Dynamic Nuclear Polarization and Electron Spin Resonance in Paramagnetic Solids at High Field

by

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in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Physics
at the
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Abstract

Recent advances in high resolution solid state nuclear magnetic resonance spectroscopy have permitted the detailed structural study of large biological systems. The feasibility of many of these experiments is limited by the inherently low sensitivity of solid state NMR, and the implementation of complex multi-dimensional homo- and hetero-nuclear recoupling pulse sequences has been restricted to small model compounds. The primary focus of this thesis is the description of investigations of dynamic nuclear polarization (DNP) at high magnetic field as a means of improving the signal to noise in solid state NMR spectroscopy.

DNP transfers the large polarization of unpaired electron spins to nuclei in a process involving sample irradiation at or near the electronic Larmor frequency. Large signal enhancements have been achieved in a nitroxide doped frozen aqueous solution at 5 T. The relevant relaxation times governing the transfer process have been measured via the application of various high frequency pulsed electron paramagnetic resonance (EPR) techniques, and a model incorporating cross-relaxation is used to explain the results. It is shown that the electronic and nuclear spin dynamics are consistent with the thermal mixing mechanism of polarization transfer.

The high frequency (139.5 GHz, 5 T) EPR spectrometer used to perform these experiments is described in detail and several other advancements in the application of high power, high frequency microwave technology to magnetic resonance are also discussed.

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I want to thank all the people who have been part of my life as a graduate student. I owe much to my advisor Bob Griffin who has been extremely encouraging and patient. I have gained immensely from him and from all of the talented people in his research group.

The work described in this thesis is the result of the collaborative efforts of the members of the DNP/EPR subgroup. I have worked most closely with Dennis Hall. We joined the lab at the same time, and learned a whole lot about magnetic resonance from Lino Becerra and Gary Gerfen. Along the way, I worked and played with a tight-knit bunch of friends and collaborators: Brendan Bellew, Marina Bennati, Chris Farrar, Melanie Rosay, and Volker Weis. I have received a great deal of technical assistance and instruction from Jeff Bryant, Ajay Thakkar, Jim Wrenn, Dave Ruben, and Ken Kreischer. Jeff’s creativity and skill helped to make this work possible.

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 пенси الجمال يتأهوّ بعَزْيّتنا
 إن الجمال جمال العلم والأدب

 - اين الوردی -
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Chapter 1

Introduction

Nuclear Magnetic Resonance (NMR) has become an important tool in the structural and functional analysis of materials. In the last twenty years, the number of solution state NMR methods used in the determination of the structures of biological macromolecules has grown substantially [1], and the use of solution state NMR in solving high-resolution structures of biomolecules is now routine. Unfortunately, the applicability of solution state NMR techniques is restricted to low to intermediate molecular weight systems\(^1\) and its utility in the study of large proteins, molecular complexes, membrane-bound systems, and other important biological moieties is limited. Recent advances in solid state NMR methodology [2, 3, 4], have begun to provide alternative means of obtaining structural information in biological systems inaccessible to solution state spectroscopy. These multidimensional homo- and heteronuclear recoupling schemes, and their associated pulse sequences, are becoming increasingly more complex, leading to decreased signal size and increased acquisition time, particularly as applications shift to "interesting" systems, i.e. those with relatively

\(^{1}\) For systems with a molecular weight less than 30–50 kD, fast molecular reorientations average dipole-dipole couplings leading to narrow lines and very highly resolved spectra. Slower tumbling rates in larger molecules leads to less efficient averaging and broad, unresolved (solid state like) lines. 1 kilo Dalton (kD) is 1000 g/mol. The average molecular weight of a protein is approximately 0.1 kD per amino acid residue.
few nuclei, low natural abundance, and/or small gyromagnetic ratios. In fact, the feasibility of many solid-state NMR experiments is often limited by the inherently low sensitivity of the technique [5, 1]. The focus of this thesis is the study of one promising method of signal-to-noise ratio improvement: the incorporation of dynamic nuclear polarization into solid state NMR spectroscopy.

Dynamic nuclear polarization (DNP) transfers the large polarization of unpaired electrons to nuclei, and thus significantly enhances the NMR signal strength. Predicted by Overhauser [6, 7] and then first demonstrated by Carver and Slichter [8, 9] in 1953, DNP involves irradiating the electron spins at or near their Larmor frequency. Its primary application has been the production of polarized targets for nuclear scattering experiments which are normally conducted at milli-Kelvin temperatures, often achieving overall nuclear spin polarizations approaching 70 to 90% [10]. Recent interest has focused on integrating DNP into solid state NMR experiments which, by comparison, exhibit nuclear polarization well below 1% under typical experimental conditions. A number of investigators have obtained significant results in the application of DNP to magic angle spinning (MAS) NMR in studies of polymer systems and carbonaceous solids at relatively low magnetic fields, i.e. 0.3–1.5 T, 13–60 MHz \(^1\)H, and 9–45 GHz electron Larmor frequencies [11, 12, 13]. This work is part of an ongoing investigation into the implementation of DNP in high resolution solid state NMR applications in the study of biological solids at a significantly higher field (5 T).

The structure of the remainder of the thesis is as follows: chapter 2 is a brief review of the basic theory of spin-spin interactions in paramagnetic, dielectric solids, and the relevant physics of polarization transfer and relaxation; chapter 3 presents high field DNP results in two systems, a model polymer system: \(\alpha,\gamma\)-bis[diphenylene-\(\beta\)-phenylallyl] (BDPA) in polystyrene, and a nitroxide doped frozen aqueous solution: 2,2,6,6-tetramethyl-
1-piperidinyloxy (TEMPO) in water:glycerol; chapter 4 describes the various experimental methods used in the acquisition of the data; chapter 5 presents the results of the pulsed EPR experiments and the analysis of the DNP results for the TEMPO system; and chapter 6 is a brief set of concluding remarks.

DNP has been extensively studied and is relatively well understood in the regime of low magnetic field. In chapter 2, this basic low field, physical theory is reviewed, with an emphasis on the derivation of the dynamical equations of polarization transfer and their steady state solutions for both the solid effect and thermal mixing, the two main DNP mechanisms of electron-nuclear polarization transfer. Unfortunately, the polarization transfer mechanisms and dynamics are generally more complex at high magnetic fields. However, this description of the theory is presented in order to construct the model used in chapter 5 for analyzing DNP at high field, where the simple low field thermal mixing model breaks down due to inhomogeneous broadening of the EPR line.

Some of my early work with the group involved performing pulsed EPR experiments to measure the electronic spin-lattice relaxation time in the BDPA/polystyrene model system, and served to confirm the estimated value of $T_{1e}$, which had been previously determined by the less accurate method of continuous wave EPR saturation experiments. The results of the BDPA/PS DNP experiments are presented in chapter 3. Although previously published [14], the data and analysis are included in order to present an example of the well-resolved solid effect at high field and to highlight the importance of spin diffusion.

Chapter 3 also contains a presentation of experimental results in the application of DNP to a biological system at high field, using the nitroxide free radical TEMPO as the polarization agent. Enhancements of nearly two orders of magnitude are achieved in the NMR spectra of both the amino acid arginine, and the 18.7 kD protein T4-Lysozyme dissolved
in this frozen solution. These results indicate that this particular system can serve as a polarization matrix for a host of biological solutes. The transfer mechanism in this system is similar to low field thermal mixing in that relatively little microwave power is required to effect polarization transfer.

The efficacy of DNP depends strongly on various external parameters such as microwave irradiation power and sample temperature, due to the competition between the ability to drive the system away from thermal equilibrium via irradiation of nominally forbidden transition, and the system's intrinsic relaxation properties. The creation and delivery of sufficient microwave power is thus essential. Standard high-frequency microwave devices do not provide sufficient power to drive polarization transfer at these frequencies, therefore we have implemented a cyclotron resonance maser, or gyrotron, as the primary high-power microwave source for our experiments.

Chapter 4 contains a discussion of some of the technical considerations involved in the incorporation of DNP into high-field solid-state NMR and the associated EPR studies necessary for the elucidation of the mechanism of polarization transfer, significantly challenging experimental endeavors. Particular emphasis is placed on the descriptions of the design and operation of the EPR spectrometer and the 140 GHz gyrotron. The design and construction of the lock system and spectrometer interface program comprise part of my contribution to this research effort.

Nuclear polarization tends to "leak" due spin-lattice relaxation via processes which slow significantly as the sample temperature is lowered; thus the DNP efficiency will, in general, improve significantly at lower temperatures. High-resolution solid-state NMR experiments are usually performed at temperatures above 120 K, where the implementation of magic-angle spinning (MAS) is straightforward. In order to take advantage of the low-temperature
efficiency of DNP, a DNP/MAS system was constructed which operates at temperatures as low as 30 K. The main features of this implementation are presented.

Chapter 5 contains a discussion of our investigations into the low power polarization transfer dynamics in the TEMPO system at 5 T. The results are shown to be consistent with a high field analog of thermal mixing. The formalism presented in chapter 2 to describe thermal mixing in a single homogeneous EPR line is shown to be inadequate in explaining these high field dynamics. The effect of cross-relaxation in the inhomogeneous TEMPO EPR line must be explicitly included in order to construct a model that adequately fits the DNP, pulsed EPR, and electron-electron double resonance (ELDOR) measurements, and the agreement between the model and the experimental results is gratifying.
Chapter 2

A Thermodynamic Description of the Magnetic Interactions in Paramagnetic Solids

2.1 Introduction

In this chapter, we will present a description of the various magnetic interactions in dielectric, paramagnetic solids. We are interested particularly in samples which have been prepared by dilution of a specific amount of stable paramagnetic molecule in a diamagnetic solvent system, which, upon freezing in the case of liquid solvents, or polymerization in the case of polymer blend solvents, result in a random distribution of paramagnetic impurities within a glass or disordered solid matrix.

The magnetic properties of such paramagnetically doped solids are due primarily to the paramagnetic impurity molecules which have a non-zero total electron spin and a magnetic moment proportional to this electron spin. Early on in the study of paramagnetic crystals
in an externally applied magnetic field, the concept of a magnetic system separate from the mechanical lattice vibrations was introduced [15], and a thermodynamic description of the observed magnetic properties was constructed. The magnetic spin system was shown to be in thermal contact with the lattice, and the process by which the spin system reached thermal equilibrium with the lattice was called electron spin-lattice relaxation. Subsequently, it was found necessary to divide the magnetic system into two parts: the Zeeman system and the interaction system [16]. The Zeeman system contains the energy of the electron spins in the applied magnetic field, and the interaction system contains the energy of the magnetic interactions between the electron spins. In weak magnetic fields a thermal contact between these two spin systems was observed and the physical process leading to this thermal contact was called Kronig-Bouwkamp relaxation.

Paramagnetic solids also contain nuclei with non-zero spin and nuclear magnetic moment. Since nuclear magnetic moments are three orders of magnitude smaller than the electron magnetic moments described above, their contribution to the total magnetization of the sample is small; however, nuclear spins can be studied separately using nuclear magnetic resonance (NMR). A third spin system was therefore introduced to describe NMR experiments, the nuclear Zeeman system containing the energy of the nuclear spins in the external field. The heat contact between the nuclear Zeeman system and the lattice is called nuclear spin-lattice relaxation and was first studied by Bloembergen [17]. In the last 50 years NMR and EPR have been powerful tools in the study of the magnetic properties of paramagnetic solids.

In this chapter we present a thermodynamic description of the magnetic interactions in paramagnetic solids, with particular emphasis on the underlying physics leading to this theory. A more or less complete picture of the theory, starting from the fundamental
Hamiltonian and building up to the full thermodynamic model, is presented. Although none of what is written in this chapter is new, it was found necessary to present the reader with our particular view of the subject to maintain clarity and coherence within the larger document. It is also necessary to show how the experimental results obtained at high magnetic field by our group compare with previous investigators’ results at lower fields; and how our choice of model is similar to, and yet is fundamentally different from, the low field theory upon which it is based.

2.2 Spin Systems and Spin Temperature

2.2.1 The spin Hamiltonians

In this section we will introduce the spin Hamiltonian which forms the basis for the construction of the thermodynamic description of the magnetic interactions in paramagnetic substances via the notions of spin systems and spin temperatures. As such it will contain the relevant magnetic interactions between the three types of constituents of the paramagnetic solid, which we will call:

1. paramagnetic impurities, or electrons: molecules exhibiting electronic paramagnetism

2. nuclear spins: molecules exhibiting nuclear paramagnetism only

3. everything else: diamagnetic molecules making up the rest of the lattice

The paramagnetic impurities

The paramagnetic impurity can be described by the following Hamiltonian $\mathcal{H}_{PI}$:

$$\mathcal{H}_{PI} = \mathcal{H}_0 + \mathcal{H}_c + \lambda \mathbf{L} \cdot \mathbf{S}_i + \beta_S(\mathbf{L} + 2\mathbf{S}_i) \cdot \mathbf{H}_0 + \mathcal{H}_{hf} + \sum_j \beta_n g_i^j \mathbf{I}^j \cdot \mathbf{H}_0$$  \hspace{1cm} (2.1)
where $\mathcal{H}_0$ is the Hamiltonian of the free molecule. The ground state of $\mathcal{H}_0$ determines the total orbital angular momentum $\mathbf{L}$ and the total electron spin $\mathbf{S}_e$. When such a paramagnetic impurity molecule is situated in a solid, the degeneracy of the ground state is lifted by the electrostatic interaction $\mathcal{H}_e$ of the molecule with its neighbors, the spin-orbit coupling $\lambda \mathbf{L} \cdot \mathbf{S}_e$, and the hyperfine interactions between the electron and the impurity’s constituent nuclei $\mathcal{H}_{hf}$. The application of an external magnetic field $\mathbf{H}_0$ produces the electronic Zeeman interaction $\beta_S (\mathbf{L} + 2\mathbf{S}_e) \cdot \mathbf{H}_0$ and the nuclear Zeeman interaction $\beta_n g_n^I \mathbf{I} \cdot \mathbf{H}_0$, where $\mathbf{I}$ is the spin, and $g_n^I \beta_n \mathbf{I}$ is the magnetic moment of the $j$th nucleus of the paramagnetic impurity molecule, and the sum runs over all the paramagnetic nuclei. Often, and in the particular experimental cases discussed in this thesis, one only needs to consider the ground multiplet of $\mathcal{H}_{FI}$. This ground multiplet can be represented by an effective Hamiltonian, called the spin Hamiltonian $\mathcal{H}_S$. For the calculation of $\mathcal{H}_S$ for any particular paramagnetic impurity, the reader is referred to Abragam and Bleaney’s book on the EPR of transition ions [18]. In many cases the result can be written as:

$$\mathcal{H}_S = -\beta_S \mathbf{S} \cdot g_S \cdot \mathbf{H}_0 + \hbar \mathbf{S} \cdot \mathbf{D} \cdot \mathbf{S} + \hbar \sum_j \mathbf{S} \cdot A_{hf}^j \cdot \mathbf{I}^j + \hbar \sum_j \mathbf{I}^j \cdot Q^j \cdot I^j + \sum_j \beta_n g_n^j \mathbf{I} \cdot \mathbf{H}_0 \quad (2.2)$$

where the ground multiplet is described by the effective electron spin $\mathbf{S}$, which has the following interactions:

1. $\beta_S \mathbf{S} \cdot g_S \cdot \mathbf{H}_0$: the Zeeman interaction of the effective electron spin $\mathbf{S}$ with $\mathbf{H}_0$

2. $\hbar \mathbf{S} \cdot \mathbf{D} \cdot \mathbf{S}$: the electronic fine-splitting

3. $\hbar \mathbf{S} \cdot A_{hf}^j \cdot \mathbf{I}^j$: the hyperfine interaction between $\mathbf{S}$ and the molecule’s $j$th nucleus

4. $\hbar \mathbf{I}^j \cdot Q^j \cdot \mathbf{I}^j$: the quadropolar interaction of the paramagnetic molecule’s $j$th nucleus
The spin Hamiltonian in equation 2.2 will have \((2S+1) \prod_j (2I_j+1)\) energy levels. These levels can be found by perturbation theory, and to zero order are given by \(|M, m_1, m_2, \ldots, m_n\rangle\), where \(M\) and the \(M_j\)'s are the eigenvalues of \(S_z\) and \(I_z^j\) with the \(z\)-axis parallel to the external magnetic field \(H_0\).

**The nuclear spins**

Now to the molecules exhibiting nuclear paramagnetism only, which we call the nuclear spins. They can be described similarly by the spin Hamiltonian \(H_1\) and the reader is referred to Abragam's book on nuclear magnetism for its derivation [19].

\[
H_1 = \beta_n g_n I \cdot H_0 + \hbar I \cdot Q \cdot I
\]

(2.3)

where \(\beta_n g_n I \cdot H_0\) is the nuclear Zeeman interaction, and \(I \cdot Q \cdot I\) is the nuclear quadropolar interaction. The Hamiltonian in equation 2.3 will have \((2I+1)\) non-equidistant energy levels and to zero order the wavefunctions corresponding to these levels are \(|m\rangle\) where \(m\) is the eigenvalue of \(I_z\). In the remainder of this thesis, we will only be interested in spin \(\frac{1}{2}\) nuclei. These nuclei possess no quadropole moment, therefore, the second term in equation 2.3 will be neglected.

**The total spin Hamiltonian**

The overall magnetic behavior of a dielectric solid containing \(N_S\) paramagnetic impurities of one type, and \(N_I\) nuclear spins of one type, can be described by the total spin Hamiltonian:

\[
H_{\text{spin}} = \sum_{j=1}^{N_S} H_S^j + \sum_{k=1}^{N_I} H_I^k \quad + \quad H_{SS} + H_{II} + H_{SI}
\]

(2.4)

where \(H_S^j\) and \(H_I^k\) are the spin Hamiltonians of the \(j\)th paramagnetic impurity and the \(k\)th nuclear spin respectively.
\[ \mathcal{H}_{SS} = \hbar \sum_{j<m} S^j \cdot A^m \cdot S^m \] is the dipolar interaction between the electron spins.

\[ \mathcal{H}_{II} = \hbar \sum_{k<l} I^k \cdot C^l \cdot I^l \] is the dipolar interaction between the nuclear spins.

\[ \mathcal{H}_{SI} = \hbar \sum_{j<k} S^j \cdot B^{jk} \cdot I^k \] is the dipolar interaction between the electron spins and the nuclear spins.

The energy levels of the total spin-Hamiltonian are determined in first order perturbation theory by the energy levels of the single spin terms \( \mathcal{H}_S \) and \( \mathcal{H}_I \) and the secular parts of the interaction terms \( \mathcal{H}_{SS}, \mathcal{H}_{II} \) and \( \mathcal{H}_{SI} \), which commute with \( S^j_z \) and \( I^k_z \). We will denote the secular parts of the interaction terms by \( \mathcal{H}'_{SS}, \mathcal{H}'_{II} \) and \( \mathcal{H}'_{SI} \), and the non-secular ones by \( \mathcal{H}''_{SS}, \mathcal{H}''_{II} \) and \( \mathcal{H}''_{SI} \). The secular terms are shown below in equation 2.5:

\[
\mathcal{H}'_{SS} = \hbar \sum_{j<m} (A^{im} S^j_z S^m_z + A^{jm} (S^j_z S^m_z + S^j_z S^m_z)) \\
\mathcal{H}'_{II} = \hbar \sum_{k<l} (C^{kl} I^k_z I^l_z + C^{lk} (I^k_z I^l_z + I^k_z I^l_z)) \tag{2.5} \\
\mathcal{H}'_{SI} = \hbar \sum_{j,k} B^{jk} S^j_z I^k_z
\]

### 2.2.2 The concept of spin temperature

In order to apply the tools of statistical mechanics and thermodynamics to systems of interacting spins, one must postulate the existence of a spin temperature. A system of interacting spins, isolated from its surroundings and described by the spin Hamiltonian \( \mathcal{H} \), will evolve toward an equilibrium state characterized by an inverse temperature \( \beta \), related to the spin temperature \( T \) via \( \beta = 1/k_B T \), where \( k_B \) is Boltzmann's constant. The entropy \( S \) of a state characterized by the density matrix \( \rho \) given by:

\[
S = -k_B \text{Tr}\{\rho \ln \rho\}. \tag{2.6}
\]

The equilibrium state (which maximizes the entropy of the system) has a density matrix
\( \rho_{eq} \) of the form:

\[
\rho_{eq} = \exp(-\beta \mathcal{H})/\text{Tr}\{\exp(-\beta \mathcal{H})\}, \tag{2.7}
\]

and an entropy \( S_{\text{max}} \) given by:

\[
\frac{S}{k_B} = -\beta \text{Tr}(\mathcal{H}) - \ln[\text{Tr}\{\exp(-\beta \mathcal{H})\}], \tag{2.8}
\]

The energy, i.e. the expectation value of the Hamiltonian, is given by \( \langle \mathcal{H} \rangle = \text{Tr}\{\rho_{eq} \mathcal{H}\} \), and the sign of the inverse spin temperature \( \beta \) depends on the value of this energy. One can choose the origin of the energies of the spin system to have \( \text{Tr}\{\mathcal{H}\} = 0 \), in which case:

\[
\langle \mathcal{H} \rangle < 0 \quad \text{for} \quad T > 0, \quad \text{and} \quad \langle \mathcal{H} \rangle > 0 \quad \text{for} \quad T < 0. \tag{2.9}
\]

If the spin system starts in some initial state described by the density matrix \( \rho_i \), it will reach thermal equilibrium by evolving into the state described by \( \rho_{eq} \). The rate at which the system evolves toward equilibrium depends on the Hamiltonian. In the next two subsections we will restrict ourselves to a discussion of the simple case of a system of \( N \) identical, coupled spins in a magnetic field, i.e. a system in which the spin Hamiltonian is a sum of Zeeman and dipolar terms only:

\[
\mathcal{H} = \mathcal{H}_Z + \mathcal{H}_D = \hbar \omega_0 \sum_i I_i^z + \mathcal{H}_D. \tag{2.10}
\]

The rate at which this spin system approaches thermal equilibrium with the lattice depends on the size of the Zeeman splitting \( \omega_0 \) relative to the size of the dipolar width \( \Delta \omega \), and the solutions for the two limiting cases \( \Delta \omega \sim \omega_0 \) and \( \Delta \omega \ll \omega_0 \), i.e. "low field" and "high field", are presented below.

We will also restrict ourselves to the so-called high temperature approximation, \( \hbar \Delta \omega, \hbar \omega_0 \ll k_B T \), for which one may use for \( \rho \) an expansion in powers of \( \beta \), limited to the
linear term:

$$\rho = A(1 - \beta \mathcal{H}),$$ \hspace{1cm} (2.11)

where the normalization constant $A = (\text{Tr} 1)^{-1}$ is used in order that $\text{Tr} \rho = 1$; note that for a system of $N$ identical spins $I$: $\text{Tr} 1 = (2I + 1)^N$.

For a system of non-interacting spins, the Hamiltonian reduces to the Zeeman term:

$$\mathcal{H} = -\gamma \mathcal{H} I_z = \sum_i -\gamma \mathcal{H} I_z^i = \sum_i \omega_0 I_z^i,$$

and the density matrix factors into the products:

$$\rho = \prod_i \rho_i$$

with

$$\rho_i = \exp(-\beta \omega_0 I_z^i)/\text{Tr}_i\{-\beta \omega_0 I_z^i\},$$ \hspace{1cm} (2.12)

where the trace is carried out with respect to the variables $I^i$ only. In this special case, the validity condition for the high temperature approximation ($|\beta \hbar \omega_0| \ll 1$) can be written as:

$$\frac{1}{N} \beta^2 \text{Tr}\{\mathcal{H}^2\} \ll 1.$$ \hspace{1cm} (2.13)

So, for a set of $N$ identical, non-interacting spins in a magnetic field, where the high temperature approximation is valid, the energy is given by:

$$\langle \mathcal{H} \rangle = -A \beta \text{Tr}\{\mathcal{H}^2\}$$ \hspace{1cm} (2.14)

and the entropy is given by:

$$\frac{S}{k_B} = N \ln(2I + 1) - \frac{1}{2} A \beta^2 \text{Tr}\{\mathcal{H}^2\}.$$ \hspace{1cm} (2.15)

Low fields

Low fields means fields such that the Zeeman splitting $\omega_0$ is comparable to the span $\Delta \omega$ of the dipolar spectrum of $\mathcal{H_D}$. The time constant for reaching equilibrium is then of the order of $(\Delta \omega)^{-1}$ which is also of the order of $T_2$, the lifetime of the free precession signal.
observed in high fields.

Although the above validity criterion (equation 2.13) applies only to non-interacting spins, we will assume that it is also valid for interacting spins, and for the simple Hamiltonian that is a sum of Zeeman and dipolar interactions, $\mathcal{H} = -\gamma H I_z + \mathcal{H}_D$, one can easily compute the energy via

$$\text{Tr}\{\mathcal{H}^2\} = \gamma^2 H^2 \text{Tr}\{I_z^2\} + \text{Tr}\{\mathcal{H}_D^2\} = \gamma^2 \text{Tr}\{I_z^2\}(H^2 + H_L^2),$$

(2.16)

where the local field $H_L$ is defined by:

$$\gamma^2 H_L^2 = \frac{\text{Tr}\{\mathcal{H}_D^2\}}{\text{Tr}\{I_z^2\}}.$$

(2.17)

The evolution towards equilibrium for a set of identical coupled spins in low field can then be described simply in the following way. Let $T_{1z}$ and $T_{1D}$ be the respective relaxation times for $I_z$ and $\mathcal{H}_D$. If both are much longer than $T_2$, which is generally the case, then the system will have a single, well-defined inverse spin temperature $\beta$ evolving toward the inverse lattice temperature $\beta_L$ at a rate $1/T_1$ given by:

$$\frac{1}{T_1} = \left[H^2 + H_L^2\right]^{-1} \left[\frac{H^2}{T_{1z}} + \frac{H_L^2}{T_{1D}}\right]$$

(2.18)

**High fields**

When $H \gg H_L$ the time $\tau$ for reaching equilibrium becomes very long. This is due to the fact that the energy spectra of the Zeeman energy and the dipolar energy are not on speaking terms: the Zeeman energy can only change by quanta $\pm h\omega_0$, much larger than the total span of the dipolar spectrum. However a time of the order of $T_2$ is sufficient for the establishment of a quasi-equilibrium state characterized by more than one spin temperature.

Consider again the system of coupled identical spins, this time at high field. Here the
dipolar Hamiltonian $H_D$ is much smaller than the Zeeman Hamiltonian $H_Z = \hbar \omega I_z$. The secular part of the dipolar Hamiltonian we will call $H'_D$, that is, $H'_D$ is the part of $H_D$ that commutes with $H_Z$. The non-secular part $H'_D = H_D - H'_D$ does not affect the energy levels of the spin system to first order and be disregarded in high field, leaving an approximate Hamiltonian sum of two commuting terms. It is important to note that it is the non-secular part of the dipolar Hamiltonian that will lead to an exchange of energy between $H_D$ and $H_Z$.

It is reasonable to assume and well verified by experiment [19], that a quasi-equilibrium state of this simple spin system can be described by a density matrix of the form:

$$\rho = \exp(-\alpha \omega I_z - \beta H'_D)/\text{Tr}\{\exp(-\alpha \omega I_z - \beta H'_D)\}, \quad (2.19)$$

where $\alpha$ and $\beta$ are the inverse Zeeman and inverse dipolar temperatures respectively. In the high temperature limit, the density matrix is reduced to:

$$\rho \approx 1 - \alpha \omega I_z - \beta H'_D. \quad (2.20)$$

Since $\text{Tr}\{I_z H'_D\} = 0$, the computation of the following expectation values becomes straightforward:

$$\langle Z \rangle = \omega_0 \langle I_z \rangle = -\alpha \omega_0^2 \text{Tr}\{I_z^2\} = -\alpha \gamma^2 H^2 \text{Tr}\{I_z^2\}; \quad (2.21)$$

$$\langle H'_D \rangle = -\beta \text{Tr}\{H'_D^2\} = -\beta \gamma^2 H^2 \text{Tr}\{I_z^2\}; \quad (2.22)$$

where the local field $H'_L$ is defined by the following relation:

$$\gamma^2 H'^2_L = D^2 = \text{Tr}\{H'_D^2\}/\text{Tr}\{I_z^2\}. \quad (2.23)$$

Note that the local fields defined in equations 2.17 and 2.23 are different.

This sort of analysis of spin systems, and the points made regarding the partitioning
of the Hamiltonian into various commuting parts which form independent thermodynamic reservoirs, each with a well defined temperature and exchanging energy with the other baths via the non-secular parts of the Hamiltonian, is the fundamental picture of spin dynamics that we use in this thesis.

2.2.3 The different thermodynamic reservoirs

In this subsection we will give a thermodynamic description of spin systems and spin temperatures for a solid having the spin Hamiltonian of equation 2.4. This method was originally introduced by Provotorov [20] and Jeener [21]. Recall the total spin Hamiltonian for $N_S$ electron spins and $N_I$ nuclear spins:

$$\mathcal{H}_{\text{spin}} = \sum_{j=1}^{N_S} \mathcal{H}^j_S + \sum_{k=1}^{N_I} \mathcal{H}^k_I + \mathcal{H}_{\text{SS}} + \mathcal{H}_{\text{II}} + \mathcal{H}_{\text{SI}}.$$  \quad (2.24)

First we define the number operators $\hat{N}_{SM'm'}$ and $\hat{N}_{Im}$ via the equations:

$$\sum_{j=1}^{N_S} \mathcal{H}^j_S = \sum_{M'm'} E_{SM'm'} \hat{N}_{SM'm'} \quad (2.25)$$

$$\sum_{k=1}^{N_I} \mathcal{H}^k_I = \sum_{m} E_{Im} \hat{N}_{Im}$$

If the system is in a state $|\Psi\rangle$, then $\hat{N}_{SM'm'}$ denotes the expectation value of the number of electron spins with energy $E_{SM'm'}$, and $\hat{N}_{Im}$ denotes the expectation value of the number of nuclear spins with energy $E_{Im}$. The number of paramagnetic impurities $N_S$ and the number of nuclear spins $N_I$ are fixed quantities; and since $\sum_{M'm'} \hat{N}_{SM'm'} = N_S = \text{constant}$, and $\sum_{m} \hat{N}_{Im} = N_I = \text{constant}$, the number operators are not independent. So we can use two slightly reduced sets of number operators: one which is $(2S+1)(2I'+1) - 1$ independent linear combinations $\hat{N}_{Sr}$ of the original $(2S+1)(2I'+1)$ set of $\hat{N}_{SM'm'}$ operators, and another
which is a $2I$ set of $\hat{N}_{1q}$ of the original $(2I + 1)$ set of $\hat{N}_{1m}$ operators. This lets me define

the corresponding energies $E_{Sr}$ and $E_{Iq}$ via the equations:

\[
\sum_{j=1}^{N_S} \mathcal{H}_S^j = \sum_r E_{Sr} \hat{N}_{Sr} \tag{2.26}
\]
\[
\sum_{k=1}^{N_I} \mathcal{H}_I^k = \sum_q E_{Iq} \hat{N}_{Iq}
\]

The above equations 2.26 defines the energies $E_{Sr}$ and $E_{Iq}$ which are linear combinations
of the energies $E_{SMm'}$ and $E_{Im}$. It is possible to chose the linear combinations $\hat{N}_{Sr}$ and
$\hat{N}_{Iq}$, such that $\text{Tr}\{\hat{N}_{Sr} \hat{N}_{Su}\} = \delta_{ur} \text{Tr}\{\hat{N}_{Sr}\}^2$ and $\text{Tr}\{\hat{N}_{Iq} \hat{N}_{Iv}\} = \delta_{qv} \text{Tr}\{\hat{N}_{Iq}\}^2$, although the
particular choice is not unique. Usually one chooses the number operators $\hat{N}_{Sr}$ and $\hat{N}_{Iq}$
such that they represent a measurable quantity such as the intensity of a resonance line.

If the number operators $\hat{N}_{Sr}$ and $\hat{N}_{Iq}$, and the dipolar energy $\langle \mathcal{H}'_{SS} \rangle + \langle \mathcal{H}'_{SI} \rangle + \langle \mathcal{H}'_{II} \rangle$
are constants of motion, then the system is considered to be in thermodynamic equilibrium.

We can then described it in the grand-canonical formalism by the following density matrix:

\[
\rho = \frac{1}{\Xi} \exp \left( - \sum_r \hat{N}_{Sr} \mu_{Sr} - \sum_q \hat{N}_{Iq} \mu_{Iq} - (\mathcal{H}'_{SS} - \mathcal{H}'_{SI} - \mathcal{H}'_{II}) \beta \right) \tag{2.27}
\]

where $\Xi$ is a normalization constant, $\mu_{Sr}$ and $\mu_{Iq}$ are the chemical potentials correspond-
ing to the number operators $\hat{N}_{Sr}$ and $\hat{N}_{Iq}$ respectively and $\beta = 1/kT_D$ where $T_D$ is the
temperature of the dipolar interaction system. In the high temperature approximation a
more suitable description is obtained by introducing the parameters $\alpha_r = \mu_{Sr}/E_{Sr}$ and
$\gamma_q = \mu_{Iq}/E_{Iq}$. They have the dimension of $1/kT$ where $T$ is a temperature. The density
matrix then becomes:

\[
\rho = \frac{1}{\Xi} \left( 1 - \sum_r \hat{N}_{Sr} E_{Sr} \alpha_r - \sum_q \hat{N}_{Iq} E_{Iq} \gamma_q - (\mathcal{H}'_{SS} - \mathcal{H}'_{SI} - \mathcal{H}'_{II}) \beta \right) \tag{2.28}
\]

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We can now calculate the expectation value of the spin Hamiltonian of equation 2.24.

\[
\langle \mathcal{H}_{\text{spin}} \rangle = \text{Tr} \{ \rho \mathcal{H}_{\text{spin}} \} = \text{Tr} \left\{ \rho \left( \sum_r \hat{N}_{Sr} E_{Sr} + \sum_q \hat{N}_{1q} E_{1q} + \mathcal{H}'_{SS} + \mathcal{H}'_{SI} + \mathcal{H}'_{II} \right) \right\} = -\frac{1}{2} \left[ \sum_r \hat{N}_{Sr} E_{Sr}^2 \text{Tr} \{ \hat{N}_{Sr} \}^2 + \sum_q \hat{N}_{1q} E_{1q}^2 \text{Tr} \{ \hat{N}_{1q} \}^2 + \beta \text{Tr} \{ \mathcal{H}'_{SS} + \mathcal{H}'_{SI} + \mathcal{H}'_{II} \} \right] \quad (2.29)
\]

Philipot [22] showed that we can interpret this result as a description involving \((2S + 1)(2I + 1) - 1\) independent systems originating in \(\mathcal{H}_S\) with spin temperatures \(T_{Sr} = 1/k\alpha_r\). Because the electron Zeeman energy is in high fields the most important contribution to \(\mathcal{H}_S\), we will call these systems the electron Zeeman systems. Furthermore we have \(2I\) independent systems originating in \(\mathcal{H}_I\) with spin temperatures \(T_{1q} = 1/k\gamma_q\). These systems will be called the nuclear Zeeman systems. Finally we have the dipolar interaction system, which is independent from the other systems and has the temperature \(T_D = 1/k\beta\).

Normally the spin Hamiltonian as given by equation 2.24 is perturbed by extra terms due to spin-lattice relaxation, a microwave field which is applied to perform ESR experiments, an RF field to perform NMR experiments and the non-secular terms in \(\mathcal{H}'_{SS} + \mathcal{H}'_{SI} + \mathcal{H}'_{II}\). As a result the number operators \(\hat{N}_{Sr}\) and \(\hat{N}_{1q}\) and the dipolar energy \(\langle \mathcal{H}'_{SS} \rangle + \langle \mathcal{H}'_{SI} \rangle + \langle \mathcal{H}'_{II} \rangle\) are no longer constants of motion. In many cases however, these perturbations are small and it is still possible to use a thermodynamic description as given above. Then the inverse temperatures \(\alpha_r, \gamma_q, \) and \(\beta\) will be time dependent. In section 2.4 the time dependence of the inverse spin temperatures due to several types of perturbations will be treated using Provotorov's theory.

In section 2.3 we discuss some of the processes which lead to spin-lattice relaxation, the thermal contact between spin systems and the lattice. The other time dependent perturbations in the spin Hamiltonian 2.24 lead to thermal contact between the nuclear Zeeman,
a) Direct electron-nuclear contact

\[
\begin{array}{c}
\downarrow \\
e
\end{array} \rightarrow \begin{array}{c}
\uparrow \\
e
\end{array} \rightarrow \begin{array}{c}
\downarrow \\
e
\end{array}
\]

b) Electron-nuclear contact via electron dipolar bath

\[
\begin{array}{c}
\uparrow \\
e1
\end{array} \rightarrow \begin{array}{c}
\downarrow \uparrow \\
e2 \ n
\end{array} \rightarrow \begin{array}{c}
\downarrow \\
e1
\end{array} \rightarrow \begin{array}{c}
\uparrow \\
e2 \ n
\end{array}
\]

Figure 2-1: Energy conserving mechanisms leading to thermal contact between the nuclear Zeeman system and the electron Zeeman and electron dipolar systems. a) One electron, one nucleus flip-flops lead to direct contact between the electron Zeeman and nuclear Zeeman baths. b) Two electrons flip-flop and the difference in their energies is taken up by the nuclear spin flip, coupling the nuclear Zeeman and electron dipolar reservoirs.

electron Zeeman and electron dipole reservoirs. There are two main classes of such energy conserving mechanisms due to perturbations in $H_{\text{spin}}$: a) one electron, one nucleus flip-flops that lead to direct contact between the electron Zeeman and nuclear Zeeman baths; and b) two electrons flip-flop and the difference in their energies being taken up by the nuclear spin flip, thus connecting the nuclear Zeeman and electron dipolar reservoirs. These spin-flip mechanisms are shown schematically in Figure 2.2.3.

In the case of paramagnetic solids, irradiation near the electron Larmor frequency leads to polarization transfer from the electron Zeeman reservoir to the nuclear Zeeman reservoir. The DNP polarization transfer can proceed via one of two pathways due to the spin flips discussed above. In the case of the solid effect, the microwave field causes the one electron, one nucleus spin flips. In the case of thermal mixing on the other hand, off-resonance irradiation of the electron spins cools the electron dipole bath (c.f. section 2.4), and subsequent two electron, one nucleus spin flips leads to contact from the electron dipole bath to the nuclear Zeeman bath; i.e. the nuclear Zeeman bath is cooled indirectly.
2.3 Spin-Lattice Relaxation

2.3.1 General theory of spin-lattice relaxation

Spin-lattice relaxation is the conversion of the energy absorbed by the spin system into thermal energy in the sample. It serves to bring the temperature of the spins into equilibrium with the surroundings. In optical spectroscopy the major relaxation process is a radiative one due to spontaneous emission and stimulated absorption of blackbody photons. A calculation of relaxation dynamics for spin systems assuming this mechanism yields relaxation rates that are many orders of magnitude slower than what is observed. This due to the fact that spin-lattice relaxation in solids is actually mediated by spin-phonon interactions, and even though phonons are at least \(10^{-15}\) times as effective in stimulating spin transitions as are photons, there are many more of them at the low energies of spin systems in magnetic fields [18].

Longitudinal spin-lattice relaxation rates can be computed by second-order perturbation theory in terms of transition probabilities \(W_{\alpha\beta}\) between the energy levels \(\alpha\) and \(\beta\) in spin systems with \(n\) eigenstates [23, 19]. The transition probabilities can be combined into an \(n \times n\) relaxation matrix \(W\) which describes the evolution of the populations \(P_\alpha\) collected in a vector \(P\)

\[
\frac{d}{dt} P(t) = W \{ P(t) - P_0 \}. \tag{2.30}
\]

This is the master equation of populations. The transition probabilities \(W_{\alpha\beta}\) are given by

\[
W_{\alpha\beta} = J_{\alpha\beta\alpha\beta}(\omega_{\alpha\beta}) \quad \text{for} \quad \alpha \neq \beta
\]

\[
W_{\alpha\alpha} = -\sum_{\beta \neq \alpha} W_{\alpha\beta} \tag{2.31}
\]
with the power spectral density $J_{\alpha\beta\alpha\beta}(\omega)$ at the zero order transition frequency $\omega_{\alpha\beta} = \langle \alpha | H_0 | \alpha \rangle - \langle \beta | H_0 | \beta \rangle$

$$J_{\alpha\beta\alpha\beta}(\omega) = \int_{-\infty}^{\infty} d\tau e^{-i\omega\tau} H_1(t)_{\alpha\beta} H_1^*(t-\tau)_{\alpha\beta}$$

(2.32)

where $H_1(t)$ is the Hamiltonian of the random perturbations due to the thermal lattice motions. Transverse relaxation cannot be described by this approach. It requires a more fundamental density operator treatment.

The semi-classical relaxation theory approach tends to be the most useful for applications. In this framework, the evolution of the spin system is described quantum mechanically by a density operator, while the influence of the surroundings is represented by fluctuating random processes [1, 19, 24].

2.3.2 Processes leading to $T_{1e}$

In paramagnetic materials the electronic spin-lattice relaxation is thought to originate in the modulation of the electrostatic crystal field $H_c$ by lattice vibrations [25]. Thus, electron spin-lattice relaxation can be described in terms of the mechanism coupling the lattice and the electron spin. There are three classes of such processes: direct, Raman, and Orbach-Aminov. In the direct process the energy required to flip the spin is provided by the creation or annihilation of a single phonon, analogous to the absorption or emission of a single photon in optical spectroscopy. In the Raman process, like its optical counterpart, the difference in energy between two phonons is used to conserve the total energy of the system. Finally in the Orbach-Aminov process higher order processes involve local vibrations or excited states to effect the spin flip, somewhat analogous to resonant Raman optical spectroscopy.

Each of these processes has a different dependence on the phonon density in crystals at low
temperature (< 10 K), and it is possible to distinguish between them on the basis of their temperature dependencies [18].

For relaxation in glasses or disordered solids, where lack of a well defined lattice invalidates the concept of lattice phonons, it is more useful to consider the nuclear motion leading to relaxation as described by a correlation function with a correlation time \( \tau_c \) which is temperature dependent. In such cases, the relaxation processes tend to be more a function of the matrix than of the radical; in particular, it is the existence of localized vibrational or tunneling modes that leads to effective spin lattice relaxation, e.g. large amplitude nuclear motion, such as methyl group rotation, modulates the electron nuclear hyperfine interaction between the radical and matrix nuclei [26].

In section 5.3.1 we discuss some of the technical considerations for measuring the electron spin-lattice relaxation time \( T_{1e} \) for \( g \approx 2 \) electrons at 5 Tesla and 140 GHz. Experimental results are also presented in that section, and highlight the importance of cross-relaxation and weak microwave irradiation in the acquisition of data at high frequency in inhomogeneously broadened spin systems.

2.3.3 Processes leading to \( T_{1n} \)

Nuclear spin-lattice relaxation in paramagnetic crystals was first described by Bloembergen [17]. A good review of this initial description and its extensions can be found in Abragam’s book on NMR [19], and a review of the more modern treatments of nuclear spin-lattice relaxation can be found in Ernst’s book on multidimensional NMR [1]. The details of any realistic calculation of spin-lattice relaxation dynamics will in general be extremely complicated, particularly in the cases relevant to DNP in solids, i.e. those systems which are disordered solids containing a substantial number of paramagnetic impurities.
We will therefore only list and briefly describe the physical processes leading to relaxation; the reader is referred to Abragam [19] and Ernst [1] for more details. These are the major causes of nuclear spin-lattice relaxation in solids doped with paramagnetic impurities:

1. Zeeman interaction. Molecular tumbling modulates the Larmor frequency through the anisotropy of the chemical shielding.

2. Spin-rotation interaction. The magnetic field created by a rotating molecule is modulated by molecular reorientations caused by collisions.

3. Dipolar interaction with “external” spins. The interaction with electronic or nuclear spins of solvent molecules is modulated by translation and molecular rotation.

4. Intramolecular dipolar relaxation. The dipolar Hamiltonian fluctuates because of the random molecular rotation.

2.4 Thermal Mixing and the Electron Dipolar Reservoir

Thermal mixing refers to the process by which microwave irradiation induces energy transfer between the electron Zeeman and electron dipolar systems. In the absence of an RF field, these two thermodynamic reservoirs are uncoupled from each other and reach states of quasi-equilibrium separately due to the large discrepancy between the electron Zeeman energy and the electron dipolar energy. The presence of the RF field is required to bring the two systems into “speaking terms” with one another in the rotating frame [27].

In this section we will show how the electron Zeeman system and the electron dipolar interaction system are coupled to each other. This can be seen immediately when one considers the case when the electron Zeeman temperature is very low, so that \( T_S \approx 0 \), that is, all the spins are parallel and dipolar interaction system has no well-defined temperature...
Generally speaking, the dipolar field $H_d^j$ at electron $j$ depends on the total number of neighboring spins up or down, as well as their spatial distribution. The number of neighbors up or down depends on the electron Zeeman temperature $T_S$, therefore the possible values of the dipolar field $H_d^j$, and hence $T_D$, depend on $T_S$. In the high temperature approximation however, the number of spins up is approximately equal to the number of spins down, and the dependence of $T_D$ on $T_S$ is negligible. Since we are restricting ourselves to the high temperature regime, we can safely assume the existence and independence of $T_S$ and $T_D$.

Consider a solid containing $N_e$ electron spins $S = \frac{1}{2}$ with a magnetic moment $\mu_e = g \beta_S S$ where $g$ is the electron $g$-value and $\beta_S$ is the Bohr magneton. Suppose that the only interaction between the electrons is their dipolar coupling, and suppose also that a magnetic field $H_0$ is applied to the sample along the $z$-axis in the laboratory frame of reference. The Zeeman energy of one of the electron spins $S^i$ is then equal to $g \beta_S H_0 S_z^i = \hbar \omega_e$, where $\omega_e$ is the electron Larmor frequency. The two resulting Zeeman levels are split into many sublevels by the dipolar interaction of $S^i$ with its neighbors.

Recalling the results from section 2.2.2 for a set of identical dipole coupled spins in high field and at high temperature, the probability $P(E)$ that $S^i$ has the energy $E$ is given by:

$$P(E) = \frac{1}{2} (1 - g_S \beta_S H_0 S_z^i \alpha - \mathcal{H}_D \beta).$$

(2.33)

The average energy per spin can also be calculated straightforwardly

$$\langle E \rangle = -\frac{1}{4} g_S^2 \beta_S^2 H_0^2 \alpha - \frac{1}{4} g_S^2 \beta_S^2 H_L^2 \beta = -\frac{1}{4} \hbar^2 \omega_e^2 \alpha - \frac{1}{4} \hbar^2 D^2 \beta$$

(2.34)

where the local field $H_L$ is the average of the $H_d^j$ fields, and $D$ is defined the same way it was in section 2.2.2.

When a microwave field of frequency $\omega_m$ close to the electron Larmor $\omega_e$ is applied to the
sample perpendicular to \( H_0 \), transitions \( \Delta S_z = \pm 1 \) of the electron spins are induced. As a result, both the electron Zeeman and the electronic dipolar interaction energies will change. Provotorov derived the equations for the time dependence of the inverse temperatures \( \alpha \) and \( \beta \), and for the time dependence of the magnitude of the EPR signal. For a system of electrons with lineshape \( g(\omega) \), it is easy to show using perturbation theory that the probability \( W_m \) that a weak field of strength \( H_1 \) and a frequency \( \Delta \) away from resonance will induce a transition \( \delta S_z^i = +1 \) is given by \( W_m = \pi \omega_i^2 g(\Delta) \) where \( \omega_i = \gamma_e H_1 \) [19]. The probability for \( \delta S_z^i = +1 \) is equal to that for \( \delta S_z^i = -1 \), and the net rate of transitions per second \( \delta S_z^i = +1 \) is equal to:

\[
W_m [P\left(-\frac{1}{2} \hbar \omega_m\right) - P\left(+\frac{1}{2} \hbar \omega_m\right)] = +\frac{1}{2} W_m \hbar (\omega_e \alpha + \Delta \beta) \tag{2.35}
\]

where \( \Delta = \omega_m - \omega_e \). A quantum \( \hbar \omega_m \) is absorbed by the spin system in every transition \( \delta S_z^i = +1 \). From this, \( \hbar \omega_e \) is used to increase the electron Zeeman energy, and the remaining \( \hbar \Delta \) is used to change the temperature of the dipolar interaction system. So the rate of change of the electron Zeeman energy is given by:

\[
-\frac{1}{4} \hbar^2 \omega_e^2 \frac{d\alpha}{dt} = +\frac{1}{2} \hbar^2 W_m \omega_e (\omega_e \alpha + \Delta \beta), \tag{2.36}
\]

while the rate of change of dipolar interaction energy is given by:

\[
-\frac{1}{4} \hbar^2 D^2 \frac{d\beta}{dt} = +\frac{1}{2} \hbar^2 W_m \Delta (\omega_e \alpha + \Delta \beta). \tag{2.37}
\]

Equations 2.36 and 2.37 are the Provotorov equations for the influence of the microwave field on the temperatures of the electron Zeeman and electronic dipolar interaction system. The steady state solution to equations 2.36 and 2.37 is given by:

\[
\beta = -\frac{\omega_e}{\Delta} \alpha, \tag{2.38}
\]

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Figure 2-2: Off-resonance irradiation of a homogeneous EPR line: cooling of the electron dipolar reservoir. An ensemble of identical, coupled electron spins in a magnetic field $H_0$ will exhibit the EPR spectrum $g(\omega)$ shown above. The EPR line has a homogeneous width $\delta$, and is centered at $\omega_c = \hbar^{-1} g \beta_S H_0$. If the sample is irradiated slightly off-resonance with a microwave field of frequency $\omega_m = \omega_c - \Delta$, for every electron spin flip transition $\delta S_z^I = +1$, a quantum $\hbar \omega_m$ is absorbed by the spin system, divided as follows: $\hbar \omega_c$ is absorbed by the electron Zeeman system, and the remaining $\hbar \Delta$ is emitted by the dipolar interaction system; i.e. the Zeeman bath gets hotter and the dipolar bath gets colder.

Figure 2-3: Population diagram of a homogeneous EPR line under off-resonance irradiation. The two bands in each figure represent the populations in the two Zeeman manifolds $S_z = \pm \frac{1}{2}$. Each Zeeman level is dipole broadened into a band, and the temperature of the dipole interaction bath leads to a population gradient within each band. In both figures the system is at thermal equilibrium. In the one on the left, the spin system is at thermal equilibrium with the lattice: $\alpha = \beta = \beta_L$. In the one on the right, the spin system is undergoing off-resonance microwave irradiation at a frequency $\omega_m = \omega_c - \Delta$. In that case the Zeeman bath is heated and the dipolar bath is cooled: $\alpha > \beta_L > \beta$. 

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so the dipolar interaction system will be much colder than the electron Zeeman system. The dipolar interactions system inverse temperature \( \beta \) will be positive for \( \omega_m < \omega_e \) and negative for \( \omega_m > \omega_e \). This is shown in Figure 2-3.

When a strong thermal contact exists between the nuclear Zeeman and electron dipolar systems due to two electron, one nucleus energy conserving spin flips, the two reservoirs will be in thermal equilibrium at all times, and their temperatures, \( \gamma \) and \( \beta \) respectively, will be equal. This is the case at low magnetic fields where the nuclear Larmor frequency is on the order of, or smaller than, the electron dipolar linewidth. See the schematic representation of this in Figure 2-4. The thermodynamic Provotorov equations 2.36 and 2.37 need to be modified slightly to include the strong intrinsic interaction between the EDS and NZS.

\[
\frac{d\beta}{dt} = -T_{een}^{-1}(\beta - \gamma) \\
\frac{d\gamma}{dt} = +T_{nec}^{-1}(\beta - \gamma)
\] (2.39)

where the time constants \( T_{een} \) and \( T_{nec} \) are related to each other via the following relation:

\[
T_{nec}^{-1} = T_{een}^{-1} \frac{N\omega_n^2}{\omega_L^2}
\] (2.40)

where \( N \) is the ration of nuclei to electrons, \( \omega_n \) is the nuclear Larmor frequency and \( \omega_L \) is the local electronic dipolar field. The time constants differ due the relative heat capacities of the two baths.

Several authors discuss thermal mixing in this low field situation, and the reader is referred to their work for further detail and for references to experimental verification of this model [27, 25, 28]. An explicit calculation of the rate of energy transfer between the electron dipolar and nuclear Zeeman systems is given by Duijvestijn in a paper on the effects of nuclear spin diffusion for a system with a distribution of electron-nucleus couplings [29].
Figure 2-4: Thermodynamic model of thermal mixing at low magnetic field. The nuclear Zeeman system (NZeS) and the electron dipolar system (EDS) are strongly coupled. Off-resonance irradiation of the electron Zeeman system (EZeS) will cool the EDS. Since the EZeS and NZeS are strongly coupled by two electron, one nucleus spin flips the NZeS will be cooled as well and β will be equal to γ.

2.5 The Solid Effect and Isolated Paramagnetic Impurities

One of the main mechanisms for polarization transfer in dilute paramagnetic solids is the solid effect, which is driven by irradiation of nominally forbidden EPR transitions and involves the simultaneous flip of both a nuclear and an electron spin. In this section we present a simple model of nuclear spin-lattice relaxation and DNP in systems where the electron spins are isolated from one another. Much of what is contained in this section can be found in Abragam and Goldman's book on order and disorder in magnetic resonance [27], and the reader is referred there for more detail. We start with the basic Hamiltonian for a coupled electron-nucleus pair, then move on to nuclear spin-lattice relaxation, and finally to the well-resolved solid effect.

2.5.1 An isolated electron-nucleus pair

The Hamiltonian

Consider a nuclear spin $I$ and a nearby electronic spin $S$ in an external magnetic field $B_0$ along the z-axis. The spins couple to the field via the Zeeman interaction Hamiltonian $\mathcal{H}_Z$
given by:

\[ \mathcal{H}_Z = -\mu_I \cdot B_0 - \mu_S \cdot B_0 = -\gamma h B_0 I_z - \gamma h B_0 S_z, \]  

(2.41)

where \( \gamma_I, \gamma_S \) are the gyromagnetic ratios. If the two spins \( I \) and \( S \) are separated by a distance \( r \) and the electronic wavefunction is well localized away from the nucleus, they will be coupled via the through-space dipolar interaction Hamiltonian \( \mathcal{H}_D \) given by:

\[ \mathcal{H}_D = \frac{\gamma_I \gamma_S \hbar^2}{r^3} (A + B + C + D + E + F), \]  

(2.42)

where the "dipolar alphabet" is given by:

\[
\begin{align*}
A &= I_z S_z (1 - 3 \cos^2 \theta) \\
B &= -\frac{1}{4} (I^+ S^- + I^- S^+) (1 - 3 \cos^2 \theta) \\
C &= -\frac{3}{2} (I^+ S_z + I_z S^+) \sin \theta \cos \theta e^{-i\phi} \\
D &= -\frac{3}{2} (I^- S_z + I_z S^-) \sin \theta \cos \theta e^{i\phi} \\
E &= -\frac{3}{4} I^+ S^+ \sin^2 \theta e^{-2i\phi} \\
F &= -\frac{3}{4} I^- S^- \sin^2 \theta e^{2i\phi}.
\end{align*}
\]

The angles \( \theta \) and \( \phi \) relate the dipolar tensor principle axis system to the \( z \)-axis defined by the external magnetic field \( B_0 \).

Energy levels

The dipolar interaction \( \mathcal{H}_D = \gamma_I \gamma_S \hbar^2 / r^3 \) between the nuclear spin \( I \) and the electronic spin \( S \) is of the order of a MHz, whereas the Zeeman interaction \( \mathcal{H}_Z = -\gamma h B_0 I_z - \gamma h B_0 S_z \) is 10's of MHz for the nucleus and 10's of GHz for the electron. Even though the case of two spin \( \frac{1}{2} \) particles can be solved exactly, the first-order perturbation theory solution will be useful for the discussion of many interacting spins.
To zero order, that is with no dipole coupling, the eigenstates of the electron-nucleus pair are given by the four pure states \(|a_0\rangle = |++\rangle, |b_0\rangle = |+-\rangle, |c_0\rangle = |--\rangle, |d_0\rangle = |--\rangle\); where \(|m_S m_I\rangle\) is the state with the eigenvalues of \(S_z\) and \(I_z\) given by \(m_S\) and \(m_I\).

The dipolar interaction mixes all of these states together, but since the electronic Larmor frequency \(\omega_e\) is so much larger than the nuclear Larmor frequency \(\omega_n\), the resulting states \(|a\rangle, |b\rangle, |c\rangle, |d\rangle\) and energy spacings are given to a very good approximation by Figure 2-5, which shows the isolated electron-nucleus pair with its four levels. The transitions are labeled electron-nucleus flip-flop, electron-nucleus flip-flip, electron single flip, and nuclear single flip. These transitions have frequencies \(\omega_e - \omega_n, \omega_e + \omega_n, \omega_e,\) and \(\omega_n\) respectively.

The mixing parameter \(q\) from perturbation theory is much less than one and has a spatial dependence arising from the \(C\) and \(D\) terms in the interaction Hamiltonian; \(q\) is given by:

\[
q = \frac{3 \gamma_e \gamma_n}{2 \frac{r^3}{\omega_n}} \sin \theta \cos \theta \exp(i\phi) \ll 1
\]

\[
p = (1 - q^*)^{1/2} \approx 1
\]

(2.43)

2.5.2 Nuclear relaxation due to isolated paramagnetic impurities

The relaxation of one nuclear spin

If the fractional level populations are denoted by \(a, b, c,\) and \(d,\) then from the definition of the electronic and nuclear polarizations, \(P_e = (a + b) - (c + d)\) and \(P_n = (a + c) - (b + d)\) respectively, and the normalization condition \(a + b + c + d = 1,\) the populations of the states can be calculated assuming that the ratios \(a/c\) and \(b/d\) are always equal. The equality of the population ratios \(a/c = b/d\) cannot be disturbed by an RF field and is a direct consequence of the equality of the relaxation probabilities \(|a\rangle \leftrightarrow |c\rangle\) and \(|b\rangle \leftrightarrow |d\rangle\). The transition probabilities between the four levels are given in the set of equations 2.44 shown
Figure 2-5: Eigenstates of a dipolar coupled electron-nucleus pair in high field to first order in perturbation theory. To zero order the states are given by $|m_S m_I \rangle$ where $m_S$ and $m_I$ are the eigenvalues of $S_z$ and $I_z$ respectively. The dipolar interaction mixes all of these states together, but since $\omega_n \ll \omega_e$, the approximate states $|a\rangle$, $|b\rangle$, $|c\rangle$, $|d\rangle$ are very good approximations to the exact states. The coefficients $p$ and $q$ are given in the text, with $p \simeq 1$ and $q \ll 1$. The previously forbidden flip-flip $|a\rangle \rightarrow |d\rangle$ and flip-flip $|c\rangle \rightarrow |d\rangle$ transitions have become slightly allowed due to the electron-nucleus dipole coupling.

below:

\[
|a\rangle \rightarrow |c\rangle \text{ and } |b\rangle \rightarrow |d\rangle \text{ with probability } W_L \\
|c\rangle \rightarrow |a\rangle \text{ and } |d\rangle \rightarrow |b\rangle \text{ with probability } \zeta W_L \\
|a\rangle \rightarrow |d\rangle \text{ and } |b\rangle \rightarrow |c\rangle \text{ with probability } 4|q|^2 W_L \ll W_L \\
|d\rangle \rightarrow |a\rangle \text{ and } |c\rangle \rightarrow |b\rangle \text{ with probability } \zeta W_L,
\]

(2.44)

where the parameters $\zeta$ and $W_L$ are set so that the spin-lattice relaxation of the electron proceeds at a rate $W_L$ with the equilibrium electronic polarization given by $P_0$:

\[
P_0 = - \tanh(\frac{1}{2} \beta_L \omega_e) = -(1 - \zeta)(1 + \zeta)^{-1},
\]

(2.45)

where

\[
\zeta = \exp(-\hbar \omega_e / k_B T) \simeq \exp[-\hbar(\omega_e \pm \omega_n) / k_B T].
\]

(2.46)

The nuclear polarization is zero in the approximation where $\omega_n \ll \omega_e$, and its spin-lattice
relaxation proceeds through its interaction with the electron only.

From the definitions of the relaxation rates between the four levels, we can write the rate equations for the time rate of change of the

\[
\frac{dP_e}{dt} = -\frac{1}{T_{1e}} (P_e - P_0) \\
\frac{dP_n}{dt} = -\frac{1}{T_{1n}} P_n \\
\frac{1}{T_{1e}} = (1 + \zeta)(W_L + 4|q|^2W_L) \approx (1 + \zeta)W_L \quad (2.47) \\
\frac{1}{T_{1n}} = (1 + \zeta)4|q|^2W_L(1 - P_eP_0) \approx 4|q|^2(1 - P_eP_0)\frac{1}{T_{1e}}
\]

If the electron's are at equilibrium with the lattice, then the polarization \(P_e\) is equal to the equilibrium value \(P_0\). If the temperature is high, in which case \(P_e \approx 0\), then \(T_{1n}^{-1} = 4|q|^2T_{1e}^{-1}\).

In any case, if the electrons are in thermal equilibrium with the lattice, a random field calculation along the lines of section 2.3 for the nucleus with a fluctuating field given by the dipolar field due to the coupling with the electron will give the same answer for \(T_{1n}\).

**The relaxation of an assembly of strongly coupled nuclear spins**

Equations 2.47 have been derived for an isolated electron-nucleus pair. For an assembly of nuclei distributed around a paramagnetic center there will be a range of electron-nuclear dipolar couplings. Each nucleus will experience a different polarization transfer rate determined by its dipolar coupling to the electron, and the total polarization transfer dynamics will then a sum of the dynamics of the individual nuclear spins. However, proton spins are generally strongly coupled to one another by mutual spin flips. The strong coupling, and resulting fast spin diffusion, tends to maintain a uniform \(^1\)H polarization throughout the sample.

To describe the polarization transfer from one paramagnetic center to the protons in
the sample, which behave as a collection of strongly coupled spins, we will use the following simple model [27]. Nuclei close to the paramagnetic center will have their resonance frequency shifted by the secular, i.e. static, part of the electron-nuclear dipole interaction, the $A$ term in equation 2.42. We define the diffusion barrier $b$ as the distance from the electron center where the shift in nuclear frequency $\Delta \omega_n$ due to the electron-nuclear dipole coupling is less than the nuclear linewidth $\Delta H_n$. The nuclei inside the diffusion barrier are uncoupled from the bulk and cannot be detected in a normal NMR experiment because their frequency shifts are so large. The rest of the nuclei outside the diffusion barrier $b$, the bulk, are very strongly coupled to each other through flip-flop interactions. The bulk nuclei will relax to the lattice with one rate $1/T_{1n}$ which is the average of $1/T_{1n}(r)$, and fast spin diffusion will maintain a uniform polarization throughout. The reader is referred to Abragam and Goldman [27] for a calculation of the size of the diffusion barrier and the resulting average relaxation rate $1/T_{1n}$ for the case of randomly distributed nuclei. The validity of the fast spin diffusion assumption for protons is discussed further in sections 3.2 and 4.3.1.

$^{13}$C has a relatively small gyromagnetic ratio as compared with protons and is only 1% naturally abundant, therefore its spin diffusion rate is several orders of magnitude slower than that of $^1$H. The assumption of uniform nuclear polarization is not applicable in this case and a range of relaxation rates will be observed. The DNP dynamics will also be non-uniform across the $^{13}$C nuclei in the sample. The polarization of nuclei closer to the electron spins will be selectively enhanced. This point is discussed further in section 3.2. For a theoretical treatment of this problem the reader is referred to the review article on the applications of DNP to $^{13}$C NMR in solids by Wind [12].
Figure 2-6: The populations of an isolated electron-nuclear spin system under microwave irradiation. The population of each level is represented schematically by the size of the corresponding circle. Irradiation at the EPR frequency, $\omega_e$, results in no change in the nuclear polarization from thermal equilibrium. Irradiation of the inner DNP (flip-flop) transition, $\omega_e - \omega_n$, equalizes the population of the states it connects, producing a positive enhancement. Conversely, irradiation of the outer DNP (flip-flop) transition, $\omega_e + \omega_n$, produces a negative enhancement.

2.5.3 The well-resolved solid effect

Consider again the four levels of the isolated electron-nucleus pair in Figure 2-5, except that now instead of a single nucleus per electron, there are $n$ nuclei per electron, and we make the following assumptions: 1) the electronic linewidth $\delta$ is much smaller than the nuclear Larmor frequency $\omega_n$, 2) the transitions with frequency $\omega_e - \omega_n$, $\omega_e + \omega_n$, and $\omega_e$ do not overlap, and 3) the nuclear spin-spin interactions maintain a uniform polarization $P_n$ across the sample as in section 2.5.2.

Figure 2-6 shows the populations of an isolated electron-nuclear spin system under microwave irradiation. The population of each level is represented schematically by the size of the corresponding circle. Irradiation at the EPR frequency, $\omega_e$, results in no change in the nuclear polarization from thermal equilibrium. Irradiation of the inner DNP (flip-flop) transition, $\omega_e - \omega_n$, equalizes the population of the states it connects, producing a positive enhancement. Conversely, irradiation of the outer DNP (flip-flop) transition, $\omega_e + \omega_n$, produces a negative enhancement.
Suppose that we irradiate the sample with a microwave field $H_1$ and drive the flip-flop transition $|b\rangle \leftrightarrow |c\rangle$, i.e. the set of levels labeled by $E > 0$ in Figure 2-6. In this case the rate equations 2.47 are modified by the inclusion of the microwave absorption term and become:

$$\frac{dP_e}{dt} = -(1 + \zeta)(WL + \sum_i 4|q_i|^2 W_L)(P_e - P_0) - \sum_i w_i (P_e - P_n)$$

$$\frac{dP_n}{dt} = -\frac{1}{n} \sum_i w_i (P_n - P_e) - \frac{(1 + \zeta)}{n} \sum_i 4|q_i|^2 W_L P_n (1 - P_e P_0) - \frac{P_n}{T_{1n}'} \quad (2.48)$$

The coefficient $w_i$ is defined relative to the strength of the coupling between the nucleus $i$ and the electron. If $W_m$ is the transition probability for the allowed transitions induced by the microwave field $H_1$, then $w_i = 4|q_i|^2 W_m$, where the $q_i$ is the coefficient from equation 2.43 for the $i$th nucleus. The last term in equation 2.48 $-P_n/T_{1n}'$ is the leakage term. It is due to nuclear relaxation to the lattice from processes other than direct coupling to the electron; c.f. section 2.3.

The reader is referred to Abragam Goldman [27] for the procedure for performing the above summation and defining the following quantities:

1. $T_{1e}^{-1}$: the electron spin-lattice relaxation rate: $(1 + \zeta) W_L = T_{1e}^{-1}$.

2. $1/T_n^0$: The nuclear spin lattice relaxation rate due to coupling with the electrons but divided by $(1 - P_0)$. Typically $\alpha$ is of the order of $10^{-3}$-$10^{-4}$: $1/T_n^0 = \alpha/nT_{1e}$.

3. $\alpha$: A dimensionless parameter defined such that $\sum_i w_i = \alpha W_m$; also if the nuclear linewidth is $\delta_n$, then $\alpha \approx \frac{8\pi}{5} \left( \frac{\tau_c}{\gamma_n} \right) \left( \frac{\delta_n}{\omega_n} \right)^2$.

4. $s$: The dimensionless saturation factor which is the saturation that would be induced by the microwave field $H_1$ if we were driving the allowed EPR transition $\omega_e$ instead.
of the forbidden flip-flop DNP transition \( \omega_e - \omega_n \). It can be quite large, although typically not as large as \( 1/\alpha \): \( s = W_m T_{1e} \).

5. \( f \): The dimensionless leakage coefficient, i.e. the ratio of direct relaxation to leakage relaxation; It can take on any value large or small: \( f = T_n^0/T_{1n}' = (T_{1e}/T_{1n}')(n/\alpha) \).

With these definitions the rate equations 2.48 become:

\[
\frac{dP_e}{dt} = -\frac{1}{T_{1e}}[\alpha s(P_e - P_n) + (P_e - P_0)]
\]  

(2.49)

\[
\frac{dP_n}{dt} = -\frac{1}{T_n^0}[s(P_n - P_e) + P_n(f + 1 - P_e P_0)]
\]  

(2.50)

Since the electron spins reach equilibrium much faster than the nuclei, we can take the electronic polarization \( P_e \) to be its quasi-equilibrium value at all times, and solve the first of the rate equations 2.49:

\[
P_e = \frac{\alpha s P_n + P_0}{1 + \alpha s}.
\]  

(2.51)

Replacing \( P_e \) with its equilibrium value in the second of the rate equations 2.50 gives:

\[
\frac{dP_n}{dt} = -\frac{1}{T_n^0}[(s + f + 1 - P_0^2)P_n - sP_0];
\]  

(2.52)

which can be written more compactly as:

\[
P_n = -\frac{1}{\tau}(P_n - P_{eq}),
\]  

(2.53)

which has the simple solution of an exponential approach to the thermal equilibrium value \( P_{eq} \) with a characteristic time constant \( \tau \):
\[ P_{eq} = P_0 \left[ 1 + f(\alpha + \frac{1}{\delta}) \right]^{-1} \]  \hspace{1cm} (2.54)

\[ \frac{1}{\tau} = \frac{1}{T_n^0} \left( f + \frac{s}{1 + \alpha \delta} \right). \]  \hspace{1cm} (2.55)

Each electron spin \( S \) is in contact with a large number of nuclear spins \( I \), and in order for DNP to be efficient, the electron must be able to flip back to thermal equilibrium after each flip-flop before any of the \( n \) nuclear spins in its sphere of influence have flipped through a nuclear spin-lattice relaxation mechanism. This is the condition \( f \ll 1 \). This condition is always true if the nuclear spin-lattice relaxation is due purely to couplings with the electron spins, and if the microwave power is high enough, the equilibrium nuclear polarization \( P_{eq} \) will be equal to the electron polarization \( P_e \), or equivalently \( P_0 \).

If, on the other hand, different 'leakage' nuclear relaxation mechanisms are present, the condition \( f \ll 1 \) may be violated; and if the leakage is very strong, i.e. \( f \sim 1/\alpha \), then the equilibrium nuclear polarization \( P_{eq} \) will be much smaller than \( P_0 \), even in the limit of infinite microwave power.

Section 3.2 contains an experimental observation of the well-resolved solid effect at high field. Note in particular Figure 3-4 which shows positive and negative enhancements in the model system BDPA doped in polystyrene at 5 Tesla. For 2% BDPA in polystyrene at room temperature the leakage is strong.

### 2.6 Broadening, Spin Packets, and Cross-Relaxation

In sections 2.4 and 2.5 we considered two idealizations of DNP for a paramagnetic solid whose EPR lineshape was characterized by a single, homogeneous EPR line: 1) thermal mixing; and 2) the well-resolved solid effect. In this section we will extend the discussion to
systems where the EPR spectrum is complicated by both homogeneous and inhomogeneous broadening, we use the terms 'homogeneous' and 'inhomogeneous' in the following way: 

*homogeneous* - those effects due to local fields fluctuating on a time scale faster than that of the magnetic resonance experiment, e.g. ones that are not refocussed by a spin echo; 

*inhomogeneous* - those effects due to local fields that are static on the time scale of the magnetic resonance experiment, e.g. ones that are refocussed by a spin echo.

Inhomogeneous broadening is generally classified into three types, all of which are relevant for the stable free radicals in disordered organic materials [27]. The first type of these inhomogeneous broadening mechanisms is dispersion in the $g$ factors of the electrons; the second is the hyperfine coupling of the electron spins with the neighboring nuclei; and the third is the electron spin-spin interactions. For example the electron spin in the nitroxide radical TEMPO (discussed in the remainder of the thesis) has a spatial wavefunction which spills over onto the adjacent $^{14}$N nucleus and is thus strongly hyperfine coupled to it. It also has a moderately large $g$-anisotropy of $6.65 \times 10^{-3}$ which corresponds to a spectral width of approximately 200 G at 139.5 GHz. Finally, at a concentration of 40 mM in a solution of water and glycerol, a sample suitable for DNP, TEMPO has a static electron-electron dipole coupling of approximately 10 G. This is discussed further in chapter 5.

Homogeneous broadening is due to the non-secular, fluctuating, random, local fields in the spin Hamiltonian. Examples include: 1) modulations of the hyperfine field due to vibrations of matrix nuclei; and 2) modulations of the dipolar field due to electron spin flips, $T_1$ or $T_2$. At low paramagnet concentrations the matrix effects dominate, and at higher concentrations the electron-electron effects dominate. The measurement of the phase memory time $T_M$ in a pulsed EPR experiment gives a good indication of the homogeneous character of the EPR line. See section 5.3.3 for more detail.
Figure 2-7: Cross-relaxation between two EPR spin packets. The spin packets can exchange energy via energy-conserving flip-flops, with the difference in their Zeeman energies $\hbar \Delta = \hbar (\omega_1 - \omega_2)$ being taken up by the electron broadening system. The cross-relaxation rate is proportional to the flip-flop rate in each packet times the overlap of the two packets.

The concept of dividing an inhomogeneous EPR line into many overlapping, homogeneous spin packets was introduced by Portis [30]. This is a further division of the spin Hamiltonian into commuting pieces in which each spin packet reaches internal equilibrium in a time $T_2$, much shorter than the spin-lattice relaxation rate $T_{1e}$, or any other process which might change the energy (temperature) of the packet.

The main process by which spin packets exchange energy is called 'cross-relaxation' and was initially described by Bloembergen [31]. Two spin 1/2 electron spins $S_i$ and $S_j$ which have overlapping spin packets can exchange polarization via energy conserving flip-flops. The rate of cross-relaxation $\tau_{cr}^{-1}$ between them depends on the magnitude of the flip-flop term $B_{ij}(S_i^+ S_j^- + S_i^- S_j^+)$ of the dipolar Hamiltonian $H_D$ (c.f. section 2.42), and on the spectral overlap between them.

Figure 2-7 shows the following example: a system consisting of two coupled electrons, each with a homogeneous EPR line and with center frequencies $\omega_1$ and $\omega_2$ respectively. Here
the cross-relaxation rate can be approximated as:

$$\tau_{cr}^{-1} = 2\pi|B_{ij}|^2 g_{ij}(\Delta),$$  \hfill (2.56)

where $\Delta = \omega_i - \omega_j$, and

$$B_{ij} = -\frac{1}{4} \frac{\gamma_e^2 \hbar}{r_{ij}^3} (1 - 3 \cos^2 \theta_{ij}),$$ \hfill (2.57)

$$g_{ij}(\Delta) = \int_{-\infty}^{\infty} d\omega d\omega' g_i(\omega) g_j(\omega') \delta(\omega - \omega' - \Delta),$$ \hfill (2.58)

and where $r_{ij}$ and $\theta_{ij}$ are coordinates of the vector between the two electrons with respect to the external magnetic field, and $g_i(\omega)$ and $g_j(\omega)$ are the normalized spin packet line-shapes \cite{31, 32, 33}. The flip-flop transition removes Zeeman energy from one spin packet and delivers to the other, and the difference in energy $\hbar(\omega_1 - \omega_2)$ which emerges from the cross-relaxation process is taken up by the electron-electron dipolar reservoir. Thus, cross-relaxation transitions perturb the Zeeman temperatures of the two spin packets and the temperature of the dipolar broadening system, leading to the following set of rate equations for the evolution of the spin temperatures of three baths:

$$\omega_i \frac{d\alpha_i}{dt} = -\frac{1}{\tau_{cr}} (\omega_i \alpha_i - \omega_j \alpha_j - \Delta \beta)$$

$$\omega_j \frac{d\alpha_j}{dt} = +\frac{1}{\tau_{cr}} (\omega_i \alpha_i - \omega_j \alpha_j - \Delta \beta)$$ \hfill (2.59)

$$\Delta \frac{d\beta}{dt} = +\frac{1}{\tau_{cr} 2\omega_L^2} (\omega_i \alpha_i - \omega_j \alpha_j - \Delta \beta)$$

where $\alpha_i$ and $\alpha_j$ are the temperatures of spin packets $i$ and $j$ respectively, $\Delta = \omega_i - \omega_j$, $\omega_L$ is the local dipole field, and $\beta$ is the temperature of the dipolar broadening system.

The above simple example of two spin packets coupled via cross-relaxation provides another path for energy to flow from the electron Zeeman system to the electron dipole
system. However, for this pathway to be effective, cross-relaxation must proceed at a rate faster than the electronic spin-lattice relaxation $T_{1e}$, otherwise the spin-packets will relax to the lattice faster than they will to each other. Another point worth making here is that cross-relaxation does not in fact lead to equalization of the temperatures (i.e. polarizations) of the packets. This can be easily seen from the rate equations. At equilibrium, we have $\omega_i \alpha_i - \omega_j \alpha_j - \Delta \beta = 0$, i.e. unless $\beta = 0$, which is not possible from the definition of dipolar temperature, we must have $\alpha_i \neq \alpha_j$.

Broadening mechanisms, homogeneity, inhomogeneity, and spin packet interactions will be discussed again in more detail in chapter 5. These concepts are central to this thesis and to the model of thermal mixing at high magnetic fields that is presented herein.
Chapter 3

DNP Results at 5 Tesla

3.1 Introduction

The experiments described herein are restricted to dilute paramagnetic solid state insulators in which there are two mechanisms for effecting polarization transfer: the solid effect and thermal mixing. The solid effect is driven by irradiation of nominally forbidden EPR transitions and involves the simultaneous flip of both a nuclear and an electron spin. Thermal mixing, on the other hand, is driven by the irradiation of allowed EPR transitions and involves transfer of energy from the electronic dipolar spin-spin interaction reservoir to the nuclear Zeeman reservoir. The DNP enhancement is defined as the ratio of the enhanced signal to the normal signal minus 1 and is maximally the ratio of the electron and nuclear gyromagnetic ratios, 657 for $^1$H, although the maximum enhancement is rarely attained experimentally due to a variety of experimental factors, including leakage and incomplete saturation. The solid effect and thermal mixing enhancements differ in their dependence on sample and experimental parameters, and a detailed description of each transfer pathway will be presented later, but a very brief introduction will be given here to provide a
framework for the discussion. In the case of a completely homogeneous EPR line under non-saturating conditions, formulae for the enhancement have been given by Wind [12]:

\[ \varepsilon_{SE} = \alpha \gamma_e N_e \frac{\gamma_n}{b^3 \delta} \left( \frac{B_1}{B_0} \right)^2 T_{1n} \]  
\[ \varepsilon_{TM} = \alpha' \gamma_e N_e^2 \frac{\gamma_n}{\delta^2} \left( \frac{B_1^2}{B_0^2} \right) T_{1n} T_{1e} \]  

where \( \alpha \) and \( \alpha' \) are physical constants, \( \gamma_e \) and \( \gamma_n \) are the electron and nuclear gyromagnetic ratios, \( \delta \) is the homogeneous EPR linewidth, \( b \) is the nuclear spin diffusion barrier, \( N_e \) is the number of unpaired electrons, \( T_{1n} \) and \( T_{1e} \) are the nuclear and electronic spin lattice relaxation times, \( B_1 \) is the microwave magnetic field strength, and \( B_0 \) is the external magnetic field strength. The values of the external magnetic field for which the maximum enhancements are achieved are given by:

\[ B_{0,SE} = \gamma_e \omega = (\omega_e \pm \omega_n) / \gamma_e, \quad \text{for the solid effect} \]  
\[ B_{0,TM} = \gamma_e \omega = (\omega_e \pm \delta) / \gamma_e, \quad \text{for thermal mixing} \]  

in which \( \omega_e \) and \( \omega_n \) are the electron and nuclear resonance frequencies respectively, and \( \delta \) is the homogeneous EPR linewidth. The sign of the enhancement is determined by the value of the external magnetic field, or equivalently the frequency of microwave irradiation: positive enhancement when the sample is irradiated with \( \omega = \omega_e + \omega_n \) or \( \omega = \omega_e + \delta \), and negative enhancement when the sample is irradiated with \( \omega = \omega_e - \omega_n \) or \( \omega = \omega_e - \delta \).

These set of equations highlight the dependence on microwave field \( B_1 \) and external field \( B_0 \) for the two polarization transfer mechanism. Since the enhancement decreases at best inversely proportional to the external field, DNP experiments could not be extended to the higher magnetic fields (5–17 T) commonly employed in modern NMR spectroscopy.
because high power (10–100 W) microwave sources operating in the 100–600 GHz range were not available and the necessary increase in $B_1$ could not be achieved. This limitation was overcome with the development of a 140 GHz gyrotron microwave source, and enhancements of 10 for $^1$H and 40 for $^{13}$C were achieved in room temperature MAS experiments of the free radical BDPA doped in polystyrene [14, 34].

In addition to extending DNP into the regime of magnetic field strengths used in modern NMR spectroscopy, it is desirable to develop a scheme to polarize a wide range of samples, unfortunately, BDPA is not soluble in aqueous media, and is therefore not useful for polarizing aqueous solutions of biological macromolecules such as membranes, proteins, or nucleic acids. Nitroxide-based free radicals are unreactive, resist oxidation by molecular oxygen, and are soluble in a wide variety of organic and aqueous media, and as such, are good candidates for use as general polarizing agents. The free radical TEMPO and one of its derivatives were employed, and enhancements of $\sim$ 100 for $^{13}$C and $\sim$ 50 for $^{15}$N were achieved in the low temperature (40 K) magic angle spinning of the protein T4-lysozyme in a solution of TEMPO in water:glycerol [35, 36].

The derivation of the above equations makes several assumptions regarding the form of the EPR line. For the equations to hold, the EPR line must be homogeneous. In the case of the solid effect, the homogeneous width $\delta$ must be less than the nuclear Larmor frequency $\omega_n$, while in the case of thermal mixing on the other hand, the homogeneous width $\delta$ must be greater than the nuclear Larmor frequency $\omega_n$. Since homogeneous line broadening mechanisms are often field independent, while inhomogenous ones scale linearly with the external field, the assumption of a homogeneous EPR line is not valid for many paramagnetic systems at high field, and the restrictions on the relationship between the homogeneous EPR linewidth $\delta$ and nuclear Larmor frequency must be reexamined. The
BDPA EPR line is narrow and homogeneous and thus the polarization transfer proceeds through the solid effect. The TEMPO EPR line, is strongly inhomogeneously broadened and the observation of DNP was at first rather surprising. As it turns out, the mechanism of cross-relaxation renders the line somewhat homogeneous, permitting thermal mixing. Much of the work described in this thesis is aimed at understanding this process and the resulting nuclear signal enhancement.

3.2 BDPA in Polystyrene at Room Temperature: the Solid Effect

DNP experiments at 5 T using the 140 GHz gyrotron as the microwave source were performed on samples of polystyrene doped with the free radical $\alpha,\gamma$-bisdiphenylene-$\beta$-phenylallyl (BDPA) at room temperature. The maximum observed DNP enhancements were $\sim 10$ for $^1\text{H}$ and $\sim 40$ for $^{13}\text{C}$, corresponding to reductions in signal averaging times of 100 and 1600, respectively. The results shown below are consistent with the well-resolved solid-effect mechanism discussed in section 2.5.3.

The BDPA/PS samples used in these experiments were prepared by dissolving 0.024 g of BDPA and 1.0 g of PS in 10 ml chloroform. The solution was spread on glass plates, then the dried polymer film was ground and packed into 7 mm rotors. This yields a glassy polystyrene matrix with paramagnetic $g \approx 2$ impurities in which electronic spin and $^1\text{H}$ concentrations are $N_e \approx 3 \times 10^{25}e^-/m^3$ and $N_H \approx 5 \times 10^{28}e^-/m^3$. The EPR spectrum, shown in Figure 3-1, consists of a single homogeneous line approximately 10 G wide.

The $^{13}\text{C}$ MAS NMR spectra are shown in Figure 3-2. They were acquired with $^1\text{H}$-$^{13}\text{C}$ cross-polarization and with the external magnetic field adjusted such that the microwave
Figure 3-1: EPR spectrum of 2% BDPA/PS at 140 GHz. The spectrum shown is the integral of the CW absorption signal. One particular resonance structure of the molecule BDPA is shown in the inset. The dot represents the unpaired electron.

Figure 3-2: $^{13}$C CPMAS spectrum of 2% BDPA/PS at 5 T and room temperature. The sample was alternatively irradiated with 139.6 GHz microwaves at $\omega_e - \omega_H$ for 8 s before acquisition of the DNP enhanced spectrum (top), or not subject to microwave irradiation (bottom).
Figure 3-3: $^{13}$C MAS spectrum of 2% BDPA/PS at 5 T and room temperature. The external field strength was adjusted such that the 139.6 GHz microwave irradiation (20 s) alternately corresponded to: (top) $\omega_e - \omega_C$; (middle) $\omega_e$; (bottom) $\omega_e + \omega_C$.

Figure 3-4: DNP enhancement profile for BDPA/PS at 5 T and room temperature. The enhancement is plotted versus the offset of the external magnetic field ($B_0$) from the center of the BDPA EPR line ($B_{res}$) for $^1$H DNP.
frequency was applied at $\omega_e - \omega_H$. The bottom trace is a spectrum recorded without microwave irradiation and a comparison with the top trace clearly illustrates the enhancement in signal intensity; the $^{13}$C spectra acquired in this manner thus provide an indirect probe of $^1$H polarization enhancements. The $^1$H NMR spectrum, an 80 kHz wide, featureless line, was also observed directly obtaining similar results. Figure 3-3 shows spectra acquired with microwave irradiation at $\omega_e + \omega_C$, $\omega_e$, and $\omega_e - \omega_C$, but without cross-polarization, providing a direct measure of $^{13}$C polarization enhancements. In the $^{13}$C spectra, the peaks at 125 and 40 ppm correspond to the polystyrene aromatic and aliphatic carbons, respectively; the remaining lines are the spinning sidebands associated with MAS at rates of 2–5 kHz. The peaks in Figure 3-3 are clearly broader than those of Figure 3-2, illustrating the difference between DNP in the two regimes of fast and slow nuclear spin diffusion. In the first case, the $^{13}$C nuclei are polarized via transfer from the abundant $^1$H nuclei which undergo fast...
spin diffusion and distribute polarization over extended polymer domains. That is, all of the $^1\text{H}$ nuclei in the sample, and by cross-polarization all of the $^{13}\text{C}$ nuclei, are polarized to the same extent. In the second case, polarization transfer proceeds directly to the $^{13}\text{C}$ nuclei. The nuclei that are enhanced are those close to the paramagnetic centers, just outside the diffusion barrier and therefore experiencing more paramagnetic dipolar broadening than those in the bulk.

The dependencies of the $^1\text{H}$ and $^{13}\text{C}$ polarization enhancements on electron spin resonance offset are illustrated in Figures 3-4 and 3-5. The dependence of the proton enhancement on irradiation frequency is in very good agreement with the well-resolved solid-effect discussed in section 2.5.3. The maximum enhancement obtained at room temperature is 10, and is similar to that obtained for BDPA/PS at 1.4 T under otherwise similar conditions [12]. It is considerably smaller than the theoretically maximum of $\gamma_e/\gamma_n$, implying substantial "leakage" of the nuclear polarization. Leakage should be negligible when the spin-lattice relaxation of a set of nuclei undergoing rapid spin diffusion is governed by their interaction with isolated paramagnetic centers. In that case

$$T_{1n}^{-1} = \frac{N_e}{N_n} \frac{\gamma_e}{\gamma_n} \left( \frac{\Delta B_n}{B_0} \right)^2 T_{1e}^{-1}$$

(3.5)

in which $N_e$ and $N_n$ are the nuclear and electronic spin concentrations, $\Delta B_n$ is the nuclear resonance linewidth and $B_0$ is the external field strength. Although the incorporation of BDPA into polystyrene leads to a substantial reduction of the proton $T_{1n}$ from 7 s for the neat PS to 1.6 s in the presence of BDPA at this concentration—the relation between $T_{1n}$ and $T_{1e}$ expressed in equation 3.5 does not apply. $T_{1e}$ was measured via pulsed EPR to be $\sim 0.5$ ms under these conditions, implying a $T_{1n}$ of $\sim 5600$ s. The large disparity between the calculated and observed values of $T_{1n}$ indicates that nuclear relaxation in the radical-
doped sample is due to processes other than electron spin-lattice relaxation, in fact when the sample is degassed the $T_{1e}$ is increased to $\sim 2$ ms at room temperature and $\sim 5$–$6$ ms at 10 K, implying that the oxygen contribution to $T_{1e}$, and by extension $T_{1n}$, is substantial. Following section 2.5.3, the leakage coefficient $f$ is defined as the ratio of the calculated and observed values of $T_{1n}$. In this case, $f \approx 3500$, corresponding to the strong leakage regime. The maximum enhancement for the resolved solid effect is then given by:

$$\epsilon = \left( \frac{\gamma_e}{\gamma_n} \right) \frac{1}{1 + \alpha s + f/s} \quad (3.6)$$

in which $\alpha = \gamma_e/\gamma_n(\Delta B_n/B_0)^2$, $s = \pi(\gamma_e B_1)^2 g(0)T_{1e}$ is the saturation factor, and $g(\omega)$ is the normalized EPR lineshape function centered at $\omega = 0$. Assuming that the microwave field at the sample is the same as inside the waveguide, $B_1 \approx 1$ G, gives a saturation factor $s \approx 70$, and a maximum enhancement according to equation 3.6 of $\sim 13$, in reasonable agreement with the observed value of 10.

Equation 3.5 is inappropriate for predicting $T_{1n}$ in the case of the large electron concentration, where the paramagnetic centers are not isolated and are coupled via spin-spin interactions. The observed nuclear relaxation rate can be estimated by identifying the electron flip-flop time $T_{ee}$, rather than $T_{1e}$, as the relevant correlation time for the fluctuations of the electron-nuclear interaction.

The $^1$H DNP profile of Figure 3-4 seems to be due only to the solid-effect. The $^{13}$C enhancement field profile in Figure 3-5 on the other hand, shows an additional thermal mixing enhancement of approximately 15. The solid-effect gives rise to slightly greater $^{13}$C DNP and amounts to roughly $\gamma_H/\gamma_C$ times the $^1$H solid-effect DNP enhancement.
3.3 TEMPO in Water:Glycerol at Low Temperature: Thermal Mixing

As previously mentioned, BDPA is not soluble in aqueous media and in general, cannot be used as a polarizing agent for biological systems. Nitroxide-based free radicals are soluble in a wide variety of organic and aqueous media, and have been chemically incorporated into polymers, membranes, peptides, and proteins for use as EPR spin labels [37]. They have also been used for DNP in liquids [38, 39, 40] and at very low temperatures (< 2 K) in polarized target generation for nuclear scattering experiments [41, 42, 43, 44]. At 5 Tesla, the field strength used in these studies, the EPR spectra of nitroxides are broad enough to support the thermal mixing mechanism. In this section, we present some DNP results obtained using the nitroxide TEMPO (2,2,6,6-tetramethyl-1-piperidinylx) in a frozen solution of water and glycerol. This particular nitroxide, and one of its close analogs 4-amino TEMPO, were used to enhance the NMR signals of both the aqueous solvent and protein solutes. Large NMR signal enhancements were achieved: 185 for $^1$H and $^{13}$C in static experiments at 10 K [35]; and $\sim$ 100 for $^{13}$C and $\sim$ 50 for $^{15}$N in magic angle spinning experiments at 40 K [36]. This section contains an overview of some of the results from these experiments, as well as initial analysis which begins to characterize the polarization transfer process as being due primarily to thermal mixing.

The DNP field dependence for two microwave power levels is shown in Figure 5-1: low power (10 mW) from a Gunn diode and high power (1 W) from a gyrotron. These field-dependent enhancement profiles provide insight into the nature of the DNP mechanism. High power irradiation produces significant enhancements at field values within the EPR powder lineshape shown in Figure 3-6. However, irradiation beyond the EPR spectrum,
Figure 3-6: Echo detected EPR spectrum of 1 mM 4-amino TEMPO in 40:60 glycerol/water at 10 K. The shape of the spectrum is due mainly to $g$ anisotropy and hyperfine coupling to $^{14}\text{N}$. There is some homogeneous and inhomogeneous broadening due to matrix protons and other interactions. The presence of $^{14}\text{N}$ hyperfine structure at the high field edge clearly demonstrates the overall inhomogeneous nature of the line. The spectra become only slightly more homogeneous at higher concentrations. The molecular structure is shown in the inset.

which drives the forbidden transitions associated with the solid effect, produces only modest enhancements. In the low power profile, no enhancement occurs at fields appreciably outside the EPR spectrum, indicating that the mechanisms which function via forbidden transitions are suppressed. This leaves the thermal mixing via allowed EPR transitions as the only mechanism contributing to the low power DNP. Furthermore, the observed maxima of both the high and low power enhancement profiles occur at field offsets $(B_0 - B_{res})/2\pi\gamma_e$, $\pm135 \text{ MHz}$ and $\pm125 \text{ MHz}$ respectively, significantly less than the $\pm\omega_H/2\pi = \pm211 \text{ MHz}$ expected if the enhancement came primarily from the solid effect. These observations give clear evidence of the predominance of the thermal mixing mechanism in this inhomogeneously broadened electron spin system.

Previous analyses of the thermal mixing mechanism generally assume that the EPR spectrum is homogeneously broadened. However, the inhomogeneous nature of this nitrox-
ide electron spin system, evidenced by the EPR spectrum shown in Figure 3-6, and by the presence of $^{14}$N hyperfine structure in the high field edges of the DNP enhancement profiles shown in Figure 5-1, does not preclude DNP via this polarization transfer mechanism. The effectiveness of the thermal mixing in this nitroxide system may be explained by the process of cross relaxation among electron spin packets, which renders the EPR line moderately homogeneous. Further experiments into the mechanism of polarization transfer and a detailed model of the dynamics of the DNP mechanism at low power are presented in section 5.4. For now, we will limit ourselves to presenting the results from one experiment which demonstrates the potential applicability of this form of DNP to the study of biological systems.

Figure 3-7 illustrates a typical result: an enhancement of $\sim 20$ in the $^{13}$C MAS spectrum of uniformly $^{13}$C, $^{15}$N labeled arginine (30 mg/ml) in frozen solution at 55 K. This experiment consists of polarization transfer from the paramagnetic TEMPO to the $^1$H nuclei via DNP, followed by cross polarization to $^{13}$C. The enhancement of both the arginine $^{13}$C labels, as well as the glycerol carbons, indicates that polarization transfer has occurred to both the solute and the solvent in this system. Peak integration confirms that both the solvent and solute carbons experience equivalent polarization transfer. Moreover, no differences in the NMR peak widths are observed when the DNP enhanced spectrum is compared to a signal-averaged unenhanced spectrum (not shown). This indicates that, following DNP of $^1$H, spin diffusion uniformly distributes polarization to nuclei which do not sustain a significant hyperfine coupling. Polarization transfer proceeds from the electron spins to proximate solvent protons via DNP and subsequently to distant solvent and arginine protons via solvent-mediated spin diffusion, as was the case for the BDPA/PS system discussed in section 3.2.
The reader is referred to Dennis Hall's thesis [45] for detailed discussion of a series of experiments exploring the applicability of these sorts of NMR signal enhancements to structure determination in large biological systems, and to the paper by Hall et. al. [36] on the observation of enhancements of approximately 50 in the CP-MAS spectrum of a 1 mM buffered solution of 15N-Ala-labeled T4-lysozyme in TEMPO/water/glycerol at 40 K. T4 lysozyme is 18.7 kD, 164 residue monomeric protein necessary for the lytic growth of bacteriophage T4. Enhancements of this magnitude may enable the utilization of newly developed solid-state NMR techniques designed for structural studies but which currently are difficult to apply to large biological systems.
Figure 3-7: DNP-CP $^{13}$C MAS spectra (3.0 kHz spin rate) of fully $^{13}$C and $^{15}$N labeled L-Arginine (30 mg/ml Arg and 40 mM 4-amino-TEMPO in 40:60 water:glycerol) at 55 K. The peaks labeled with an asterisk are spinning sidebands. The peaks labeled with G are the natural abundance $^{13}$C glycerol peaks. Microwave irradiation was performed using the 139.6 GHz gyrotron in 15 s pulses with $\sim$ 1 W at the sample. Sixteen acquisitions were averaged with a 1 s recycle delay. The bottom spectrum was recorded under identical conditions with no microwave power. The magnetic field was set to maximize the positive enhancement, which is approximately 20. The molecular structure of arginine is shown above, with labels indicating spectral position.
Chapter 4

Experimental Methods for DNP

and EPR at 5 Tesla and 140 GHz

4.1 Electron Paramagnetic Resonance at 140 GHz

4.1.1 Introduction

The implementation and characterization of DNP experiments requires a knowledge of
the EPR spectrum and relaxation rates of the paramagnetic center used as the polarizing
agent. A significant fraction of the work for this thesis is centered around high field EPR
spectroscopy. The free radicals used for the DNP experiments described in chapters 4 and
5 are typical of most organic $g \approx 2$ free radicals in that their ground-state electron spin
distribution can be characterized by the Zeeman and electron-nuclear hyperfine interactions.
In frozen solutions their EPR spectra consist of overlapping powder patterns arising from
anisotropy in both $g$ and the hyperfine interactions. At conventional field strengths ($\sim
0.3$ T), the spectra are complicated since spectral dispersion from the Zeeman anisotropy
is comparable in size to dispersion from the hyperfine couplings. At high fields ($\sim 5$ T),
Figure 4-1: 139.5 GHz CW-EPR spectra at 110 K of organic free radicals which exhibit varying degrees of $g$-anisotropy.

(a) BDPA doped 2% by weight into polystyrene ($g_{\text{max}} - g_{\text{min}} = \Delta g = 2.7 \times 10^{-4}$).
(b) Galvinoxyl doped 2% by weight into polystyrene ($\Delta g = 3.83 \times 10^{-3}$).
(c) Frozen solution of 2.5 mM of TEMPO in 1:1 water/glycerol ($\Delta g = 6.65 \times 10^{-3}$).
however, the $g$ value anisotropy tends to dominate and leads to simple spectra; $g$ values can be readily obtained and the hyperfine splittings can then be related to the Zeeman axis frame. These experimentally determined parameters, in conjunction with molecular orbital calculations, can provide a detailed picture of the electronic wave functions. Figure 4-1 depicts the 140 GHz EPR spectra of three free radical samples which exhibit varying degrees of $g$-anisotropy and nuclear hyperfine couplings: BDPA doped into polystyrene, galvinoxyl in polystyrene and a frozen solution of TEMPO in water:glycerol.

Implementation of pulsed methods greatly expands the scope of possible high frequency EPR experiments. These time domain techniques include Fourier transform EPR, electron spin echo envelope modulation (ESEEM), echo detected EPR, and electron relaxation rate measurements. Electron nuclear double resonance (ENDOR) and electron double resonance (ELDOR) can also be extremely useful in the characterization of radical species.

Typically, biological samples studied via high-frequency EPR spectroscopy are dilute solutions with long electronic spin-lattice relaxation times $T_1$. Saturation and adiabatic passage effects are thus common in modulation-detected EPR signals, leading to increased line broadening and decreased spectral resolution compared to conventional, unsaturated absorption EPR signals [46]. These saturation problems are not encountered in echo detected spectra and as a result greatly enhanced resolution can be obtained. Pulsed methods are also useful for obtaining EPR spectra of transient species or for species with very broad resonances, and also provides a means of separating overlapping spectra based on differences in the relaxation rates of the species involved.

Since the publication of the paper by Becerra and Gerfen describing the 140 GHz spectrometer [14] several interesting radical systems have been studied [47, 48, 49, 50, 51]. Several major changes to the spectrometer have been implemented, including a new mag-
Figure 4-2: The EPR spectrometer: Two Gunn diode sources (139.50 and 139.95 GHz, 20 mW) provide heterodyne detection capabilities. Quadrature detection is used in both the CW and the pulsed mode. The gyrotron can also be used as a high power source. The magnetic field is varied using superconducting sweep coils which are separated from the main superconducting coil. See the text for more information regarding the components and operations of this spectrometer.

...net with a larger sweep range, an improved lock system, a new computer interface, a four phase, switched 140 GHz amplifier, etc.; an overview of the spectrometer and its capabilities will be presented in the next section.

4.1.2 The EPR spectrometer

A block diagram of the 140 GHz EPR spectrometer is shown in Figure 4-2. The main features of the EPR spectrometer include a quadrature detection scheme in both the pulsed...
and CW modes, a large bore magnet with a superconducting sweep coil which is separated from the main superconducting coil, and a field sweep system based on a $^2H$ lock channel signal. A brief description of the components follows, while the magnet and sweep system are discussed in greater detail in section 4.2.

**The EPR bridge**

Microwaves are generated by two phase locked Gunn diode oscillators, one at 139.5 GHz and the other at 139.95 GHz. The sample is irradiated with microwaves from the 139.5 GHz source in a reflection mode configuration. The 450 MHz intermediate frequency is created by mixing 139.5 GHz with 139.95 GHz, amplifying and splitting through a quadrature hybrid. The signal is also mixed with 139.95 GHz, amplified, split, and mixed with the reference channel. The resulting quadrature channels are sent to matched lock-in detectors tuned to detect the first or second harmonic of the field modulation frequency in CW mode or sent to a box car integrator and oscilloscope in pulsed mode. The voltage at the lock-in detectors or the integrator is sampled and transmitted over the IEEE bus (GPIB) to a Power Mac computer where it is stored and analyzed.

Each of the microwave sources (139.5 and 139.95 GHz) consists of a double stage injection-locked Gunn oscillator and a passive varactor tripler with a final output power of 13 dBm. The input to both of these assemblies is derived from solid state oscillators operating at 15.50 and 15.55 GHz respectively. The solid state oscillators in turn are locked to the output of a single 50 MHz crystal oscillator. Two Gunn diode stages are necessary in order to achieve enough amplification to overcome the losses in the final tripler stage.

The noise figure of the CW EPR spectrometer, approximately 42 dBc at 1 kHz (the frequency of the field modulation), is largely determined by the phase noise of the sources [52].
Analysis of the frequency multiplication used to generate the 140 GHz radiation predicts a noise figure of 81 dB at 1 kHz from carrier: the phase noise of the 50 MHz crystal oscillator \( NF_1 \) is -152 dBC at 1 kHz; the theoretical noise figure due to multiplication is then given by \( NF = NF_1 + 20 \log(MF) \), where \( MF \) is the multiplication factor. The phase locking and mixing scheme however, produces a phase correlation between the signal and reference channels which reduces the resulting overall spectrometer noise figure. The sensitivity in continuous wave mode for low loss samples has recently been measured to be \( 2-3 \times 10^9 \) spins/G; pulsed mode sensitivity is approximately ten times worse [48].

**Spectrometer interface and computer control**

The EPR spectrometer is controlled from a PowerMac 7100 personal computer running LabVIEW, a data acquisition and analysis package from National Instruments. Programs are created in two parts: the front panel, which serves as a user interface, and the code, which is in the form of block diagrams. The data flow programming style forces efficient, organized coding, and the extensive libraries of functions and development tools designed specifically for data acquisition, GPIB instrument control, data analysis, presentation and storage provided make the task of creating a user interface relatively straightforward.

The spectrometer control software was initially written by Un [53] in FORTRAN on a VAX workstation, and the windows-like user interface was built on GKS routines for display hardware that is no longer supported. With the purchase of the new, larger sweep magnet, it became necessary to separate the EPR spectrometer from the DNP/NMR one. The implementation of pulsed experiments also required a flexible platform, and the ease and speed with which LabVIEW programs can be modified made this configuration a logical alternative.
In writing the set of programs that serve as the user interface and spectrometer control, we built upon the logical construction of field control and data acquisition that had been written for the VAX. Commands are sent to, and data is read from the various devices via GPIB, and a data acquisition card serves as the analog to digital converter for the sweep system as discussed in section 4.2.

We have written a substantial set of flexible routines for performing CW and pulsed experiments. Once the data is acquired it is then ported to other commercial software packages like Matlab and Kaleidagraph for more sophisticated analysis and presentation.

Cylindrical resonators

Both cylindrical and Fabry-Perot resonators have been used as resonant microwave structures for high frequency EPR [14, 54, 55, 56, 57, 58].

However, the cylindrical resonators are used exclusively in all of the experiments discussed in this thesis, because of their decreased sample size requirement, greater filling factor, stability and ease of use. A cylindrical resonator is depicted in Figure 4-3. The cavity is tuned to the $TE_{011}$ mode by moving the spring-loaded plunger. Variable coupling is achieved by rotating the waveguide with respect to the axis of the resonator. Estimated loaded $Q$ values are approximately 1000–3000, depending on the sample. Slots are cut 0.25 mm wide and 0.25 mm apart to allow RF penetration in ENDOR experiments and modulation field penetration in continuous wave EPR experiments, and do not significantly degrade the $Q$. Both silver and copper resonators have been used, with the silver ones providing somewhat higher $Q$ ($\sim 2$ times greater) due to silver’s lower resistance, however, because of this increased $Q$ the silver resonators tended to be more sensitive to mechanical vibrations, particularly before the EPR board mounting scheme was improved. The EPR
Figure 4-3: Cylindrical resonator: slots allow the penetration of RF for ENDOR experiments and field modulation. The adjustable plunger provides remote tuning. Quartz capillary sample tubes (0.3–0.8 mm o.d.) are fed through the hole in the center of the fixed plunger and held along the axis of the resonator.

data collected for this thesis was acquired primarily in copper cavities.

The conversion factor of microwave power into $B_1$ in the cylindrical resonator is given by:

$$B_1 = c\sqrt{QP}$$  \hspace{1cm} (4.1)

where $c$ is a constant including sample volume, filling factor and other constants, $Q$ is the resonator quality factor and $P$ is the incident power at the resonator. The conversion factor for the resonators used in these experiments is $\sim 36 \mu T/W$. 

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Temperature control

Variable temperature operation (4–300 K) is achieved by boiling liquid helium or nitrogen and transferring the cold gas through a vacuum jacketed transfer line into a dewar housing the probe, and over the resonator and sample. The temperature is measured using a silicon diode sensor and active control is achieved using heater tape in conjunction with a Lakeshore temperature controller unit. The temperature can be controlled to within ±1° Kelvin, but we found that for most experiments active temperature control was unnecessary. With cold helium gas as the cryogen, the system tended to reach thermal equilibrium between 8–12° Kelvin depending on the room temperature and the volume of liquid in the helium reservoir. Once at equilibrium was reached, the temperature was constant to within a degree or two for the duration of most experiments (4–6 hours). This method only worked for the low temperatures used in the studies described in this thesis. In other experiments where the measurement of some temperature dependent quantity over a large temperature range is desired, active thermal control is necessary.

4.1.3 Pulsed operation

Pulse programmer

Timing for the pulsed experiments is provided by an Interface Technologies 250 MHz, model RS 690, digital word generator capable of 4 ns resolution. Pulse programs are loaded from the PowerMac over the GPIB bus, and although tedious, the programming is fairly straightforward. Both ECL and TTL control pulses are used to gate the microwave switches, blank the RF-amplifier, and trigger the oscilloscope, integrator, and analog to digital converter.
High speed, low power switches

The 140 GHz low power, high speed, microwave switches (Donetsk Physico-Technical Institute of the Ukraine Academy of Science) used in pulsed mode have an insertion loss of 7 dB, an isolation of 57 dB, rise/fall times of 4 ns and are capable of handling powers up to 0.5 W. When used in the EPR bridge to gate the output of the 139.5 GHz Gunn diode (as shown in Figure 4-2), 90° pulse lengths in the cylindrical resonator were approximately 400 ns.

Figure 4-4 shows the result of a typical electron spin echo experiment at 140 GHz. The dead-time of the spectrometer is approximately 80 ns and is determined not by resonator ringdown, but by the time necessary for the dissipation of the standing wave power in the long piece of waveguide between the board and cavity [59].

High power, slow speed switches

Several prototype switches from Capital Technology Corporation (CTC) have been used to gate the gyrotron output in two high frequency pulsed experiments: pulsed electron double resonance (ELDOR) and rotating frame dynamic nuclear polarization (RFDNP). The ELDOR experiments and results are discussed in section 5.3.2.

Briefly, RFDNP can be viewed as a transfer of polarization from the laboratory frame of the electron spins to the rotating frame of the nuclear spins, which under certain conditions, can offer significant advantages over conventional electron lab frame to nuclear lab frame DNP experiments. RFDNP transfers polarization in milliseconds rather than seconds, allowing for much faster experimental repetition rates and decreasing the duty cycle of the microwave source. The RFDNP experiments will be described in detail by Farrar in a forthcoming publication [60].
Figure 4-4: Electron spin echoes at 140 GHz: a three pulse, stimulated echo sequence applied to BDPA/PS at room temperature using the high speed, low power switches.

The behavior of a typical CTC microwave switch as controlled by the prototype switch drivers is shown in Figure 4-5: excellent isolation (30 dB), moderate insertion loss (< 3 dB), long rise time (3μs), and short fall time (< 7 ns). Test switch drivers have been modified by voltage biasing and voltage spiking in order to increase the speed of carrier extraction and reduce the rise time to <1.5 μs and improve the insertion loss to ~ 1 dB. The diode array design is in the process of being modified to allow for fast (< 10 ns) rise and fall times. The phase shift of a particular switch can be adjusted by the addition of thin spacers (λ ~ 2 mm at 140 GHz), and the insertion loss can be optimized by a slight adjustment of the switch driver voltage.

A combination of two switches, a circulator, and a resistive load has been used to assemble a switch capable of producing short (50 ns) pulses with both fast rise times and fast fall times (< 10 ns). The scheme is shown in Figure 4-6. When switch A is on (insertion loss mode), the microwave power is deposited in the load; and when it is off (isolation mode),
Figure 4-5: The switching characteristics of a CTC switch. The isolation is 30 dB. The microwave pulse fall time is short (< 7 ns) and the microwave rise time is long (3µs) due to slow carrier extraction from the semiconductor substrate.

Figure 4-6: The formation of a pulse with fast rise and fall times using two switches and a circulator. The CTC switches have long rise times and short fall times.
the microwaves are reflected to switch B. Switches A and B are both initially on, and the power is deposited into the load. When switch A is turned off (< 10 ns), microwaves exit the device. When switch B then turns off (< 10 ns), microwave transmission ceases. This produces a pulse of arbitrary length with short rise and fall times. The insertion loss of the combined switch is the sum of the reflection loss of switch A (≪ 1 dB), the insertion loss of the circulator (≈ 1 dB), and the insertion loss of the switch B, for a total insertion loss ≈ 1 dB greater than that of one switch alone. The isolation of the combined switch is comparable to that of one switch. The minimum possible interpulse delay is twice the rise time of one switch, a severe restriction for pulsed EPR experiments, however proposed experiments, such as electron-nuclear cross-polarization, where the pulse sequence consists of two pulses of different phases closely spaced in time should be possible using such a combination switch. The implementation of this scheme at high power will require the replacement of the low power circulator with a high power 3 dB short slot hybrid.

Four phase switched amplifier

A four phase, switched 140 GHz amplifier has been recently incorporated into the EPR bridge as shown in Figure 4-7. The amplifier consists of a series of IMPATT diode amplifiers, switches and phase shifters and was manufactured in the Ukraine by V. Krymov. The IMPATT is injection-locked to the output of the old 139.5 GHz Gunn diode. This new source produces 33 mW of power and can be switched between one of the four available phases in 5 ns. This source represents a factor of ≈ 15 increase in power over the old configuration of the Gunn diode and single switch, and a corresponding increase of a factor ≈ 4 in $B_1$ in the cavity reducing the 90° pulse length to 100 ns. The use of shorter pulses will, in general, substantially increase the signal size in echo detected experiments.
Figure 4-7: Four phase switched amplifier: This is the new configuration of the EPR bridge. The IMPATT diode is injection-locked to the output of the 139.5 GHz Gunn diode (which is also used to generate the IF). The source produces 33 mW output power and can switch between the four phases \((0, 90, 180, 270)\) in 5 ns. The phases can be independently adjusted \(\pm 5^\circ\) with trim screws. The mixing and detection schemes are unchanged from Figure 4-2.

Transverse magnetization decays exponentially with a short transverse relaxation time \(T_2\), therefore short pulses will make it possible to reduce the total time between the first pulse, which creates the transverse magnetization, and the echo, which arising from mangetization refocussed by the subsequent pulses.

The mixing scheme is the same as that in the old bridge, with a small amount of the Gunn diode’s output being used to generate the IF, so the noise figure of the spectrometer is not affected by the introduction of the amplifier even though IMPATT diodes are noisier than Gunn diodes. This new microwave source allows for phase cycling of pulses and makes possible sophisticated pulsed EPR experiments that have been performed previously at lower frequencies, such as spin-locking, COSY, SECSY, double quantum 2D correlation, and other multiple quantum coherence methods.
4.2 Precision Field and Frequency Sweep System

4.2.1 Introduction

The Griffin group's high-field EPR spectrometer uses a high Q cavity to detect resonances at a fixed frequency of 139.5 GHz, therefore in order to acquire spectra reliably the magnetic field must be swept in a reproducible and measurable fashion. Since one of the principal advantages of high-field EPR lies in its detection and resolution of resonances due to the electronic $g$ factor, issues of field control and stability are particularly important. In this section we will describe the precision field and frequency lock system that we have constructed. It is based on the one described by Un et. al. [53] and uses existing technology from both NMR and X-band EPR.

For X-band EPR spectrometers the electromagnet field strength can be measured by using NMR magnetometers [61], i.e. measuring the resonance frequency of some nuclear spin like deuterium and comparing it to its gyromagnetic ratios which is known to high precision from high resolution NMR experiments. Modern NMR spectrometers, on the other hand, use a variant of the NMR magnetometer to "lock" the superconducting field by using the dispersive component of a $^2$H NMR signal from a deuterated solvent as an error signal in a feedback loop. On resonance, the dispersive signal is zero, while for small field deviations its amplitude is proportional to the field offset. Therefore, this signal may be used to drive shim coils which compensate for small DC field changes. The EPR spectrometer lock system described in this thesis uses a somewhat similar scheme to measure the field precisely, lock it to a particular value, and then sweep it in order to acquire EPR spectra.

We will now go through the various field control components of the spectrometer in some detail, and present the results of a series of benchmark EPR experiments demonstrating
various system performance characteristics including field stability, resolution, homogeneity and sweep rate capability.

4.2.2 Field and frequency sweep system hardware

In this subsection we describe the following sweep system hardware components in some detail: the magnet, the sweep power supply, the NMR lock spectrometer, the auto-tuned NMR lock probe and the NMR lock sample. The emphasis is on the overall state and action of sweep system which are controlled by the Power Macintosh 7100 computer, also the controller for the rest of the EPR spectrometer as described in section 4.1.2.

EPR magnet

Although not technically part of the field lock system, the EPR spectrometer's superconducting magnet characteristics are discussed in this section because of the intimate connection between them.

The polarizing field consists of a two-coil superconducting magnet designed and constructed by Magnex Corporation with a room temperature 130 mm bore. The larger main coil, operating in persistent mode, produces a static 5 T field. The smaller superconducting sweep coil located inside the main coil bore produces an additional field of ±0.4 T. This sweep range provides access to paramagnetic systems with electron $g$-values in the range 1.84–2.16, sufficient for most protein based free radicals. The inductance of the main coil is 48.3 H and the operating current is nominally 96.6 A. The inductance of the sweep coil is 19.6 H with a field to current ratio of 339 G/A. There is a 4.5 H mutual inductance between the main and sweep coil which manifests itself as a sweep coil effective inductance of 24.1 H, and a reduced effective field to current ratio of 292 G/A. This is one of the main
reasons for constructing a field lock system as the coupling between the two coils leads to some variability in the field to current ratio as a function of sweep coil current.

Another potential cause of hysteresis in the field vs. current profile is residual magnetization in the superconducting wires. This problem used to be more common in older magnets where fields of several 10’s of Gauss would remain in a superconducting coil after current discharge. Advances in magnet technology have reduced this effect considerably, but it cannot be neglected altogether.

The split-coil magnet configuration has the feature that only 14 A are necessary for full field sweep, thereby minimizing resistive heating losses in the leads, and reducing liquid He consumption. The low current requirements allow for the use of a high quality, solid state, bipolar current amplifier, and reduce the quenching risks associated with sweeping the main superconducting coil in the vicinity of 100 A.

Power supply

Since only ±14 A are required for full field sweep we have chosen to use a very stable bipolar current amplifier as the sweep power supply. It is model BOP20-20M-4882 manufactured by KEPCO Corporation. This supply is rated at 400 W maximum output power at 20 A and 20 V. A GPIB card allows for communication with the computer, and a 12 bit D/A card provides programmability with a resolution of 4.88 mA per step. The current stability is rated at 0.01 % of the current value although the size of the sweep coil inductance renders this effect negligible, c.f. see the later discussion on field resolution and stability.

We have made the following two small modifications to the power supply: 1) the circuit in GPIB controller card which normally would disable analog input has been bypassed to allow for simultaneous digital and analog programming, and 2) the programming input
capacitance was increased to increase the feedback time constant since the load (sweep coil) is very inductive.

The digital resolution of the power supply is insufficient for high precision EPR experiments. This can be seen by computing the difference in magnetic field when the programmed output is incremented by a single step: 4.88 mA x 292 G/A = 1.42 G, much larger than the linewidth of some of the narrow single crystal EPR spectra observed at this field, e.g. the spectrum of perylene shown in Figure 4-12 which is only 0.5 G wide. By summing the digital and analog programming inputs it is possible to achieve arbitrary current, and therefore arbitrary field values. Ultimately the field resolution and stability are limited by the width of the NMR signal used in the field lock feed-back loop.

NMR spectrometer and field locking

Now that we have described the magnet and power supply, we will move on to a discussion of the lock system's NMR spectrometer which is used first to measure the field, then to "lock" it to a fixed value, and finally to sweep it in a controlled fashion.

A schematic representation of the spectrometer is shown in Figure 4-8. The spectrometer is a single phase excitation, single sideband homodyne detection device. A square wave lock-in detector is used to detect whatever NMR signal happens to be 4.8 kHz above or below the carrier. The pulse generator, which produces the control pulses gating the transmitter, receiver and lock-in detectors, runs on a 1 MHz TTL clock obtained by converting the output of a 10 MHz single crystal oscillator to TTL, and then dividing by 10. The RF frequency is generated by a Program Test Sources 040 oscillator. By phase locking the PTS to the same 10 MHz oscillator as the pulse generator, one obtains an NMR signal which is always the same phase with respect to the carrier.
Figure 4-8: This is a simplified diagram showing the main components of the lock system NMR spectrometer. This is a single phase excitation, single sideband, homodyne detection device. The TTL gating and square wave lock-in detection switches are controlled by a digital delay generator (not shown) which is locked to the same 10 MHz crystal oscillator source as the PTS. The reader is referred to the text for more detail.

Figure 4-9: Field and frequency lock system schematic showing how the various components depend on one-another. The NMR spectrometer is shown in Figure 4-8. The reader is referred to the text for more detail.
The output of the PTS040 frequency synthesizer is divided. Half of it is used to generate the LO channel, and the other half is amplified and gated to produce small flip angle excitation pulses. During the pulse, the receiver is blanked to avoid saturation of the pre-amplifier. During detection the NMR signal returns from the probe, is amplified, and then mixed down to audio with the LO channel.

The audio output of the mixer is passed through a 4.8 kHz band pass filter and the signal is square wave lock-in detected at 4.8 kHz in two channels 90° out of phase with one another. The real and imaginary signals are nearly DC and are proportional to the absorption and dispersion components of the NMR signal, ±4.8 kHz from the PTS frequency. A variable delay between the unblanking of the receiver and the start of the lock-in detection period provides the global phase adjustment which makes the real (absorption) signal purely positive.

The field strength can be measured using this NMR magnetometer by finding the deuterium resonance frequency of of a $^2$H$_2$O sample (see below) and comparing to the known gyromagnetic ratio of deuterated water. The superconducting field can now be locked to a chosen value using the dispersive component of the $^2$H NMR signal as an error signal in a feedback loop. On resonance, the dispersive signal is zero, while for small field deviations its amplitude is proportional to the field offset. Therefore, this signal may be integrated and amplified by the power supply, and used to drive the superconducting sweep coil to compensate for small DC field changes. This is shown schematically in Figure 4-9. Once the field is locked, a small change in the RF frequency is equivalent to a slight field error, and the lock system will compensate adjusting the field to match the new resonance frequency.

The actual sweep procedure during data acquisition is somewhat more complicated in order to achieve fast sweep and take advantage of the programmability of the power supply.
This is discussed below in the section on field sweep rates.

Lock probe and auto-tuning circuit

The full sweep range of the magnet places a large constraint on the design of the lock system’s deuterium NMR probe. The magnetic field range of $5 \pm 0.4$ T corresponds to a deuterium frequency range of $32.5 \pm 2.6$ MHz, i.e. a 5.2 MHz frequency sweep range. This bandwidth is considerably larger than the 0.5 MHz bandwidth of a typical 32.5 MHz deuterium probe with a $Q$ of 60. Therefore either the bandwidth must be increased, or the probe must be remotely tunable with the tuning adjusted during the sweep to have the circuit and NMR resonance frequencies equal. Since we wish to retain a high $Q$ we have chosen the latter option.

Figure 4-10 shows a schematic diagram of the lock probe which can be remotely tuned over the range 23.5–45.2 MHz, much greater than what is required in our application. The probe’s $Q$ is nearly 50 and remains approximately constant over the 32.5±2.5 MHz frequency range. The circuit is a relatively basic resonant tank circuit with a 7 turn, 5 mm diameter NMR coil of 22 gauge copper wire and a set of high $Q$ tuning and matching capacitors. The impedance of the tank circuit is matched to the 50 Ohm transmission line using a single 9–35 pF ceramic capacitor. Matching remains sufficiently constant over the total frequency range so as not to adversely affect the performance of the lock channel. The tank circuit is tuned by biasing the set of silicon tuning diodes, Motorola MV2115, which have a tuning range of 70–200 pF for a bias voltage range of 10–0.1 V, although 1.72–3.63 V is sufficient to provide tuning over the range 30.5–34.5 MHz which is nearly the entire sweep range of the magnet. The bias voltage for the silicon tuning diodes is derived from the the auto-tuning feedback circuit described below. An RF choke and a capacitor to ground are used
to keep noise traveling on the bias voltage control line from interfering with acquisition of the deuterium NMR signal.

The probe tuning is controlled by the following feedback system. A square wave generator oscillating at approximately 400 Hz is used to modulate the tuning diode bias voltage, thereby slightly modulating the probe tuning. The amplitude of the real NMR signal is detected in lock-in fashion with reference to the square wave generator. Consider a cycle of the square wave generator: first the bias voltage is increased slightly, increasing the resonant frequency of the NMR tank circuit, then the bias voltage is decreased slightly, decreasing the resonant frequency of the NMR tank circuit. The signal during the positive step is added to the negative of the signal during the negative step, and the result is used to adjust the probe tuning. Of course, this procedure only works if there is an NMR signal; the probe must be tuned manually once.
Lock NMR sample

The source of the deuterium NMR signal is a sealed sample of 99% $^2$H$_2$O doped with a small amount of MnCl$_2$ which serves to reduce the spin-lattice relaxation time and increase the NMR linewidth. The sample holder is a standard 5 mm diameter glass NMR tube cut to approximately 25 mm in length and oriented with the tube axis perpendicular to the magnetic field.

At a Mn$^{2+}$ concentration of 100 mM or so, the linewidth from this sample is typically 200 Hz. This represents a compromise between field stability and sweep step size. A broader line would result in reduced field stability since the magnitude of the correction signal for a given change in the field - that is, the amplitude of the dispersion signal as a function of field - is inversely related to the linewidth. By the same token, this same broader line would allow for faster sweeping and larger step sizes since the field can only be incremented in steps on the order of the lock linewidth so as to not disrupt the lock feedback loop. Reasonable sweep rates require that the linewidth not be too narrow. This particular choice of $^2$H$_2$O doped with 120 mM Mn is a compromise between a narrow line for field resolution and broad line for faster sweep. This is discussed further in the following section on sweep rate.

4.2.3 Performance characteristics

We will now discuss the following performance and operational characteristics of the EPR spectrometer's precision field and frequency lock system: field calibration, sweep rate, resolution, stability, and homogeneity.
Figure 4-11: MnO/MgO spectrum used for field calibration: Mn_{0.0002}Mg_{0.9998}O, which has \( g_e = 2.00101 \) and \( A_{Mn}^M = 8.71 \) mT. The observed resonant values are compared to calculated values to determine the magnitude of the field correction.

**Field calibration**

The magnetic field was calibrated by recording the EPR spectrum of a manganese \( g \) value standard. The sample consisted of a powder of Mn_{0.0002}Mg_{0.9998}O, which has \( g_e = 2.00101 \) and \( A_{Mn}^M = 8.71 \) mT. The resonant values of the observed spectrum, shown in Figure 4-11, were then compared to calculated values to determine the magnitude of the field correction.

**Sweep rate**

Moving the field from one value to another proceeds in a stepwise fashion. In the first part of the sweep procedure, the current and the NMR lock frequency are stepped concurrently under computer control. The time taken to change the PTS frequency synthesizer output and the power supply current set point is extremely short (< 30 ms). On the other hand, the current in the superconducting sweep coil changes more slowly due to the coil's large inductance, therefore time must be taken for the circuit current and voltage to stabilize.

Once the difference between the present field value and the desired field value is smaller than one digital step of the power supply, the final field steps are taken by changing the
PTS frequency alone. In these small steps analog adjustment is made to the current by the
lock system until the desired final field value is achieved.

The size of a digital step in the power supply output is given by the resolution of the
digital to analog converter card and the relationship between the current in the sweep
coil and the field at the sample. The DAC card has 12 bits of resolution for 20 Amperes
of current (positive or negative), that is each digital step corresponds to 4.88 mA. The
effective field to current ratio for the superconducting sweep coil is 292 G/A. Each step
is then 1.425 G, therefore the PTS frequency must be incremented by 917 Hz in order to
keep the deuterium signal on resonance, to within ~ 100 Hz of the center of the NMR
line. The system requires approximately 300 ms to stabilize after a simultaneous step of
the current and lock frequency, meaning that the sweep rate for the majority of the field
sweep is ~ 5 G/s.

For the final portion of the sweep procedure the PTS frequency is incremented by 100 Hz
steps to reach the desired field value. The system requires approximately 75 ms to stabilize
for each small step, i.e. a sweep rate of approximately 0.2 G/s, significantly slower than the
rate above, hence the desire for the two tiered sweep procedure.

At the end of a series of experiments which may take anywhere from 4 to 10 hours, the
current in the sweep coil is reduced to zero in order to minimize Helium boiloff during the
time that the spectrometer is idle. At this point, knowledge of the precise field value is not
required, therefore the lock system can be disengaged, and the power supply current set
point can be stepped down to zero as quickly as possible. The limit on ramping speed is
given ultimately by the magnet specifications (5 G/s), mainly so that no unnecessary stress
is placed on the coils and potentially leading to a quench of the main superconducting
magnet. Since the time taken for the ramp-down is short - at most 14 minutes if the
sweep coil is charged to the maximum current of 14 A - we have chosen to set the magnet ramp-down rate to the recommended maximum rate of 5 G/s.

The older sweep system described by Un [53] was operated only in the slow step mode with a sweep rate of approximately 0.5 G/s. The lock system, power supply and magnet configuration for the new EPR spectrometer provide for a 10 fold increase in sweep rate and a 5 fold increase in sweep range allowing for the rapid acquisition of broad spectra.

Field resolution and stability

At first glance, one would think that the field resolution would be limited by the stability of the sweep coil power supply, i.e. 0.01% of the current value according to the KEPCO BOP20-20M specifications. For a sweep coil current of about 1 A (on resonance for $g \approx 2$), this would lead to a current stability of 0.1 mA or, alternatively, a field stability of 31 mG, or 600 ppm. Another possibility is that the field stability could be determined by the width of the deuterium NMR lock signal $\sim$ 200 Hz, which would correspond to a field resolution of 6 ppm. Fortunately, neither of these is the case, in fact, the measured EPR spectrometer field stability, and therefore maximum resolution, was measured to be 10 mG, corresponding to only $\sim$ 0.5 ppm of the central field value.

Field stability and resolution were tested experimentally by acquiring a high resolution EPR spectra of perylene-PF$_6$, an organic conductor radical cation salt. A 140 GHz CW EPR spectrum of a small single crystal of perylene measuring 0.1x0.15x0.1mm$^3$ was recorded with a field step size of 10 mG and is shown in Figure 4-12. It consists of two components with a total width of 0.5 Gauss, or 0.2 ppm of the central field. In order to determine the origin of this 0.5 G broadening a pulsed EPR experiment was required.

The pulsed EPR signal of perylene in the time domain was measured by applying a
Figure 4-12: 140 GHz CW EPR spectrum of a small perylene-PF₆ single crystal 0.1x0.15x0.1mm³ recorded with a field step size of 10 mG. Two components with a total width of 0.5 Gauss are visible. This sample was used to assess the overall field stability of the spectrometer.

A single 139.5 GHz pulse and then recording the FID. The FID envelope decayed with a time constant of 2–3 μs in agreement with the estimate of $T_2$ from the CW linewidth of the entire line. The FID frequency fluctuated approximately 30 kHz between consecutive shots, implying that the signal decay was due to $T_2$ only, and that the field fluctuations were on the order of 10 mG. Apparently the large inductance of the superconducting sweep coil is sufficient to dampen nearly all of the oscillations in the power supply and lock feedback system filtering everything except a small 10 mG field jitter.

Field homogeneity

In order that the $^2$H₂O linewidth be constant, the NMR lock probe must be placed in the “sweet spot” of the magnet, where the field homogeneity is best. The EPR and NMR probe geometries are such that if the NMR sample is in the sweet spot, then EPR sample is in
a region approximately 2 inches above the one with the most homogeneous magnetic field. Although the field is not sufficiently homogeneous across the NMR lock sample (5 mm diam. x 25 mm long) at this more elevated position, it is sufficiently homogeneous across the much smaller EPR sample (0.4 mm diam. x 1 mm long). The verification of this statement is shown in Figure 4-13.

A single crystal \( \text{Cu}^{2+} \) in a glycine host was made by dissolving \( \text{CuNO}_3 \) with glycine in warm \( ^2\text{H}_2\text{O} \) and then allowing the solution to sit quietly for several days. The crystals contained approximately 5:1 by percent weight \( \text{Cu} \) to Glycine. Two CW EPR spectra of the crystal were recorded at two different orientations of the crystallographic axis with respect to the external magnetic field. These are shown in Figure 4-13. The large \( g \)-anisotropy in the sample leads to the two resonances appearing at widely separated field values. Although the spectra are 750 G apart, the resolution of the individual hyperfine lines is identical, demonstrating that the field homogeneity is better than the width of any particular single crystal EPR resonance. This experiment was performed over a large fraction of the total sweep width, indicating that the particular geometry of the lock sample at the sweet spot and the EPR a few centimeters above it does not lead to any measurable degradation in the quality and resolution of EPR spectra over a large fraction of the range of the sweep coil.
Figure 4-13: Two single crystal CW EPR spectra of Cu$^{2+}$ in a glycine host matrix recorded at two orientations of the crystallographic axis with respect to the external magnetic field, demonstrating that the field homogeneity is sufficient for EPR experiments across the full range of the sweep coil. The reader is referred to the text for more detail.
4.3 DNP and Solid State NMR

4.3.1 General considerations

Recently developed multi-dimensional high-resolution solid-state NMR methodology allows extraction of detailed structural information. Techniques like rotational resonance [62], rotational echo double resonance [63], and their offspring rely on rotor or RF driven recoupling which interferes with the averaging process of magic angle spinning. The dozen or so such methods currently in use have been employed to measure homo- and heteronuclear distances in proteins [64, 4, 65, 66, 67], but the spectra require unacceptably long periods of signal averaging, and to date most applications have been limited to small model compounds. The low sensitivity of solid-state NMR makes the application of these multidimensional techniques difficult, and unless the signal strengths are increased by one to two orders of magnitude, it will not be possible to apply them in a general way to large biomolecules.

Several demonstration experiments have been performed incorporating DNP into the high-resolution solid-state NMR spectroscopy of biomolecules in frozen solution. NMR signal enhancements of 50-100 were obtained in the spectra of the amino acid glycine and the 18.7 kD protein T4-Lysozyme dissolved in a frozen TEMPO/water/glycerol solution [36, 35]. These enhancements, obtained through microwave irradiation of the TEMPO EPR line to transfer electron polarization to proton spins, followed by cross-polarization to low-γ nuclei for detection, demonstrate conclusively the feasibility of using DNP as a signal enhancement tool. The next step would be to combine DNP with some multi-dimensional solid-state NMR experiments, and eventually, the high signal enhancements which DNP provides could open the possibility of performing structural studies on extremely large macromolecular systems. However, for this method to be applicable in a general manner that is compatible with
the current state-of-the-art, high-resolution, multi-dimensional solid-state NMR methodology, several issues need to be resolved and/or investigated further. NMR probes capable of sustained, stable, low temperature, magic angle spinning, without extraordinary helium consumption must be constructed, and the ideal paramagnet/solvent system and sample preparation protocol maximizing the DNP enhancement and minimizing the inhomogeneous broadening must be found. This section describes preliminary experiments aimed at addressing these questions and serves to describe the experimental methods used in the acquisition of the NMR data presented in chapters 4 and 5. Of course, as research in this area progresses, and then interest shifts towards the development of a commercially viable implementation of this technique, issues of cost effectiveness and ease of use will no doubt need to be addressed.

The efficiency of DNP in TEMPO/water/glycerol increases greatly at low temperature, primarily because of the longer nuclear relaxation times at those temperatures, hence DNP experiments should be performed at the lowest possible temperature. Static experiments have been performed at $\sim 10$ K and MAS experiments at $\sim 50$ K Sections 3.3 and 4.3.2 describe the static and MAS experiments at low temperature. At these low temperatures, the water:glycerol solvent system forms a glassy solid in which the TEMPO and solute of interest are assumed to be uniformly dispersed. The disorder in the glass and the presence of the paramagnetic molecules lead to line broadening which is briefly discussed below. A comment on the possible limitations imposed by spin diffusion on the applicability of DNP to the SSNMR of very large biomolecules is also made.

A minor point should also be made regarding the introduction of magic angle spinning into DNP experiments. Because the homogeneous linewidth of the electron spin packet is large (MHz) compared to typical rotor spinning speeds (kHz), the MAS should not
significantly affect the polarization transfer process. The electrons are not "decoupled" from the nuclei by MAS at any achievable spinning speed.

Disorder and line broadening in glasses

The accuracy of most measurements in solids via NMR is severely attenuated in non-crystalline systems due to inhomogeneous broadening of the resonances, and in fact most solid-state NMR pulse sequences are optimized on polycrystalline model compounds which have narrow linewidths. A big concern for the integration of DNP into SSNMR is the line broadening introduced by the incorporation of paramagnetic impurities, sample preparation, etc. The broadening may be due to both the electron-nuclear dipolar interaction [19], and the inhomogeneous broadening arising from the non-crystalline nature of the system [68]. For example, amino acids in the frozen TEMPO/water/glycerol solution, used in the DNP experiments described in chapter 5, suffer moderate line broadening (on the order of 100-150 Hz for $^{13}$C at 211 MHz $^1$H); but it has been shown that under MAS, the NMR linewidth of the amino acids in this paramagnet/solvent system varies minimally with radical concentration [36], and thus, that the electron-nuclear dipolar interaction is not the major broadening mechanism in this case.

The main cause of inhomogeneous broadening in these frozen solutions seems to be the formation of ice domains in the solvent which place stress on the solute and cause denaturing. These ice domains cause variation in local order in the host matrix and a result in a distribution in the chemical shifts of the nuclei of the solute of interest as they experience different local chemical environments from one site to another in the sample. Rapid sample freezing helps to prevent the formation of these ice domains and has recently been applied in solid-state NMR [69], alliaviating the inhomogeneous broadening problem.
Freezing effects can also depend on nature of the solute, and line broadening tends to be reduced in larger biomolecules whose interiors have minimal exposure to the ice domains in the solvent; that is, some large systems can act as their own cryoprotectant.

Much work still needs to be done to find the ideal paramagnet/solvent system and sample preparation protocol that maximizes the DNP enhancement and minimizes the inhomogeneous broadening due to disorder.

**Spin diffusion**

A second important issue related to the incorporation of DNP into SSNMR is that of the upper limit on the size of systems which can be explored by DNP. The current implementation of the technique relies on proton spin diffusion for polarization transfer: protons near the radicals are polarized first, and then spin diffusion distributes that polarization across the entire sample, see sections 2.5.2 and 3.2. Non-uniform enhancements of a solute can occur if its interior protons, which are furthest from the solvent, relax to thermal equilibrium via spin-lattice relaxation $T_1$, faster than polarization can be delivered to them via spin diffusion [70]. Although the initial T4-lysozyme experiment [36] shows uniform enhancement, and the estimate of spin diffusion rates indicate that even systems larger than 100 kD should be uniformly enhanced, a quantitative confirmation of this size limit needs to be performed in order to assess the ultimate usefulness of this enhancement technique in the study of very large systems. An example of a confirmation of the uniformity of DNP efficiency, and independence of particular solute conformation, would be the measurement of the enhancements in a large biomolecular system with several isotopic labels at different distances from the solvent.
4.3.2 Solid state NMR methodology

The NMR spectrometer used for the DNP experiments was designed and built in-house. The RF section provides variable phase and amplitude capability for four channels for cross polarization and decoupling experiments using a heterodyne mixing scheme with an IF of 120 MHz. The NMR signals are digitized with a 125 MHz LeCroy 9400 digital oscilloscope and the data is transferred to a VAX workstation for storage and processing. Figure 4-14 shows the typical pulsed NMR experiments used in conjunction with the solid effect and thermal mixing, the two versions of CW DNP that we have considered. By CW DNP, we mean the kind of incoherent polarization transfer which proceeds via continuous, or nearly continuous irradiation of the electron spins, as opposed to pulsed coherent transfer mechanisms like Hartmann-Hahn cross-polarization. All of the experiments start with a long microwave pulse near the electron Larmor frequency. Since the polarization transfer times in the solid effect and thermal mixing are of the order of the nuclear spin-lattice relaxation time $T_{1n}$, this pulse is often longer than 10 s. During the microwave pulse the high polarization of the electrons is transferred to the protons or the low-$\gamma$ nucleus depending on the frequency of irradiation. If the low-$\gamma$ nuclei are polarized, then a simple $90^\circ$ RF pulse is applied followed by detection and $^1$H decoupling as shown in (b). If the $^1$H nuclei are polarized then the enhancement can be detected indirectly via simple Hartmann-Hahn cross-polarization and detection on the low-$\gamma$ channel as shown in (a), or directly via a two-pulse echo experiment applied on the $^1$H channel. Although these pulse sequences are extremely simple in comparison to those used in sophisticated recoupling schemes, they are sufficient for measuring signal enhancement and for recording 1-D spectra. They contain the fundamental building blocks of nearly all SSNMR sequences: $^1$H excitation, cross-polarization, and decoupling.
Figure 4-14: Typical NMR pulse sequences used for the DNP experiments. Note that the time axis is not to scale: the microwave pulse is on the order of seconds. Cross-polarization times are of the order of a few ms. The free induction decay lasts 1–100 ms depending on the width of the NMR line. $^{13}\text{C}$ is representative of any low $\gamma$ nucleus. To date experiments have been performed with $^{15}\text{N}$, $^{13}\text{C}$, and $^{31}\text{P}$.

(a) DNPCP: following DNP to the $^{1}\text{H}$, a standard cross-polarization sequence transfers the $^{1}\text{H}$ polarization to $^{13}\text{C}$.
(b) DNP-FID: following DNP to the $^{13}\text{C}$, a simple 90° pulse (4μs) is applied to the $^{13}\text{C}$ with decoupling of the $^{1}\text{H}$ to directly detect $^{13}\text{C}$.
(c) DNPECHO: following DNP to the $^{1}\text{H}$, a standard two-pulse echo sequence is used to detect $^{1}\text{H}$. 
Figure 4-15: Static DNP probe assembly. The Teflon insulated NMR coil is housed in a Kel-F block to provide mechanical stability. The sample is contained in a 4 mm o.d. quartz EPR tube.

4.3.3 Static low temperature experiments

A schematic of the DNP probe head is shown in Figure 4-15. The probe is housed in a low temperature cryostat and functions in the 5–300 K regime by boiling liquid helium or nitrogen and transferring the cold gas through a vacuum-jacketed transfer line into the dewar. The RF coil consists of 5 turns of Teflon insulated copper wire with an inner diameter of 4 mm. Since He gas has a low breakdown voltage, Teflon coated wire and silicone base sealant on all solder joints are used to prevent arcing. A semirigid coaxial cable, approximately \( \lambda \) for the \(^1\)H resonance, connects the coil to the tuning and matching circuit at the top of the probe. The RF circuit is tuned and matched for both \(^1\)H and \(^{13}\)C externally by variable capacitors to eliminate the temperature dependent change in the capacitance and to reduce the exposure of the circuit to He gas; typical 90° pulse lengths are
2 and 3.5 \( \mu s \) for \(^1\text{H}\) and \(^{13}\text{C}\) respectively. The microwaves are transmitted to within 10 cm of the sample using K-band waveguide (WR-42, 0.23 dB/foot attenuation at 140 GHz) which is used rather than the fundamental D-band (WR-8, 1 dB/foot) to minimize losses. Near the coil, the WR-42 waveguide is tapered to WR-8. A piece of WR-8 waveguide is positioned vertically above the coil such that microwaves are launched between the turns of wire. A concave mirror is placed below the coil and positioned for maximum enhancements. The microwave \( Q \) is low, \( \sim 1-5 \), which limits the microwave \( B_1 \) field and hence the DNP enhancement. However, the advantage of this design is that it retains the optimal filling factor and \( Q \) of the NMR system. The components used to introduce the microwaves do not perturb the RF circuitry or perturb the static magnetic field at the sample. The sample is contained in a quartz EPR tube (4 mm o.d.); quartz is used for its relatively low absorptivity at millimeter wavelengths.

### 4.3.4 Low temperature magic angle spinning

DNP experiments are performed optimally at low temperatures ranging from 10 K to 100 K. In order to incorporate DNP into MAS dipolar recoupling NMR experiments will therefore require a low temperature spinning probe that can maintain not only stable temperatures, but also stable spinning speeds. Low temperature spinning in the temperature range 80–120 K is currently achieved by blowing nitrogen gas through copper coils mounted in a pressurized copper can that is submerged in a Lo-Boy liquid nitrogen dewar. The nitrogen pressure (and hence spinning speed) can be controlled with a regulator resulting in stable spinning speeds. The pressurized can surrounding the copper coils prevents liquefication of the nitrogen gas in the coils and helps maintain a stable temperature of the spinning gas. In the future a spinning speed controller will be used to control the regulator to maintain very
high spinning speed stability for extended periods of time ($\Delta \nu_s < 1$ Hz for many hours).

Standard low-temperature MAS techniques [71, 72] use nitrogen as the spinning gas, and are therefore limited to temperatures above 77 K. A dewared DNP/MAS probe which can reach temperatures as low as 30 K using pressurized cold helium as the spinning gas has been constructed. This new probe is a significant improvement over the one used to perform the room temperature DNP MAS experiments [14] on BDPA/PS described in chapter 3. Aside from the minor difference in microwave delivery (parallel vs. perpendicular to the spinning axis), this probe can reach significantly higher spinning speeds (12 kHz vs. 6 kHz), and significantly lower temperatures (30 K vs. 150 K). The reader is referred to Hall's thesis [45] for details regarding its construction and performance, along with other discussion regarding low temperature MAS in general. A short description and some minor comments are given below.

The spinning assembly is a standard 5 mm Chemagnetics system and the RF section is almost identical to the one for the static probe described above. The probe sensitivity and pulse lengths are comparable to standard room temperature MAS probes, i.e. $^1$H and $^{13}$C 90$^\circ$ pulse lengths are typically 2 and 3.5 $\mu$s respectively. The pressurized cold gas is generated by heating a resistor submerged in the liquid helium. The helium boil-off gas is then forced into a helium transfer line and the gas is cooled further in passing through the liquid helium before being transferred from the helium dewar to the low temperature spinning probe. Once a stable boiloff rate and a stable spinning speed have been achieved, the temperature fluctuations tend to be very small and slow ($\sim 2$ deg C/hr). The temperature can be crudely controlled by manual adjustment of the current delivered to the resistor submerged in the liquid helium, i.e. controlling the boiloff rate.

The rotors are zirconia and the endcap and drive tip are plastic, leading to mechan-
ical instability due to the large difference in coefficients of thermal expansion of the two materials. The drive tip is matched carefully to a rotor and inserted at liquid nitrogen temperatures. It is also sometimes necessary to slightly deform the section of the drive tip which is inserted into the rotor. The use of quartz rotors proved to be impractical at very low temperature due to their fragility, particularly as the sample was brought back to room temperature at the end of an experiment. Ideally the drive tips should be made of the same material as the rotor, unfortunately ceramics are very brittle and machining zirconia drive tips is exceedingly difficult.

If very low temperature MAS is to be used routinely, several modifications to the probe and spinning assembly need to be made. We have successfully spun samples for several hours at speeds up to 5 kHz, unfortunately a large amount of helium is consumed (10–15 liters/hour), especially lower temperatures (<50 K). At $4/liter for liquid helium, the cost quickly becomes prohibitive. Furthermore, the spinning speed needs to be controlled very precisely for long times (~1 day) in order to perform most types of multi-dimensional SSNMR experiments.

A helium recirculating system has been designed and is currently under construction. It should greatly decrease helium consumption, and allow for precise spinning speed control. A schematic is shown in Figure 4-16, along with a summary of the flow/cooling cycle. The connections between the heat exchanger, cryo-cooler and the probe will consist of rigid vacuum jacketed stainless steel transfer lines to the extent possible, because these lines are less lossy (by a factor of ~2) than flexible transfer lines. Care must also be taken in the construction of the probe as well, heat loss needs to be minimized, and He leaks must be avoided. It is estimated that temperatures of 50 K could be sustained for approximately a week with this system, with a high degree of spinning speed control, $\Delta \nu_s < 1$ Hz.
Figure 4-16: Proposed Helium recirculating system: The helium gas flow cycle can be divided into a five step cycle as follows:

1) Compressed room temperature helium gas enters the heat exchanger. It relinquishes heat to the gas flowing in the opposite direction and cools to ~ 55 K.
2) 55 K gas is cooled by a Gifford-McMahon cryo-cooler operating at 50 K.
3) 50 K gas enters the dewared probe where it is divided between the drive and bearing lines of the MAS probe. After passing through the spinner module, the gas exits the probe having absorbed approximately 20 W from the various heat sources in the probe: microwave irradiation, RF irradiation, and conduction losses.
4) 55 K exhaust gas enters the heat exchanger and exchanges heat with the incoming flow and warms to room temperature.
5) Room temperature gas is compressed to the required pressure. The compressor generates the pressure necessary to overcome the drops through the spinner module and the various tubing and transfer lines.
4.4 A High Frequency High Power Microwave Source: the Gyrotron

4.4.1 Introduction and overview

Since the sensitivity per unit time and resolution of a typical Nuclear Magnetic Resonance experiment scale with the external field as $B_0^3$ and $B_0$ respectively [1], conventional NMR systems operate at the largest practical field (5–18 T), where practicality relates to the limitations imposed by homogeneity requirements, current magnet technology, and cost. Until recently, the utility of Dynamic Nuclear Polarization as a tool for NMR signal enhancement has been limited to relatively low magnetic fields (≤1.5 T) by microwave technology. DNP requires high power irradiation of the sample near the electronic Larmor frequency, hence the need for reliable, high power, high frequency microwave sources. There are very few millimeter and submillimeter wave sources capable of generating the necessary power. Typical millimeter wave sources such as the ElO (Extended Interaction Oscillator) or the BWO (Backward Wave Oscillator) are slow wave devices, requiring small, fragile structures to generate radiation; as the frequency increases, the size of these structures decreases, and the resulting higher power densities limit device power output and lifetime. The gyrotron, or electron cyclotron resonance maser, is a coherent millimeter wave source based on a fast wave interaction that can provide the necessary power to perform DNP experiments at typical NMR field strengths. Since gyrotrons can be operated at frequencies up to 600 GHz [73, 74] it is in principle possible to perform DNP at fields up to 20 T using gyrotrons as microwave sources.

The gyrotron principle was first discovered by Gapanov [75] in the late 1950’s, and then extensively studied in the 60’s by the Russians. In the past fifteen years its main application
has been the heating of magnetically confined plasma in fusion research [76]. This has led

to the development of very high power sources (100 kW – 1 MW) at frequencies between 28
and 170 GHz [77]. Other promising applications include material processing, high resolution
radar, and plasma diagnostics [78].

The gyrotron is a device which emits radiation at a frequency near the electron cyclotron
frequency. Like other millimeter wave sources, such as the magnetron and the klystron, it
relies on a resonant structure to extract energy from an electron beam. Typical beams have
energies of tens of kilovolts and currents of a few milliamperes to 50 A. The gyrotron differs
fundamentally from other microwave tube devices however, in its use of a high magnetic
field to achieve the resonance effect. Since it is the strength of this static magnetic field that
determines the frequency of the emitted radiation, and not the dimensions of some small
slow wave structure, the gyrotron offers a distinct advantage, allowing for the production
of high powers (10^2–10^6 W) at high frequencies (30–600 GHz) without the generation of
damagingly large energy densities.

Gyrotrons come in a variety of flavors: oscillators (gyromonotron), traveling wave amplifiers (gyro-TWT), or klystron amplifiers (gyrokystron) [79]. Discussion will be limited
to the gyromonotron, or gyrotron for short. The 139.6 GHz MIT gyrotron which has been
applied to DNP at 5 T, and is capable of producing 100 W pulses for 1–20 seconds, or 10 W
continuously [34, 14], will be described in the following sections in more detail.

4.4.2 Operational description of a typical gyrotron

The basic components of the MIT gyrotron, shown in Figure 4-17, include a magnet, an
electron gun, and a vacuum tube which consists of a beam tunnel, a resonator, mode
converters, a collector, and a miter elbow.
Figure 4-17: The main components of the 140 GHz gyrotron. Refer to the text for a description of its components and an explanation of its operation.

The 5.4 T super-conducting magnet manufactured by Cryomagnetics, Inc. is arranged in a split-coil configuration, and generates a field along the positive $\hat{z}$ direction. Electrons are emitted at the cathode of the magnetron injection gun (Varian Associates) in an annular beam. Voltages of -42 kV and -28 kV are applied to the cathode and to the focusing anode respectively. The electric field in the region between the cathode and “modulating” anode focuses the electron beam along the beam tunnel axis. The emitted current ranges from 10 to 100 mA, and is controlled by varying the temperature, and hence the work function, of the cathode. The electron gun and vacuum tube pressure is maintained below $10^{-7}$ Torr by VacIon pumps (Varian), not shown.

**The electron beam trajectory**

The beam is magnetically compressed as it exits the gun and travels through the beam tunnel, which consists of alternating copper and beryllia rings, and serves both as a ground plane and as a microwave absorber. The beam tunnel focuses the beam, helps to suppress oscillations, and prevents power that leaks from the cavity from reaching the gun and
disrupting its operation.

The annular electron beam is further compressed in the cavity to a radius of 1.82 mm at the center of the resonator, where it interacts with the second radial maximum of the resonant 139.60 GHz TE_{031} mode. This geometry avoids the higher current and space charge densities required to interact with the first (innermost) radial maximum. Beam compression is related the strength of the magnetic field as \( R(z) \sim \sqrt{B(z)} \), and since the beam is emitted with a radius of 9.1 mm, the ratio of the magnetic field strength at the interaction region to that at the emission region must be \( \sim 25 \). In the current configuration the gun is far from the optimal position, and the field at the cathode is too small. The necessary field profile is achieved by applying current to two water-cooled coils placed around the gun each producing an additional field of up to 0.2 T. The resonator consists of circular waveguide with a straight section of 15 wavelengths, a down-taper (cutoff) angle of 2°, and an up-taper (output) angle of 4°, and is designed such that backwards emission of microwaves into the gun region is cut off. The theoretical diffractive \( Q \) is \( \sim 5000 \).

The electron beam decompresses upon exiting the magnet and is collected on a section of 1.27 cm diameter waveguide. Remnant beam electrons are deflected into the collector by a transverse magnetic field generated with permanent “kicker” magnets.

Microwave mode conversion and transmission

Microwaves are produced in the cavity in the circular TE_{03} mode and propagate toward the collector. Before exiting the tube, the microwaves undergo two mode conversions: TE_{03} \( \rightarrow \) TE_{02} \( \rightarrow \) TE_{01}. The two step mode conversion is necessary to avoid excitation of spurious modes. After these mode conversions the microwaves are reflected 90° by a miter elbow, which blocks any remaining uncollected electrons from striking the quartz window, and exit
the tube. The window’s thickness is equal to an integral number of half-wavelengths (10) to avoid the generation of standing waves between the resonator and the window.

Once outside the tube, the microwaves undergo another mode conversion in a “wriggler” which takes the circular TE_{01} into circular TE_{11}. All of the mode converters have been measured to have an efficiency of ≥ 99%.

The microwaves are transmitted in the circular TE_{11} mode through low-loss oversized waveguide (1.25 cm i.d., 0.01 dB/foot) to a circular downtaper and mode converter assembly which converts the radiation to the fundamental TE_{10} rectangular mode. The taper and final mode converter have an effective insertion loss of about 7 dB, but this is due mainly to incomplete conversion of power in spurious modes which are excited by the miter elbows along the transmission and mode conversion path. Transmission losses from the gyrotron output to the sample total approximately 10 dB.

**Water cooling and vacuum systems**

Conventional plasma physics applications of gyrotrons require low duty cycle operation (< 1%), short pulses (msec), and relatively long inter-pulse delays (1 sec) [80]. Dynamic Nuclear Polarization experiments, on the other hand, require nearly continuous operation since the microwave pulses effecting polarization transfer are of the order of the nuclear spin-lattice relaxation time, T_{1n}(sec), and inter-pulse delays allowing for electron spin polarization recovery are of the order of T_{1e}(msec). High duty cycle operation leads to significant resonator and collector heating. Since the tube is at high vacuum (10^{-8} Torr), heat must be transferred by conduction through the walls and then dissipated by circulating chilled water through cooling jackets brazed to the outside of the resonator and collector. The heat deposited in the cooling water, approximately 1 kW, is transferred to the central
laboratory chilled water supply via a Neslab water-water heat exchanger.

Another issue related to high duty cycle operation is vacuum integrity. Sufficient pumping and conduction are necessary to maintain the pressure below $10^{-7}$ Torr. When electrons are emitted from the hot cathode or collide with the collector walls, gas particles are emitted. This outgassing degrades gyrotron performance by interfering with current production; a “poisoning” of the gun occurs over time. Sufficient pumping capacity must be provided by the Vaclon pumps, and the geometry of the tube holes and vacuum connections must not diffusion limit the system. The tube is essentially a long piece of pipe with a very narrow constriction in the middle, the interaction region, which separates the two halves of the tube. Hence two pumps are necessary: one near the gun, and one near the collector.

4.4.3 Simple explanation of the gyrotron gain mechanism

Microwave generation in a gyrotron is based on the cyclotron resonance maser instability of electrons “gyrating” in a static magnetic field. The following is an elementary explanation of the gyrotron gain mechanism, the transfer of energy from the electron beam to the microwave field [79]. For more complete descriptions, including non-linear effects such as the interaction between the beam and the RF field, the reader is referred to the literature [81, 82].

Consider an electron moving relativistically in a static magnetic field. The particle will undergo cyclotron motion, that is, it will move in a helical trajectory with a frequency of gyration $\Omega_0$

$$\Omega_0 = \frac{eB_0}{\gamma mc} \quad (4.2)$$

where $e$ and $m$ are the charge and rest mass of the electron, $c$ is speed of light, $B_0$ is the
Figure 4-18: Snapshot of two electrons undergoing cyclotron motion in a field $B_0$, and interacting with a perpendicular electric field. $B_0$ is the static external field. $E$ and $H$ are the oscillating RF electric and magnetic fields in the cavity. The electrons moving in a direction opposite to the electric field will be accelerated and their gyrofrequency will decrease ($\Delta \gamma > 0, \Delta \Omega < 0$), whereas those moving parallel to the field will be decelerated and their gyrofrequency will increase ($\Delta \gamma < 0, \Delta \Omega > 0$). In this diagram Particle 1 will have $\Delta \Omega < 0$ and will be decelerated, while particle 2 will have $\Delta \Omega > 0$ and will be accelerated. If the electric field is oscillating at the appropriate frequency, the electron’s will bunch in their orbits.

strength of the applied field, and $\gamma$ is the relativistic factor

$$\gamma = \sqrt{1 - \frac{v^2}{c^2}}$$  \hspace{1cm} (4.3)

where $v$ is the velocity of the electron in the chosen reference frame. In this frame, the energy of the particle is given by

$$\xi = \gamma mc^2$$  \hspace{1cm} (4.4)

If the particle now interacts with an electric field $E$ for a short time $\Delta t$, its energy $\xi$ and therefore $\gamma$ will change by a small amount

$$\Delta \gamma = \frac{-eE \cdot v \Delta t}{mc^2}$$  \hspace{1cm} (4.5)

Using 4.2 and 4.5, the change in the particle’s energy can be related to the change in its
cyclotron frequency

$$\Delta \Omega = -\frac{eB_0}{\gamma^2 mc} \Delta \gamma = \frac{e^2 B_0}{\gamma^2 m^2 c^3} \mathbf{E} \cdot \mathbf{v} \Delta t \quad (4.6)$$

Hence those electrons moving in a direction opposite to the electric field will be accelerated and their gyrofrequency will decrease ($\Delta \gamma > 0$, $\Delta \Omega < 0$), whereas those moving parallel to the field will be decelerated and their gyrofrequency will increase ($\Delta \gamma < 0$, $\Delta \Omega > 0$). This is shown schematically in Figure 4-18. Particle 1 has $\Delta \Omega < 0$ and will be decelerated while particle 2 has $\Delta \Omega > 0$ and will be accelerated. If the beam is composed of electrons initially randomly distributed in their helical orbits, then the electric field oscillating with a frequency $\omega$, near the cyclotron frequency $\Omega_0$, will cause the electrons to bunch together over a number of cycles. This azimuthal, or transverse, phase bunching causes the electrons to radiate coherently. If the microwave field frequency is slightly higher than the electron cyclotron frequency $\Omega_0$, energy will be transferred from the electrons to the microwave field, and with optimal bunching, as much as 70% of the transverse energy of the beam can be converted into radiation.

4.4.4 A continuous wave 140 GHz gyrotron

Dynamic Nuclear Polarization requires nearly continuous microwave irradiation of the sample since the polarization transfer times are of the order of the nuclear spin-lattice relaxation time $T_{1n}$. A gyrotron operating in continuous wave mode is unusual, and issues of RF frequency stability and bandwidth become important. The dependence of the MIT 140 GHz gyrotron's frequency pulling on system parameters has been measured experimentally [83], and is shown in table 4.1.

These parameters are directly related to the physics of microwave generation, although
Magnetic field 200 MHz/T
Beam voltage 20 MHz/kV
Cavity temperature 2.2 MHz/°C
Beam current 30 kHz/mA

Table 4.1: 140 GHz Frequency pulling parameters

an explicit calculation of the measured values requires non-linear theory and is beyond the scope of this discussion. Roughly speaking however, for a given beam current, the observed frequency shift resulting from changes to the magnetic field $B$ or the cathode voltage $V_c$ is due to their effect on the cyclotron frequency $\omega_c = eB/\gamma m$, where $\gamma = 1 + V_c/511$ and $V_c$ is in kV. The shift due to cavity temperature is calculated by considering the effect of thermal expansion of the copper cavity on its resonant frequency in the $TE_{03}$ mode.

The magnetic field constraint sets a limit on the field homogeneity at the interaction region while running in persistent mode, and is easily satisfied by the Cryomagnetics superconducting magnet. The Spellman high-voltage DC power supply used to accelerate the beam has a 50 ppm ripple, and sets the short term frequency stability of the gyrotron. Temperature control of the cavity is achieved by circulating chilled water through the braised cooling jacket as discussed in 4.4.2. The long term oscillations ($\pm 2^\circ$/minute) due to the temperature regulation circuitry of the Neslab water-water heat exchanger are handled through a ballasting scheme. Water from a large tank is used to cool the cavity. The tank is then cooled by the heat exchanger, so the temperature fluctuations inherent in the operation of the heat exchanger are reduced by the ratio of the volume of the ballast tank to the volume of the water used in cooling the tank.

The 140 GHz gyrotron exhibits a 30 MHz, or 200 ppm, transient drift during the first three seconds of operation as the cavity reaches thermal equilibrium, see Figure 4-19. The final CW frequency stability is 40 kHz, or 0.3 ppm, due to AC ripple on the high voltage power supply output (50 ppm ripple$\times$42 kV$\times$20 MHz/kV = 42 kHz $\approx$ 0.3 ppm). Although
Figure 4-19: The frequency as a function of time for the 140 GHz gyrotron demonstrating the initial 30 MHz/200 ppm approach to thermal equilibrium. The triangles are the measured values. The solid curve is a spline fit to the data, and serves as a guide to the eye.

Figure 4-20: Free induction decays of neat BDPA obtained at room temperature using the gyrotron as the microwave source. (a) Single scan. (b) Average of 1000 scans. Experimental parameters include microwave frequency = 39.6 GHz; incident power = 23 mW; pulse width = 56 ns; repetition rate = 1 KHZ; dwell time = 10 ns
Figure 4-21: Spectrum analyzer display of the gyrotron output mixed with the output of a 139.95 GHz Gunn diode. This is a log-log plot of power vs. frequency offset in kHz from 139.57 GHz. The intensity of the central peak is approximately 40 dB above the baseline. Power supply ripple of 200 ppm leads to the observed 400 kHz bandwidth.

The resulting frequency bandwidth can be detected in a pulsed EPR experiment as shown in Figure 4-20 and directly by mixing the gyrotron output with 139.5 GHz microwaves from a Gunn diode as shown in Figure 4-21, it is small enough that the gyrotron is a satisfactory CW source for DNP and EPR experiments at 140 GHz. To perform the experiment shown in Figure 4-20, the gyrotron was operated at 1 W CW and replaced the 139.5 GHz Gunn diode in the EPR bridge, see 4-2. A small crystallite of neat BDPA, which has an EPR linewidth of about 2 G, was used to gauge the gyrotron's stability. A single FID was detected and compared to an average of 1000 scans. The noise reduction from 1 scan to the average of 1000 scans was measured to be 24, less than the ideal value of 32. In both the direct measurement and the demonstration experiment however, the gyrotron was operated using an older power supply with 200 ppm ripple. The newer power supply (50 ppm ripple) renders this RF bandwidth effect negligible for this and most other EPR samples, since the
typical intrinsic unresolved hyperfine broadening is of the order of 1 G (2.8 MHz) for $g = 2$
paramagnetic systems; that is the RF bandwidth is approximately a factor of 70 less than
the homogeneous linewidth.

In its current form, the gyrotron output is about 200 W of microwaves at the quartz
window under quasi-CW (60% duty cycle) and 10 W under CW operation with stability
suitable for phase coherent magnetic resonance spectroscopy. A 250 GHz gyrotron is cur-
rently under construction; it is designed be compact (< 1 m height, 50 cm diameter), and to
provide for similar CW power levels and frequency stability at higher efficiency by operating
at lower beam voltages.
Chapter 5

The Mechanism of DNP in an Inhomogeneous EPR Line

5.1 Introduction

In this chapter we discuss the mechanism of DNP in an inhomogeneously broadened EPR line at high field, particularly as it applies to the system consisting of a frozen solution of TEMPO in water and glycerol. The formalism which we had presented in chapter 2 to describe thermal mixing in a single homogeneous EPR line at low field is insufficient for a satisfactory explanation of DNP in the inhomogeneous TEMPO EPR line at high field. We show how the effects of cross-relaxation must be explicitly included in order to construct a model that adequately fits the DNP, pulsed EPR, and electron-electron double resonance (ELDOR) measurements. Although our attention is focussed on the TEMPO system exclusively, many other systems should behave the same way. Frozen solutions of TEMPO in water:glycerol are representative of an entire class of paramagnet-host combinations compatible with the implementation of DNP to the solid-state NMR
study of biological systems at high field.

The remainder of this chapter is divided into several sections, the first of which, section 5.2, is a summary of the preliminary DNP results previously mentioned in section 3.3. The efficiency of DNP at low power microwave irradiation was rather unexpected, and the results from initial investigations into the dependence of the NMR signal enhancement on various experimental parameters comprise the remainder of section 5.2.

Section 5.3 contains a detailed description of a series of pulsed high frequency EPR experiments probing the electronic side of the low power polarization transfer process. These experiments highlight the importance of electron-electron cross-relaxation within the inhomogeneously broadened TEMPO EPR line. One is forced to extend the simple model of thermal mixing presented in section 2.4 in order to construct a model suitable for analysis of low power DNP at high field. A description of the extended model incorporating inhomogeneous broadening and cross-relaxation is given in section 5.4, along with the results of numerical simulations based upon it. The simulations are shown to be in good agreement with experiment.

5.2 High Field DNP Results for TEMPO in Water:Glycerol

In this section we present the results of a series of DNP experiments which begin to address the question of the mechanism of polarization transfer in the nitroxide doped frozen aqueous solution TEMPO in water:glycerol.

5.2.1 DNP field vs. enhancement profiles

The magnetic field dependence of the $^1$H DNP enhancement of a 40 mM solution of 4-amino TEMPO in 60:40 glycerol:water at 14 K under both low and high power microwave irra-
diation is shown in Figure 5-1. The enhancement was determined by comparing the NMR signal from $^1$H Hahn echoes at a repetition rate of $1/150 \, \text{s}^{-1}$ with microwave irradiation to without. The high power enhancement curve was obtained using the 139.6 GHz gyrotron in CW mode with 1 W of power incident on the sample, while the low power curve was obtained using the 139.5 GHz Gunn diode source in CW mode with 10 mW of power incident on the sample. The TEMPO echo detected EPR spectrum is shown above the enhancement curves. High power irradiation produces significant signal enhancement at field values within the EPR powder lineshape, as well as modest enhancement at fields outside the EPR spectrum. Low power irradiation on the other hand, produces reasonable enhancements at field values within the EPR spectrum, and no detectable enhancement at field values outside the spectrum. The reader is referred sections 4.1 and 4.4 of the text for more detail on the experimental apparatus and to section 3.3 for applications of this DNP in this system to signal enhancement in biological systems.

These enhancement vs. field curves provide a great deal of insight into the nature of the DNP mechanism. The observed maxima of both the high and low power enhancement profiles occur at field offsets $(B_0 - B_{re})/2\pi\gamma_e$, ±135 MHz and ±125 MHz respectively, significantly less than the $\pm\omega_H/2\pi = \pm211$ MHz expected if the enhancement were to be due primarily to the solid effect. The fact that low power irradiation produces signal enhancement only within the EPR line indicates that only mechanisms which function via allowed transitions can be mediating polarization transfer in that case. The enhancement obtained from high power irradiation on the other hand, seems to be due to two processes, one which proceeds via allowed transitions giving the enhancement within the EPR line, the other which proceeds via forbidden transitions giving the enhancement outside the line.

These observations indicate the existence of some form of thermal mixing in this system,
Figure 5-1: $^1$H DNP enhancement profile for 40 mM 4-amino-TEMPO in 40:60 water:glycerol at 14 K. The enhancement was determined by comparing Hahn echoes at a repetition rate of 1/150 s$^{-1}$, with and without microwave irradiation. Squares and left axis: $^1$H enhancement profile using the 139.6 GHz gyrotron in CW mode with 1 W incident power. Circles and right axis: $^1$H profile using a 139.5 GHz Gunn diode source in CW mode with 10 mW output power. The lines drawn through the points are cubic spline fits to guide the eye. The TEMPO echo detected spectrum is shown above. High power irradiation produces significant enhancements at field values within the EPR powder lineshape; however, irradiation beyond the EPR spectrum produces only modest enhancements. In the low power profile, no enhancement occurs at fields appreciably outside the EFR spectrum, indicating that the mechanisms which function via forbidden transitions are suppressed.
even though the EPR line is rather inhomogeneous as seen in the EPR spectrum at the top of Figure 5-1. We would call the reader's attention to the high field side of the spectrum where the $^{14}$N hyperfine splitting is clearly visible. The fact that DNP occurs at all at low powers in this case is then surprising. Whatever cross-relaxation exists among the electron spins is insufficient to give the line enough homogeneity to support the low field version of thermal mixing described in section 2.4, hence the use of the phrase "some form of thermal mixing" above, yet it is sufficient to provide a pathway between the electron Zeeman reservoir and the electron dipolar reservoir with some intrinsic relaxation process allowing energy to flow from the nuclear Zeeman reservoir to the electron dipolar one.

5.2.2 The dependence of DNP efficiency on experimental parameters

Several experiments were performed in order to gauge the relative importance of various experimental parameters on the maximum value of the signal enhancement achievable in the TEMPO in water:glycerol system. The three Figures 5-2, 5-3, and 5-4 show the results of these experiments on the dependence of the enhancement on microwave power, radical concentration and temperature. The reader is referred to chapter 4 for more details on the spectrometer and static, low temperature DNP probe used in these experiments.

Microwave power

By varying the microwave irradiation power, and hence the strength of the resonant RF magnetic field which induces transitions among the spin energy levels, one gains some insight into the dynamics of DNP. The results of such an are shown in Figure 5-2 for 50 mM TEMPO in 40:60 water:glycerol at 12 K. The low power data, represented by the closed circles, were obtained using the 139.5 GHz Gunn diode in CW mode. The high power data, represented
Figure 5-2: The power dependence of the DNP enhancement for 50 mM TEMPO in 40:60 water:glycerol at 12 K. The closed circles were obtained using the 139.5 GHz Gunn diode as the source in CW mode. In each case, the magnetic field was set to maximize the positive enhancement. The open circles were obtained using the 139.6 GHz gyrotron as the source. The curves drawn through the points are guides to the eye. Although the growth of the enhancement is similar in the two power regimes, the point at which the low power pathways begin to saturate is obvious, and the power needs to be increased substantially before the effect of driving forbidden transitions becomes important.

by the open circles, were obtained using the 139.6 GHz gyrotron as the source. The curves drawn through the points are guides to the eye. For each power level the magnetic field was set to maximize the positive $^1$H enhancement.

The approach to saturation of the relevant spin transitions effecting polarization transfer is similar in the two power regimes. The point at which the low power pathways begin to saturate is obvious, and a substantial increase in microwave power is required before DNP via nominally forbidden transitions becomes important. The fact that both low and high power processes exhibit an exponential approach to saturation is consistent with the theory of DNP outlined in chapter 2, where it was shown that the nuclear polarization depends linearly on the transition probability, which in turn depends linearly on the RF power, i.e. quadratically on the RF magnetic field.

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Radical concentration

A series of samples were prepared by successive dilution in a solution of 40:60 water:glycerol in order to investigate the dependence of the NMR signal enhancement on radical concentration. The results of these experiments are shown in Figure 5-3. Each sample was cooled to 14 K and irradiated with microwaves from the Gunn diode, as described in section 4.3.3. The magnetic field was then set to maximize the $^1$H NMR signal enhancement.

The results shown in Figure 5-3 demonstrate how the dependence of the enhancement on paramagnet concentration is a balance of effects. As the concentration increases, the nuclear spin-lattice relaxation time $T_1$ decreases due to faster electron flip-flops, resulting in increased "leakage" of nuclear polarization to the lattice via the electron dipolar reservoir and consequently reducing the maximal enhancement. At low concentrations on the other hand, the electrons are few, and hence only weakly coupled to one another. The cross-relaxation rate between them is then too slow to permit efficient thermal mixing, and the signal enhancement is small. A concentration of 20–30 mM seemed to lead to maximal enhancement under low power irradiation. The points made regarding the necessity of an intermediate amount of electron-electron interaction will be elaborated on further in the remainder of this chapter.

Temperature

The dependence of the signal enhancement on temperature was measured for 40 mM 4-amino TEMPO in 40:60 glycerol water under. The results of these experiments for temperatures easily accessible to magic angle spinning (MAS) are shown in Figure 5-4. The $^1$H spin-lattice relaxation time $T_{1n}$ for this sample was also measured and is plotted in Figure 5-4 as well. Note closely the enhancement is parametrized by $T_{1n}$. 

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Figure 5-3: The DNP dependence on radical concentration for TEMPO in water:glycerol at 10 K. As the radical concentration increases the nuclear spin-lattice relaxation time $T_1$ decreases due to faster electron flip-flops. The resulting increase in nuclear polarization leakage to the electron dipolar bath reduces the enhancement. At low concentrations cross-relaxation is too slow to permit efficient thermal mixing.

Figure 5-4: DNP temperature dependence for TEMPO in water:glycerol. Molecular motions increase with temperature and reduce the nuclear spin-lattice. This leads to greater leakage of nuclear polarization directly to the lattice and reduced DNP enhancements. The $^1$H NMR signal enhancement is represented by the open circles. The $^1$H spin-lattice relaxation time $T_{1n}$ is represented by the closed triangles. The curves are cubic spline fits to the data and serve as a guide to the eye.
5.3 Pulsed EPR Spectroscopy of the TEMPO System

A series of experiments was undertaken in order to characterize the electron spin dynamics of the TEMPO in water:glycerol system. Several common pulsed EPR techniques were applied in order to measure the electronic relaxation times: spin-lattice $T_{1e}$, phase memory $T_{M}$, cross-relaxation $T_{e\tau}$, etc. These results are described in this section.

Pulsed EPR spectroscopy provides a powerful set of tools for studying the electronic relaxation dynamics of paramagnetic systems in the solid state. Electron spin echoes are frequently used because the strong inhomogeneous broadening of EPR lines in solids leads to very rapid decay of transverse magnetization, or equivalently, very short free induction decays which are often largely undetectable due to instrumental dead time. Unlike NMR experiments, in which the entire spin system can be excited with a single pulse, the excitation bandwidth of high-frequency EPR sources is orders of magnitude narrower than the spectral width of the EPR line. The $g$-factor anisotropy leading to most of the large dispersion in high frequency EPR spectra allows one to selectively excite molecules with a particular orientation with respect to the external magnetic field, and hence extract orientationally dependent relaxation rates.

The remainder of this section is divided into three parts, each of which describes the results from one type of pulse EPR experiment. The first one is a measurement of spin-lattice relaxation using the method of long pulse, saturation recovery. The second is a direct measurement of cross-relaxation between different parts of the line using ELelectron DOuble Resonance (ELDOR). The third is a measurement of the phase memory time in the transverse plane, which is equivalent to measuring the Fourier transform of the spin packet shapes, using Hahn echoes. The EPR spectrometer used to perform these experiments described below is described in detail in chapter 4.
5.3.1 Electronic spin-lattice relaxation

The simplest way to measure the electron spin-lattice relaxation rate $T_{1e}^{-1}$ is to create a non-equilibrium population distribution by saturating, or partially saturating, the EPR transition, and then to monitor the return to equilibrium. Practically speaking however, this procedure is difficult to implement due to lack of microwave power. In fact, in most cases the microwave power is so low - and consequently, the bandwidth is so narrow - that only a small part of the EPR line will be saturated. Irradiation at a particular frequency will result in the formation of a narrow hole centered at the irradiation frequency while the rest of the line remains unaffected. The 'burned' hole will not recover strictly with $T_{1e}$; rather, the hole will become broader and shallower as spectral diffusion transports magnetization from adjacent parts of the spectrum. Therefore, the observed magnetization recovery will be a combination of concurrent processes, one due to spin-lattice relaxation, the other due to spectral diffusion.

The effects of spectral diffusion can be reduced by the use of a very long saturation pulse [84, 85, 86], or by the application what is known in the literature as a "picket fence", a series of short pulses over a long period of time [84, 85]. In cases where $T_{x} \approx T_{1e}$ the hole burning in conjunction with FID detection, as described by Schweiger and coworkers [87], can be used to fully discriminate between spectral diffusion and spin lattice relaxation.

In the TEMPO system, $T_{1e} \gg T_{x}$ and the long saturation pulse method can be used effectively. Figure 5.3.1 shows the typical result of an approximately bi-exponential recovery of the magnetization with two time constants: $T_{1e}$ which is due to true spin-lattice relaxation, and $T_{x}$ which is due to spectral diffusion. By varying the duration of the saturating pulse, the recovery kinetics change from being dominated by spectral-diffusion to being dominated by solely $T_{1e}$. The 1 mM concentration recovery curve is plotted on the same
Figure 5-5: T1e measurement 40 mM 4-amo TEMPO in 40:60 water:glycerol at 10 K. The RF source was the 139.5 GHz Gunn diode, and the magnetic field was set to 49690 G, on resonance with the first moment of the TEMPO EPR line. The pulse sequence consists of a long saturation pulse, an evolution time, and then three pulse stimulated echo detection. The pulses are 400 ns long with a 500 ns interpulse delay. The reader is referred to chapter 4 for more details on the operation of the pulsed EPR spectrometer. The figure above shows the typical result of an approximately bi-exponential recovery of the magnetization with two time constants: T1s which is due to true spin-lattice relaxation, and T2 which is due to spectral diffusion. By varying the duration of the saturating pulse, the recovery kinetics change from being dominated by spectral-diffusion to being dominated by solely T1s. The 1 mM concentration recovery curve is plotted on the same graph showing a nearly single-exponential recovering and no effects from cross-relaxation.

The saturation-recovery curves for the 40 mM solution are strongly field dependent as well. At the edges of the line the magnetization recovers in a mono-exponential fashion even for short saturation pulses, significantly different from the bi-exponential, pulse length dependent curves at the center of the line. These observations are indicative of a variation in the cross-relaxation rate, and hence the homogeneity, across EPR line: faster cross-relaxation and more homogeneity in the center than the edges. It is also worth mentioning that these preliminary saturation recovery experiments indicate some small degree of anisotropy in T1e across the TEMPO EPR line (~ 10%).
5.3.2 Electron double resonance (ELDOR) and cross-relaxation

ELDOR (EElectron DOuble Resonance) is a magnetic resonance technique often used to determine motional dynamics, relaxation properties, and molecular structure in paramagnetic systems. As the name suggests, it involves the study of the interaction between two distinct electron resonances. The version of ELDOR described here accomplishes this through the irradiation of the sample with microwaves from two sources operating at different frequencies.

The pulse sequence is shown in the upper part of Figure 5-6. The preparation pulse is generated by gating the output of the gyrotron using one of the high power switches from Capital Technology. The gyrotron is operated in CW mode and provides a few watts at a frequency of 139.63 GHz. The saturating effect of this long pulse is then observed using three short pulses generated by gating the output of the 139.50 GHz Gunn diode source on the EPR bridge using the Ukrainian low-power, high-speed switch. A second high power switch is used to protect the mixer during the preparation pulse. The reader is referred to sections 4.1.3 and 4.4 for descriptions of the pulsed EPR hardware and the gyrotron. The preparation pulse is applied to the sample for 1–100 msec, then a time $\Delta \sim 1$ ms later, the three pulse stimulated echo sequence is used to detect the effect on the spins which are on resonance 130 MHz upfield. The detection pulses are a few hundred nanoseconds long. The two microwave sources provide fixed frequencies, hence it is necessary to vary the external magnetic field to bring different parts of the EPR spectrum into resonance. The results of this experiment applied to two different concentrations of TEMPO in water:glycerol are shown in Figure 5-6. To our knowledge this is the first high frequency pulsed ELDOR experiment ever performed.

The open circles show the significant field-dependent ELDOR effect for a relatively
Figure 5-6: ELDOR of TEMPO in water:glycerol and 10 K: Experimental data and simulations of the ELDOR echo attenuation as a function of magnetic field for two concentrations of 4-amino TEMPO in 60:40 glycerol:water at 10 K. The pulse sequence is shown above the data: a 100 ms 139.63 GHz gyrotron pulse is used for excitation, and a 139.50 GHz stimulated echo sequence is used for detection. The open circles show the significant field-dependent ELDOR effect for a relatively high concentration (40 mM) of TEMPO, while the open triangles show the lack of an ELDOR effect at a relatively low electron concentration (1 mM). The low concentration result provides a control demonstrating that the effect is not due to sample heating. The filled points are the result of simulations which are discussed later in section 5.4. The 139.5 GHz echo-detected TEMPO EPR spectrum is plotted with a solid curve.
high concentration (40 mM) of TEMPO, while the open triangles show the lack of an ELDOR effect at a relatively low electron concentration (1 mM). The low concentration result provides a control demonstrating that the effect is not due to sample heating. The filled points are the result of simulations which are discussed later in section 5.4. The echo-detected EPR spectrum at 139.50 GHz is also shown. The reader's attention is drawn to the large anisotropy in echo attenuation across the ELDOR curve, consistent with the idea that cross-relaxation rates vary considerably across the 40 mM TEMPO EPR line.

The results of the pulsed ELDOR experiment provide direct evidence for cross-relaxation in the TEMPO EPR line at high concentrations due to strong electron spin-spin interactions. The cross-relaxation, which is essential to the DNP mechanism in this system, is eliminated at lower concentrations where electron-electron dipolar contact is weak.

The results of the ELDOR and saturation-recovery suggest that the electron spins behave neither as a single purely homogeneous line, nor as a collection of non-interacting spin packets.

5.3.3 Phase memory time $T_M$ and spin packet shapes

The strong inhomogeneous broadening in the TEMPO EPR line at 5 T leads to a very short FID, and one must use spin echoes to refocus the static inhomogeneities, particularly $g$-anisotropy, in order to study the homogeneous aspect of the spin system. We have used Hahn echoes to measure the phase memory time and the homogeneous spin packet shape. The EPR spectrometer used to perform these experiments described below has been described in detail in chapter 4.

The Fourier transform of the echo envelope decay can be used to define the homogeneous line shape of an individual spin packet with a half-width at half-intensity that corresponds
to the reciprocal of the phase memory time. Since the homogeneous spin packet width is several orders of magnitude smaller than the width of the inhomogeneously broadened EPR line, it is more sensitive to time-dependent local fields.

One usually divides the electron spin system into two types of spins, A and B [88, 89]. The A spins are on resonance with the microwave pulses and contribute to the echo signal, while the B spins are off resonance and do not. The static contributions to the local fields result in reversible dephasings that are refocussed at $t = 2\tau$ by the second microwave pulse. We list here the possible mechanisms by which the field at A can change causing the echo height to decay:

1. A-A coupling fluctuates

2. A-B coupling fluctuates

3. A-I hyperfine couplings fluctuate

The dipolar interactions of the A spins with each other and with the B spins affect the electron spin echo signal decay differently. The local dipolar field from the B spins is a kind of external field for the A spins. If during experiment the B spins do not change their orientation, then the local field at A due to the B spins is just another source of inhomogeneous broadening for the A spins and will be refocussed by the second microwave pulse. If the B spins have enough time to change their spin orientations, on the other hand, then an irreversible dephasing of the spin precession occurs. Changes in B spin orientation occur because of either spin-lattice interactions ($T_1$-type samples), or mutual spin flip-flops ($T_2$-type samples). This mechanism of echo height attenuation is called spectral diffusion. The resonance frequency of the spin under consideration (the A spin) is shifted by the fluctuating local field, and its spectrum "diffuses".

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The spin-spin interactions between the A spins is reflected in the echo signal decay in one other way. A 180° pulse changes the sign of the local magnetic field created by the A spins, and causes an "instantaneous" resonant frequency shift of the spins for the duration of the pulse. This dephasing mechanism is called instantaneous diffusion. However, if the A spins are flipping quickly due to $T_1$ or $T_2$, then during the time microwave pulse is on they will have changed their spin orientation several times, and the one extra flip caused by the 180° pulse is inconsequential against the background of the random flips. For the mechanism of instantaneous diffusion to work then, the A spin flips must be sufficiently infrequent. If the A spin-flip rates are comparatively fast, the interaction between the A spins will contribute to the echo signal decay not by instantaneous diffusion, but by the spectral diffusion mechanism outlined above. By varying the power levels of the microwave pulses in the Hahn-echo sequence one can study the instantaneous diffusion effects [89].

Finally, one more process contributing to the electron spin echo decay is due to the I-S interaction of the electrons with the matrix nuclear spins. Here, the nuclei act as B-type spins. As they undergo spin flips, generally from $T_{2n}$, they cause dephasing of the electron spins by random modulation of the local magnetic field they create at the electron.

The case where the local field fluctuations are caused by the spin-lattice relaxation of the B spins is the one that has received the most attention both theoretically and experimentally, mainly because it is tractable to mathematical analysis. Two models have been proposed: the sudden-jump model of Klauder and Anderson [90] and the Gauss-Markovian model of Mims [88].

In the sudden jump model, the $z$-component of the $B$-spin magnetic moment can undergo random sudden jumps between the values $\mu_z = \pm \frac{1}{2} g_B \beta_e$, at an average rate $R = 1/T_1$. Klauder and Anderson have shown that in the limit that the echo time is much small than
the spin-lattice relaxation time, i.e. that \( \tau \ll T_1 \), the two-pulse echo envelope decay function \( E(2\tau) \) is given by:

\[
E(2\tau) = \exp \left[ -\left( \frac{2\tau}{T_M} \right)^2 \right],
\]

(5.1)

with

\[
T_M = \left( \frac{2}{R\Delta \omega_{\text{dip}}} \right)^{1/2},
\]

(5.2)

and where \( \Delta \omega_{\text{dip}} \) is the half-width from dipolar broadening from a dilute, random distribution of static B spins. The reader is referred to Abragam [18] for calculation for spin \( \frac{1}{2} \). The result for a concentration \( C_B \) of B spins is given by:

\[
\Delta \omega_{\text{dip}} = \frac{4\pi^2}{9\sqrt{3}} \gamma_A \gamma_B \hbar C_B
\]

(5.3)

In the Gauss-Markovian model, Mims assumed that the magnetic moments of the B spins behaved as Gaussian random variables with an exponentially decaying Markovian correlation function. This model predicts that in the same \( \tau \ll T_1 \) limit, the echo decay function is given by:

\[
E(2\tau) = \exp \left[ -\left( \frac{2\tau}{T_M} \right)^{3/2} \right]
\]

(5.4)

with

\[
T_M = 1.9[R(\Delta \omega_{\text{dip}})^2]^{1/3}.
\]

(5.5)

One simple model for \( T_2 \)-type samples is the same as that used for \( T_1 \)-type samples with some mean effective time of spin flips as the correlation time \( \tau_c \). The problem is actually much more complicated in magnetically dilute solids since the spins have local
environments that differ from point to point in the lattice and thus, their spin orientations change with different frequencies during spin diffusion, that is there is a set of flip-flop rates. Numerical calculations [88] of the kinetics of the two-pulse electron signal decay due to spectral diffusion in $T_2$-type samples show however than in the limit where the most probable rate of mutual flip-flops $W_{\text{max}}$ is less than $10^3 s^{-1}$, the decay is similar to that for spectral diffusion in $T_1$-type samples,

$$E(2\tau) \approx \exp(-m\tau^2)$$  \hspace{1cm} (5.6)

with

$$m \approx \Delta \omega_{\text{dip}} W_{\text{max}}.$$  \hspace{1cm} (5.7)

Even though the parameters are similar, the rate constants depend on the concentration of the $B$ spins differently: in $T_1$-type samples $m \sim C_B$; in $T_e$-type samples $m \sim C_B^2$ or higher. As the $B$ spin flip-flop rates increase, the time dependence of the decay changes from $\exp(-m\tau^2)$ to $\exp(-m\tau)$ and then to $\exp(-m\sqrt{\tau})$.

Instantaneous diffusion results in a two-pulse signal decay that obeys the equation

$$E(2\tau) = \exp(-2\Delta \omega_{1/2} \rho \tau),$$  \hspace{1cm} (5.8)

where $\Delta \omega_{1/2}$ is the dipolar broadening and $\rho$ is the fraction of $A$ spins.

Nuclei, on the other hand, form a concentrated spin system, so the mode of averaging the spatial distribution is different from the dilute case above. Also, one must take into account the diffusion barrier that surrounds the electron spins. Spin diffusion can take place only outside this barrier. Only nuclei in shells outside a radius $b$ undergo mutual spin flips, where $b$, the diffusion barrier, is defined as the distance from the electron at which the electron-nuclear interaction equals the interaction causing the mutual spin-flip processes.
Figure 5-7: The phase memory time $T_M$ of three concentrations of TEMPO in 40:60 water:glycerol at 10 K. The height of the spin echo from the two pulse sequence shown above, is plotted as a function of inter-pulse spacing $\tau$. All three decay curves fit very well to a gaussian: the two low concentration samples, 300 $\mu$M and 1 mM, with a time constant $\tau_L = 4.0 \mu$s; and the high concentration with a time constant $\tau_H = 2.8 \mu$s. From the low and high concentration decay curves, the 40 mM electron flip-flop rate $T_{2e}$ is estimated to be $\sim 7 \mu$s. See text for details.

See section 2.5.2 for further discussion of nuclear spin diffusion. The time constant of the echo signal decay for the due to nuclear flip-flops is given by:

$$T_{MN}^{-1} = \Delta \omega_c = 0.49 \left( \frac{\gamma_e}{\gamma_n} \right)^{1/2} \frac{\Delta \omega_{nn}}{[I(I+1)]^{1/4}},$$

where $\Delta \omega_{nn}$ is the mutual spin-flip rate and is of the order of $1/T_2$, the homogeneous NMR linewidth.

The results of a series of experiments measuring the decay of the Hahn echo as a function of interpulse delay $\tau$ for three concentrations of TEMPO in a frozen solution of 40:60 water:glycerol at 10 K are shown in Figure 5-7. For all three concentrations, the shape of
the decay fits very well to a Gaussian. From the low and high concentration decay curves, the 40 mM electron flip-flop rate $T_{2e}$ is estimated to be $\sim 7 \mu s$ as discussed below.

To estimate the electronic flip-flop rate in the frozen solution of 40 mM TEMPO in 40:60 water:glycerol, we fit the echo decay curves shown in Figure 5-7. All three curves fit very well to gaussians with the two low concentration samples, 300 $\mu$M and 1 mM, having nearly identical curves which decayed with a time constant $\tau_L = 4.0 \mu s$, and the high concentration sample, 40 mM, having a curve which decayed with a time constant $\tau_H = 2.8 \mu s$.

Since 300 $\mu$M is such a low concentration of electron spins, we can assume that all of the echo decay curve is due to the nuclear spin diffusion process. The electrons are too far apart to have much of an impact on one another. The 1 mM sample behaves almost identically to the 300 $\mu$M sample, and since its electronic spin-lattice relaxation time is moderately fast at this temperature $T_{1e} \sim 100$ ms nearly the same as for 40 mM, we can safely assume that $T_{1e}$ is not a particularly important process for the spin echo decay in this system. Therefore we will say that the extra decay in the 40 mM sample is due to the electronic flip-flop processes, and since the decay remains gaussian, we will model it using the Klauder and Anderson model with equations 5.1 and 5.2. From the measurement of the phase memory time $T_M$ and the knowledge of the concentration of spins, we can calculate the rate of jumps leading to echo decay. This is the inverse of the electronic $T_2$, which turns out to be $\sim 7 \mu s$.

### 5.4 Spin systems and thermodynamics in TEMPO

In this section we will discuss the thermodynamic model used for analyzing the TEMO in water:glycerol ELDOR and DNP data. It is an extension of the one presented in section 2.4 which was used to describe thermal mixing at low magnetic field in a system with a sin-
Figure 5-8: Thermodynamic model of the spin dynamics in the TEMPO in water:glycerol system. The EPR spectrum is divided into many spin packets which exchange energy with each other and with the electron dipolar reservoir via cross-relaxation. The spin packets exchange energy with the nuclear Zeeman system via energy conserving spin flips of one nuclear spin and two electron spins from packets $\omega_n$ apart. Spin-lattice relaxation and microwave irradiation are also explicitly included.

Single homogeneous EPR line and a very strong coupling between the electronic dipolar and nuclear Zeeman systems. We will restrict ourselves to a discussion of low-power microwave irradiation, and defer the analysis of the high-power DNP results to future studies.

5.4.1 Thermodynamic description of the TEMPO system

To describe the mechanism of low-power DNP and ELDOR in the TEMPO in water:glycerol system, we split the inhomogeneous EPR line into $N$ homogeneous spin packets [30] as shown in Figure 5-8. Each packet $i$ is centered at a frequency $\omega_{ci}$ shifted slightly from the central electron Zeeman resonance $\omega_e$ by the static, inhomogeneous effects of $g$-anisotropy and hyperfine couplings. The width of each packet is much less than the overall width of the EPR spectrum, and on the time scale of DNP and ELDOR will be in internal equilibrium with a well defined inverse Zeeman spin temperature $\alpha_i = 1/k_{BT_i}$.

There are two additional spin energy reservoirs, the electron dipolar system (EDS) and the nuclear Zeeman system (NZS), each with its own well defined inverse temperature $\beta$ and $\gamma$ respectively. We are restricting ourselves to experiments at high field and high
temperature, and assume that these \( N + 2 \) temperatures fully characterize the system, that is, we will ignore the nuclear dipolar interaction reservoir. The validity of these assumptions is discussed extensively in section 2.4. These \( N + 2 \) thermodynamic reservoirs exchange energy with each other, with the lattice, and with the microwave irradiation field as listed below:

1. Cross-relaxation between adjacent spin packets \( i \) and \( j \) will change the temperature of the two packets and also change the temperature of the electron dipolar reservoir as outlined in section 2.6.

2. Each of the systems will relax to the lattice via the mechanisms outlined in chapter 2. I will assume that all of the \( N \) electron spin packets have the same relaxation rate \( T_{1e}^{-1} \). The other two reservoirs relax to the lattice with rates \( T_{1D}^{-1} \) and \( T_{1n}^{-1} \) for the EDS and NZS respectively.

3. The temperature of the nuclear Zeeman system can change via a three spin process. A nuclear spin and two electron spins from packets \( \omega_n \) apart can undergo energy conserving flips allowing for energy exchange to the NZS.

4. Since we are describing the effect of a relatively weak microwave irradiation field, we will only consider the absorption of energy by the packets exactly on resonance. We ignore the "forbidden" transitions that are involved in the solid effect discussed in section 2.5.

The Provotorov equations governing the dynamics of the spin system temperatures can be written using the prescription developed in chapter 2. We will take some of the parameters like the nuclear and electronic \( T_1 \) to be specified by the experiments discussed in section 5.3, and other parameters, like the strength of cross-relaxation, to be adjustable in the numerical
simulation of the ELDOR and DNP data. These simulations comprise the remainder of this chapter.

5.4.2 Numerical results

Numerical simulations of the spin dynamics in a frozen solution of TEMPO in water:glycerol system are discussed in this subsection. They are based on the thermodynamic model shown schematically in Figure 5-8.

We define a vector $\vec{X}$ consisting of the $N+2$ inverse spin temperatures that characterize the state of the system. The normalized EPR line $g(\omega)$ is divided into $N$ bins each of width $\omega_B$, and centered at $\omega_{ei}$. The first $N$ elements of $\vec{X}$ are the inverse temperatures of the $N$ bins: $X_i = \alpha_i$ for $i \leq N$. The final two elements of $\vec{X}$ are the temperatures of the electron dipolar and nuclear Zeeman baths: $X_{N+1} = \beta, X_{N+2} = \gamma$.

Suppose that we irradiate the $m^{th}$ bin with a microwave field of frequency $\omega_m$ and strength $\omega_1$, we can write the Provotorov equations which describe the system's approach to thermal equilibrium. This set of differential equations, which includes the effect spin-lattice relaxation, microwave irradiation, and cross-relaxation on the evolution of the $N+2$ spin temperatures, can be written compactly in the following form:

$$\frac{d\vec{X}}{dt} = M\vec{X} + \vec{B}$$  \hspace{1cm} (5.10)

where the elements of $M$ and $\vec{B}$ are derived from the following relations:

$$\omega_{ei} \frac{d\alpha_i}{dt} = -M^{m}_i - M^{SL}_i - M^{ee}_{i-1,i} + M^{ee}_{i,i+1} - M^{een}_{i-2,i} + M^{een}_{i,i+a}$$  \hspace{1cm} (5.11)

$$\omega_B \frac{d\beta}{dt} = -M^{SL}_B - \frac{\omega_B^2}{\omega_{Loc}^2} \sum_i M^{ee}_{i,i+1}$$  \hspace{1cm} (5.12)

$$\omega_n \frac{d\gamma}{dt} = -M^{SL}_n - \frac{1}{N} \sum_i M^{een}_{i,i+a}$$  \hspace{1cm} (5.13)
and the $M$'s are given by

\[ M_i^m = W_m \hbar \omega_{ei} a_i \delta_{i,m} \]
\[ W_m = \frac{\pi}{2} \hbar \omega_1^2 g(\omega - \omega_m) \]
\[ M_i^{SL} = \frac{1}{T_{1e}} (\alpha_i - \beta L) \]
\[ M_i^{SL} = \frac{1}{T_{1D}} (\beta - \beta L) \]
\[ M_n^{SL} = \frac{1}{T_{1n}^e} (\gamma - \beta L) \]
\[ M_{ij}^{ee} = W_{cr} [n_i n_j \alpha_i \omega_{ej} - n_j n_i \alpha_i \omega_{ei} + n_i n_j \beta \omega_{Bin}] \]
\[ M_{ij}^{en} = W_{een} [n_i n_j \alpha_i \omega_{ej} - n_j n_i \alpha_i \omega_{ei} + n_i n_j \gamma \omega_n] \]

We have used the following notation: $n_i$ is the fractional population of bin $i$; $\alpha$ is the number of bins separating spin packets $\omega_n$ apart; $\beta L$, is the inverse temperature of the lattice; $W_{cr}$ is the cross-relaxation time between neighboring bins; $W_{een}$ is the three spin cross-relaxation rate; $T_{1e}$ and $T_{1D}$ are the electronic and dipolar spin-lattice relaxation times; $T_{1n}^e$ is the nuclear spin-lattice relaxation time due processes other than electron-electron-nuclear relaxation; and $\omega_{Loc}$ is the average electron-electron dipolar field. The notation is based loosely on those used by Hoogstraate [91] and Wenckebach [25]. We will now discuss the parameters in equations 5.11 and the assumptions we have made in their construction.

**Number of bins:** We divide the TEMPO spectrum into $N$ bins each $\omega_{Bin}$ wide. $N$ must be chosen carefully. It must be large enough that the intensity of the TEMPO spectrum is nearly constant across any bin, yet not so large as to make calculation times unwieldy.

For the simulation results presented below, we have chosen $N = 400$, corresponding to $\omega_{Bin} = 1.8$ MHz. In this case, the width of a bin is substantially wider than the width of a homogeneous spin packet ($\sim 0.25$ MHz) as measured by pulsed EPR experiments in section 5.3.3.
Microwave saturation: Since we are restricting ourselves to describing low-power experiments only, we can safely assume that only allowed transitions are excited. The ELDOR experiment is performed in a high $Q$ cavity, while the DNP experiment is performed in a non-resonant, low-$Q$ structure as described in sections 4.1.2 and 4.3.3. In either case the strength of the RF field $\omega_1$ is difficult to measure directly. However, reasonable estimates can be made from the known Rabi frequency ($\omega_1/2\pi \sim 8\text{ MHz}$) of the 139.5 GHz pulsed EPR spectrometer when using the 10 mW Gunn diode source in conjunction with a cylindrical resonator. In the ELDOR experiment, the cavity is tuned to 139.50 GHz, and therefore somewhat less than half of the 139.64 GHz gyrotron power enters the cavity and interacts with the spins. In the DNP experiment, the microwaves are launched directly at the sample from a WR-8 waveguide. The power distribution in the NMR sample is therefore highly anisotropic, resulting in a wide distribution of field strengths. We will assume that the The microwave field will only affect the bin with which it is microwave field is considered to only perturb the bin with which it is on resonance. We will also neglect the effect of microwaves on the electron dipolar temperature $\beta$. This can be justified by considering the bandwidth of the microwave field in comparison with the size of a bin. Since we have chosen bins a factor of 5 larger than the homogeneous linewidth, and an order of magnitude or more larger than the microwave bandwidth, we can assume that the processes that bring the spin packets in the bin being irradiated into thermal equilibrium with one another, will serve to redistribute the energy evenly. This will happen on a time scale of the order of the inverse of the bin width, significantly faster than the rate of energy transfer between bins.

Spin-lattice relaxation: The electronic spin-lattice relaxation rate $T_{1e}^{-1}$ was assumed to be constant across the TEMPO line. It was measured to be approximately 80 ms at 12 K for the 40 mM solution (c.f. section 5.3.1). $T_{1D}$ is difficult to measure directly, but is
typically taken to be a factor of one to three times shorter than \( T_{1e} \) [28]. The nuclear spin-lattice relaxation process can be divided into its constituent mechanisms, and since relaxation rates are additive, the observed \( T_{1n}^{-1} \) can be taken to be \( T_{1n}^{-1} = (T_{1n}^{e})^{-1} + T_{nee}^{-1} = (T_{1n}^{e})^{-1} + NT_{een}^{-1} \). Measurements of the nuclear relaxation rate without any electrons present gives a reasonable estimate of \( T_{1n}^{e} \) for moderate concentrations of paramagnet, although it is important to recognize that the direct electron-nuclear relaxation processes also contribute to \( T_{1n}^{e} \).

**Electron-electron cross-relaxation:** Since the bin size chosen is large in comparison with the homogeneous linewidth, we can restrict our attention to cross-relaxation between adjacent bins. The assumption that only communication between adjacent bins is important rests on the observation of the Gaussian shape of the spin packets measured in section 5.3.3. If the spin packets have long tails, a property difficult to measure due to spectrometer dead-time, then cross-relaxation between spin-packets far apart in frequency may need to be included. The cross-relaxation rate \( W_{cr} \) is difficult to predict a priori, therefore it is estimated by fitting the experimental ELDOR and DNP measurements.

**Electron-electron-nucleus cross-relaxation:** The equations predicting the dynamics of the three-spin cross relaxation process are very similar to those for the electron-electron process as described in section 2.6. Only those spin packets which differ in frequency by exactly \( \omega_n \) will be able to undergo energy conserving mutual spin-flips and contribute to three-spin electron-nuclear polarization transfer. The reader is also referred to the section on nuclear spin-lattice relaxation 2.3, and Figure 2.2.3 for more discussion of these energy conserving spin flip processes.
Hole burning simulation

Figure 5-9 shows a series of simulations of EPR hole-burning experiments under a reasonably strong microwave saturation. Equations 5.11 were used to compute spectra with varying degree of cross-relaxation using parameters for the 40 mM TEMPO in 40:60 water:glycerol frozen solution at 12 K. The cross-relaxation rate $W_{cr}$ is varied over several orders of magnitude. The microwave irradiation frequency was chosen to correspond to resonance at a magnetic field of 49670 G for a 139.50 GHz source.

There is very little spectral diffusion when the cross-relaxation is small and the line behaves effectively inhomogeneously. Microwave irradiation burns a narrow hole. As the cross-relaxation rate increases, the line becomes progressively more homogeneous and the hole broadens. In the limit of a nearly homogeneous line, the hole becomes delocalized and the portion of the EPR line downfield from the microwaves becomes emissive.

ELDOR simulation

We will now discuss the simulation of the results of the ELDOR experiment shown in Figure 5-6 in section 5.3.2.

The simulation in Figure 5-6 is computed by comparing the steady-state EPR intensity at 139.5 GHz, under microwave irradiation at 139.64 GHz, to the EPR intensity without microwave irradiation. The experiment was actually performed using a long saturating pulse instead of CW irradiation, but it was observed that increasing the microwave pulse length did not affect the experimental results; therefore the steady-state approximation should be considered adequate.

We found that a cross-relaxation rate of $W_{cr} = 25$ MHz and a microwave transition frequency of $W_m = 0.1$ MHz, along with the other relaxation parameters obtained from
Figure 5-9: Simulations of hole-burning in the 4-amino TEMPO EPR line. Spectra with varying degree of cross-relaxation are computed using parameters for the 40 mM TEMPO in 40:60 water:glycerol frozen solution at 12 K. The solid line shows the 1 mM echo-detected EPR spectrum. See the text for further details on the procedure used to compute the curves. The parameters were: \( N = 400 \) bins, \( \omega_m = 49670 \) G, \( T_{1e} = 75 \) ms, \( T_{1D} = 25 \) ms, \( \omega_{Loc} = 20 \) MHz, and \( W = .1 \) MHz.
experiment, discussed above, and shown in the hole burning simulation shown in Figure 5-9, resulted in an adequate fit of the experimental ELDOR curve for the frozen solution of 40 mM TEMPO in 60:40 glycerol:water.

As a consistency check, an ELDOR curve was calculated using the same parameters as those for the 40 mM TEMPO solution, except for the cross-relaxation rate $W_{cr}$ which was reduced by a factor of 40. Since the cross-relaxation rate is proportional to the average dipolar coupling between the electronic spins, and hence proportional to the concentration of electron spins (c.f. reference [17] and section 2.6), this curve is expected to fit the 1 mM TEMPO experimental ELDOR curve. The satisfactory results of this exercise are also shown in Figure 5-6.

**DNP simulation**

The magnetic field dependence of low-power DNP in a frozen solution of 40 mM 4-amino TEMPO in 60:40 glycerol:water at 12 K is shown in Figure 5-10. The experimental data, represented by the circles, were acquired by measuring the $^1$H NMR signal enhancement under microwave irradiation with the 139.50 GHz Gunn diode source as described in section 4.3.3. The simulation (solid curve) was computed using the same parameters as for the ELDOR curve in Figure 5-6 and the hole burning simulation in Figure 5-9, except that the microwave saturation parameter was reduced to $W = 5^{-4}$ MHz. The cross-relaxation rate $W_{cr} = 25$ MHz was the same as for the ELDOR simulation.

We must briefly mention one somewhat unsatisfactory aspect of the DNP simulation procedure. Because of the difficulty in measuring $T_{1n}$ and $W_{cen}$, we do not calculate the absolute value of the DNP enhancement directly. Instead we compute an enhancement curve as a function of magnetic field using the same procedure outlined above for hole-burning.
Figure 5-10: The magnetic field dependence of low-power DNP in a frozen solution of 40 mM 4-amino TEMPO in 60:40 glycerol:water at 12 K. The data (circles) were acquired by measuring the $^1$H NMR signal enhancement under microwave irradiation with the 139.50 GHz Gunn diode source. The simulation (solid curve) was computed using the same parameters as in Figure 5-9, except that $W = 0.005$ MHz and $W_{ef} = 25$ MHz. See text for more details.

and ELDOR, and then scale the resulting curve to the experimental data. Therefore we make no real attempt at analyzing the consistency of our calculated enhancements with the magnitude of the observed enhancement.
Chapter 6

Conclusion

Recent advances in methodology, such as multidimensional homo- and heteronuclear recoupling schemes, have allowed solid-state NMR to become an important tool in the structural and functional analysis of materials; unfortunately, these new methods require complex pulse sequences which increase acquisition time and decrease signal size, and the feasibility of many solid-state NMR experiments is limited. This has led to an intense research interest toward the development of techniques to increase signal and overcome NMR's inherent insensitivity. My graduate work has been part of an ongoing investigation by the Griffin group into the implementation of DNP at high magnetic fields in high resolution solid-state NMR, with the eventual goal being the development of a collection of signal enhancement tools applicable to the study of biological solids. As such, the advances in methodology and understanding of DNP described in this thesis are steps towards that ultimate goal, and represent one chosen avenue of research. This chapter contains a brief summary of the thesis and some thoughts regarding possible extensions of this work.

The basic theory of spin-spin interactions in paramagnetic solids was discussed in chapter 2. The relevant physics of polarization transfer and relaxation was presented, with
particular emphasis on DNP in the low magnetic field regime. The dynamical equations of polarization transfer were given for the two main DNP mechanisms of electron-nuclear polarization transfer: the solid effect and thermal mixing.

High field DNP results in two systems, a model polymer system (BDPA in polystyrene), and a nitroxide doped frozen aqueous solution (TEMPO in water and glycerol), were presented in chapter 3. The BDPA results were shown to be consistent with the solid effect. The nitroxide results, on the other hand, were shown to be somewhat similar to low field thermal mixing, even though the simple low field thermal mixing model breaks down at high field due to inhomogeneous broadening of the EPR line. The polarization transfer mechanism and dynamics are generally more complex at high magnetic fields and a model suitable for analyzing the nitroxide DNP results was presented in chapter 5.

The various experimental methods used in the acquisition of the data were described in chapter 4. Included were descriptions of the design and operation of the 140 GHz gyrotron, the primary high-power microwave source for our experiments, and of the EPR spectrometer, the device upon which the necessary studies for the elucidation of the mechanism of polarization transfer were performed. Also discussed were some of the technical considerations involved in incorporating DNP into high-field solid-state NMR, including the low-temperature static and magic angle spinning probes.

The results of the pulsed EPR experiments and the analysis of the DNP results for the TEMPO system were presented in chapter 5. It was shown that the results are consistent with a high field analog of thermal mixing. The formalism presented in chapter 2 to describe thermal mixing in a single homogeneous EPR line was shown to be inadequate in explaining these high field dynamics, and the effect of cross-relaxation in the inhomogeneous TEMPO EPR line had to be explicitly included in order to construct the model that adequately fits
the DNP, pulsed EPR, and electron-electron double resonance (ELDOR) measurements.

Several questions need to be addressed in future investigations, including issues pertaining to the role of the solvent in mediating polarization transfer, the utility of other paramagnet/solvent systems, optimization of the linewidth and relaxation characteristics of the paramagnet, and extension of the theoretical model to include the effect of high microwave power on the DNP dynamics. For example, some other class of polarization transfer scheme may turn out to be more efficient than the one presented here. The overall utility of DNP is limited by the polarization transfer time which is on the order of the nuclear spin-lattice relaxation time $T_{1n}$ for continuous wave type schemes such as the one discussed in this thesis; on the other hand, the polarization transfer time is on the order of the electronic spin-lattice relaxation time $T_{1e}$ in coherent transfer schemes such as electron-nuclear cross polarization.

The model developed here should be applicable to DNP at higher magnetic fields as well, where the EPR line even less likely to be in the fast cross-relaxation limit due to the dominance of inhomogeneous broadening mechanisms. In the cases where one knows the EPR lineshape and the values of the relaxation times, it will be possible to assess the probable utility of a particular radical/solvent system as a polarization matrix. The possibility of constructing a gyrotron operating at higher frequencies (200–600 GHz) allows for the relatively straightforward implementation of this methodology at higher fields, where NMR resolution and sensitivity improve markedly.

In my opinion, the results and analysis presented herein are sufficiently compelling to justify continuation and extension of this research to higher fields, as well as to more practical applications development at 5 Tesla, with an eye towards addressing structural and functional questions in biochemistry, material science, and physics.
Bibliography


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