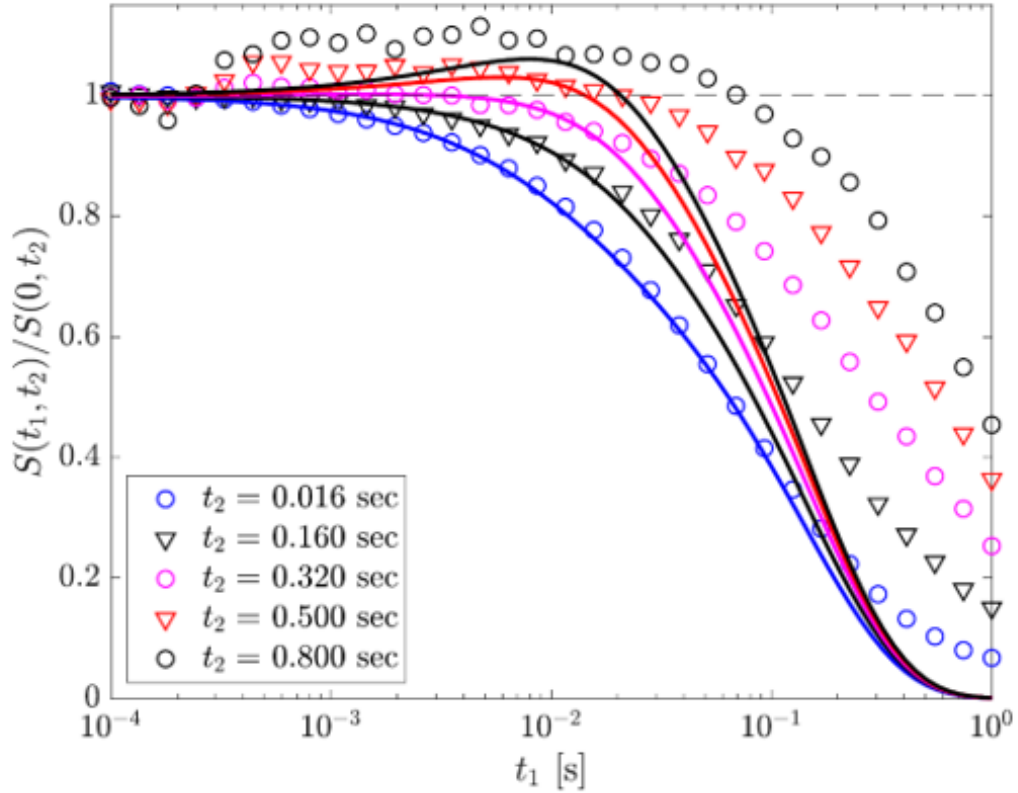


Figure 5.7: Comparison of the two-pore model (solid lines) with the microporous bead data of Figure 6 (symbols). Model parameter values were $L_A = 20\mu\text{m}$, $D = 2500\mu\text{m}^2/\text{s}$, $\delta_{A1} = 0.01$, $\delta_{A2} = 100$, $\delta_{B1} = 100$, $\delta_{B2} = 1000$, $\kappa_c = 10$, and $\beta = 0.4$. Values of $S(0, t_2)$ were estimated from the first 5 t_1 values at any given t_2 value in the signal array, $s(t_{1,m}, t_{2,n})$. The time scale used for the $t_1 = t_D \tau_1$ dimensional axis of the model is the diffusion time for the experimental spherical particle system ($t_D = 0.16\text{ s}$). The dimensional time-scale for the model t_2 curves was increased by a factor of 100 over the diffusion time $t_2 = 100t_D \tau_2$ to provide the registration of the data and model as shown.

As is evident from Figure 5.7, a reasonable fit of the model to measurements is possible, especially for the t_1 interval for which the rise develops. Given the level of

estimation of the parameters involved, we find the correspondence shown here to be quite encouraging as it strongly suggests that pore-pore interactions are key to the anomalous rise behavior in this system.

There are, however, some important discrepancies between the model and the data. The model misses the long- t_1 behavior in the data at any value of t_2 . Further analysis and experimentation with other model systems will be required to get insight into the reason for this discrepancy. One possibility is that the system is actually best described by a three region-model (Region 1: external water between particles, Region 2: internal water in “large pores”, Region 3: internal water in “small pores”). A light coupling between Region 1 and the other regions would leave the spins in Region 1 largely unimpacted by interactions with the solid and hence would lead to a portion of the system with longer relaxation times. This alternative view of the system would also imply that the model predictions in Figure 5.7 are associated with the material heterogeneity or the microstructure of the beads themselves. Indeed, such a revised conception of the system would also help make sense of the value of $\beta = 0.4$ used in the computations and suggest that the “larger” pores are about 2-3 times as large as the “smaller” pores. Though these extensions to the model are outside the scope of the current study, they could provide the basis for further work on the model and its application.

Finally, the comparison in Figure 5.7 also involves an inflation of the scaled times in the t_2 dimension that is 100 times greater than that of the required diffusion-time-scale inflation (*i.e.*, $t_2 = 100t_D\tau_2$ for the curves shown). We do not have a clear idea of why

such a rescaling is needed. Part of the issue might be related to the ideas mentioned above for the t_1 axis but the order of magnitude of this rescaling seems to be much too large for an addition of a non-interacting region. The large values of the Damköhler numbers ($\gg 10$) could also be a part of the issue since these values are key to establishing the observed relaxation times in this system. However, rise behavior in the current model depends on the fast/slow behavior of a pore and so reducing the values of $\delta_{i,2}$ in order to increase the predicted lifetime of the spins would eliminate the prediction of the observed effect. We leave this issue unresolved and look to further work in this area to provide insights.

5.4.2: Organic Hydrate Formation – Cyclopentane/Water

During the formation of hydrates, a porous hydrate shell consisting of hydrate cage agglomerates will form around thawing ice particles dispersed in the organic cyclopentane phase. The exchange between the regions of high mobility excess cyclopentane and the cyclopentane entrained in the porous structure formed by the hydrate agglomerate generates a time domain T_1 - T_2 signal increase due to the coupling of the diffusion dynamics, see Figure 5.8. The structure of the hydrate cage agglomerate porous media evolves from a porosity of ~95%, when hydrate formation begins, to 5% over the hydrate formation time [16, 30]. The hydrate system exhibits complex diffusion dynamics on the mesoscale of the agglomeration of hydrate cage structures as shown by the signal increase with t_1 . On the scale of microseconds and Angstroms, water molecules cage one single cyclopentane molecule and the cages agglomerate to form

structure III hydrates [31]. The rise in the time domain signal is from the coupling of the bulk pentane and pore scale pentane, an analogous system to the microporous bead pack [8]. To apply the model to this system, the porosity of the hydrate shell would be represented by the model pore connectivity term ϕ . However due to the evolution of the hydrate shell the model would need to be extended to allow a time dependent pore connectivity. Appropriate choice of model parameters to capture the diffusion dynamics during hydrate formation is further complicated by the magnetization loss from water and cyclopentane molecules which are not detectable once entrained in the hydrate cage structure. The T_1 - T_2 time domain signal rise occurs at intermediate ~ 14 hrs formation times, but not at early times when the high porosity of the porous agglomerate does not significantly impact cyclopentane diffusion and relaxation, nor interestingly at long time equilibrium after all water is consumed. Studies with anti-agglomerate chemicals, which keep the cages in a nanoparticle slurry state, showed no signal increase [16].

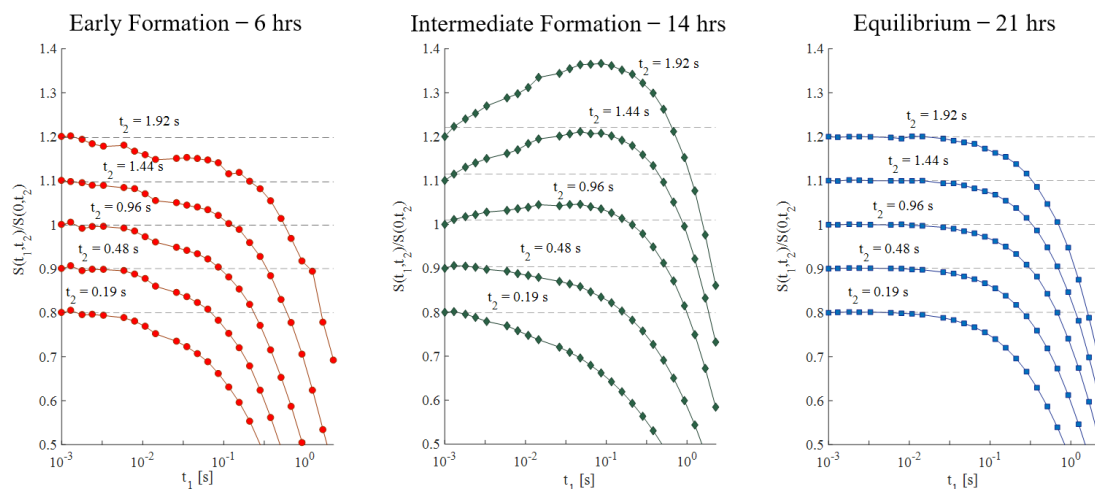


Figure 5.8: T_1 - T_2 time domain signal for a model hydrate forming system of water and excess cyclopentane. The signal rise indicates diffusive coupling between bulk cyclopentane and cyclopentane in micron size pores of agglomerates of hydrate cages. At early times (6 hrs) the agglomerate is of very low porosity and does not impact the signal. After hydrate formation is complete, the signal rise is not present as the very high density $\phi < 5\%$ of the porous structure effectively prevents exchange through the hydrate shell.

5.4.3: Multi-Component Wax – Beeswax

Beeswax, a mixture of long chain alkanes, esters with smaller amounts of free acids and alcohols, exhibits a polymorphic crystalline structure at room temperature [32]. Solid-solid phase transitions occur throughout heating, breaking the conformation of the polymorphic lattice and introducing mobility into the system. These transitions occur at temperatures from 36.4°C up to the beginning of the softening point at 50.0°C which is due to the transition from a crystalline state to an intermediate state [32]. The softening of the lipid matrix has been characterized through ^{13}C ssNMR [32-34] and is described as a transition from a dominated *trans* configuration with short T_2 with small populations of *gauche* structures of longer T_2 , to the shift toward a primarily *gauche* lattice. This initial breaking of the symmetry toward a *gauche* structure is captured by the T_1 - T_2 measurements shown in Figure 5.9. Unlike the microporous bead pack and the hydrate

system, this system does not have mass transfer occurring. It provides an example of the observed time domain signal increase as a function of physico-chemical state due to flip-flop spin diffusion [35] between the *trans* and *gauche* domains [18]. Spin diffusion ssNMR measurements use double quantum, or other RF filters, to selectively excite regions of strong dipolar coupling and use diffusion models to quantify spin diffusion between crystalline and amorphous domains [36]. The spin diffusion model of Buda *et al* [36] posits an interfacial domain between the crystal domain magnetization source and amorphous domain magnetization sink, through which the magnetization diffuses. Their model is analogous to that introduced here, with $\kappa_c = D_I/d_I$, where D_I is the flip-flop spin diffusivity of the interface which occurs through a thickness d_I , without surface relaxation effects.

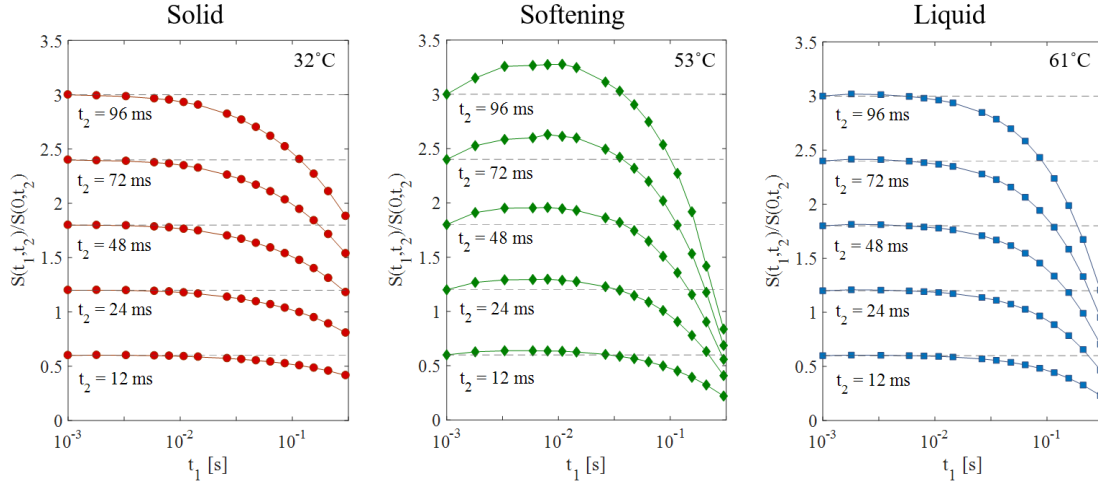


Figure 5.9: T_1 - T_2 time domain signal for beeswax, a complex wax, is shown. The signal rise is not present in the crystalline solid state at 32°C, nor at the homogenous liquid state at 61°C. In the softening state at 53°C, the signal rise indicates exchange occurring which is not brought on through mass transfer. Rather spin diffusion is the mechanism of exchange causing the anomalous time domain signal rise.

5.5: Conclusions

Song *et. al* [8] demonstrated an increase with experimental time τ_1 in the reconditioned time domain signal in T_1 - T_2 correlation experiment. In this paper the observation of an increase in the time domain signal from T_1 - T_2 correlation experiments is demonstrated to depend on exchange of magnetization between domains of different surface relaxivity in a simple 1D diffusion and relaxation model [14, 19]. The model indicates that the presence of negative eigenmodes is necessary to induce a signal increase in the time domain, however, the negative modes must also be of sufficient amplitude. The negative value of the eigenmode can come from the projection of the initial condition onto the third T_1 eigenfunctions, from the coil response function projected on the first T_2 eigenfunctions and the eigenfunction overlap or correlation

between the third longitudinal and first transverse eigenfunctions. It is the amplitude of overlap and its rate of change with τ_1 that generates the time domain signal rise. This provides novel insight into the specific origin in the coupled diffusion problem and the role of negative eigenmodes on T_1 - T_2 measurements [7, 10, 12, 15].

The signal rise effect on the time domain T_1 - T_2 signal provides a robust means to determine coupling or exchange between regions of different diffusion and relaxation behavior. Experimental examples of the signal increase have been demonstrated in a microporous glass bead packed beds [8], where coupling between large and small pores due to molecular diffusion is present. In a high concentration polymer-solvent system [17], where molecular diffusion exchange between solid and liquid like domains of a glassy state is present and in hydrate formation where organic phase cyclopentane diffuses through an evolving porous structure of agglomerated hydrate [16]. These systems are consistent with the model developed in terms of varying fast and slow diffusion modes among domains and mass transport mediated magnetization exchange. The signal rise effect is also shown for a solid-solid phase transition in a complex lipid system, beeswax, where no mass transport between different domains occurs [18]. Further analysis of the underlying physics mechanisms generating this effect in the context of the model presented here and other extensions could provide a robust time domain method for the characterization of magnetization exchange in complex systems.

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CHAPTER SIX

OBSERVING SOLID-SOLID PHASE TRANSITIONS IN TWO ALKANES AND
NATURAL WAX WITH MULTIDIMENSIONAL NMR RELAXOMETRY

Contribution of Authors and Co-Authors

Manuscript in Chapter 6

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Contributions: Designed and implemented NMR experiments. Analyzed data and interpreted data. Wrote the manuscript.

Co-Author: Linn W. Thrane

Contributions: Helped with data interpretation. Provided feedback and comments on manuscript.

Co-Author: Sarah L. Codd

Contributions: Helped with data interpretation. Provided feedback and comments on manuscript.

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Contributions: Helped with data interpretation. Provided feedback and comments on manuscript.

Co-Author: Christopher D. Craig

Contributions: Helped with data interpretation. Provided feedback and comments on manuscript.

Co-Author: Amanda Pluntze

Contributions: Helped with data interpretation. Provided feedback and comments on manuscript.

Co-Author: Joseph D. Seymour

Contributions: Assisted in design and implementation of NMR experiments. Helped with data interpretation. Assisted in writing of manuscript. Provided feedback and comments on manuscript.

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OBSERVING SOLID-SOLID PHASE TRANSITIONS IN TWO ALKANES AND NATURAL WAX WITH MULTIDIMENSIONAL NMR RELAXOMETRY

Abstract

Nuclear magnetic resonance (NMR) spin-spin T_2 relaxation is applied to characterize structural rearrangements in solid-solid phase transitions of lipids. 1D T_2 dispersion distributions and measurements and 2D T_2 - T_2 exchange are demonstrated to be sensitive to spin-diffusion, allowing for the potential to directly measure morphological length scales by NMR relaxometry. The ability to probe and characterize spin-diffusion through NMR relaxometry techniques is an additional method to explore causes of complex dynamics within matter. The NMR relaxometry data is highly complementary to differential scanning calorimetry (DSC) and has potential for bench top low field NMR implementation.

Introduction

Lipids are universal building blocks throughout pharmaceutical drug delivery, food science and membrane biology. They exhibit complex solid phase transition behavior under temperature variations near the liquid point in addition to a wide variety of chemical makeup and structural complexity. Certain lipids are the base component for drug delivery systems. Drug release rate is correlated to interactions between the polymorphic domains of lipid, polymer and API components. Advancing noninvasive techniques to probe these complex macromolecular mixtures is important in improving the relationship between material properties and material function.

Nuclear magnetic resonance (NMR) relaxometry provides a method to probe a sample's ordered versus disordered structure through determination of the molecular mobility of molecular populations with differing molecular structure[1]. This allows exploration of molecular dynamic changes in solid-solid phase transitions as well as solid-liquid phase transitions. Solid state NMR (ssNMR) methods are the standard tools used to characterize materials where amorphous and crystal structure are present[2-8]. Those ssNMR methods, including magic angle spinning (MAS), have been implemented to characterize the solid-solid polymorphic transitions within lipids[9-12]. NMR relaxometry allows for the potential for measurement of these phase transition dynamics in low field bench top and non-laboratory settings with simpler equipment than ssNMR. In this article, multidimensional NMR relaxometry is implemented to study lipid phase transitions for two model simple lipid systems and a complex lipid system. The systems studied are eicosane, a straight chained alkane with no solid-solid transition, octacosane, a C₂₈ straight chain alkane with known solid-solid transitions, and a composite natural wax with more complicated solid-solid transitions, beeswax. The data indicate the ability of NMR relaxometry to characterize solid-solid phase transitions in lipids, making our method complementary to differential scanning calorimetry (DSC).

Theory

In general, lipids are complex molecular mixtures with polymorphic crystal states. These polymorphic states include rotator states, with domains sharing similar meso-structure, but differing in terms of molecular mobility[12, 13]. Interactions between domains of varying dynamics change with temperature and chemical composition.

Developing NMR relaxometry techniques to quantify solid-solid phase transitions in terms of molecular dynamics provides means for improving understanding of the molecular structure of these materials.

A solid-solid phase transition involves changes of the crystalline lattice. For example, rotator phases are marked by long-range positional order and the breaking of long-range rotational order[13, 14]. This destruction of the symmetry causes a degradation of the structural order, creating a weakly ordered crystal[15]. Because of this, the lattice structure of the rotator phases undergo relatively large conformational changes with increasing temperature when compared to well-ordered crystalline configurations[14]. However, other transitions occur through rearrangement of the molecular structure which affects the packing structure creating these polymorphic domains. Depending on the history of the lipid, a memory may be instilled within the structure. This structure can be less ordered at lower temperatures, than at higher temperatures, enough energy is put into the system to allow reorganization to attain a more stable configuration. This can be seen for sub- α to α transitions within glyceryl behenate lipids[16]. Increasing temperatures does not always create more mobility and disorder within a domain. Sometimes it allows for a lower energy state to be occupied, thermal energy overcomes kinetic energy barriers, allowing a transition to a more stable phase. NMR has the unique ability to quantify and discern the changes in the rotational and translational molecular mobility due to the physico-chemical structure.

A traditional manner of probing these transitional structures is through DSC methods in which measurement of the enthalpic changes in the sample gives information

about the structure and phase of the system[13]. The rate of temperature variation changes the resolution of phase transitions occurring within the sample[17]. In using this technique, the information gleaned is highly dependent on this rate variable, and if not optimized, some transitions may be missed. Subtle lattice transitions, such as rotator transitions, can be overlooked and it may prove difficult to differentiate between a strong pre-transitional second-order fluctuation, and a first order transition[15]. Additionally, for larger or more complex systems, DSC can be invasive due to either irreversible melting of certain polymorphic domains or breaking apart larger samples.

Solid state ^{13}C NMR measurements have observed dynamics and interactions of the polymorphic transitions in beeswax with temperature, which corroborated and enhanced DSC measurements[9]. However, NMR spin-spin T_2 relaxation measurements have not been broadly applied to study such transitions, due in part to very short relaxation times. In using a purpose built, high-powered radio frequency (rf) probe, measurements of rotational mobility show configurational variations indicative of molecular packing changes within the system, yielding information about phases and interaction between these domains.

Mass transport can occur through molecular diffusion or chemical exchange between molecules. If there is magnetization exchange without mass transport between domains of different relaxation, spin diffusion is the mechanism[18-20]. Traditionally, spin diffusion, or flip-flop secular dipolar interactions, has been probed using ssNMR in systems with no mass transport. Spin diffusion during longitudinal magnetization storage due to energy state transitions is the primary mechanism used to interrogate sample

structure[21, 22]. However, transverse magnetization can also undergo spin diffusion via gradients in frequency $\nabla\omega(\mathbf{r})$ in contrast to the spin-lattice spatial variation $T_1^{-1}(\mathbf{r})$ dependence for longitudinal magnetization[23]. This is done through using spin diffusion as the evolution mechanism after selection based on dipolar coupling[1, 6, 21, 24]. Despite there being no mass transfer, this exchange can be described as a diffusive propagation dependent on the frequency variance of the spin states and the distance between nearest neighbors squared[25], with spin diffusion coefficient, $D_{sp} \sim (\overline{\Delta\omega^2})^2 a^2$. The flip-flop of spins occurs between nearest neighbors and then propagates out. Since the variance of the spin states is inversely proportional to T_2 spin-spin relaxation [22, 23, 26], the detection of this spin diffusion can occur through relaxometry measurements. Mellinger *et. al.*[22] coupled these two processes through ssNMR techniques for block copolymer samples, selecting the same spins based on dipolar coupling with both spin diffusion and relaxation experiments and found, $D_{sp} \sim \frac{1}{T_2} + b$, with b a constant. Through this relationship, and with the knowledge of a T_2 time, an approximate spin diffusion coefficient value can be obtained. Length scales over which magnetization exchange takes place are given by $d \sim (2D_{sp}t)^{1/2}$, in one dimension.

Spin-spin T_2 relaxation time is short (<1ms) for solids and more ordered structures, while it is longer (>100ms) for liquids. This is due to the dependence on the proton dipolar coupling which also determines the line distribution and width of spin populations[1, 27]. Frequency spectra of the time-dependent free induction decay (FID) for solids are Gaussian. This is due to the long correlation time between the dipolar couplings which generates a rapidly decaying Gaussian FID[25, 28]. The slow nature of

the random modulation due to the solid lattice structure of the spins causes this. This creates a broad Gaussian distribution in the frequency domain. Unlike solids, the FIDs characterizing liquids are Lorentzian [25, 28]. The short correlation time due to fast tumbling and motion between spins generates an exponential decay in the time domain due to motional averaging, leading to the motionally narrowed Lorentzian in the frequency domain. T_2 relaxation dispersion *i.e.*, variation in measured effective T_2 with variation in CPMG echo time interval 2τ (see Fig. 1 below), is a well-established method to understand chemical exchange in proteins, carbohydrates, and biomolecules with exchangeable protons ^1H , typically on -OH groups[29, 30]. In these types of experiments the longer T_2 of water has traditionally been measured and as τ increases, the effective measured T_2 decreases, due to exchangeable protons experiencing stronger dipolar coupling while on the biomolecule. To our knowledge, T_2 dispersion measurements using inversions to determine multiple populations in a system have not been reported. The presence of τ dependence on T_2 measurements on solids without exchangeable protons and no mass transfer indicates the presence of spin diffusion of transverse magnetization due to spatial gradients in frequency[23].

Multidimensional relaxometry has been well established as a method to explore mass transport exchange between domains [19, 31, 32]. This mechanism's impact on T_2 - T_2 experiments has been indicated in systems of aqueous protein gels[33], glyceride gels[34], and kerogen in shales[20]. For T_2 - T_2 exchange experiments, off diagonal peaks indicate magnetization exchange or transfer occurring between two populations. This may occur due to physical mass transfer via molecular diffusion or chemical exchange, or

through magnetization exchange by spin diffusion[21]. If the system is not in a liquid or mobile state, molecular diffusion driven mass transfer cannot be the mechanism for exchange. The storage of the magnetization in the longitudinal T_1 domain allows spin diffusion to occur during the mixing time τ_m . If spin diffusion is the primary mechanism for exchange, through T_2 - T_2 experiments, the relationship between T_2 and D_{sp} can be used to obtain length scales due to the τ_m times over which the exchange peaks appear.

Methods

Samples of 99% pure eicosane (Sigma Aldrich), 99% pure octacosane (Sigma Aldrich), and bleached beeswax (Sigma-Aldrich) were used in these experiments. DSC data was taken in a Mettler Toledo DSC 3+ (Columbus, OH, USA) equipped with an IntraCooler. Samples were weighed out into 40 μ L aluminum-crucibles (part number 1/3 ME-51119870) and crimped using the aluminum-piercing lids (part number 1/5 ME-51119873). For eicosane and beeswax the temperature was varied at 1°C/min while octacosane was varied at 5°C/min.

Sample of each were loaded into 5 mm NMR tubes through melting and pipetting to ensure no voids or air bubbles formed upon solidification at room temperature. T_2 relaxation and T_2 - T_2 exchange measurements were done in a Bruker 250 MHz superconducting magnet using a custom built high-powered rf probe and 5mm rf coil (Bruker). Sample temperature was controlled through the Bruker BTU system with N₂ gas flow. The sample temperature control system (Bruker BTU) was calibrated by measuring frequency spectra for methanol in a 5 mm NMR tube. The frequency shift was

measured during an increasing temperature ramp at $1^{\circ}\text{C}/\text{min}$ and the temperature calibrated according to Ammann *et al.*[35].

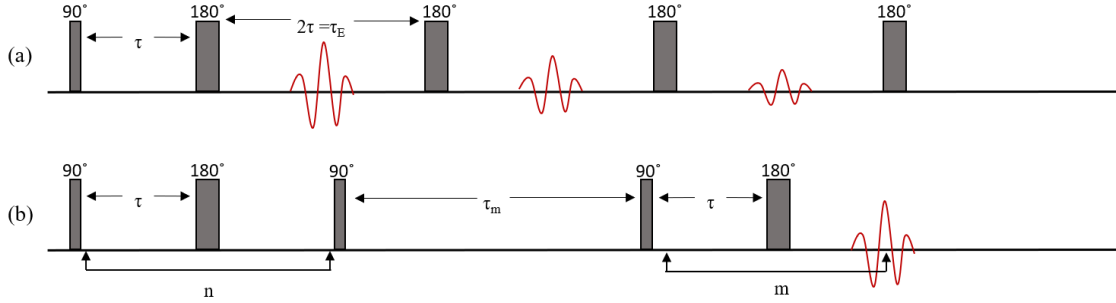


Figure 6.1: Pulse sequences used in this work are shown, (a) Carr-Purcell-Meiboom-Gill (CPMG) and (b) T_2 - T_2 exchange correlation.

For the eicosane sample, temperature was increased from 24°C up to 41°C . A standard Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence (Figure 6.1(a)) was implemented for the T_2 relaxation experiments with $\tau = 12\mu\text{s}$ for an echo time of $\tau_E = 24\mu\text{s}$, 10,000-15,000 echoes acquired dependent on the longest T_2 present, acquisition sample dwell time of $1\mu\text{s}$, and $3.7\mu\text{s}$ 90° rf pulses at a power of 100W. Each relaxation measurement had 32 averages. The sample began at 24°C and equilibrated for 10 minutes to ensure a homogenous temperature distribution throughout the plug of solid eicosane, after which, a T_2 relaxation measurement was performed. Temperature was then raised to 28°C , allowed to equilibrate for 10 minutes, then experiments were repeated. This process continued for a range of temperatures between 32 - 41°C where at 41°C the sample is in the liquid state. T_2 relaxation dispersion distributions with increasing τ time were measured at 24°C for τ times of $12\mu\text{s}$, $24\mu\text{s}$, $48\mu\text{s}$, $96\mu\text{s}$, and $240\mu\text{s}$ using the CPMG pulse sequence described above and shown in Figure 6.1(a).

The octacosane sample investigated over a temperature range from 54°C up to 66°C to explore the orthorhombic to hexagonal solid-solid transition then the hexagonal to liquid melt transition[13]. A CPMG pulse sequence (Figure 1(a)) was used for the T_2 relaxation experiments with echo time of $\tau_E = 96\mu\text{s}$, 40,000 echoes acquired, acquisition sample dwell time of $2\mu\text{s}$, and $3.5\mu\text{s}$ 90° rf pulses at a power of 100W with 64 averages. A similar equilibration scheme was implemented as for eicosane. The T_2 relaxation dispersion measurements with increasing τ for 56°C, 58°C and 60°C were obtained for τ times of $24\mu\text{s}$, $48\mu\text{s}$, $96\mu\text{s}$, $144\mu\text{s}$, $240\mu\text{s}$, and $480\mu\text{s}$ using the CPMG pulse sequence. T_2 - T_2 relaxation correlation data (Figure 1(b)) was also measured for temperatures 56°C up to 60°C. Four mixing times of $\tau_m = 100\mu\text{s}$, 1ms, 5ms, and 10ms were observed at each temperature.

The beeswax sample, temperature was varied from 19°C to 62°C. A CPMG pulse sequence (Figure 6.1(a)) was again implemented for the T_2 relaxation experiments with an echo time of $\tau_E = 24\mu\text{s}$, 10,000 echoes acquired, acquisition sample dwell time of $1\mu\text{s}$, and $3.7\mu\text{s}$ 90° rf pulses at a power of 100W. Each relaxation measurement had 32 averages. A similar temperature equilibration scheme as used in the alkane samples was implemented. T_2 relaxation dispersion measurements with increasing τ time were observed at 32°C and 53°C for τ times of $12\mu\text{s}$, $48\mu\text{s}$, $96\mu\text{s}$, and $240\mu\text{s}$ using the same CPMG pulse sequence. T_2 - T_2 relaxation correlation data (Figure 6.1(b)) was also acquired with two CPMG pulse sequences separated by a storage or mixing time, τ_m , during which the magnetization is stored in the longitudinal mode and exchange between domains occurs. Five mixing times, $\tau_m = 100\mu\text{s}$, 1ms, 5ms, 10ms and 30ms, were

examined for various sample temperatures of interest determined by endothermic peaks in the DSC data. For 32°C and 45°C, seven additional mixing times were observed to explore exchange correlation times.

The T_2 and T_2 - T_2 relaxation correlation experimental data for all samples were processed through a Fredholm integral or, inverse Laplace transform analysis[36].

Results and Discussion

DSC Measurements

DSC was performed on representative samples of eicosane, octacosane, and beeswax (Figure 6.2). Phase transitions are marked by the rapid change in heat flow presenting as a peak. Eicosane and octacosane are simple straight chain alkanes, whereas beeswax is a blend of various alcohols, esters, free fatty acids, hydrocarbons and other materials [9-11]. This is reflected in the DSC data by the single solid-liquid transition for eicosane (Figure 6.2(a)) occurring at 36°C with a transition temperature range of approximately 4°C compared to beeswax exhibiting a transition with three distinct peaks (Figure 6.2(c)). For octacosane (Figure 6.2(b)), a slightly more complex straight chain alkane system, a shoulder appears during the melting at 58°C with a full melt transition at 65°C. There is a very minor liquid component solidifying at -31°C which we attribute to a <1% decane impurity in the sample. In the beeswax, intermediate solid-solid phase transitions are indicated by peaks in heat flow intensity occurring at 28°C and 52°C with the solid-liquid transition at 61°C (Figure 6.2(c)), the melting temperature of beeswax[37]. Notably, the transition peaks in beeswax occur over a significantly larger temperature range than eicosane or octacosane, due to the multiple chemical components.

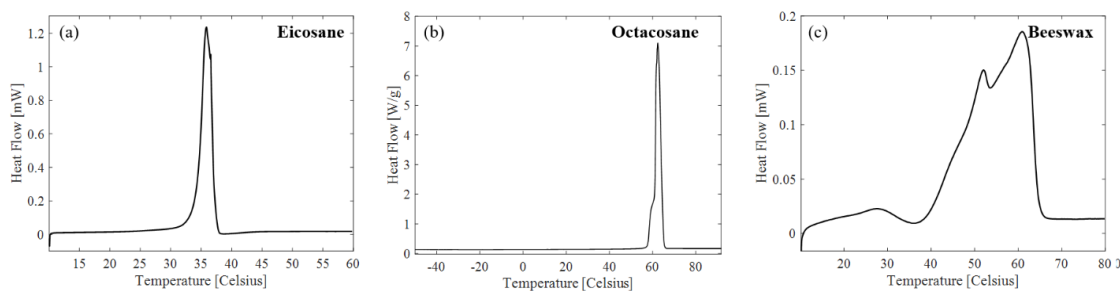


Figure 6.2: DSC measurements of (a) eicosane at $1^{\circ}\text{C}/\text{min}$, (b) octacosane at $5^{\circ}\text{C}/\text{min}$, and (c) beeswax at $1^{\circ}\text{C}/\text{min}$ temperature ramping. According to DSC, eicosane (a) undergoes a simple, first order solid-liquid phase transition indicated by the single peak. Octacosane (b) has a solid-solid transition just before the solid-liquid transition, evidenced by the small energy absorbance at 53°C . Beeswax (c), shows intermediate structural rearrangements, one at lower temperatures, then softening and pre-melting rearrangements at higher temperatures prior to melting.

Eicosane

After melting and pipetting the eicosane into a 5mm NMR tube, it was held at room temperature for over 24 hours before measurements were taken. The sample was placed in the rf probe in the magnet and T_2 distributions measurements were taken at temperatures ranging from 24°C to 41°C , with a temperature equilibration delay between each temperature increase. The sample underwent a solid to liquid phase transition over this range, being solid at 24°C and liquid at 41°C .

Increasing Temperature T_2 Relaxation Measurements

Detailed information regarding the observed phase transitions can be obtained through the T_2 relaxation distributions in Figure 6.3. In Figure 6.3(a), the full temperature range is shown from 24°C (blue) up to 41°C (red). At 24°C , three distinct populations are seen, the most restricted population at 0.249ms, an intermediate population at 12.2ms, and the most mobile at 281.6ms. We hypothesize the presence of two extrema

populations are due to the measurement of the $-\text{CH}_2$ rigid backbone and the more mobile $-\text{CH}_3$ methyl end groups. The intermediate population at 12.2ms may be due to a pseudo interface of $-\text{CH}_2$ groups between these two extremes with more mobility than the center of the backbone chain but less than the rotationally mobile end groups (Figure 6.4). As temperature is increased, the most restricted population decreases in intensity and shifts from 0.249ms toward 0.819ms by 34°C. By 36°C, this population is lost, and by 38°C the sample has liquefied indicated by the collapse onto a single peak centered at 669.1ms. At 41°C, the distribution has narrowed and shifted to slightly longer T_2 , consistent with higher rotational diffusion at higher temperature. The temperatures of melting agree within the width of the DSC melting range of 31°C-39°C.

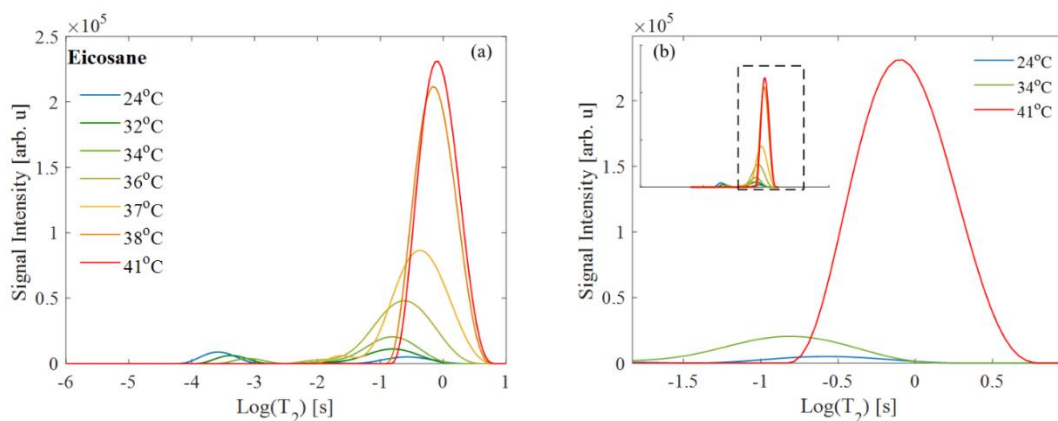


Figure 6.3: T_2 distributions for eicosane over temperatures from 24°C to 41°C (a). The liquification of the wax is indicated by the collapse of the distribution to one peak centered at 0.831s. A zoomed in view of the longest T_2 peak is shown at 24°C, 34°C, and 41°C (b). A shift toward shorter T_2 relaxation values is seen prior to melting which occurs at 41°C.

Interestingly, there appears to be pre-melting changes in molecular mobility behavior indicated by the relaxation data that does not appear in the DSC measurement.

In Figure 6.3(b), the longest T_2 population is zoomed in on for temperatures 24°C, 34°C and 41°C. At 24°C (blue), the population has a maximum at 281.6ms. Upon increasing temperature and melting, what is expected is a shift toward longer T_2 relaxation times indicating more mobility of the spins. Unexpectedly, what is seen is a shift toward shorter T_2 times prior to melting. At 34°C, the population has a maximum at 147.2ms and at 41°C the population shifts back toward longer relaxation times of 830.7ms as expected of a liquid. This move toward shorter T_2 times indicates a shift toward a more restricted mobility structure. This indicates an annealing effect where with increasing temperature, energy allows the system to rearrange at the molecular level, creating a more orderly arrangement of hydrocarbon chains and stronger dipolar coupling[1]. It has been shown eicosane experiences either a metastable triclinic or rotator phase transition [38, 39] between 32°C (rotator) and 34°C (triclinic) depending on past cooling history. The move toward shorter, then longer T_2 relaxation times, is attributed to moving from a pseudohexagonal phase to a hexagonal structure in which the configuration is marked by a tighter, closer packing configuration[13].

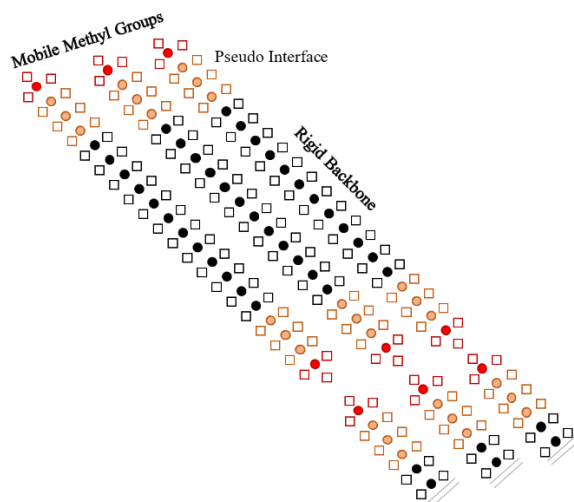


Figure 6.4: Packing schematic of eicosane. Filled circles indicate carbon atoms while open squares indicate hydrogens. The mobile methyl end groups are shown in red, the rigid backbone is shown in black, and the pseudo interface between the two with intermediate mobility is shown in orange.

T_2 Relaxation Dispersion Measurements

Exchange between T_2 populations was probed at 24°C (the solid phase) through T_2 measurements with increasing τ time [29, 40, 41]. If exchange is occurring between T_2 populations, as τ is increased, a shift or averaging effect between the two populations in question should be observed. This occurs regardless of filtering out of the short relaxation time signal from the more restricted environment with increasing τ , when $T_2 < \tau$. At $\tau = 12\mu\text{s}$, three populations are shown as discussed in Figure 6.3 at $T_2 = 0.249\text{ms}$, 12.2ms and 281.6ms respectively. With increasing τ there appears to be exchange occurring simultaneously between the populations to the left of the red dotted line and between the populations to the right of the red dotted line (Figure 6.5). This exchange is exhibited through shifting of the long time 281.6ms population toward shorter relaxation times and merging/disappearance of intermediate relaxation time populations which are not being

filtered out due to the increasing τ time. By a τ time of $240\mu\text{s}$, the distributions have merged to essentially a single peak at $T_2 = 295\text{ ms}$, signifying magnetization exchange is occurring at a more rapid rate than the CPMG sequence is sampling the system.

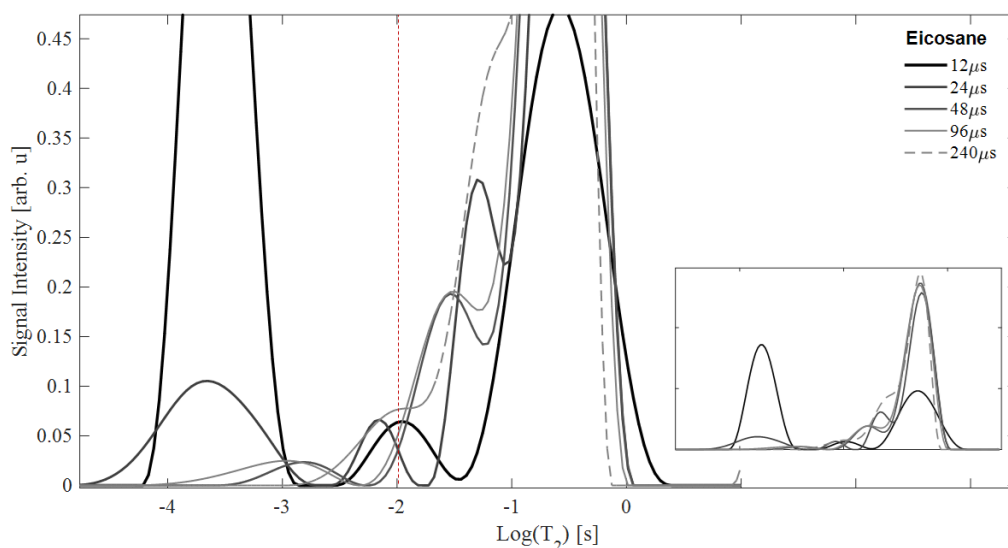


Figure 6.5: Normalized T_2 distributions are shown as a function of increasing τ time for eicosane at 24°C . Exchange can be seen between the shortest and mid T_2 populations and between the mid and longest T_2 populations indicated by the averaging of the population peaks between the two. The dotted red line bisects the intermediate peak of $T_2 = 10.96\text{ ms}$ measured for $\tau = 12\mu\text{s}$.

Increasing the τ time from $12\mu\text{s}$ to $24\mu\text{s}$, the intermediate population decreases T_2 from 12.2 ms to 7.2 ms indicating exchange between the rigid $-\text{CH}_2$ backbone and the more mobile pseudo interface spins discussed above (left of dotted line). At $\tau = 24\mu\text{s}$, the rigid backbone component $T_2 = 0.249\text{ ms}$ is not filtered out and should remain stationary. Additionally, to the right of the dotted line, there is an emergence of another peak between this intermediate population and the more mobile end groups at $\tau = 24\mu\text{s}$ with $T_2 \sim 49.9\text{ ms}$. This is likely due to exchange occurring between the pseudo interface

and the mobile end groups. Increasing τ to $48\mu\text{s}$ and $96\mu\text{s}$, the shortest T_2 population increases in relaxation time while the intermediate population present at $\tau = 12\mu\text{s}$ vanishes, suggesting averaging and distribution merging which occurs when the τ time is greater than the time between multiple exchange events. To the right of the dotted red line, the peak that emerged at $\tau = 24\mu\text{s}$ with $T_2 \sim 49.9\text{ms}$ has decreased in relaxation time to $T_2 \sim 29.0\text{ms}$. This shift to shorter T_2 once again indicates exchange between the pseudo interface and the ridged backbone spins. By $\tau = 240\mu\text{s}$, the populations have merged and centered on the longest T_2 relaxation time. This exchange is occurring on short timescales; however, chemical exchange is not present in normal alkanes nor is mass transport exchange occurring in the solid state at this temperature. This indicates a coherent process such as spin diffusion is the mechanism responsible for the magnetization exchange. Further quantification of the spin diffusion magnetization exchange occurring between multiple populations in the T_2 distribution requires extension of current analysis models to multiple site exchange[40, 42]. The data clearly indicates the enhanced information available from NMR relaxometry relative to the single energy absorption in DSC.

Octacosane

The octacosane sample was held at room temperature after melting and pipetting into the 5mm NMR tube for 24 hours. After, it was placed in the magnet and T_2 , T_2 dispersion, and T_2 - T_2 relaxation exchange measurements were taken for temperatures between 56°C and 66°C with a similar temperature equilibration scheme as used in eicosane.

Increasing Temperature T_2 Relaxation Measurements

T_2 relaxation measurements with a $\tau = 48\mu\text{s}$ were taken for temperatures of 56°C, 58°C, 60°C, and 66°C shown in Figure 6. At 56°C (blue, Figure 6.6), there are four populations, a restricted mobility at 0.204ms, intermediate mobility at 8.3ms and 53.1ms, and the most mobile population at 491.2ms, at this temperature octacosane is in a orthorhombic packing structure[43]. The population at 0.204ms are the hydrogens on the backbone with the population at 491.2ms being the liquid decane high mobility impurity. A transition between an orthorhombic structure to a hexagonal structure occurs between 56°C and 60°C [13, 43]. This can be seen as the distribution of signal shifts from the restricted population toward a more equal distribution at 58°C (green), then an increase in the more mobile population at 491.2ms with a shift of the most restricted population to 0.5474ms at 60°C (yellow). At 66°C the distribution collapses to a single peak at 805.5ms indicating a liquid. The transition seen through T_2 relaxation measurements is complementary to the specific volume plots observed by Templin *et. al.*[43] indicating the orthorhombic to hexagonal transition, prior to the hexagonal to liquid melt transition.

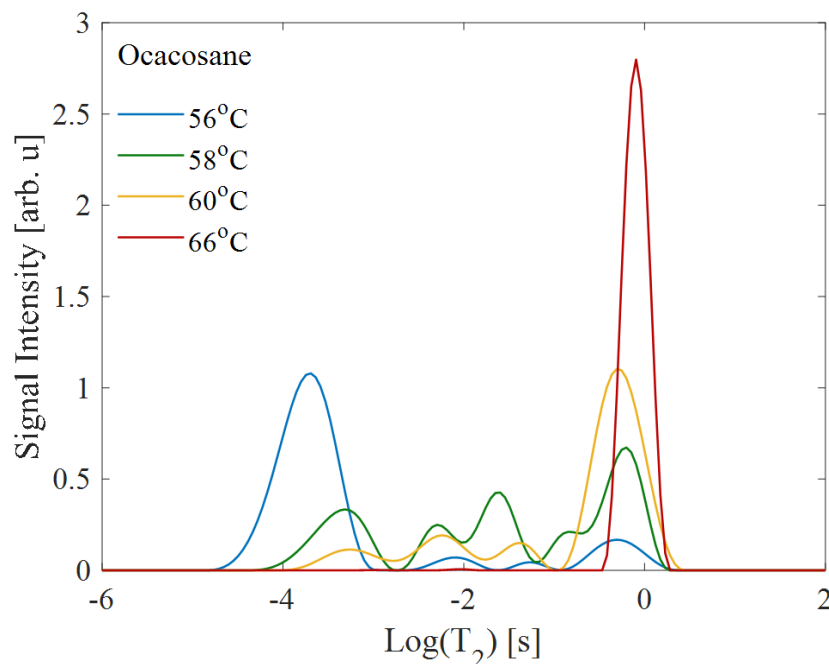


Figure 6.6: T_2 distributions with $\tau = 48\mu\text{s}$ for octacosane are shown for temperatures ranging from 56°C (blue) up to 66°C (red). An increase in mobility can be seen moving starting at 56°C, a transition at 58°C, and ending at 60°C correlating to a orthorhombic to hexagonal solid-solid transition[43]. The sample is melted by 66°C.

The most mobile population is from the decane impurity as T_2 measurements taken at 19°C (not shown) indicated the mobile population was still present suggesting a liquid component while the sample is known to be solid. Decane has a melting point of -31°C which corresponds to the small peak in the DSC measurements in Figure 6.2(b) and the presence of a liquid species within the sample.

T_2 - T_2 Relaxation Exchange Measurements

T_2 - T_2 relaxation exchange experiments at 56°C, 58°C and 60°C for mixing times of 100 μs , 1ms, 5ms and 10ms are shown in Figure 6.7. At temperatures prior to and during the orthorhombic to hexagonal transition, asymmetric exchange can be seen

between the backbone of the molecule and the methyl end groups. These are saturated at short mixing times. After the solid-solid transition, 60°C, these off diagonal exchange populations vanish, indicating a loss of exchange.

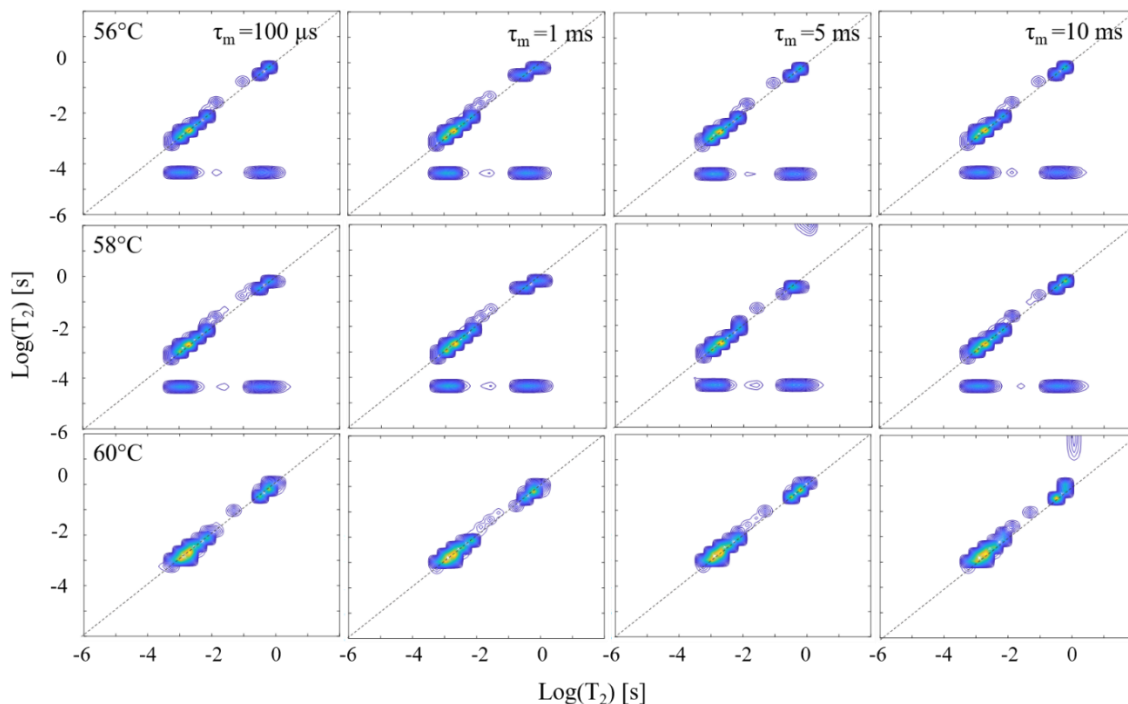


Figure 6.7: $T_2 - T_2$ relaxation exchange measurements are shown for mixing times of 100 μs , 1ms, 5ms and 10ms at temperatures of 56°C, 58°C, and 60°C taken with a $\tau = 12\mu s$. The exchange seen in the dispersion measurements is mirrored in the lower temperatures. The increase in temperature at 60°C causes the disappearance of the exchange populations.

At these temperatures the octacosane is still solid, the exchange cannot be due to physical mass exchange due to diffusion given the small liquid content of decane. Thus, this magnetization exchange is due to spin diffusion, or secular magnetization flip-flop, during the longitudinal magnetization storage in the mixing time. This is supported by the disappearance of the exchange after 60°C since the temperature change overlaps the

transition from an orthorhombic crystalline structure to a hexagonal structure. Templin *et.al.*[43] found the coefficient of expansion of the orthorhombic packed solid to be $0.0007 \text{ cm}^3/\text{g per } ^\circ\text{C}$, this would be represented by the data at 56°C . After the hexagonal transition, at 60°C , the coefficient of expansion of the hexagonal crystalline packing is $0.0020 \text{ cm}^3/\text{g per } ^\circ\text{C}$, the density of the molecular packing decreases. Spin diffusion is due to dipolar coupling and depends on $\frac{1}{r^6}$, meaning as the distance r between neighboring nuclei increases, the propagation of magnetization through spin diffusion decreases[44]. This manifests itself in the data of Figure 6.7 as the vanishing of exchange cross peaks after the solid-solid transition toward looser packing. Additionally, as temperature increases, the dipolar fluctuations caused by thermal energy increases as well. This causes the magnetization propagation denoted as spin diffusion to be averaged out. The combination of the change in the crystalline packing and the magnetization flip-flop rate causes the dissipation of spin diffusion within the system.

Figure 6.7 indicates a three site exchange and, in general the magnetization dynamics can be described as

$$\mathbf{M}(t) - \mathbf{M}^{(eq)} = \exp \{-(\mathbf{R} - \mathbf{K})(t - t_0)\} c[\mathbf{M}(t_0) - \mathbf{M}^{(eq)}] \quad (6.1)$$

where $\mathbf{R}$ is the relaxation matrix and $\mathbf{K}$ is the exchange matrix[42]. Unlike in two site exchange where the exchange maps are symmetric, three site exchange can exhibit asymmetry as evident in Figure 6.7. This is because of detailed balance. Each sum of each column of $\mathbf{K}$ must vanish, $k_{11} - k_{21} - k_{31} = 0$. An asymmetric exchange map means the exchange times differ strongly from each other suggesting there are both fast and slow exchange occurring within the system[42].

T₂ Relaxation Dispersion Measurements

T_2 dispersion measurements were taken at 56°C, 58°C, and 60°C to capture the change in magnetization exchange before, during and after the orthorhombic to hexagonal transition (Figure 6.8). At 56°C (Figure 6.8(a)), exchange is clearly occurring from the decrease in T_2 time of the most mobile population from 629ms at a τ time of 24 μ s down to 161.5ms at a τ time of 480 μ s. This exchange is seen at 58°C but disappears at 60°C (Figure 6.8 (b) and (c) respectively).

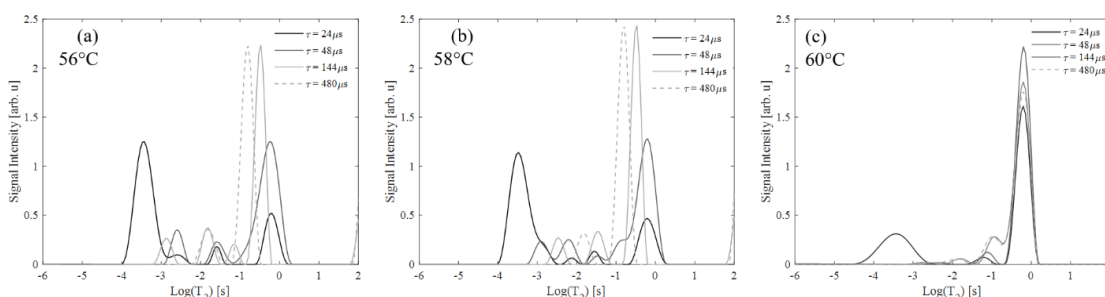


Figure 6.8: T_2 dispersion measurements are shown at 56°C (a), 58°C (b), and 60°C (c). Before and during the transition at 56°C and 58°C, exchange is observed. However, at 60°C, after an increase in mobility in the hexagonal structure, the exchange stops.

Spin diffusion is traditionally seen as something fixed within the system in ssNMR techniques. This is primarily due to the crystalline structure and lattice packing being stationary and held constant throughout experiments. However, with increasing temperature and allowing the system to undergo a crystalline rearrangement, the neighbor packing and magnetization propagation line is disrupted. This disruption and change in the system is seen clearly in Figures 6.7 and 6.8 through the change in exchange observed.

Beeswax

The beeswax sample was placed in the magnet T_2 relaxation measurements were taken in a similar manner as the eicosane sample with a starting temperature of 19°C increasing up to 62°C.

Increasing Temperature T_2 Relaxation Measurements

T_2 relaxation measurements using $\tau = 12\mu\text{s}$ were taken at increasing temperatures (Figure 6.9) to gain molecular motion information regarding the solid-solid phase transitions occurring. Figure 6.9(a) shows data over the temperature range of 19°C to 45°C. Comparing to the DSC data in Figure 6.2(c), this temperature range encompasses the first detected transition appearing between 10°C and 36°C where an orthorhombic to rotator phase transition occurs and the energy absorption DSC increase region approaching the pre-melting/softening transition occurring at 52°C[11]. At 19°C (blue), there is a bimodal distribution, as the temperature is increased from 19°C up to 41°C there is an emergence of an additional distribution peak at 0.723ms, between the two populations. As temperature increased from 19°C to 28°C, the most mobile distribution's maximum increases from 2ms up to 4.6ms. This is associated with the pre-melt transition shown in the DSC (Figure 6.2(c)). Additionally, starting at 28°C, the longer relaxation peak broadens to encompass longer relaxation times, while the local minimum of the bimodal distribution at 19°C becomes shallower. This transition and emergence of an additional peak indicates the breakdown of lattice symmetry and order, generating more molecular mobility due to changes in structural organization. Then, at 45°C, the distribution is broad, reflecting the broad range of molecular mobility in the system.

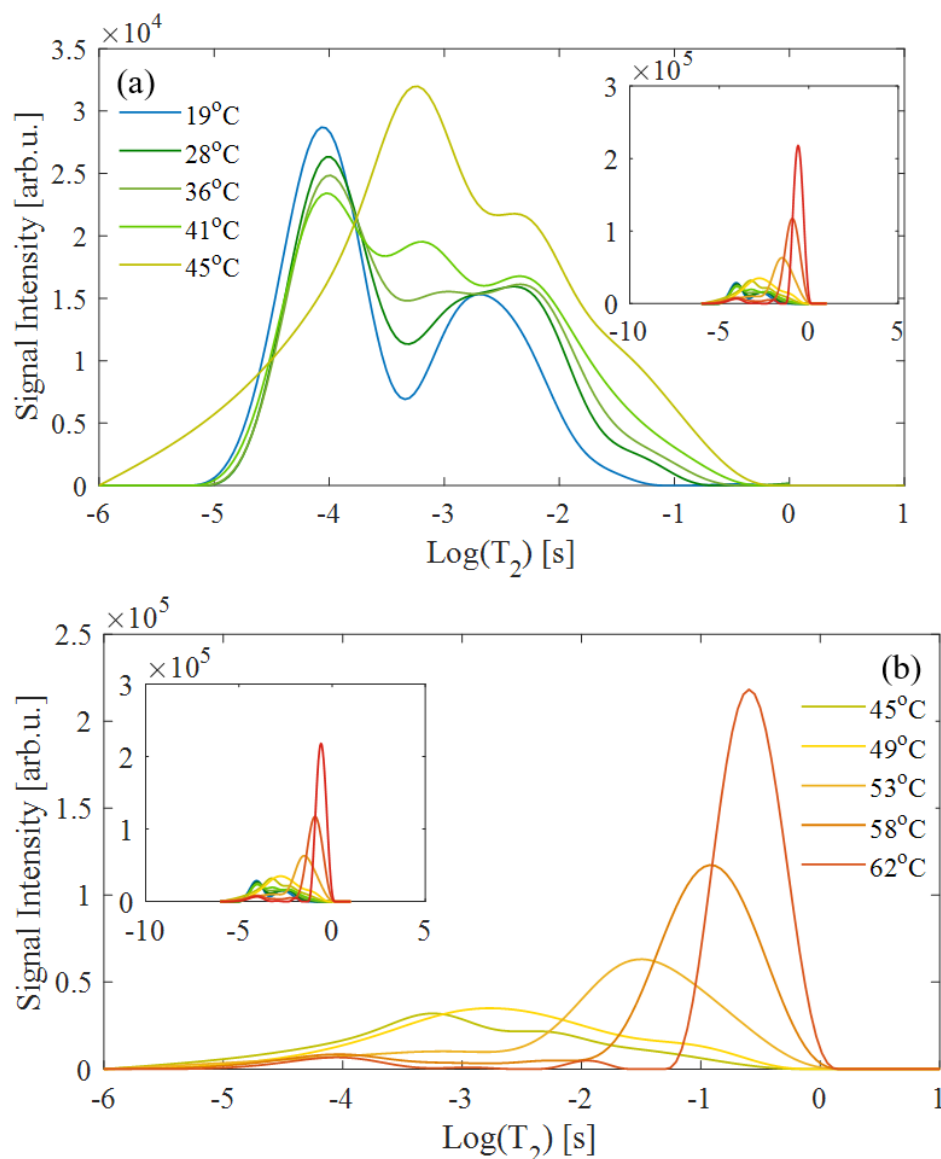


Figure 6.9: T_2 distributions for beeswax over increasing temperatures, low temperatures (a) and high temperatures (b) with the full distribution in the inset. There is an overall increase in signal, collapsing of the distribution and shift from short relaxation values to longer values indicating melting. In the temperature range of 19°C-45°C (a) there is a transition from a more rigid structure to one with more mobility indicated by the change in the T_2 distribution. The softening of the wax at 45°C (yellow-green), is marked by the T_2 population at $\sim 10^{-4}$ s shifting toward longer times of $\sim 10^{-3}$ s indicating an increase in molecular mobility of the bulk wax structure. In the temperature range of 45°C-62°C (b), the softening and melting of the wax is evident as merging of populations.

The data can be understood by comparison with Kameda *et. al.* where ssNMR techniques [9-11] have been used to investigate intermediate lattice structures of beeswax. At temperatures within the range of the first endothermic peak in the DSC data, 10°C to 36°C, it was reported the beeswax underwent an orthorhombic to rotator phase transition[11]. This solid-solid transition manifests itself as a broadening of the relaxation distribution and a breakdown of the bimodal distribution with the emergence of the central peak starting at 28°C. This phenomenon is stable and consistent until 45°C when a larger configurational change alters molecular mobility further. The temperature range in which the initial transition occurs agrees with the DSC data shown in Figure 6.2(c).

Additionally, in Kameda *et. al.*, it was found between 42.4°C and 47.5°C beeswax undergoes a solid-solid phase transition in which *gauche* and *trans* lattice configurations are mixed throughout the sample, being dominated by the *gauche* configuration within this temperature range[11]. Notably, the *gauche* structure is still present at 29.6°C but increases in magnitude with temperature increase until the structure is dominated by the *gauche* configuration[11]. This shift toward a more disordered lattice structure was determined to indicate a phase transition from crystalline to intermediate states[11]. The increase in *gauche* components can be seen in Figure 9(a) through the emergence of the peak at 0.723ms until it dominates the distribution at 45°C, in agreement with the ¹³C ssNMR data results.

The higher temperature range data shown in Figure 6.9(b) confirms the increased molecular mobility of the softening transition prior to melting. Figure 6.2(c) shows a

large pre-melting temperature range starting at 40°C up until the first transition at 52°C. Between these temperatures, the shift toward a *gauche* dominated structure occurs, Figure 6.9(a). By 53°C, the short relaxation time tail of the distribution decreases and the maximum peak is centered at 29ms accompanied by a signal increase of 180% for the maximum peak amplitude. This directly captures the softening of the sample occurring around 50°C[11, 45]. According to Figure 6.2(c) the solid-liquid melting transition is at 61°C. The pre-melting transition is evident in the T_2 relaxation distribution (Figure 6.9(b)) as a continual shift toward longer relaxation times, collapsing into a dominant long T_2 relaxation peak at 267ms and increase in signal intensity. By 62°C (red), the system has liquified and the relaxation distribution shows a dominant high mobility population at 267 ms and small persistent populations at 11 ms and 0.094 ms due to strong dipolar coupling of central protons on large chain molecules.

T_2 - T_2 Relaxation Exchange Measurements

T_2 - T_2 exchange experiments were performed on beeswax for mixing times between $\tau_m = 100\mu\text{s} - 50\text{ms}$ at temperatures spaced throughout the transition of 32-62°C (Figure 6.10).

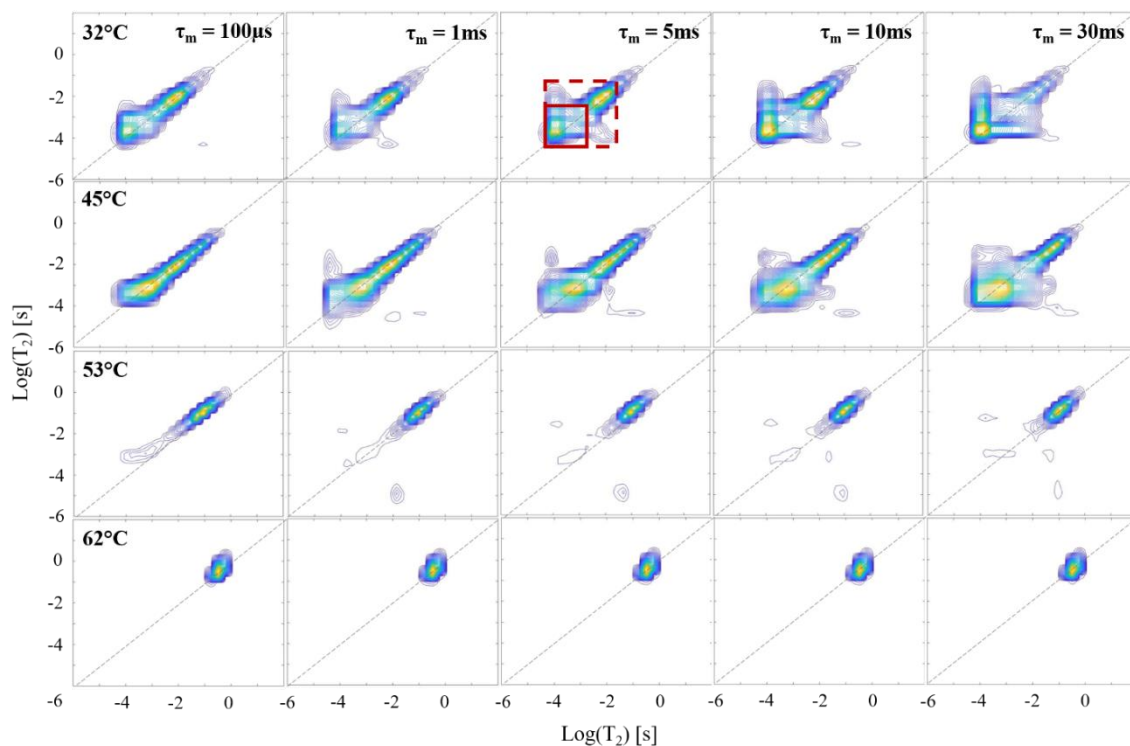


Figure 6.10: T_2 - T_2 exchange measurements for (rows) 32°C, 45°C, 53°C and 62°C with mixing time (columns) 100 μ s, 1 ms, 5 ms, 10 ms and 30 ms. At lower temperatures, two types of exchange can be seen, exchange between the two short T_2 peaks (solid red box) and exchange between a short T_2 and a longer T_2 (dotted red box).

As required, there is direct agreement between the T_2 relaxation distributions on the dotted (diagonal) line Figure 6.10 and the 1D distributions shown in Figure 6.9. Comparing the distribution intensities at 32°C, the primarily bimodal distribution corroborates the presence of a more rigid *trans* with traces of more mobile *gauche* lattice structure. Increasing to 45°C, the merging of the distributions and the structure becoming primarily *gauche* in nature is again seen along the diagonal. The phase transition between *trans* and *gauche* structures is manifest in the exchange data in Figure 10 as well. The non-diagonal exchange peaks indicate there are two distinct exchange processes occurring. One occurs between shorter T_2 relaxation environments creating a “box-like”

exchange pattern (solid box Figure 6.10), and a second produces a “wing-like” exchange pattern (dotted box Figure 6.10) between the shorter T_2 populations and a longer T_2 population. Both exchange phenomena occur at the lower temperature range and disappear by 53°C.

The “box” exchange pattern at 32°C is already established at the shortest mixing time of 100 μ s and grows in intensity and spreads with increasing mixing time. Due to the semi-solid nature of the system at this temperature and the short τ_m when the exchange occurs, mass transport translational molecular diffusion cannot be the cause of the exchange. Chemical exchange between the *trans* and *gauche* configurations will be present due to the close proximity of the hydrogens on the molecules and the presence of alcohols and any other chemically exchangeable protons. Given a typical timescale for chemical exchange in polysaccharide and alcohol solutions of 0.5 ms[41] and faster in proteins in solution and gels[29, 46], in a lipid solid state where any exchanging protons must be in direct spatial proximity and will have even faster exchange, impacts from chemical exchange will have partially saturated in the “box” cross peak intensity and pattern by the shortest exchange time of $\tau_m = 100\mu$ s. Hence the evolution of the exchange peaks on the longer timescales is attributable to spin diffusion, as is known to occur in beeswax[9]. Spin diffusion occurring between proton spins in mesoscale domains of differing structure allows calculation of domain length scales[1, 21, 22, 24]. Using calculated spin diffusion coefficients tabulated for block copolymer samples by Mellinger *et al*[22], for a T_2 on the order of 0.1ms and 5ms, the “box” exchange pattern, the spin diffusion coefficients are approximately 0.5nm²/ms and 0.02nm²/ms

respectively. Defining a spin diffusion length scale $d \sim (2D_{sp}\tau_m)^{1/2}$ based on the spin diffusion coefficient D_{sp} and the mixing time τ_m at which the exchange peak appears, we calculate the exchange occurring between sites with a distance on the order of magnitude of Angstroms for the “box” pattern between the shortest T_2 times present. This length scale could correlate to either inter-lamellar magnetization exchange within the crystalline structure or intramolecular since a single carbon bond is approximately 1.5 Angstroms. If the exchange is intramolecular, the disappearance with increasing temperature could be due to averaging with increase in molecular mobility. Inter-lamellar exchange would correlate to be between *trans* and *gauche* defects, or between interfaces of domains of *trans* and *gauche* oriented molecules. By 45°C, the “box” pattern is still present, but less distinct. This is due to the system being primarily dominated by *gauche* configurations. The presence of smaller amounts of *trans* oriented molecules, responsible for the broad 1D relaxation distribution, is also evidenced by the broadening of the 2D relaxation exchange distribution in the non-diagonal direction due to continuing exchange at 45°C. At 53°C, the system is entirely *gauche* and the “box” exchange pattern is lost. However, the assignment for the specific molecular or spin populations undergoing the exchange are ambiguous. These results do provide valuable information about the relative length scales and timescales of spin diffusion and will aid in future investigations to identify the structural nature of spin-exchangeable sites.

The “wing” exchange also occurs only at lower temperatures, but unlike the “box” exchange it appears and is prominent at longer mixing times. At 32°C, the exchange is occurring between T_2 populations on the order of 1ms and 50ms relaxation

times. The exchange timescale at $\tau_m = 10\text{ms}$ is still too short to allow for significant mass exchange due to translational diffusion in the solid and is therefore due to magnetization exchange from spin diffusion. Calculating a length scale of the “wing” exchange, yields a length scale between Angstroms and nanometers. This length scale suggests the observed spin diffusion is transferring magnetization across mesoscale domains of the crystalline lattice or between functional groups of the various components of beeswax. It has been shown through ^{13}C ssNMR, spin diffusion occurs within beeswax between the $-\text{CH}_3$ groups and the orthorhombic crystalline phase[9]. While it has been established[33] that spin diffusion can be probed through T_2 - T_2 relaxation techniques, these measurements indicate ^1H 2D T_2 - T_2 exchange experiments can characterize solid-solid structural transitions with temperature using exchange due to spin diffusion.

Additional measurements for 32°C and 45°C were taken at mixing times between $100\mu\text{s}$ and 50ms . Integration of the exchange populations of the “box” pattern, “wing” pattern and the total exchange with increasing mixing time is shown in Figure 6.11.

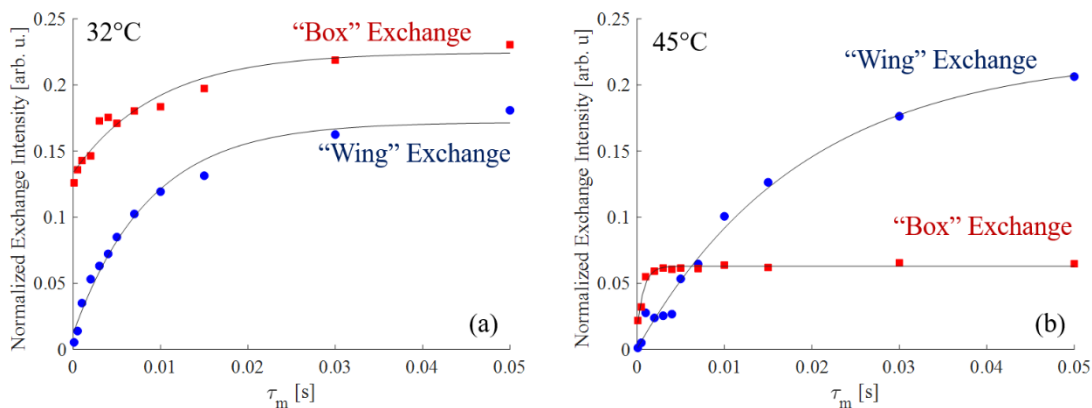


Figure 6.11: Normalized exchange intensities of each population for 32°C (a) and 45°C (b). Each population increase was fitted with $I = A - B \exp[-\frac{\tau_m}{C}]$ where C is the exchange correlation time.

A more quantitative approach is found in Figure 6.11. At 32°C and 45°C (Figure 6.11(a-b)), both exchange populations are following an exponential plateau as expected from a simple theory[19, 32]. At 32°C, the correlation timescales for both the “box” and “wing” exchange are 9.76ms and 8.68ms respectively. Using the diffusion coefficients scaled with $1/T_2$ as before, of $0.5\text{nm}^2/\text{ms}$ and $0.02\text{nm}^2/\text{ms}$ as a range for the “box” exchange, the length scale is approximately $9.8\text{\AA} - 3\text{nm}$. Exploring the “wing” exchange with diffusion coefficients of $0.025\text{nm}^2/\text{ms}$ to $0.5\text{nm}^2/\text{ms}$, the exchange length scale is a larger range of $6.6\text{\AA}$ to 2.94nm .

At 45°C the times scales are 0.76ms and 18.9ms for “box” and “wing” exchange patterns. Following the same method but using the diffusion coefficient calculated for this temperature of $0.5\text{nm}^2/\text{ms}$ for the “box” exchange, the length scale is approximately $8.7\text{\AA}$. For the “wing” exchange, diffusion coefficients could span the range of $0.025\text{nm}^2/\text{ms}$ to $0.5\text{nm}^2/\text{ms}$ which gives a length scale range of $9.7\text{\AA} - 4\text{nm}$.

What can be seen is as the temperature increases to 45°C, the “box” like exchange reaches saturation much quicker than the exchange at 32°C and the “wing” exchange. This may be due to the increase in $k_B T$ energy in the systems allowing for more chemical exchange and spin diffusion to occur quicker with that range. This is reflected with the decrease in correlation time scale. The length scales at which the exchange occurs for each of the exchange patterns is in relative agreement across these temperatures.

T_2 Relaxation Dispersion Measurements

Exchange was probed through normalized T_2 dispersion relaxation distributions with increasing τ times at 32°C and 53°C shown in Figure 6.12. At 32°C, Figure 12(a), the bimodal distribution collapses and merges together at $\tau = 96\mu\text{s}$. The initial distribution of relaxation times, at $12\mu\text{s}$, gives two T_2 populations at 0.940ms and 3.7ms. These merge into a single population at 2ms when $\tau = 96\mu\text{s}$. Note the slight shift from 3.7ms toward 2ms of the longest relaxation population and the vanishing of the shortest relaxation peak.

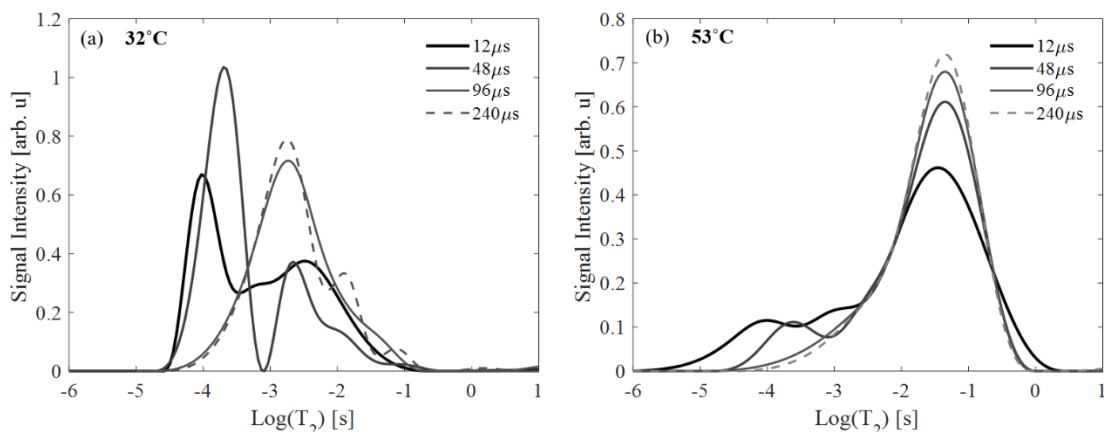


Figure 6.12: Normalized T_2 distributions are shown as a function of increasing τ time for beeswax at 32°C (a) and 53°C (b). At 32°C (a), a shift toward shorter relaxation times is seen corroborating the exchange seen in the T_2 - T_2 exchange plots. At 53°C (b), no exchange is observed, the longest relaxation time peak does not move, the shortest relaxation times are only filtered out by the increasing τ time.

At 53°C, no exchange was seen in the T_2 - T_2 exchange plots shown in Figure 6.10.

In Figure 6.12(b), there is no shift toward shorter relaxation times or merging of peaks.

The increase in τ time results only in the filtering out of the shortest T_2 populations.

Conclusions

Solid-solid phase transitions were explored for a normal alkane, eicosane, a longer normal alkane octacosane and a complex heterogeneous lipid, beeswax through ^1H NMR spin-spin relaxation measurements. T_2 relaxation distributions show structural rearrangements in both samples as a function of temperature. T_2 distributions of eicosane showed a reorganization toward tighter packing during the melting process that DSC alone did not indicate. Additionally, transitions between *trans* and *gauche* configurations were observable in beeswax through T_2 measurements and T_2 - T_2 exchange measurements.

Exchange was probed and shown to occur in all samples. Intriguingly T_2 relaxation dispersion measurements with increasing τ times exhibited spin diffusion of transverse magnetization. Eicosane was shown to have exchange between a pseudo interface of differing mobility due to crystal packing variations[21] and both the rigid backbone and the end methyl groups. Octacosane was shown to have spin diffusion before and during a solid-solid transition only to be disrupted by a hexagonal crystalline structure. T_2 - T_2 exchange in octacosane exhibited asymmetric exchange patterns that were saturated by 100 μ s. Beeswax T_2 - T_2 exchange measurements and T_2 relaxation measurements with increasing τ times indicate complex dynamics, including chemical exchange and spin diffusion, impacting the data. The spin diffusion occurred across two distinct length scales, one on the order of Angstroms indicating exchange between lamella, the other on the order of nanometers indicating exchange across mesoscale domains of differing mobility. The magnetization exchange behavior as a function of temperature correlates with DSC transitions.

The data presented demonstrate the ability of ^1H NMR relaxometry to characterize solid-solid phase transitions in lipid systems. Data such as this provides complementary information to DSC and ssNMR methods. In particular, experiments such as these can be implemented on low-field benchtop NMR systems for quality control and R&D applications.

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CHAPTER SEVEN

MULTIDIMENSIONAL NMR RELAXOMETRY OF MULTISITE EXCHANGE IN
GLYCERYL BEHENATES

Contribution of Authors and Co-Authors

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MULTIDIMENSIONAL NMR RELAXOMETRY OF MULTISITE EXCHANGE IN GLYCERYL BEHENATES

Abstract

Nuclear magnetic resonance (NMR) spin-spin T_2 relaxation is used to characterize transport phenomenon with temperature within three lipids, mono-, di- and tribehenin. With temperature changes, structural rearrangements, in the form of solid-solid phase transitions, are also characterized. $T_2(\tau)$ dispersion distributions and T_2 - T_2 exchange are shown to be sensitive to transverse and longitudinal magnetization spin diffusion respectively. Length scales this magnetization transport is occurring over are calculated based on the exchange correlation times found. The NMR relaxometry data has the potential for effectively characterizing complex dynamics and structure within lipids.

Introduction

The importance of glycerides is universal throughout food science, biology, the pharmaceutical industry, and other industries. [1] The pharmaceutical industry implements excipients to effectively release active ingredients in solid doses. Glycerides, a group of molecules, are often used due to their hydrophobic nature and the ability to have sustained drug release. Glycerol behenate is used among many due to being a mixture of mono-, di-, and tribehenin (18%, 52%, and 28% respectively). [2] The release rate of the active drug, while are often sustained in nature, is informed by the interactions and dynamics between polymorphic domains within the excipient, polymer and API component. [3] Being able to noninvasively

probe these dynamics is important to improving the relationship between function and material properties.

Nuclear magnetic resonance (NMR) relaxometry is a noninvasive technique that probes the dynamics between ^1H populations. [4] The structural order versus disorder of a sample is explored through determination of the molecular mobility of molecular populations within differing molecular structures *e.g.* mono- versus di- versus tribehenin, and their mesoscale packing structures. [1] These include lamellar and domain structures which impart longer range order on the sample. [1] Elucidation of mesoscale structure provides insights into the material function and material properties. Small-angle X-ray spectroscopy (SAXS) and wide-angle X-ray spectroscopy (WAXS) are often used to determine polymorphic dynamics, being able to explore the microstructures of a system. [2, 5] NMR relaxometry techniques allow for complementary data to explore the material dynamics further. Not only can information about what polymorphic domains are present in a lipid be obtained, but information about interactions between polymorphic domains can be teased out as well. Monobehenin, dibehenin, and tribehenin are explored as individual components and two mixtures, one composed of dibehenin and tribehenin and the other composed of monobehenin, dibehenin and tribehenin. [2] The data presented indicate the ability of NMR relaxometry to explore multisite magnetization exchange and polymorphic changes in lipids. Length and timescales of the magnetization exchange processes are probed using $T_2(\tau)$ dispersion measurements of varying echo time, and T_2 - T_2 exchange experiments. [4, 6, 7]

Theory

Lipids are macromolecular mixtures which can exhibit polymorphic crystalline states or domains. [1] Within each of these states, there can be differences in structures which cause variation in molecular mobility. [1, 8] The molecular structural arrangement can change with temperature and chemical composition. Understanding the structure and interaction of domains and provides means for more quantitative understanding of these materials.

Solid state NMR (ssNMR) techniques [9] and spectroscopy techniques such as WAXS and SAXS [2, 5] have been implemented to understand these polymorphic transitions with changing temperature and chemical composition. [8, 10-12] NMR spin-spin T_2 relaxation measurements have primarily been implemented to explore microscale molecular packing. [8, 13] While WAXS and SAXS data yield high resolution of polymorphic length scales, little information regarding transport phenomenon within or between these domains can be obtained due to no time dependence of the measurement. Transport in the form of magnetization exchange can occur through physical mass transport such as molecular diffusion or chemical exchange between sites. [4, 6, 14-17] However, if there is no mass transport, magnetization exchange occurs through a mechanism called spin diffusion. [9, 18-23]

Spin diffusion is a dipolar coupling-based magnetization exchange process, which as the name implies, can be described as a diffusive process. Thus, follows the following diffusion equation [19]

$$\frac{\partial \mathbf{M}_\alpha(\mathbf{r}, t)}{\partial t} = D \nabla^2 \mathbf{M}_\alpha(\mathbf{r}, t) - A_\alpha(\mathbf{r}) \mathbf{M}_\alpha(\mathbf{r}, t) \quad (7.1)$$

where $A_\alpha(\mathbf{r})$ is the relaxation scalar field known *a priori* as the following

$$A_\alpha(\mathbf{r}) = \begin{cases} T_1(\mathbf{r})^{-1}, & \alpha = Z \\ -i\omega(\mathbf{r}), & \alpha = T \end{cases} \quad (7.2).$$

$A_\alpha(\mathbf{r})$ depends on whether the magnetization is in the longitudinal ($\alpha = Z$) or transverse ($\alpha = T$) domain. [19] This will have ramifications for interpretation of $T_2(\tau)$ dispersion and T_2 - T_2 exchange experiments.

Two limits are useful to explore, the fast diffusion limit where $\frac{D}{l^2} \gg (A_\alpha^{Max} - A_\alpha^{Min})$, and the slow diffusion limit where $\frac{D}{l^2} \ll |A_\alpha|$, where D is the diffusion coefficient and l the average distance between A_α^{Max} and A_α^{Min} . [19] Physically, the fast diffusion limit is when the spin carrying molecule is diffusing much faster than relaxing, meaning it has time to fully explore the spatial and relaxation gradients of the system. In the slow diffusion limit, the spins are relaxing faster than diffusing. The spin will not experience or explore as many spatial locations within the same time as in the fast diffusion limit and will not experience as many relaxation gradients.

In the fast diffusion limit, magnetization has ample time to explore various spatial locations and their respective relaxation rates, $A_\alpha(\mathbf{r})$. A cumulant expansion or a second-order perturbation theory method can be used to find what the magnetization evolution depends on. [19] Either method leads to the magnetization evolution depending on $\langle \delta A_\alpha(\mathbf{r}) \delta A_\alpha(\mathbf{r}') \rangle$, the spatial correlation of fluctuations of $A_\alpha(\mathbf{r})$. This relates the spatial correlation of the fluctuations in the spin-lattice relaxation rate, T_1^{-1} , for the longitudinal component, and $\langle \Delta\omega^2 \rangle^{1/2}$, which is T_2^{-1} , the spin-spin relaxation for the transverse component to the spin diffusion process. Transverse magnetization spin

diffusion depends explicitly on the T_2 variations of the system while longitudinal magnetization spin diffusion depends explicitly on the T_1 variations of the system.

In the slow diffusion limit, the magnetization does not sample each location as in the fast diffusion limit. In this limit, the magnetization evolution depends on $|\nabla A_\alpha(\mathbf{r})|^2$ and $\nabla^2 A_\alpha(\mathbf{r})$. [19] This dependence may be combined into one function, $h(\mathbf{r}, t)$, which serves as a memory function for the evolution of the magnetization. [19] With this memory function, concrete dependence of the transverse and longitudinal magnetization components may be found. The equation of motion becomes [19]

$$\dot{M}(\mathbf{r}, t) = -A_\alpha(\mathbf{r}) M(\mathbf{r}, t) + D \int_0^t h(\mathbf{r}, t - \tau) M(\mathbf{r}, \tau) d\tau \quad (7.3).$$

Separating $A_\alpha(\mathbf{r})$ into the transverse and longitudinal components, it must first be noted $A_T(\mathbf{r})$ is imaginary and the linear approximation used in Cheung [19] is only appropriate for large frequencies. Because of this, it exhibits poles in the complex plane. While spin diffusion occurs, it leads to dephasing of the magnetization which correlates to these poles shifting toward more negative and more imaginary locations. Unlike this, the longitudinal scalar field, $A_Z(\mathbf{r})$, is linear and has poles along the real axis. Through spin diffusion, these poles may have slight imaginary components added while shifting, however, the T_1^{-1} dependence dominates. Ultimately, the equations of magnetization evolution become [19]:

$$\begin{aligned} M_T(\mathbf{r}, t) &= \exp[i\omega(\mathbf{r})t - D|K|^2 t^3/3] \\ M_Z(\mathbf{r}, t) &= \exp[-t/T_1(\mathbf{r})] \cosh|\beta_2|t \end{aligned} \quad (7.4)$$

where $|K|$ is independent of $\mathbf{r}$ and is due to random frequency fluctuations and $\beta_2 \equiv (D\nabla^2 \frac{1}{T_1(\mathbf{r})})^{1/2}$. The transverse magnetization depends on the frequency of the system

which is related to T_2 through the frequency fluctuations. The longitudinal magnetization depends on T_1 of the system. Ultimately, in each diffusion regime, transverse and longitudinal magnetization exchange, due to spin diffusion, evolve due to different relaxation mechanisms.

Methods

Samples of 99% pure monobehenin, dibehenin, and tribehenin (Sigma Aldrich, Burlington, MA) were used in these experiments. Individual native samples were measured out and loaded into 5mm NMR tubes and placed into a LiCl salt controlled relative humidity of 11% prior to melting. Each sample was melted in a bath of Fluorinert FC-43 at 120°C prior to measurements, once melted the sample sat for 30 minutes at this temperature and then the temperature was incrementally decreased at 1°C/min until the bath rested at 75°C. The sample was then moved to a Bruker 250 MHz superconducting magnet using a custom built high-powered rf probe and 5mm rf coil (Bruker). $T_2(\tau)$ dispersion measurements and T_2 - T_2 exchange experiments (Figure 7.1) were taken at 74°C after an equilibration time of 10 minutes in the magnet. After finishing measurements at 74°C, the temperature was incrementally decreased at 1°C/min until at 20°C where the measurements were repeated. Sample temperature was controlled through the active control Bruker BTU system with N₂ gas flow.

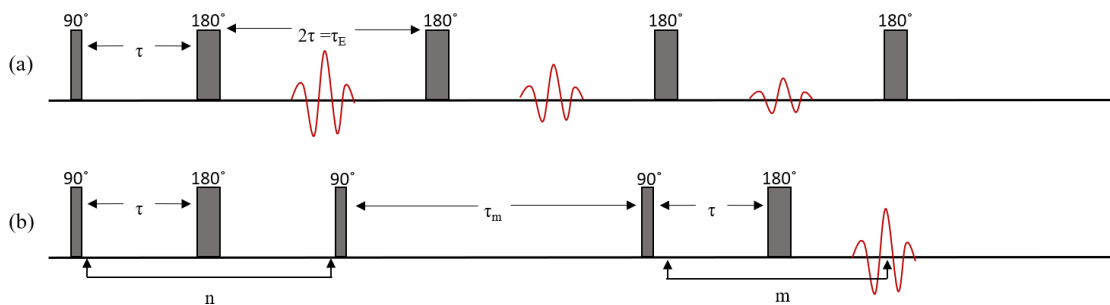


Figure 7.1: The pulse sequences used in this work are shown, (a) Carr-Purcell-Meiboom-Gill (CPMG) and (b) T_2 - T_2 exchange correlation.

A 67/33 wt% sample of dibehenin and tribehenin was made by weighing out each component and placing both into a 5mm NMR tube. This was then placed into the 11% RH container until the protocol was repeated. The melting and experimental procedure was the same as the individual samples. This process was repeated for the 20/50/30 wt% sample.

The $T_2(\tau)$ dispersion measurements had τ times of 12 μ s, 24 μ s, 36 μ s, 48 μ s, 60 μ s, 72 μ s, 84 μ s, 96 μ s, 108 μ s, 120 μ s, 144 μ s, 240 μ s, 360 μ s, 480 μ s, 576 μ s, 600 μ s, 720 μ s, 1200 μ s, 1440 μ s, 1920 μ s, 2160 μ s, 2400 μ s, 2640 μ s, 3120 μ s, 3360 μ s, and 3600 μ s for all samples. Each measurement was taken with 96 averages with 16 phase cycles per average. The repetition time was 10 s. The T_2 - T_2 exchange measurements were taken with mixing times of 100 μ s, 200 μ s, 300 μ s, 500 μ s, 700 μ s, 1 ms, 2 ms, 3 ms, 5 ms, 7 ms, 8 ms, and 10 ms with an echo time, τ_E , of 24 μ s. Each measurement was taken with 64 averages with 16 phase cycles per average. The repetition time was 10 s.

The $T_2(\tau)$ dispersion and T_2 - T_2 relaxation correlation experimental data for all samples were processed through a Fredholm integral or, inverse Laplace transform analysis. [24, 25]

Results and Discussion

Individual Components: Monobehenin, Dibehenin, and Tribehenin

After the melting and temperature equilibration protocol, each individual sample T_2 dispersion was measured with increasing τ from 12 μ s up to 2400 μ s (Figure 7.2 (b, d, f)). Then, T_2 - T_2 relaxation correlation data were acquired. The temperature was decreased at 1°C/min until 20°C where additional T_2 dispersions (Figure 7.2 (a, c, e)) and T_2 - T_2 exchange measurements were taken.

T_2 Relaxation Dispersion Measurements

If exchange is occurring during the dispersion measurement, long T_2 populations will have a decrease in T_2 time due to exchanging populations as τ increases regardless of filtering out shorter T_2 populations, when $T_2 < \tau$. [6, 14, 26-28] For each sample (Figure 7.2), the most mobile population with longest T_2 is characterized by this decrease in relaxation time.

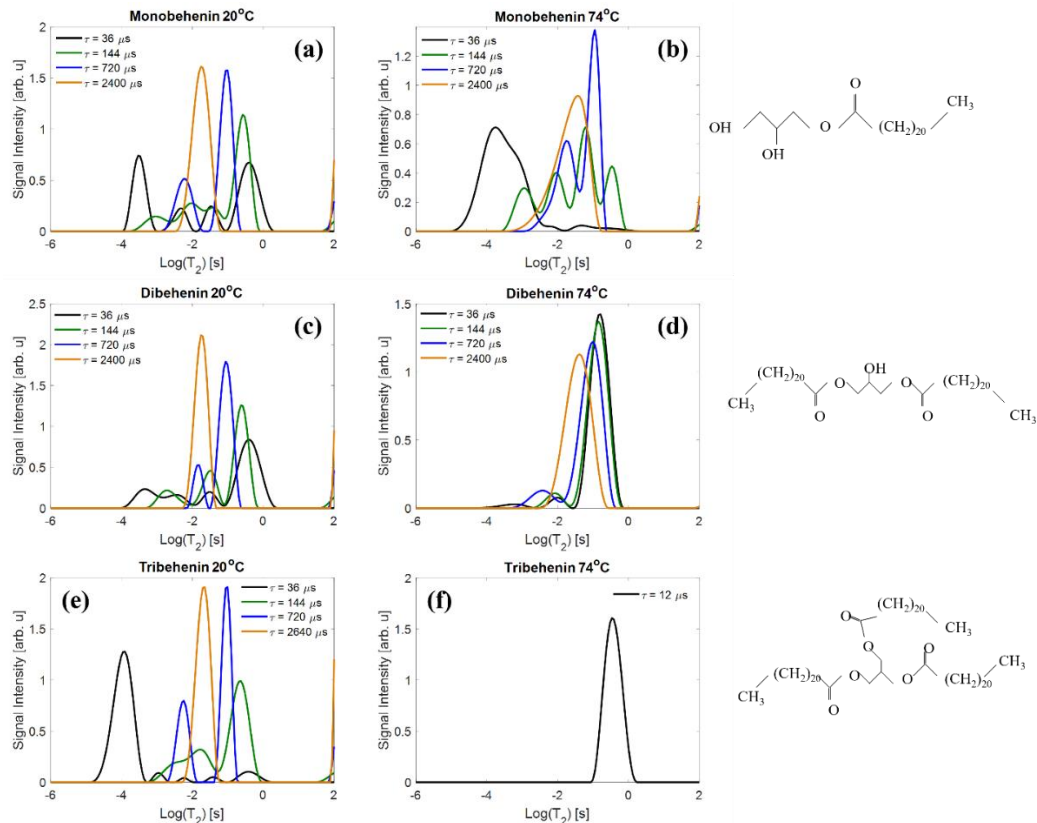


Figure 7.2: T_2 dispersion data for monobehenin (a, b), dibehenin (c, d), and tribehenin (e, f) are shown for 20°C and 74°C along with their respective chemical structure. The most mobile component of monobehenin at 20°C (a) decreases in T_2 relaxation time from 0.3836 s at a 36 μs τ time (black) down to 0.0175 s at a 2400 μs τ time (orange). At 74°C (b), the relaxation time decreases from 0.3836 s to 0.0366 s for an increasing τ from 36 μs (black) to 2400 μs (orange) respectively. For dibehenin, at 20°C (c), the most mobile component decreases from 0.3836 s to 0.0175 s for increasing τ of 36 μs (black) to 2400 μs (orange) respectively. Similarly at 74°C (d), a decrease is seen from 0.1615 s to 0.0414 with increasing τ time (36 μs = black and 2400 μs orange respectively). Tribehenin exhibits the same exchange behavior at 20°C (e) with a decrease in relaxation time starting at 0.3836 s down to 0.0223 s (36 μs = black and 2400 μs = orange respectively). At 74°C (f) tribehenin does not exhibit exchange staying at a T_2 relaxation time of 0.3392 s due to being completely melted.

The simplest glyceride studied is monobehenin (Figure 7.2 (a-b)), with a single carbon chain connected to a glycerol. The most mobile component at 20°C (Figure 7.2(a)), starts with a relaxation time of 0.3836 s with a τ time of 36 μs . As the echo time increases, the population distribution collapses down to a single peak (orange) centering at 0.0366 s with a τ time of 2400

μs . In a similar fashion, at 74°C (Figure 7.2(b)), the most mobile component begins at 0.3836 s and decreases down to a relaxation time of 0.0175 s with a τ time of 36 μs and 2400 μs respectively. As the temperature increases, the distribution morphology changes, broadening at the shortest τ time distribution. This could be due to the increase in rotational mobility within the sample, more explicitly the increased flexibility of the backbone. As the temperature increases, monobehenin begins in a sub- α_2 structure at 20°C and then begins transitioning to a sub- α_1 structure at 37°C . [2, 29, 30] This then reorients again beginning at 66°C settling at an α packing structure until melting at 86°C . [2] While similar, the sub- α_2 , sub- α_1 , and α structural rearrangement can be discerned from the distributions. Broadening of the individual peaks, seen in the T_2 distributions, is caused by a structural rearrangement. The sample is becoming more amorphous prior to melting due to the increase in temperature.

Dibehenin (Figure 7.2(c-d)), has an additional carbon chain creating a V-like packing structure [1] adding another layer of complexity onto the monobehenin base structure. Exchange is still prevalent, as seen by the converging to one peak at a shorter T_2 time at both 20°C (Figure 7.2(c)) and 74°C (Figure 7.2(d)). However, unlike monobehenin, large structural rearrangement can be seen through the change in the T_2 distribution with increasing temperature. Beginning at 20°C , it is known there is a mixture of β and sub- α domains. [2, 5] However, the sub- α domains are already beginning to transition to α at 20°C . [2, 5, 31] At 74°C , the α domains are melted (with melting temperature of 70°C) but the β domains are still solid, having a melting temperature of 86°C . This transformation is seen in the distributions. At 20°C , the morphology is complex with multiple populations, however, this disappears at 74°C , matching the simplification in unique polymorphic domains. At 20°C , the most mobile component for a τ time of 36 μs begins at 0.3836 s. After increasing the τ time up to 2400 μs , the population once again collapses to a single population at 0.0175 s. At 74°C , the T_2 population moves from 0.1615 s down to 0.0414 s with τ being 36 μs and 2400 μs respectively.

Finally, tribehenin (Figure 7.2(d-e)) is melted at 70°C which is seen in Figure 7.2(e) as a single peak at 0.3392 s. However, at the lower temperature of 20°C, there is exchange occurring once again. The most mobile peak begins at 0.3836 s and ends at 0.0223 s for a τ time of 36 μ s and 2400 μ s respectively. Unlike the other two components, tribehenin has a single polymorphic structure the entire time, α . [2, 32]

Interestingly, all individual components have the same T_2 value for the most mobile component at 20°C. This population could be due to the most mobile components of the molecule, in this case, is the -CH₃ methyl end groups. The collapsing of the distribution down to a single peak with a shorter relaxation time indicates exchange is occurring in the system between these methyl groups and other components of the molecule or domain. Regardless of the changes in packing, the addition of more hydrocarbon chains and oxygen sinks onto the glycerol, and the polymorphic domains, the mobility of what we hypothesize as the methyl groups stays the same at this temperature.

Following the method from Hills *et. al* [26], the inverse of the T_2 of the most mobile population, for each sample at each temperature, was plotted against the inverse of the τ time (Figure 7.3).

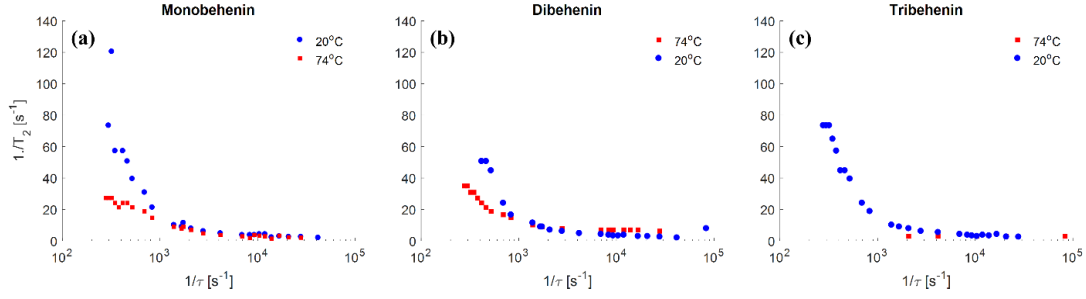


Figure 7.3: Plots of $1/\tau$ vs $1/T_2$ for each component are shown for 20°C (blue, circle) and 74°C (red, square). Exchange rate is shown in the slope of the plots. For all components, at 20°C (blue, circle), there is a larger exchange rate compared to 74°C (red, square). Monobehenin (a) has the largest exchange rate shown through the largest value of inverse T_2 , then tribehenin (c), and finally dibehenin (b) having the smallest exchange rate occurring at 20°C. At 74°C, tribehenin has no exchange while dibehenin has a larger exchange rate than monobehenin. The plateau correlating to $k_{ex} \gg 1/\tau$ is not found at 20°C for monobehenin, indicating it having the largest k_{ex} .

The two limits of the $1/\tau$ vs $1/T_2$ plots to take note are as $1/\tau$ approaches zero, the plateau could be just starting in dibehenin (Figure 7.3 (b)) and tribehenin (Figure 7.3 (c)) correlates to the regime in which $k_{ex} \gg 1/\tau$. [6] In all three components, the plateau which arises at τ approaching τ_{max} correlates to the regime in which $k_{ex} \ll 1/\tau$. [33]

Monobehenin (Figure 7.3 (a)) exhibits different magnetization exchange behavior in the transverse plane at 20°C (blue, circle) and 74°C (red, square). In the regime in which $k_{ex} \ll 1/\tau$, we are able to resolve the individual T_2 populations with little exchange averaging out the relaxation times. However, as the τ time increases, moving toward the $k_{ex} \gg 1/\tau$ regime, we are not able to reach this limit seen as the absence of the upper limit plateau. Even with this, qualitative observations may be made. At 74°C, the slope of the plot is significantly less than that at 20°C. This suggests the exchange in the transverse plane is temperature dependent, meaning the exchange is closely related to the packing structure of the lipid which changes as a function of temperature. The rigidity

of the system appears to enhance the exchange allowing more to occur. At 20°C, monobehenin is completely solid with no liquid phases. The exchange occurring must be due to some magnetization transfer not physical molecular diffusion. Dibehenin (Figure 7.3 (b)) also displays different exchange behavior at each temperature indicating the structural rigidity of the lipid impacts the ability for the exchange to occur again. At both temperatures, the plateau is barely reached with two consecutive τ values ending with the same T_2 value. Assuming this is the beginning of the $k_{ex} \gg 1/\tau$ regime, the 20°C (blue circle) has a quantitative higher exchange rate than the exchange occurring at 74°C. Tribehenin (Figure 7.3 (c)), has no exchange at 74°C. Exchange is occurring at 20°C with similar beginnings of a plateau in the $k_{ex} \gg 1/\tau$ regime as dibehenin.

Comparing all three components, there is an overall increase in transverse magnetization exchange at 20°C compared to 74°C. Also, monobehenin appears to have stronger exchange occurring indicated by the lack of acquiring the $k_{ex} \gg 1/\tau$ regime. The increase in the complexity, or addition of more carbon chains and oxygens, does alter the exchange occurring. The addition of another oxygen sink in the glycerol center could be the reason behind this.

The exchange occurring throughout this measurement is occurring in the transverse plane. Due to the lipids being solid, except for tribehenin at 74°C, physical molecular motion and diffusion cannot be the driving force of the exchange seen. Because of this, another coherent exchange phenomenon is the source. On the molecules, there are -OH groups within the glycerol. These could be undergoing chemical exchange. However, the amount of exchange seen in these systems cannot be only due to the exchange between

the hydrogens on the -OH. Spin diffusion, or magnetization flip-flop, could be a source of the exchange seen as well. This type of exchange is traditionally probed through storing the magnetization in the longitudinal plane, such as in a T_2 - T_2 experiment. However, Cheung explains while spin diffusion is seen in the longitudinal plane, it does occur in the transverse plane as well, dependent on the variance of the frequencies, or the T_2 of the system [19].

One may suggest the exchange in the transverse plane is due to cross relaxation no spin diffusion [27]. The techniques used here are unable to spectrally resolve the molecule, so in theory the spins are precessing at approximately the same frequency. However, this process is stochastic in nature and is unable to be refocused with the CPMG pulse sequence. Thus, we suggest spin diffusion as the primary exchange mechanism.

T_2 - T_2 Relaxation Exchange Measurements

After the $T_2(\tau)$ dispersion measurements, T_2 - T_2 relaxation correlation experiments were taken for each component at 74°C and 20°C (Figure 7.4).

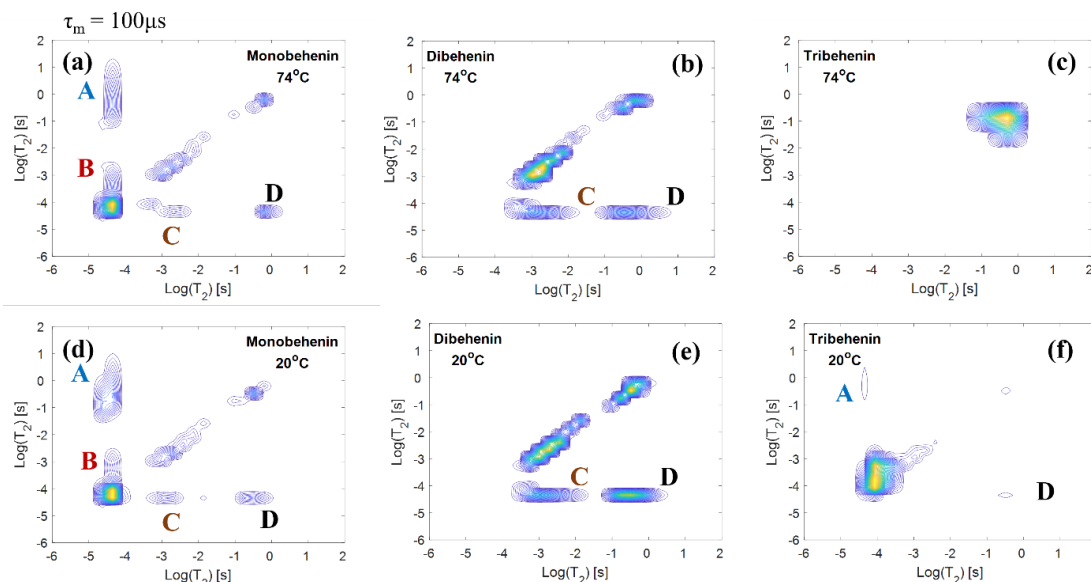


Figure 7.4: T_2 - T_2 exchange plots for a mixing time of 100 μs are shown for monobehenin (a, d), dibehenin (b, e), and tribehenin (c, f) for 74°C (top column) and 20°C (bottom column). Symmetric exchange is seen in monobehenin for both temperatures (a, d) with exchange between the most and least mobile populations (A and D) along with exchange between more restricted environments (B and C). Dibehenin (b, e), however, exhibits asymmetric exchange moving from the most restricted environments to the most mobile (C, D) for both temperatures. At 74°C (c), tribehenin is melted, but small symmetric exchange is seen at 20°C (f) between the backbone and mobile end groups (A, D). Note the first encoding period is on the y axis and the second encoding period is on the x axis.

As the base molecule increases the number of carbon chains, the amount of exchange populations decreases at each temperature. Monobehenin (Figure 7.4(a, d)), at 74°C has four, symmetric exchange populations denoted A, B, C, and D (Figure 7.4(a)). A is centered at (4.58×10^{-5} s, 0.820 s) indicating the spin began in an environment with a T_2 of 0.820 s and moved during the mixing time to an environment with a T_2 of 4.58×10^{-5} s. The magnetization moved from a more mobile environment to a less mobile environment. Similarly, the symmetric counter part is shown in D, being centered at (0.680 s, 4.30×10^{-5} s). The exchange pair of B and C are centered at (4.58×10^{-5} s, 3.67×10^{-4} s) and (1.25×10^{-3} s, 5.18×10^{-5} s) respectively. At 20°C, the symmetric exchange

is also seen with magnetization in the A exchange population moving from 0.223 s to 3.38×10^{-5} s and its counterpart D starting at 4.58×10^{-5} s and ending in an environment with a T_2 of 0.256 s. Exchange population B transfers from a local environment of having a T_2 of 6.77×10^{-3} s to 4.583×10^{-5} s. The symmetric pair C exchanges between a location having a T_2 of 4.87×10^{-5} s to one of 1.33×10^{-3} s. Exchange populations A and B have magnetization moving from a more mobile environment to a more restricted environment while C and D have magnetization moving in the opposite direction., from a more restricted environment to a more mobile one.

Interestingly, dibehenin shows an asymmetric exchange distribution. Using a simple interpretation of all longitudinal magnetization moving directionally from the more restricted environment to more mobile groups, this result is consistent with ‘flip-flop’ spin diffusion in polymers. [34] Additionally, the asymmetry could be due to a three-site exchange model. [35] The exchange populations are denoted at C and D (Figure 7.4 (b, e)). At 74°C the spins ending up at C begin with a T_2 relaxation time of 4.87×10^{-5} s and end up in a location with a relaxation time of 1.12×10^{-3} s. Similarly, in exchange population D, the spins began with a T_2 of 4.87×10^{-5} s and moved to a more mobile environment indicated with a T_2 of 0.502 s. At 20°C, a similar situation is seen with movement from 4.58×10^{-5} s to 1.25×10^{-3} s T_2 times at C and 4.05×10^{-5} s to 0.308 s T_2 times at D. However, other causes of asymmetry in T_2 - T_2 exchange experiments, such as T_1 variations, could be responsible and the interpretation as directional must be confirmed through modeling.

Tribehenin (Figure 7.4 (c, f)), with three carbon chains on glycerol, is liquid at 74°C. No exchange is measured due to motional averaging in the liquid sector. [27] At 20°C, it has a symmetric exchange distribution with exchange peaks denoted A and D. The magnetization ending at A migrate from 0.682 s relaxation environments to 4.87×10^{-5} s relaxation environments. Magnetization in exchange population D began at 5.18×10^{-5} s and ended with a T_2 of 0.307 s.

Quantitative information about the exchange rate and exchange length scale can be obtained through plotting the normalized intensity of each exchange peak against the increasing mixing time, τ_m (Figure 7.5). [7, 36]

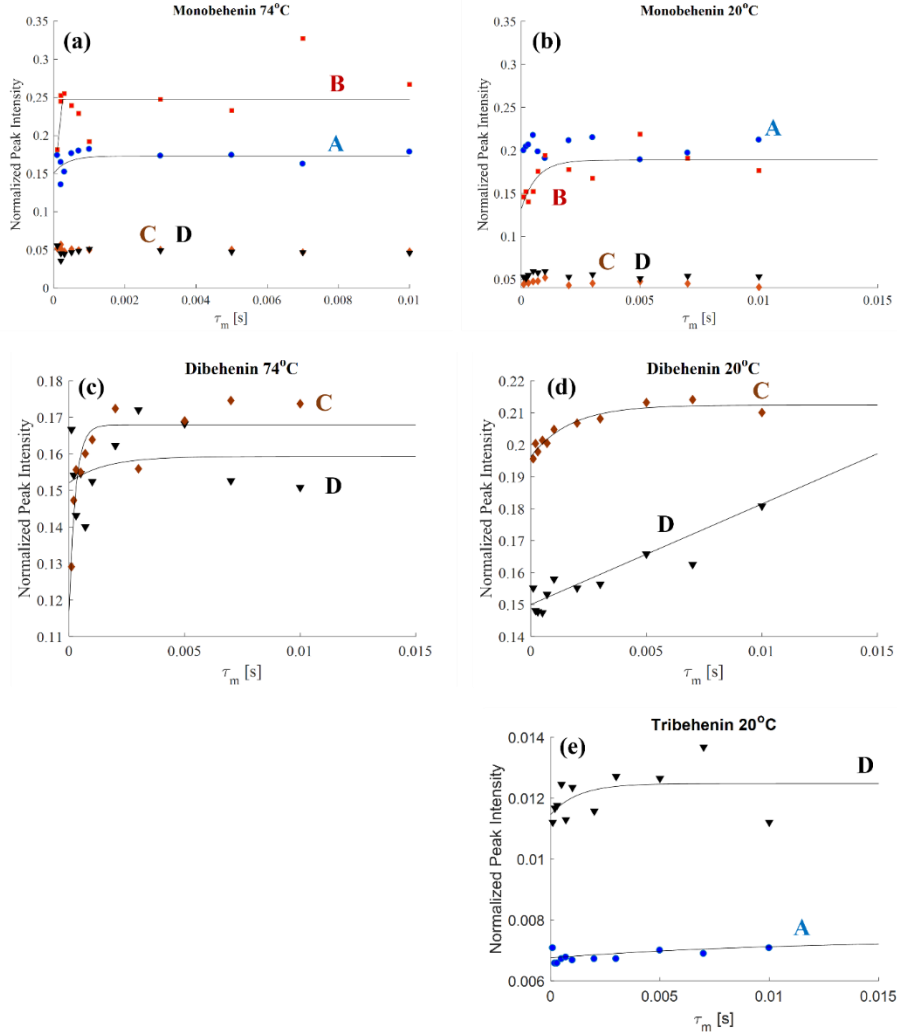


Figure 7.5: Normalized intensity of each exchange population (defined in Figure 7.4) are plotted against the increasing mixing time, τ_m , for each behenate component at 74°C and 20°C. The peaks not saturated are fitted with $I = A - B \text{Exp}[-\tau_m/C]$ where C is the exchange correlation time. Monobehenin (a, b) has four symmetric exchange populations at each temperature with C and D peaks saturating at short mixing times. At 74°C, A and B are fit with a correlation time of 3.71×10^{-4} s and 2.17×10^{-5} s respectively. At 20°C, A saturates, and B is fit with a correlation time of 6.43×10^{-4} s. Dibehenin (b,c) has anti-symmetric exchange populations at both temperatures. At 74°C the correlation times are 33 s and 1.71×10^{-3} s for C and D respectively. Finally, tribehenin is melted at 74°C, no exchange is measured. At 20°C, the symmetric exchange is shown with D and A having correlation times of 9.32×10^{-3} s and 1.30×10^{-3} s.

Fits of the form $I = A - B \text{Exp}[-\tau_m/C]$ where C is the exchange correlation time, or $1/C = k_{ex}$, were applied to each intensity plot shown in Figure 7.5. [7, 36]

Monobehenin at 74°C (Figure 7.5 (a)) has populations C and D (defined in Figure 7.4) saturated at the mixing times measured, shown by the constant normalized peak intensity. The fast exchange generating these peaks is averaged out and saturated by 100 μ s. However, populations A and B have increasing intensities over the mixing times chosen. After fitting, the magnetization in exchange peak A is exchanging with a correlation time of 3.71×10^{-4} s or having an exchange rate of 2.70×10^3 s⁻¹. For exchange peak B, the correlation time of exchange is given by 2.17×10^{-5} s or an exchange rate of 4.61×10^4 s⁻¹. Once the temperature drops to 20°C (Figure 7.5 (b)), populations A, C, and D are all exchanging on a timescale faster than the mixing times measured. The exchange rates increase at this temperature. This is indicated by the saturation of the plots leaving a constant peak intensity. However, population B is still exchanging with a correlation timescale of 6.43×10^{-4} s or an exchange rate of 1.50×10^3 s⁻¹. Comparing percentage of magnetization in each population at each temperature, population A has an increase from approximately 18% of the total population up to 21% of the total population exchanging along this pathway. B decreases from 27% down to 18%. C and D stay constant with 5% of the population exchanging to these environments.

Applying the method from Mellinger *et. al* [37] to calculate the spin diffusion coefficient from the measured T_2 values at 74°C, populations A and D have the range of diffusion coefficients between 7×10^{-3} nm²/ms and 1.24 nm²/ms. B and C correlate to diffusion values between 0.3830 nm²/ms and 1.24 nm²/ms. Using the relationship $\langle z^2 \rangle = \sqrt{2Dt}$, where t is the correlation time from the fits, a length scale is obtained. [7, 36] Populations A and D exchange over length scales of 0.711 nm to 0.960 nm, or

approximately 7 Å to 9 Å. Previous research has characterized the domain and lamella length scales through WAXS and SAXS data where for monobehenin, the polymorphic structure at 74°C has a length scale on the order of 4 Å. [2, 29] This is in agreement with our results. This length scale is associated with the polymorphic phase and not the entire polymorphic 2L period. [2] Following this analysis approach for other exchange populations at 74°C, the diffusion coefficient is approximated as 0.383 nm²/ms to 1.24 nm²/ms which correlates to an exchange length scale of 0.129 nm or approximately 1 Å. This suggests the exchange seen in these samples over these mixing times are the magnetization transfer over the more tightly packed α structure. [2] At 20°C, the only correlation time obtained was from the B exchange populations. The length scale is on the order of 9 Å. At 20°C, the monobehenin sample is in the sub- α_2 polymorph which has a length scale of 4.5 Å. [2] The length scales estimated from the T_2 - T_2 exchange data analysis are consistent with the X-ray scattering data.

Dibehenin (7.5 (c, d)), does not have the symmetric exchange that monobehenin exhibits, it only has exchange populations C and D in the quadrant of moving from a more restricted environment toward a more mobile environment. At 74°C (Figure 7.5 (c)), the exchange correlation time for C was found to be 1.53×10^{-3} s and D was found to have a timescale of 2.74×10^{-4} s. As exchange rates, these correlate to 654 s^{-1} and 3650 s^{-1} for population C and D respectively. This means magnetization is moving toward the more mobile environments (D) quicker than to the less mobile environments (C) unless there is another cause of the asymmetry. The length scales for these exchange rates between T_2 populations, using the correlation times calculated from the T_2 - T_2 intensity

fits, can be calculated for each exchange population. C corresponds to an exchange length scale of approximately 10 Å to 20 Å while D corresponds to an exchange length scale of approximately 0.6 Å to 20 Å. Dibehenin at 74°C has a liquid α polymorph and a solid β polymorph. [2, 31] The length scales found from SAXS and WAXS data are two polymorphs of length scales 4.63 Å and 4.55 Å with lamellar phase repeat distance of 58.3 Å. [2] This suggests the magnetization exchange occurring in populations C and D are on the order of the 2L periodic structure of these polymorphs. Decreasing the temperature down to 20°C (Figure 7.5(d)), the exchange parameters from C and D change with correlation times of 33.26 s and 1.71×10^{-3} s respectively. This correlates to C and D traversing the respective lengths of 150 nm to 280 nm and 1 Å to 20 Å. The value calculated for population C is not physical in this system. Because of this, this population is disregarded in future discussions. The exchange occurring in population D traverses across the 2L period. In percent of the total magnetization population, going from 74°C down to 20°C, population C has an increase from 15% up to 18%. Similarly, population D increases as well from 17% up to 21%. More of the magnetization is exchanging toward more mobile environments at lower temperature indicative of increased longitudinal magnetization exchange from spin diffusion in the more rigid, ordered structure.

Finally, tribehenin (7.5 (e)) has no exchange at 74°C. However, at 20°C there is symmetric exchange between populations A and D. The correlation times are 9.32×10^{-3} s and 1.30×10^{-3} s for A and D respectively, corresponding length scales of 4 Å to 47 Å in the A population and 1 Å to 17 Å in D. The population A magnetization exchange is

across the β lamellar phase ($\sim 3\text{-}5\text{ \AA}$) up to 2L length scale ($\sim 53\text{ \AA}$) while population D magnetization is approaching the full 2L length scale ($\sim 53\text{ \AA}$). [2, 32]

Across all individual behenate components, there is a systematic decrease in correlation time (or increase in exchange rate) of magnetization moving away from restricted environments (shorter T_2). At 20°C , all samples are solid with no possibility for physical molecular diffusion as the driving exchange transport force, indicating the exchange mechanism is spin diffusion.

67/33 wt% Dibehenin/Tribehenin Mixture

T_2 Relaxation Dispersion Measurements

At 20°C (Figure 7.6 (a)), the dibehenin/tribehenin mixture has T_2 distributions which appear to be a mixture of the individual dibehenin and tribehenin T_2 distributions, as expected. This suggests the components are partitioned into individual domains with limited intermixing. Transverse magnetization exchange is indicated by the decrease in the most mobile T_2 population from 0.183 s with a $36\text{ }\mu\text{s}$ τ to 0.0366 s with a $2400\text{ }\mu\text{s}$ τ , a 5.81% decrease in T_2 relaxation time. Comparing to the percentage decrease in relaxation time of the individual components, dibehenin had a 5.56% decrease and tribehenin had a 5.81% decrease in T_2 . At 74°C , the individual tribehenin component is melted while the dibehenin component is still mostly solid. This is seen by the T_2 distribution being dominated by the dibehenin structure (compare Fig. 7.2(d)). The magnetization exchange occurring appears to be mostly due to the interactions between the dibehenin domains with the most mobile component beginning with a T_2 relaxation time of 0.183s and decreasing down to 0.0366 s with a τ time of $36\mu\text{s}$ and $2400\mu\text{s}$ respectively. The presence

of the melted tribehenin decreases the magnetization exchange process with a 20.0% decrease in relaxation time compared to the pure dibehenin exhibiting a 25.6% decrease in relaxation time.

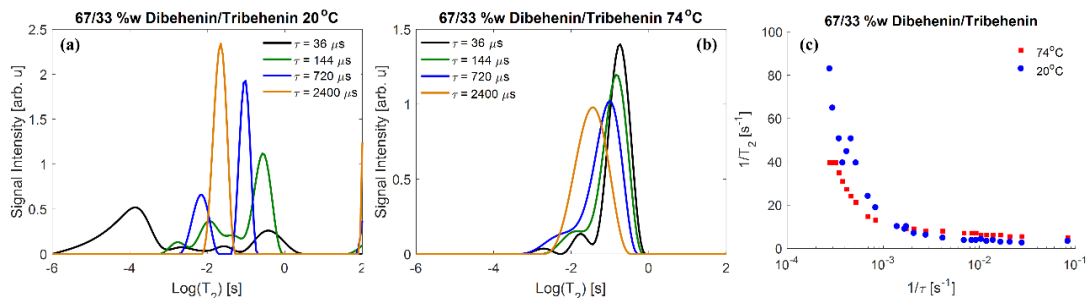


Figure 7.6: T_2 dispersion distributions (a, b) and plot of $1/T_2$ vs $1/\tau$ (c) are shown for the 67/33 wt% mixture of dibehenin/tribehenin for 20 °C (a) and 74 °C (b). At each temperature, exchange is evident with decrease in relaxation time of the most mobile component as τ increases. At 20 °C (a), the most mobile component starts with a T_2 time of 0.384 s for a τ time of 36 μ s (black) only to decrease down to 0.0223 s for a τ time of 2400 μ s (orange). At the higher temperature of 74 °C (b), the decrease is less, going from 0.183 s down to 0.0366 s as τ goes from 36 μ s up to 2400 μ s. For the $1/T_2$ vs $1/\tau$ plot (c), more exchange occurs at 20 °C (blue, circle) compared to 74 °C (red, square). The plateau correlating to $k_{ex} \gg 1/\tau$ is not found at 20 °C indicating an increase in k_{ex} with decreasing temperature.

Plotting the inverse of the τ time against the inverse of the relaxation time of the most mobile component (Figure 7.6 (c)), a higher exchange rate is occurring at 20 °C than at 74 °C indicated by the larger slope. First, the plateau occurring in the regime of $k_{ex} \gg 1/\tau$ is barely achieved at 74 °C and is not realized at 20 °C. This indicates the exchange occurring at higher temperatures is slower than that at lower temperatures. As was seen in the individual components, the transverse magnetization exchange is dependent on the molecular structure of the sample. The exchange occurring at 74 °C is closely related to the exchange occurring within the dibehenin sample, which is the remaining solid lipid phase.

This suggests the magnetization exchange is not due to physical molecular transport of liquid tribehnenin, but rather to spin diffusion in the solid dibehnenin phase. As the temperature decreases, the lattice organization becomes more rigid with less rotational mobility and a closer packing structure. The magnetization transfer occurring in spin diffusion is proportional to r^{-6} , where r is the nearest neighbor dipolar distance [19, 20], meaning the more rigid and tighter the packing structure of a system in which spin diffusion occurs in, the more magnetization transfer can occur.

T_2 - T_2 Relaxation Exchange Measurements

Figure 7.7 shows the T_2 - T_2 relaxation correlation plots (left) for the dibehenin/tribehenin mix at 74°C (top) and 20°C (bottom) for a mixing time of 100 μ s.

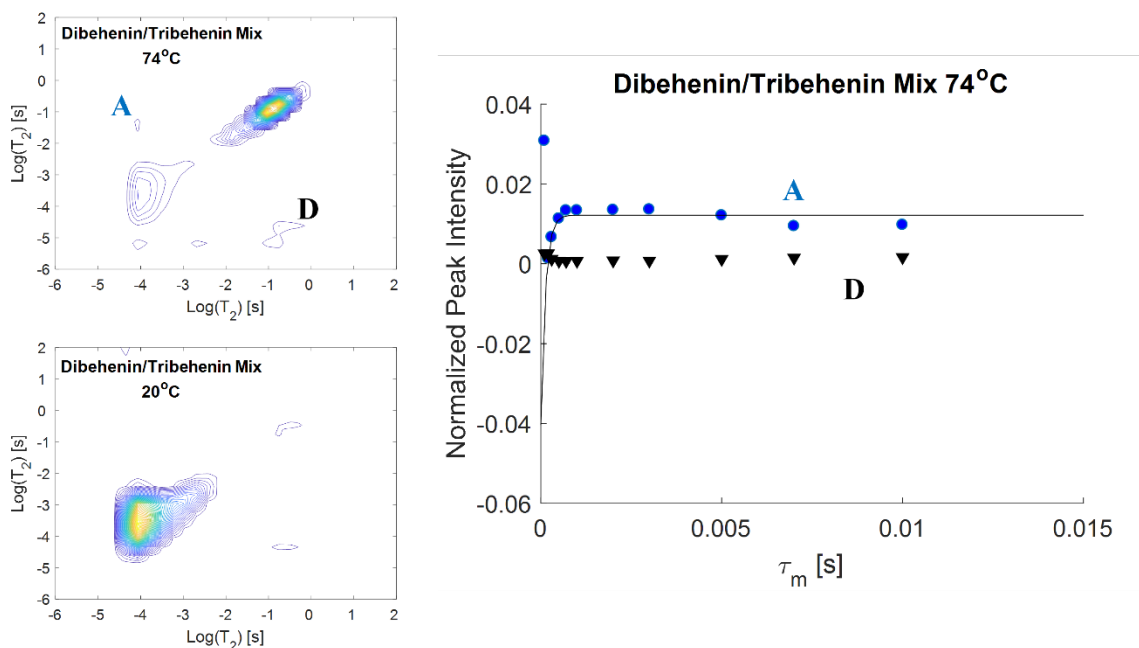


Figure 7.7: T_2 - T_2 exchange plots (left) for a 67/33 wt% dibehenin/tribehenin mixture at a mixing time of 100 μ s. At 74°C, two exchange populations are observed and labeled A (blue) and D (black). No significant exchange is seen at 20°C. The normalized intensity of the exchange peaks at 74°C is plotted against mixing time, τ_m (right). D (black, triangle) is saturated at the mixing times observed. A (blue, circle) is fitted with $I = A - B \exp[-\tau_m/C]$ where C is the exchange correlation time. The correlation time for A was 1.28×10^{-4} s.

Interestingly, no exchange is seen at 20°C when the lattice is at its most rigid, however, at 74°C slight symmetric exchange is seen with exchange pairs labeled A and D with centers at (1.22×10^{-4} s, 0.0754 s) and (0.227 s, 1.66×10^{-4} s) respectively. Plotting the most mobile component against increasing mixing time, the exchange population D is saturated for all mixing times, presenting as a constant. However, A, with obtaining only 1% of the total magnetization population at a mixing time of 10ms, has a correlation time

of 1.28×10^{-4} s, or an exchange rate of 781 s^{-1} . The length scale associated with population A is between $0.4 \text{ \AA}$ and $40 \text{ \AA}$. This range traverses the full $2L$ period length scale within an order of magnitude ($53 \text{ \AA}$ [2, 32]). The exchange occurring in the longitudinal domain in this mixture is significantly less than that of the individual components. This suggests the structural packing of each component hinders the ability to exchange longitudinal magnetization.

20/50/30 wt%

Monobehenin/Dibehenin/Tribehenin Mixture

T_2 Relaxation Dispersion Measurements

$T_2(\tau)$ dispersion distributions for the 20/50/30 wt% mixture of monobehenin/dibehenin/tribehenin is shown in Figure 7.8. Exchange is apparent at each temperature indicated by the decrease in T_2 relaxation times as τ .

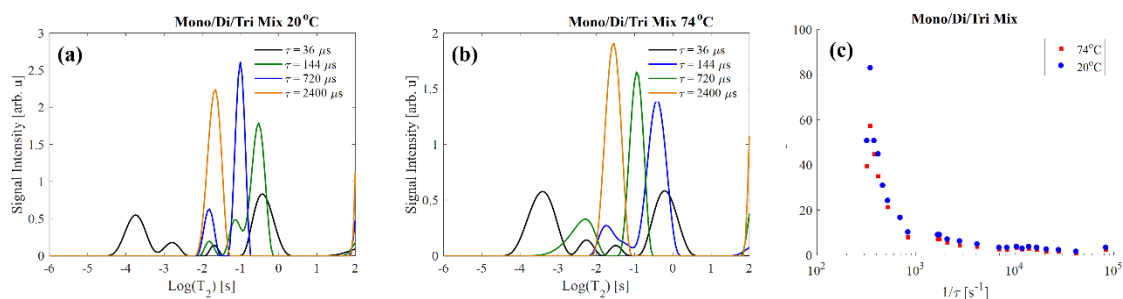


Figure 7.8: T_2 dispersion distributions (a, b) and plot of $1/T_2$ vs $1/\tau$ (c) are shown for the 20/50/30 wt% monobehenin/dibehenin/tribehenin mixture for 20°C (a) and 74°C (b). At each temperature, exchange is evident with decrease in relaxation time of the most mobile component as τ increases. At 20°C (a), the most mobile component starts with a T_2 time of 0.3836 s for a τ time of $36 \mu\text{s}$ (black) only to decrease down to 0.0223 s for a τ time of $2400 \mu\text{s}$ (orange). At the higher temperature of 74°C (b), the decrease is more, decreasing from 0.6291 s down to 0.0286 s as τ goes from $36 \mu\text{s}$ up to $2400 \mu\text{s}$. For the $1/T_2$ vs $1/\tau$ plot (c), equal exchange occurs at 20°C (blue, circle) compared to 74°C (red, square).

The addition of monobehenin can be seen clearly as the shorter T_2 populations at 74°C (Figure 7.8 (b)) compared to that of the dibehenin/tribehenin mixture shown in Figure 7.6 (b). The higher melting point of monobehenin, compared to tribehenin, is reflected by the more ordered structure, shorter T_2 , seen at this temperature.[2] Relative to the individual samples and the dibehenin/tribehenin mixture, the addition of monobehenin inhibits exchange at 20°C while enhancing exchange at 74°C (Figure 7.8 (c)). The exchange at each temperature is approximately equal shown as the similar slope of the plot of $1/T_2$ vs $1/\tau$ (Figure 7.8 (c)). The enhanced exchange at 74°C, compared to the previous samples, is due to the lamellar phase solid structures induced by monobehenin allowing spin diffusion transverse magnetization. This change in magnetization exchange behavior correlates to the monobehenin and dibehenin components interacting in the mixture as has been shown using X-ray scattering and is well known for monoglycerides. [2, 38]

The tribehenin molecules crystallize separately upon sample creation while the monobehenin and dibehenin lose the separate polymorphic phases and crystalize into a mixed phase.[2] Interestingly, the similar exchange rates at each temperature indicate this through the transverse magnetization exchange due to spin diffusion not decreasing with increasing temperature. This is due to the new polymorphic phase created by the dibehenin/monobehenin components, which at temperatures of 20°C and lower arrange into a sub- α sub-cell while at 30°C and higher transition to an α sub-cell.[2] A change of length scale from 4.21 Å and 3.80 Å to 4.16 Å. [2] This is a very small change in

polymorphic length scale compared to the single component and di/tri mixture samples.

[2]

T_2 - T_2 Relaxation Exchange Measurements

Figure 7.9 shows T_2 - T_2 exchange plots for the 20/50/30 wt% monobehenin/dibehenin/tribehenin sample at 74°C (Figure 7.9 (a)) and 20°C (Figure 7.9 (c)).

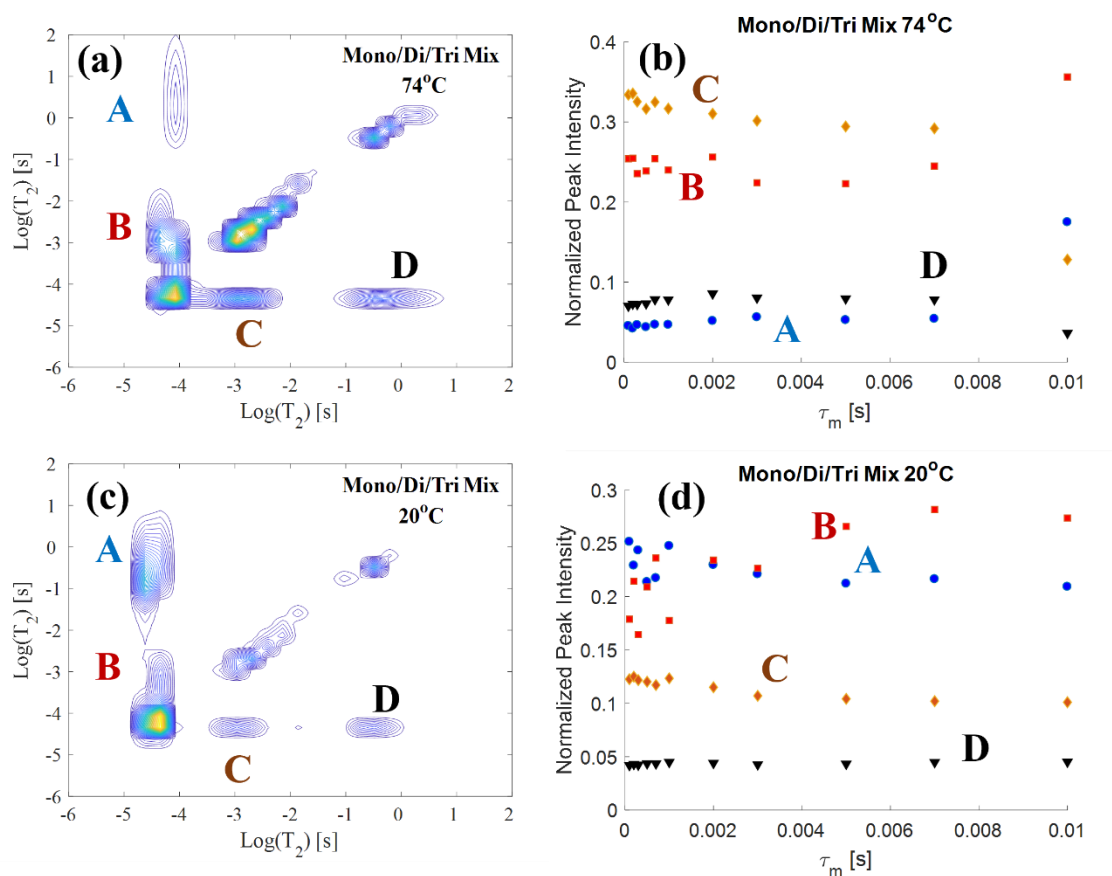


Figure 7.9: T_2 - T_2 exchange plots for the 20/50/30 wt% monobehenin/dibehenin/tribehenin mixture are shown for 74°C (a) and 20°C (c) at a mixing time, τ_m , of 100 μs . Symmetric exchange is shown, and each exchange population is labeled as A, B, C, and D. The intensity of each exchange population is normalized and plotted against increasing mixing time for temperature 74°C (b) and 20°C (d). By the shortest mixing time of 100 μs , all exchange populations are saturated.

The symmetric exchange morphology shown in the T_2 - T_2 exchange plots is indicative of the addition of the monobehenin. At 74°C (Figure 7.9 (a)), symmetric exchange populations A and D have centers at (2.64×10^{-5} s, 0.196 s) and (0.302 s, 4.18×10^{-5} s) respectively. The exchange pair A and D represent exchange between the most mobile and rigid, or weakest and strongest dipolar coupled populations. An asymmetric exchange pair of B and C have centers at (4.36×10^{-5} s, 5.30×10^{-4} s) and (1.04×10^{-3} s, 4.18×10^{-5} s) and indicate exchange between the most rigid and intermediate mobility populations. No exchange peaks between the most mobile and intermediate mobility population were present. Plotting the intensity of each exchange population against the increasing mixing times (Figure 7.9 (b)), every population is fully saturated by the shortest mixing time of 100 μ s. At 20°C (Figure 7.9 (c)), symmetric exchange is seen again with cross peaks A and D centered at (2.64×10^{-5} s, 0.11 s) and (0.395 s, 3.88×10^{-5} s) respectively. The exchange pairs B and C are centered at (3.33×10^{-5} s, 2.68×10^{-4} s) and (8.96×10^{-4} s, 4.34×10^{-5} s). Similar to 74°C, all exchange populations are saturated at the shortest mixing times.

Due to hardware limitations a shorter mixing time cannot be realized, yet some quantitative information of percentage for total magnetization exchanging can be found. There is an increase in magnetization exchange into population A from 17% up to 21% from 74°C to 20°C. However, all other populations have a decrease or no change in longitudinal magnetization exchange. In cross peak B from 74°C to 20°C, there was a decrease from 36% down to 27% of total magnetization. Similarly, exchange population C had a slight decrease from 13% to 10% and D had no change staying at 4% of the total

magnetization of the system. This is different than in previous samples where an increase in exchange was seen as the temperature decreased, indicating no significant increase in ordering and commensurate stronger dipolar coupling at 20°C than at 74°C.

Conclusions

Glycerol behenate was decomposed into its base components of mono-, di-, and tribehenin. NMR magnetization exchange was measured at two temperatures of 20°C and 74°C, over which lattice structural variations occur. Both transverse magnetization exchange analyzed through T_2 dispersion experiments and longitudinal magnetization exchange by T_2 - T_2 experiments are observed. Transverse magnetization exchange at 20°C, while each sample was in a highly ordered solid phase, indicates a coherent exchange process, spin diffusion, as the primary physics behind this magnetization transport. Length scales estimated from the longitudinal magnetization exchange indicates magnetization propagated across polymorphic phases and along the entire repeat period of each domain.

A 67/33 wt% dibehenin/tribehenin mixture and a 20/50/30 wt% monobehenin/dibehenin/tribehenin mixture were examined as well. It was found the addition of tribehenin to dibehenin enhanced exchange at higher temperatures but inhibited exchange at lower temperatures. This was also found with the addition of monobehenin into the mixture. This research lays the groundwork for future NMR relaxometry characterization of lipid mixtures. This research has potential for quantitative characterization of lipid structure stability and variation in the pharmacological industry, as well as the food science industry due to the pervasiveness of lipids in food.

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CHAPTER EIGHT

CONCLUSIONS

The novel research presented in this thesis advances the ability of nuclear magnetic resonance (NMR) to explore exchange and diffusion dynamics within soft-condensed matter. The method of NMR is uniquely qualified to non-invasively and non-destructively probe not only macroscale dynamics, such as first-order phase transitions, but also meso- and microscale dynamics, such as transport, exchange, and structural rearrangement. The goal of this dissertation was to characterize if these macro-, meso-, and microscale dynamics through NMR relaxation and diffusion measurements. The experiments performed in this body of work were reviewed and explained in chapters 4-7. Systems analyzed: (1) xylose biomolecular transport within zeolite beads in the presence of water, (2) characterization of exchange in T_1 - T_2 experiments through a simulation of magnetization evolution in coupled pores, (3) exchange dynamics driven by spin diffusion within two straight-chained alkanes and a natural beeswax in which multiple exchange mechanisms are present, and (4) deconstruction of glycerol behenate into its base components. The macro-, meso-, and microscale dynamics were all able to be successfully detected and monitored in real time through multidimensional NMR techniques.

Each system explored posed different and unique experimental and analytical challenges. The ability to explore structures and dynamics of various length scales through NMR was exploited through diffusion and relaxation experiments to measure water dynamics in zeolite beads (Chapter 4 [1]). Zeolite beads are used to increase yields

in substrate chemical reactions. Often, the macroscale processes are known, such as how much yield is given, but the microscale understanding of how this process is occurring within or on the bead is limited. LTA 5A beads were measured in both pure water and a 20% wt. water xylose solution to explore how xylose affects transport dynamics within these zeolite beads. Through diffusion measurements, it was found the introduction of xylose decreases the translational motion throughout the bead by altering the permeability between zeolite crystals of the porous structure. The xylose inhibits the solution from moving toward larger pores within the system and hinders leaking out of the bead itself. This result indicates NMR's ability to explore various structural scales through simple pulse sequences.

Spin dynamics within an interconnected system prove to add additional complexity to the mathematical governing equations for magnetization evolution. The coupling of diffusion, exchange, and relaxation of magnetization all must be explicitly counted for in simulating experimental results (Chapter 5 [2]). Experimental results from systems such as forming model laboratory organic hydrates, softening and melting lipids, and microporous bead packs, all resulted in a non-physical T_1 - T_2 relaxation correlation signal response. This was replicated after modeling through the Bloch-Torrey equations. The anomalous signal increase was due to a product of coupling of diffusive eigenmodes in two regions. While this model allowed for a physical explanation of the non-physical signal behavior, it does so in a limited fashion. Extension of this work is currently being explored by simulating multisite exchange through a similar eigenmode analysis, in

hopes of quantifying exchange dynamics via mechanisms where no physical mass transfer occurs, such as in spin diffusion.

Magnetization evolution dynamics, impacted by exchange and diffusion, are also closely connected with the molecular and mesoscale structure and temperature of the system. This was explored in Chapter 6 through relaxation measurements at increasing temperatures for two natural, straight-chained alkanes (eicosane and octacosane), and a natural wax (beeswax). [3] NMR was shown to be just as sensitive to solid-solid phase transitions as differential scanning calorimetry (DSC) techniques, one of the main methods of observing these transitions. In all systems, the transitions seen in DSC measurements were seen in addition to some microscale structural rearrangements in the form of solid-solid phase transitions. Exchange was then explored as a function of temperature through $T_2(\tau)$ dispersion measurements and T_2 - T_2 exchange correlation measurements. Due to the solid-like nature of the lipids throughout most of the temperature increase, observed exchange could not be due to physical molecular motion induced mass transfer between domains. Thus, a coherent magnetization exchange, spin diffusion, was determined to be the driving force behind the transport. Additionally, magnetization exchange was seen in both the transverse and longitudinal magnetization based on two separate relaxation encoding pulse sequences, $T_2(\tau)$ dispersion measurements and T_2 - T_2 exchange correlation measurements respectively. To our knowledge, probing transverse spin diffusion magnetization exchange is a novel extension of exchange interpretation and measurement for spin diffusion.

The decoupling of magnetization exchange dynamics into the transverse, or spin-spin T_2 domain, and the longitudinal, or spin-lattice T_1 domain, was extended to another lipid in Chapter 7. [4] Glycerol behenate, a common lipid utilized in pharmaceuticals, was decomposed into its base components of mono-, di-, and tribehenin. This allowed exploration of individual exchange dynamics and structural polymorphism in each individual component as a function of temperature. In addition, a 67/33 dibehenin/tribehenin percent weight mixture was analyzed to see how molecular interactions impact mesoscale (nm) structures. Significant transverse exchange was seen in all individual components at both temperatures, 20°C and 74°C. Due to the coherent refocusing of the signal, spin diffusion was determined to be the mechanism behind the exchange dynamics at both temperatures. This magnetization transfer was also seen in the dibehenin/trbehenin mixture as well. The exchange was measured in the longitudinal domain as well through T_2 - T_2 exchange measurements where the magnetization exchange occurred in the T_1 domain during the allowed mixing time. Significant exchange was shown to occur in this domain as well for the individual samples while interestingly, the introduction of multiple chemical domains in the mixture inhibited significant exchange from occurring. Length scales of the propagation of magnetization were determined to be in good agreement with the length scales of the polymorphic domains present at each temperature, in addition to the periodic repeat distance of these domains. Since the addition of multiple chemical domains inhibited significant exchange, a final mixture of monobehin/dibehenin/tribehenin will be made to recreate a glycerol behenate sample prior to journal submission where transverse and longitudinal exchange will be explored.

Multidimensional NMR relaxation and diffusion techniques have been shown to successfully explore the macro-, meso-, and microscale structures within not only porous media but also in soft-condensed matter systems in this dissertation. The work shown here indicates a new and exciting frontier of exploring non-mass transfer-based magnetization exchange in not only the longitudinal domain but also the transverse domain as well.

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APPENDICES

APPENDIX A

SOLUTION TO THE COUPLED, TWO-PORE MODEL

A one-dimensional, Cartesian version of the diffusion equation is used for computations of magnetization dynamics in a pore. The problem is scaled in order to simplify the notation and bring out the key dimensionless parameters [37]. Scaling position by the size of the larger pore, L_A , time by the diffusion time, L_A^2/D , magnetization by M_{eq} , and factoring out the homogeneous relaxation terms, we arrive at the dimensionless version of the two-pore problem, Eq. (5.4), with boundary conditions given by Eqs. (5.5) and (5.8) as

$$\frac{\partial u_A}{\partial \tau} = \frac{\partial^2 u_A}{\partial z^2} \quad (\text{A.1a})$$

$$u_A(z, 0) = u_{A0}(z) \quad (\text{A.1b})$$

$$-\frac{\partial u_A}{\partial z} \Big|_0 = -\delta_A u_A(0, \tau) \quad (\text{A.1c})$$

$$\frac{\partial u_A}{\partial z} \Big|_1 + \delta_{cA} u_A(1, \tau) = \frac{\partial u_B}{\partial z} \Big|_1 - \delta_{cB} u_B(1, \tau) \quad (\text{A.1d})$$

$$\frac{\partial u_A}{\partial z} \Big|_1 = \kappa_c (u_B(1, \tau) - u_A(1, \tau)) \quad (\text{A.1e})$$

$$\frac{\partial u_B}{\partial z} \Big|_{1+\beta} = -\delta_B u_B(1 + \beta, \tau) \quad (\text{A.1f})$$

$$u_B(z, 0) = u_{B0}(z) \quad (\text{A.5.1g})$$

$$\frac{\partial u_B}{\partial \tau} = \frac{\partial^2 u_B}{\partial z^2} \quad (\text{A.1h})$$

This problem statement holds for both the longitudinal and the transverse magnetization components. Computations presented in Section 5.3 will use the relation between the Damköhler numbers at the pore-pore interface and the pore-surface

Damköhler numbers given in Eq. (5.9) to eliminate the need to specify the δ_{ci} -values separately from the δ_i -values.

A normal-modes method can be applied to solve the coupled-pore diffusion problem given in Eqs. (A.1) [12]. The approach posits that the solution for the interval $0 < z < (1 + \beta)$ takes the form

$$u(z, \tau) = \sum_{k=1}^{\infty} a_k e^{-\lambda_k \tau} \phi_k(z) \quad (\text{A.2})$$

where the elements of the eigensystem $\{\lambda_k, \phi_k(z)\}$ are λ_k , the eigenvalues, and $\phi_k(z)$, the eigenfunctions. Note the eigenfunctions are *composites* of two distinct separate component eigenfunctions that separately cover each of the pores, *A* and *B*, *i.e.* $\phi_k(z) = \phi_{A,k}(z) + \phi_{B,k}(z)$, where $\phi_{A,k}$ is defined for $0 < z < 1$ and $\phi_{B,k}$ is defined for $1 < z < \beta$. The coupling boundary conditions, Eqs. (A.1d) and (A.1e), ensure the solution obtained by adding the two component functions is a solution to the problem. Additionally, the inter-pore transfer boundary condition in Eq. (A.1e), allows for a discontinuity in the composite eigenfunction at the interface. The component eigenfunctions are continuous outside of the interface.

The elements of the eigensystem for the problem are obtained from the coupled set of ordinary differential equations extracted from the spatial portions of the model, Eqs. (A.1).

$$\frac{d^2\phi_{A,k}}{dz^2} + \lambda_k\phi_{A,k} = 0 \quad 0 < z < 1 \quad (\text{A.3a})$$

$$\left. \frac{d\phi_{A,k}}{dz} \right|_0 = \delta_A\phi_{A,k}(0) \quad (\text{A.3b})$$

$$\left. \frac{d\phi_{A,k}}{dz} \right|_1 + \delta_{cA}\phi_{A,k}(1) = \left. \frac{d\phi_{B,k}}{dz} \right|_1 - \delta_{cB}\phi_{B,k}(1) \quad (\text{A.3c})$$

$$\left. \frac{d\phi_{A,k}}{dz} \right|_1 + \delta_{cA}\phi_{A,k}(1) = \kappa_c(\phi_{B,k}(1) - \phi_{A,k}(1)) \quad (\text{A.3d})$$

$$\left. \frac{d\phi_{B,k}}{dz} \right|_{1+\beta} = -\delta_B\phi_{B,k}(1+\beta) \quad (\text{A.3e})$$

$$\frac{d^2\phi_{B,k}}{dz^2} + \lambda_k\phi_{B,k} = 0 \quad 1 < z < 1 + \beta \quad (\text{A.3f})$$

The eigenfunctions for different eigenvalues are orthogonal on the interval $[0, (1 + \beta)]$ ([38], [39]) as quantified by the inner product

$$\begin{aligned} \langle \phi_m, \phi_n \rangle &= \int_0^{1+\beta} \phi_m(z)\phi_n(z)dz \\ &= \int_0^1 \phi_{A,m}(z)\phi_{A,n}(z)dz + \int_1^{1+\beta} \phi_{B,m}(z)\phi_{B,n}(z)dz \\ &= 0 \quad m \neq n \end{aligned} \quad (\text{A.4})$$

Any initial conditions for the problem (Eqs. (A.1b) and (A.1g)) can be used to provide the constants a_k in the solution (Eq. (A.2)) as $a_k = \frac{\langle u_0, \phi_k \rangle}{\langle \phi_k, \phi_k \rangle}$ with $u_0(z) = u_{A0}(z) + u_{B0}(z)$ as the (composite) initial condition.

The problem presented in Eqs. (A.3) is linear and so there is it is possible to obtain analytical expressions for the eigenfunctions [14]. However, there is substantial algebraic complexity to finding the eigenvalues for the general case which precludes

finding simple, closed-form expressions for the eigenvalues. For eigensystem computations presented in this study, solutions to Eqs. (A.3) were found using MATLABTM and the CHEBFUN toolbox [40].

To obtain the code used for the eigensystem computations and system simulation please contact the author, James E. Maneval, Bucknell University, maneval@bucknell.edu.

APPENDIX B

DETAILS OF THE DEVELOPMENT OF THE SIGNAL FOR THE T_1 - T_2 PULSE
SEQUENCE

The system is assumed to be at equilibrium before the start of the pulse sequence so that $M_z(z, 0) = M_{eq}$. The initial π -pulse inverts the magnetization to form the initial condition for the first interval as $M_z(z, 0) = -M_{eq}$. Setting $m = M_z - M_{eq}$ for the inversion-recovery interval gives $m(z, 0) = -2M_{eq}$ and, in terms of u , this initial condition is $u(z, 0) = -2$. The eigensystem in this interval is the T_1 eigensystem, $\{\lambda_m^{(1)}, \phi_m^{(1)}\}$, and is computed using the longitudinal relaxation parameters, δ_{i1} and δ_{ci1} along with the parameters κ_c and β . The system evolves according to,

$$u(z, \tau) = \sum_m a_m^{(1)} e^{-\lambda_m^{(1)} \tau} \phi_m^{(1)}(z) \quad 0 < \tau < \tau_1 \quad (\text{B.1})$$

with $\tau_1 = t_1/t_D$ as the scaled duration of the T_1 evolution interval. The coefficients in the expansion above are computed from the T_1 eigensystem by using the initial condition $u(z, 0) = -2$ to give

$$a_m^{(1)} = -2 \frac{\langle 1, \phi_m^{(1)} \rangle}{\langle \phi_m^{(1)}, \phi_m^{(1)} \rangle} \quad (\text{B.2})$$

The $\frac{\pi}{2}$ -pulse then converts the longitudinal magnetization to transverse magnetization to provide the initial condition for the spin-echo portion of the sequence as

$$u(z, \tau_1) = \sum_m a_m^{(1)} e^{-\lambda_m^{(1)} \tau_1} \phi_m^{(1)}(z) \quad (\text{B.3})$$

The eigensystem in this second interval is the T_2 eigensystem, $\{\lambda_n^{(2)}, \phi_n^{(2)}\}$, and is computed using the longitudinal relaxation parameters, $\delta_{i,2}$ and $\delta_{ci,2}$ but with the same values of κ_c and β used for the T_1 eigensystem. With the state of the system at the end of

this interval, $u(z, \tau_1)$, providing the initial conditions, the system then evolves during the second, spin-echo interval $\tau > \tau_1$ according to

$$u(z, \tau) = \sum_n a_n^{(2)} e^{-\lambda_n^{(2)}(\tau - \tau_1)} \phi_n^{(2)}(z) \quad (\tau > \tau_1) \quad (\text{B.4})$$

The coefficients in Eq. (B.5.4) are obtained from the initial condition for the interval as

$$a_n^{(2)} = \frac{\langle 1, \phi_n^{(2)} \rangle}{\langle \phi_n^{(2)}, \phi_n^{(2)} \rangle} - 2e^{-r_1 \tau_1} \sum_m \frac{\langle 1, \phi_n^{(1)} \rangle}{\langle \phi_n^{(1)}, \phi_n^{(1)} \rangle} \frac{\langle \phi_m^{(1)}, \phi_n^{(2)} \rangle}{\langle \phi_n^{(2)}, \phi_n^{(2)} \rangle} e^{-\lambda_m^{(1)} \tau_1} \quad (\text{B.5})$$

Note the coefficients for this solution involve the details of the T_1 eigensystem results as well as the duration of the inversion-recovery interval, τ_1 due to the initial condition for the solution in the second interval that is set by the final condition of the inversion-recovery interval (Eq. (B.3)).

The signal during the post- $\frac{\pi}{2}$ pulse is obtained by integration over the whole system, weighted by the coil-response function $h(z)$. Using the solution for the spin-echo interval, we have the signal for the experiment as,

$$s(\tau_1, \tau_2) = e^{-r_2 \tau_2} \int_0^{1+\beta} u(z, \tau_2) h(z) dz = e^{-r_2 \tau_2} \sum_n a_n^{(2)} h_n^{(2)} e^{-\lambda_n^{(2)} \tau_2} \quad (\text{B.6})$$

with $h_n^{(2)} = \int_0^{1+\beta} h(z) \phi_n^{(2)}(z) dz$ proportional to the projection of the response function onto the T_2 eigensystem and $\tau_2 = \tau - \tau_1$ is the (scaled) time for signal acquisition during the CPMG decay. Simplifications in the expression are possible by defining the difference of the final signal based on the equilibrium magnetization [12]. Writing $S(\tau_1, \tau_2) = s(\tau_1 \rightarrow \infty, \tau_2) - s(\tau_1, \tau_2)$, Eqs. (B.3) - (B.6) lead to the signal for the experiment as

$$\begin{aligned}
S(\tau_1, \tau_2) &= \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} \frac{\langle 1, \phi_m^{(1)} \rangle \langle \phi_m^{(1)}, \phi_n^{(2)} \rangle}{\langle \phi_m^{(1)}, \phi_m^{(1)} \rangle \langle \phi_n^{(2)}, \phi_n^{(2)} \rangle} h_n^{(2)} e^{-r_m^{(1)} \tau_1} e^{-r_n^{(2)} \tau_2} \\
&= \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} p_m^{(1)} G_{mn} h_n^{(2)} e^{-r_m^{(1)} \tau_1} e^{-r_n^{(2)} \tau_2} \\
&= \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} P_{mn} e^{-r_m^{(1)} \tau_1} e^{-r_n^{(2)} \tau_2}
\end{aligned} \tag{B.7}$$

where $r_k^{(i)} = r_i + \lambda_k^{(i)}$ are the observed relaxation rates, combinations of the homogeneous rate constants and the eigenvalues for the problem.