THE OFFSET-SATURATION NMR EXPERIMENT
FOR MEASURING SPIN RELAXATION TIMES
AND APPLICATION TO CHEMICAL EXCHANGE

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THE OFFSET-SATURATION NMR EXPERIMENT
To Xiang and Michael, and to the Memory of my Father
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TITLE: The Offset-Saturation NMR Experiment for Measuring Spin Relaxation Times and Application to Chemical Exchange

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ABSTRACT

NMR spin relaxation times $T_1$ and $T_2$ provide useful information regarding molecular motions and dynamics. The spin-spin relaxation time $T_2$ in particular is important for measuring rates of chemical exchange, but has proven difficult to measure. A facile offset-saturation experiment for the simultaneous measurement of $T_1$ and $T_2$ NMR spin relaxation times is presented in this thesis. Errors determined as the 95% confidence limits for the relaxation times measured by offset-saturation experiments are on the order of 5%, provided that the saturating field $B_2$ is well calibrated, and the sampling points are judiciously chosen. The relaxation times for acidified distilled water determined by a non-linear least-squares fit to offset-saturation data are in good agreement with the results obtained by inversion-recovery and CPMG techniques. A qualitative guideline for the optimal choice of sampling points for offset-saturation experiments is illustrated.

The offset-saturation experiment is shown to be a viable method for the study of fast chemical exchange processes. Applications to the chair-chair interconversion of cyclohexane, and the hindered C-N bond rotation in N-acetylpyrrole, show that the offset-saturation method can be used to obtain rate constants on the order of $10^4$ s$^{-1}$. Exchange rates can be determined over several orders of magnitude when the offset-saturation
method is used in combination with other dynamic NMR methods for the fast and slow exchange regimes.

The single-spin double resonance experiment is shown to be a simple and precise method for the calibration of the saturating field $B_2$ used for offset-saturation experiments. The use of pulsed homodecoupling radiofrequency sources to provide the saturating rf field has entailed a theoretical study regarding the equivalence of pulsed and continuous-wave radiofrequency radiation. An analytical expression for the $z$ magnetization as a function of irradiation resonance offset for a series of repeated soft pulses is obtained, which in the limit of small duty cycles and pulse flip angles is equivalent to the Bloch equations.
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CHAPTER 1
INTRODUCTION

1.1 Introduction

The use of nuclear magnetic resonance (NMR) spectroscopy as a tool for investigating molecular structure and dynamics has become widespread in many diverse areas of chemistry, physics, biology, medicine and materials science since its co-discovery in 1946 by the groups of Bloch\textsuperscript{1-3} and Purcell\textsuperscript{4}. A steadily increasing number of NMR experiments has provided the researcher with a variety of means to probe the structures, conformations and motions of chemical and physical systems through information from NMR parameters such as chemical shifts, scalar and dipolar coupling constants, and spin relaxation times.\textsuperscript{5-8}

Nuclear spin relaxation times are valuable in providing information on molecular motions or dynamics, especially since the advent of pulsed Fourier transform (FT) NMR has facilitated the measurement of NMR spin relaxation times. Investigations of molecular motions may be accomplished exclusively by measurements of the spin-lattice relaxation time $T_1$. As $T_1$ is sensitive only to rapid motions with a timescale on the order of the Larmor frequency,\textsuperscript{5-9} such a procedure often requires determinations of $T_1$ over a range of temperatures or magnetic field strengths in order to characterize motion in both the high and low frequency regions of the particular system under examination. Information
concerning the low frequency motions can more readily be obtained by measuring either
the spin-spin relaxation time $T_2$, or by measuring the spin-lattice relaxation time in the
rotating frame ($T_{1R}$), which provides information similar to $T_2$. The spin-lattice relaxation
time $T_1$ is readily obtained from the robust two-pulse inversion-recovery experiment$^{10-12}$,
but no facile analogue to the inversion-recovery experiment exists for the determination
of the spin-spin relaxation time $T_2$. The measurement of $T_2$ relaxation times from
linewidth measurements is hindered by static magnetic field inhomogeneities, while pulsed
spin-echo techniques designed to facilitate $T_2$ measurement by eliminating field
inhomogeneity effects lack robustness, requiring careful control over experimental
conditions and are accordingly subject to errors from various sources.$^{13-16}$

The ratio of the spin-lattice ($T_1$) to spin-spin or transverse relaxation time ($T_2$) can
thus be used to yield important information on molecular motion or fluxionality in certain
chemical systems.$^{8,9,17}$ This may entail the use of two separate experiments in order to
obtain the two distinct relaxation times. Experiments have been developed to measure
both $T_1$ and $T_2$ or their ratio$^{18-21}$ or hybrid relaxation times which are some mathematical
combination of $T_1$ and $T_2$$^{22,23}$ but these experiments are in general somewhat cumbersome
in terms of implementation and data analysis, and may require experimental apparatus
which is non-standard on many NMR spectrometers.

The study of chemical exchange, or dynamic NMR, represents one of the
longstanding applications of NMR. Many NMR techniques have been developed for the
measurement of exchange rates, which ultimately permits the determination of
thermodynamic parameters for fluxional or dynamic processes including intramolecular conformational changes such as bond rotations or ring inversions. In the simplest case of a nucleus exchanging between two sites, the ratio of the exchange rate to the frequency difference between the resonance position for each site in the absence of exchange results in three distinct exchange timescales, namely, the slow, intermediate, and fast exchange regimes.

In the fast exchange regime, when the exchange rate may dominate the chemical shift difference between exchanging sites, determination of the rate constant from the value of $T_2$ measured for the single line resulting from the exchange-averaged position for the two sites often proves challenging, due to inherent difficulties in measuring $T_2$. Rates of exchange in the fast exchange regime may be beyond the upper limit of accessibility for methods of line-shape analysis which are commonly employed as a means of obtaining rate data for exchanging systems. Spin-echo and $T_{1p}$ techniques have been developed to extend the upper range of exchange rate measurement. The use of these methods, has been somewhat restricted however, due to the requirement of careful control over experimental parameters or the need for non-standard spectrometer hardware, thereby reducing their suitability for general application.

An alternative method for the measurement of the spin-spin relaxation time $T_2$, which also provides simultaneous measurement of the $T_1/T_2$ ratio, has been proposed. This experiment is based on the steady-state solution of the Bloch equations, and involves irradiation of a spin system at various resonance offsets prior to an acquisition pulse,
which measures the resulting $z$ magnetization. If this experimental procedure is repeated for several different values of the irradiation resonance offset, the $T_1/T_2$ ratio can be obtained from the width of the curve of $M_z$ as a function of irradiation offset. This particular technique is thus relatively uncomplicated and robust in comparison with other methods for the measurement of $T_2$ or the $T_1/T_2$ ratio, and can be performed on any high resolution NMR spectrometer equipped with a homodecoupler.

The rather simple experiment described above for measuring the $T_1/T_2$ ratio has been termed the offset-saturation method$^{35,36}$, and can be modified by the insertion of a pre-acquisition delay time, which is some fraction of the $T_1$ of the nucleus under study, after gating off the homodecoupler.$^{35,36}$ A three-parameter non-linear least-squares fit to the data set of irradiation offsets and corresponding observed intensities ($M_z$), permits the facile determination of absolute values for $T_1$ and $T_2$ from a single experiment. Significantly, the $T_2$ obtained from an offset-saturation experiment may be independent of magnetic field inhomogeneities which normally restrict the determination of $T_2$ values from linewidth measurements$^{17,35}$ which has important implications with respect to the determination of fast exchange rates. The offset-saturation method accordingly has potential for possible use as a dynamic NMR method for systems undergoing fast chemical exchange.
1.2 Objectives and Scope of the Study

The difficulties encountered in measuring the spin-spin relaxation time $T_2$, as well as the apparent utility of a method with the capability of simultaneously measuring both $T_1$ and $T_2$, make the offset-saturation method outlined above a potentially attractive alternative to the existing established methods for measuring NMR spin relaxation times. In addition, the difficulties inherent in measuring $T_2$ hinder measurements of exchange rates in the fast exchange regime, thereby restricting the upper limit of accessible rate determination. The purpose of this thesis is accordingly to undertake a detailed analysis of the offset-saturation experiment as a technique for the simultaneous determination of $T_1$ and $T_2$ nuclear spin relaxation times, and as a possible method for studying systems undergoing fast chemical exchange.

The present study essentially consists of two main areas of research. In the first part, several aspects of the basic offset-saturation experiment will be addressed, including experiment design and optimization, the analysis of data and evaluation of errors, and methods for accurately calibrating the saturating rf field. The offset-saturation method is then applied to determine $T_1$ and $T_2$ for a relatively simple system in which both relaxation times are predicted to be approximately equal, namely, water at low pH, in order to demonstrate the application, accuracy and precision of the technique.

Additional important aspects relevant to the understanding and successful application of offset-saturation experiments, such as the calibration of the irradiating rf
field, are examined. The minimization of errors in relaxation times from offset-saturation experiments requires that the irradiating radiofrequency (rf) field be calibrated as accurately and precisely as possible. The conventional method of calibrating rf field strengths by measuring a 90°, 180° or 360° pulse length does not meet this criteria. The classic single-spin double-resonance experiment\textsuperscript{37,38} was demonstrated some years ago as a method for measuring rf field strengths, and this experiment is examined in the present work as a facile and precise means of calibrating the strength of the irradiating rf field used in offset-saturation experiments.

An additional facet addressed in this work arises as a consequence of the use of pulsed rf sources to provide the irradiating rf field for single-spin double resonance and offset-saturation experiments. The use of pulsed homodecoupling sources in modern high-resolution NMR spectrometers requires that irradiation and acquisition be time-shared,\textsuperscript{5} so that the irradiation appears as an essentially infinite series of closely-spaced soft pulses. The traditional single-spin double resonance experiment assumes a continuous-wave (cw) source of rf radiation, and the theoretical basis of the offset-saturation experiment is the steady-state solution of the Bloch equations which assumes a cw rf source. The equivalence of continuous-wave (cw) and pulsed sources of rf irradiation thus must be assumed and perhaps subjected to some otherwise unknown limitations, or examined in a quantitative fashion. In this thesis, the equivalence of cw and pulsed rf sources is addressed theoretically through an extension of the steady-state calculations of Waldstein and Wallace\textsuperscript{39} to include the off-resonance z component of the magnetization.
during a series of closely-spaced pulses. An analytical expression containing pulse parameters such as flip angle, pulse length, precession time and dwell time which is analogous to the Bloch equation for $M_z$ as a function of irradiation resonance offset can be derived to demonstrate the equivalence of pulsed and cw rf radiation.

The second main area of research is concerned with the application of the offset-saturation experiment to chemical exchange, as a possible viable alternative method for measuring fast chemical exchange rates which can be used with some facility to extend the upper region of accessible rates. The offset-saturation method is accordingly applied to some relatively simple two-site intramolecular exchange processes. A preliminary investigation regarding the viability of the offset-saturation experiment for the study of fast fluxional processes will be undertaken by measuring rates of exchange for the classic chair-chair ring interconversion in cyclohexane. A detailed application of the offset-saturation method will then be presented for investigation of the hindered rotation for the C-N bond in N-acetylpyrrole, in combination with lineshape analysis and selective inversion methods for obtaining exchange rates in the intermediate and slow exchange regimes respectively.

Prior to the investigation of the offset-saturation method for measuring spin-spin and spin-lattice relaxation times and the subsequent application of this technique to systems undergoing fast chemical exchange, discussions of basic NMR theory and the effects of chemical exchange on NMR spectra are presented in the following chapters.
CHAPTER 2

NMR THEORY AND MEASUREMENT OF SPIN RELAXATION TIMES

2.1 Resonance Theory and Spin Relaxation in NMR

An overview of basic NMR theory pertinent to the topic of spin relaxation times and methods for their measurement is presented in this chapter. The concepts of relaxation times and pulsed NMR are introduced through the classical Bloch equations formalism for NMR. Experiments for the separate measurement of $T_1$ and $T_2$, as well as for their simultaneous determination in various manifestations, including the offset-saturation experiment, are reviewed.

The basic physics involved in a nuclear magnetic resonance experiment is well established. Presentations of magnetic resonance theory essential to an understanding of the technique may be found in any of the numerous textbooks on NMR. For sound discussions on the basic theory of NMR, the classic texts of Abragam, Slichter, Pople, Schneider and Bernstein or more recent works such as those by Harris and Ernst and co-authors should be consulted. An overview of the basic NMR theory pertinent to a discussion of relaxation times and their measurement is presented here. Texts such as those cited above can be consulted for more detail.

When a sample containing magnetic nuclei is placed in an external magnetic field, the magnetic moments of the nuclei interact with the field in such a way that the field
attempts to force the moments to align along the field. From classical electromagnetic theory, a magnetic moment in a magnetic field will experience a torque which is equal to the rate of change of its angular momentum. The applied magnetic field thus exerts a torque on the individual magnetic moments. The nuclei possess angular momenta $\mathbf{I}$, where $I$ is the spin angular momentum quantum number and $\hbar$ is Planck’s constant $\hbar$ divided by $2\pi$. Each such nucleus will thus precess about the static magnetic field $\mathbf{B}_o$ with an angular velocity $\omega_o$ known as the Larmor velocity which is proportional to the strength of the magnetic field, as given by $\omega_o = \gamma B_o$ where $\gamma$ is the magnetogyric ratio which is characteristic of the type of nucleus. The corresponding Larmor frequency $\nu_o$ is thus given as $\nu_o = \omega_o/2\pi$.

In accordance with quantum mechanics, the orientation of a magnetic moment with respect to an applied magnetic field is quantized. There will be $(2I + 1)$ such orientations or Zeeman energy levels, and $I=1/2$ for commonly-observed nuclei in NMR such as protons $^1\text{H}$, $^{13}\text{C}$, $^{19}\text{F}$, and $^{31}\text{P}$. The two allowed orientations for spin 1/2 nuclei are not collinear with the applied magnetic field due to the torque and angular momentum, but instead assume some constant angle such that the precession of the moments appears to occur around the surface of a cone with axis either parallel (low energy orientation) or antiparallel (high energy orientation) to the magnetic field $\mathbf{B}_o$. In classical terms, the energy of a magnetic moment $\mu$ (where $\mu = \gamma I(I+1)$), in a magnetic field $\mathbf{B}_o$ is $-\mu \cdot \mathbf{B}_o$, which implies that the lowest energy orientation for a positive magnetic moment such as a proton will be parallel to the magnetic field.
Thermal effects in the sample will counteract the effect of the magnetic field, so that there will be a difference in population of the two orientations or energy levels. The population difference between the two energy levels, which have nuclear spin quantum numbers $m_1 = \pm 1/2$ for nuclei with $I=1/2$, is determined by the Boltzmann factors for the states, $\exp(-m_1 \gamma B_0 / k_B T)$, where $k_B$ is the Boltzmann constant, and $T$ is the temperature. At room temperature, this Boltzmann factor may differ from one by only $10^6$, but this difference is a sufficient enough population excess so that a net or bulk magnetic moment $M$ will be created in the sample which will proportional to the resultant of the individual nuclear moments $\mu_i$, or $M = \sum \mu_i$. The magnitude of the thermal equilibrium value of the macroscopic magnetic moment is given by equation (2.1) as

$$M_o = \frac{N\mu^2 B_o}{3k_B T}$$  \hspace{1cm} (2.1)

where $N$ is the number of nuclei per unit volume, and the other symbols are as previously defined.

The macroscopic nuclear magnetization $M_o$ obeys the Larmor relation, and the effect of an applied magnetic field can be described using classical mechanics. In accordance with the Larmor theorem mentioned above, the interaction of the macroscopic magnetic moment $M$ with a static magnetic field $B_o$ will result in a torque on the system, changing the angular momentum, as described by equation (2.2):
\[
\frac{dM}{dt} = \gamma (\mathbf{M} \times \mathbf{B})
\]  

(2.2)

Equation (2.2) states that for the magnetic moment \(\mathbf{M}\) per unit volume in a stationary coordinate system, the rate of change of magnetization vector is proportional to both the magnetization and the applied magnetic field, with the direction of change perpendicular to both aforementioned vector quantities. Thus, the magnetic moment \(\mathbf{M}\) will precess about \(\mathbf{B}_0\) with angular velocity \(\gamma B_0\) or Larmor precession frequency \(\gamma B_0/2\pi\), which for a nucleus with specific \(\gamma\) in a laboratory field \(\mathbf{B}_0\) will be the observed NMR frequency.

The classic phenomenological equations of Bloch\(^{1,17}\) describe the balance between the input of energy and relaxation in a basic NMR experiment with the assumption of a cw rf source. Bloch appended relaxation terms to the equations of motion for each of the nuclear magnetization components, to obtain

\[
\frac{dM_z}{dt} = -\frac{M_z - M_0}{T_1}
\]  

(2.3a)

\[
\frac{dM_x}{dt} = \omega \rho x \frac{M}{T_2}
\]  

(2.3b)
\[
\frac{dM_y}{dt} = -\omega_s M_z - \frac{M_z}{T_2} \tag{2.3c}
\]

In equations (2.3a-2.3c), $T_1$ and $T_2$ are the time constants for the two relaxation processes which will be discussed in further detail shortly.

In an NMR experiment, a radiofrequency (rf) field $B_1$ is applied orthogonally to the static magnetic field $B_0$. The application of the rf field alters the direction of the bulk nuclear magnetization vector $M$. The interaction of the rf field and the net magnetic moment results in the absorption of energy by the nuclei, which rotates the magnetization $M$ away from the $z$ axis, and into the $x$-$y$ plane. The absorption of energy by the nuclei causes transitions between the nuclear spin energy levels created by placement in $B_0$. The quantum of work or energy required to reverse the orientation of the magnetic moment according to Planck's law is $\hbar \omega$. The dissipation of this absorbed energy or the re-establishment of thermal equilibrium is accomplished by relaxation processes by which the nuclei exchange energy with their environments and with themselves, and which Bloch assumed were of exponential time dependence.\(^1\)

After the rotation of the magnetic moment $M$ by the application of the rf field, the component of magnetization in the $x$-$y$ plane perpendicular to $B_0$ precesses about the $z$ direction. This motion of the magnetic moment results in the creation of a current which is then detected as a voltage by a receiver coil with its axis in the $x$-$y$ plane, giving rise to the observed NMR signal. The effects of the rf field $B_1$ rotating with an angular velocity $\omega$ with counter-rotating components
\[(B_1)_x = B_1 \cos \omega \]  \hspace{1cm} (2.4a)

\[(B_1)_y = -B_1 \sin \omega \]  \hspace{1cm} (2.4b)

are then added to equations (2.3a-c) to give the complete Bloch equations.

The rf field $B_1$ is time dependent, so that the Bloch equations will take a simpler form if the time dependence of $B_1$ is removed by transforming to a rotating coordinate system (known as the rotating frame) in which the $z$-axis is parallel to $B_0$, and the $x$ and $y$ axes (now labeled as the $x'$ and $y'$ axes respectively) rotate about the $z$ axis with the frequency of $\omega_1 (=\gamma B_1)$, as indicated in Fig.2.1. The components of $M$ in the plane perpendicular to $B_0$ (in the directions of $x'$ and $y'$ respectively, directed along and perpendicular to the $B_1$ field), are then designated $U$ and $V$, where

\[U = M_x \cos \omega - M_y \sin \omega \]  \hspace{1cm} (2.5a)

\[V = M_x \sin \omega + M_y \cos \omega \]  \hspace{1cm} (2.5b)

The Bloch equations in the rotating frame thus become

\[\frac{dU}{dt} = + (\omega_z - \omega)V - \frac{U}{T_2} \]  \hspace{1cm} (2.6a)
Figure 2.1. Definition of axes in the stationary (x,y,z) and rotating (x',y',z) frames.
\[
\frac{dV}{dt} = -(\omega_0 - \omega)U + \gamma B_1 M_z - \frac{V}{T_2}
\]  
(2.6b)

\[
\frac{dM_z}{dt} = - \frac{M_z - M_z^0}{T_1} - \gamma B_1 V
\]  
(2.6c)

When the rf field has been on for a sufficiently long time, the spin precession will assume a steady state. The equilibrium magnetizations thus remain constant in the rotating frame so that the derivatives in equations (2.6a-2.6c) can be equated to zero. The stationary or steady-state solution to the Bloch equations can thus be written as

\[
U = M_o \frac{\gamma B_1 T_2^2(\omega_0 - \omega)}{1 + T_2^2(\omega_0 - \omega)^2 + (\gamma B_1)^2 T_1 T_2}
\]  
(2.7a)

\[
V = M_o \frac{\gamma B_1 T_2}{1 + T_2^2(\omega_0 - \omega)^2 + (\gamma B_1)^2 T_1 T_2}
\]  
(2.7b)

\[
M_z = M_z^0 \frac{1 + T_2^2(\omega_0 - \omega)^2}{1 + T_2^2(\omega_0 - \omega)^2 + (\gamma B_1)^2 T_1 T_2}
\]  
(2.7c)

Phase-sensitive detection has traditionally been used to detected the NMR signal in the x-y plane,\(^5^8^9\) which means that either the U or V component may be detected. Thus, for B\(_1\) applied along the x axis in the laboratory frame, the component 90° out of phase with B\(_1\) (V) is recorded. The V mode signal gives the absorption bandshape, with the signal proportional to the power absorbed from the electromagnetic field. The U mode
signal gives the dispersion spectrum. The $U$ and $V$ components are illustrated in Fig.2.2.

For normal operating conditions of NMR spectrometers, especially for those spectrometers operating in the cw mode, the magnitude of the rf field $B_1$ is kept small in order to avoid saturation effects which may result in the distortion or even disappearance of NMR signals. This implies that the term $(\gamma B_1)^2 T_1 T_2$ in the Bloch equations above, known as the saturation factor, may be neglected for small values of $B_1$ since $(\gamma B_1)^2 T_1 T_2 \ll 1$. Thus, from eq.(2.7(b)), the absorption mode lineshape will be proportional to

$$g(v) = \frac{2T_2}{1 + 4\pi^2 T_2^2 (v_e - v)^2}$$  \hspace{1cm} (2.8)

in frequency units. The absorption mode lineshape predicted by the Bloch equations and described by eq.(2.8) is known as Lorentzian, and the width of the line at half-maximum height is equal to $(1/\pi T_2)$ in frequency units.

There are essentially two distinct types of nuclear spin relaxation processes. In one of these relaxation processes, spin lattice relaxation, the absorbed nuclear spin energy is dissipated to the nuclear environment, which is essentially the electrons and other nuclei of the sample. The process of spin lattice relaxation re-establishes the Boltzmann excess of nuclei in the lower state. An alternative name for this relaxation process is longitudinal relaxation. In the Bloch equations, the spin lattice relaxation time is denoted as $T_1$. Thus, $T_1$ is essentially the time constant for the component of the nuclear magnetization along
Figure 2.2. Absorption (solid line) and dispersion (dashed line) components of the nuclear magnetization predicted by the Bloch equations.
the direction of $B_0$, i.e. the $z$ magnetization, to decay back to the equilibrium magnetization value $M_0$.

In the other distinct spin relaxation process, the absorbed energy is dissipated within the spin system itself by dephasing of the individual spin vectors. This process is called spin-spin or transverse relaxation (since the dephasing process takes place in the plane orthogonal to the $z$ axis) and is designated by the time constant $T_2$ in the Bloch equations. The amplitude of the NMR signal is assumed to decay exponentially with a rate $\exp(-t/T_2^*)$, where $T_2^*$ includes the natural spin-spin relaxation time $T_2$ as well as the contribution to the NMR linewidth from magnetic field inhomogeneities.

The general requirement for the occurrence of nuclear spin relaxation is the presence of a fluctuating or time-dependent field at the Larmor frequency\textsuperscript{8,9,17,41} which generally results from molecular motions. This fluctuating field can be a magnetic field produced by several types of interactions or processes. For example, in the case of quadrupolar nuclei (with spin $I > 1/2$) the relaxation results from interaction of an electric field gradient at the nuclear site with the nuclear quadrupole moment.

Much of the pioneering work of nuclear spin relaxation theory was undertaken by Bloembergen, Purcell and Pound,\textsuperscript{41} who formulated a theory for nuclear spin relaxation, which is commonly referred to as BPP theory. While the BPP is valid only in the weak collision case where the molecular correlation time is considerably less than $T_2$, it nevertheless gives an adequate picture of the relaxation process as encountered in high resolution NMR in solution with random and isotropic magnetic fields. Bloembergen,
Purcell and Pound demonstrated that the spin-lattice relaxation time $T_1$ is affected only by high-frequency components, while $T_2$ is uniquely affected by low-frequency motions. Such processes include diffusion and chemical exchange, as will be seen shortly. Bloembergen, Purcell and Pound\textsuperscript{41} also showed that for most cases for motions in the extreme narrowing range, characterized by $\omega \tau_c < 1$, where $\tau_c$ is the correlation time which can be defined as the average time for a molecule to move through one radian, the relaxation times $T_1$ and $T_2$ are equal. This is the case commonly encountered or assumed for small molecules in non-viscous liquids.

A number of different processes exist which can produce a fluctuating magnetic field at the nucleus, thereby inducing spin relaxation. One relatively common process for spin $I=1/2$ nuclei which has been mentioned is the dipole-dipole (dd) interaction which involves the creation of fluctuating magnetic fields from the random tumbling motions of nuclear magnetic dipoles in close proximity. Quadrupolar relaxation (qd) for nuclei with spin $> 1/2$ has also been mentioned. Other relaxation mechanisms include scalar relaxation (sc) of the first and second kind, which occur when one spin is coupled to another through an electron-moderated interaction. In the first instance, a nucleus may exchange in and out of a site in which it is scalar coupled to a magnetically inequivalent neighbour, and the modulation of the coupling may result in relaxation. In scalar coupling of the second kind, the modulation of the field at one spin is due to the relaxation effects of the spin to which it is coupled, such as a quadrupolar nucleus undergoing rapid relaxation. Chemical shift anisotropy relaxation (csa) results from an
anisotropic distribution of the shielding orbital electrons surrounding the nucleus in a static magnetic field, such that molecular tumbling modulates the local field and induces relaxation.

A spin rotation (sr) relaxation process may arise from a coupling of the angular momenta of a nuclear spin and the magnetic moment arising from the angular momentum of a rotating molecule. Interruption of this coupling by collisions with other molecules may cause fluctuations of the angular momentum which ultimately induces relaxation. The presence of unpaired electrons or paramagnetic species also creates a relaxation mechanism (pr) through dipolar or scalar interaction of a nucleus with the rather large magnetic moment of the unpaired electron. The relaxation rates due to different mechanisms for spin 1/2 nuclei are additive, so that the total spin-lattice relaxation rate \((T_1)^{-1}\) can be defined as

\[
(T_1)^{-1} = (T_{1 \text{ vel}})^{-1} + (T_{1 \text{ sc}})^{-1} + (T_{1 \text{ cor}})^{-1} + (T_{1 \text{ pr}})^{-1}
\]  

(2.9)

The relative contributions of the various mechanisms to the observed relaxation rate depend of course on the system studied and one mechanism, such as a quadrupolar or paramagnetic interaction, may dominate. The explicit forms for each of the relaxation processes in terms of the spectral density functions, as well as more detailed discussions on the relaxation mechanisms, can be found in various NMR texts\(^8,17\).

The earliest NMR experiments as performed by Bloch, Purcell and co-workers\(^12,4,17\) utilized a cw rf source. In the introduction to his paper, Bloch\(^2\) suggested that a nuclear
induction signal could be observed in the absence of the rf field by subjecting the spin system to a pulse of rf energy, while Torrey\textsuperscript{42} examined solutions to the Bloch equations when the rf field is suddenly applied, as in the form of a pulse. In 1950, the experimental effects of the free nuclear precession about a static magnetic field after removal of the rf field were first reported by E.L. Hahn,\textsuperscript{43} and in that same year, Hahn published his classic paper on the measurement of nuclear relaxation times by employing a pulsed rather than a cw rf field.\textsuperscript{44} Pulsed methods were subsequently used to determine relaxation times.\textsuperscript{17} Lowe and Norberg\textsuperscript{45} demonstrated that the Fourier transform of a signal obtained in the time domain as a free induction decay (FID) after a pulse was related to the frequency domain spectra as obtained by cw NMR. The advantages of Fourier transform (FT) pulse NMR in multiline spectra were then shown by Ernst and Anderson,\textsuperscript{46} and pulse methods have since become standard in NMR.\textsuperscript{5,8}

In a simple pulsed nmr experiment, an rf pulse of amplitude $B_1$ with frequency close to the resonance or Larmor frequency of the nucleus of interest, is turned on for a short period of time $\tau_w$, usually on the order of 1-50\,\mu s. For an rf field $B_1$ applied along the $x'$ axis in the rotating frame, the magnetization will precess about the $x'$ direction at an angular velocity $\gamma B_1$ during the pulse, with the pulse rotating the magnetization vector $M$ away from the $z$ axis in the rotating frame by an angle $\theta$, where $\theta = \gamma B_1 \tau_w$. The extent to which the magnetization is directed away from the $z$ axis can thus be arbitrarily set by changing either the rf field strength $B_1$, or more readily, the pulse duration $\tau_w$. If $B_1$ and $\tau_w$ are adjusted such that $\gamma B_1 \tau_w = \pi/2$, the magnetization vector will accordingly be rotated
exactly into the $x'$-$y'$ plane. Specifically, when the rf field is applied along the $x'$ axis, the magnetization will lie along the $y'$ direction in the rotating frame. The pulse is thus accordingly termed a 90° pulse. Following the 90° pulse, the component of magnetization in the $x$-$y$ plane precesses about the $z$ direction. A signal will accordingly be observed, and the intensity of the signal will diminish with time as spin-spin relaxation occurs.

As mentioned earlier, the amplitude of the signal is assumed to decay exponentially as a function of time $t$ with a rate $\exp(-t/T_2^*)$. The effects of magnetic field inhomogeneities actually result in a distribution of Larmor frequencies $\omega_o$ so that there will be ensembles of nuclei with the same Larmor frequency called spin isochromats by Hahn. Thus, as these spin isochromats experience slightly different $B_o$ fields, the magnetization vectors will dephase and fan out in the $x'$-$y'$ plane at the end of the pulse, since some nuclei will precess faster or slower than the mean Larmor frequency (frequency of the rotating frame). The vector sum of the spin isochromats is detected by the receiver coil of the spectrometer, and the individual magnetization vectors decay back to their equilibrium position along the $z$ axis by $T_1$ relaxation. Thus, a decay of the signal (FID) with time constant $T_2^*$ is observed.

A third nuclear spin relaxation time also exists under certain circumstances. This is known as the spin-lattice relaxation time in the rotating frame ($T_{1p}$) and in many ways can be thought of as a hybrid relaxation time, with characteristics similar to both the spin-lattice relaxation time $T_1$ and the spin-spin relaxation time $T_2$. Following a 90° pulse, spin-spin or transverse relaxation occurs as mentioned above. If, however, the phase of
the rf is changed by 90° instead of switching it off immediately following the pulse, a different situation occurs. The magnetization becomes aligned parallel to the rf field, such that the magnetization and the $B_1$ field rotate together in the $x$-$y$ plane of the laboratory frame. The magnetization may then be described as being spin-locked. When the rf is applied exactly on resonance, the rf field $B_1$ in the rotating frame becomes analogous to the $B_0$ field in the laboratory frame, with the magnetization experiencing only the $B_1$ field and not the $B_0$ field. As the magnetization prior to the rf pulse was developed in the static field $B_0$ which is normally several orders of magnitude greater than $B_1$, then after the phase shift of the rf field, the magnetization is polarized along the much smaller $B_1$ field such that the magnitude of the magnetization cannot be maintained by the rf field $B_1$. The magnetization will therefore relax or decay with time to an equilibrium value, on the order of $(B_1/B_0)M_0$ with an exponential time constant $T_{1p}$.

$T_{1p}$ can be obtained by measuring the height of the resulting FID or its Fourier transform following the end of the spin-lock period, and repeating the experiment for various lengths of the spin-locking pulse period. In most circumstances for mobile liquids, $T_{1p}=T_1=T_2$, except in the presence of chemical exchange, as previously mentioned. For solids and viscous liquids, $T_{1p}$ may differ considerably from $T_1$ and $T_2$. Since the magnetization experiences only the $B_1$ field, the relaxation rate in the rotating frame will be affected most strongly by motions occurring at the rf or Larmor frequency in the rotating frame, $\omega_1=\gamma B_1$. Thus, $T_{1p}$ is sensitive to ultraslow motion in the kHz region rather than the considerably greater MHz timescale of the laboratory frame.
2.2 Measurement of Spin Relaxation Times $T_1$ and $T_2$

2.2.1 Measurement of the Spin-Lattice Relaxation Time $T_1$

The spin-lattice or longitudinal relaxation time $T_1$ is defined as the time constant for the $z$ magnetization to return to its thermal equilibrium value. Experimental measurements of $T_1$ therefore normally involve monitoring the intensity of the magnetization as a function of time after its displacement from its equilibrium value. Assuming that the relaxation is a first-order exponential process, the intensity of the magnetization recovering from zero to thermal equilibrium is given by

$$M_z = M_o [1 - \exp(-\tau/T_1)]$$

(2.10)

where $\tau$ is the time at which the magnetization $M_z$ is sampled.

While cw methods for measuring $T_1$ were employed in the early days of NMR such methods will not be dealt with here as they have long since been superceded by pulse methods. One of the simplest and most versatile pulse experiments has been used for the measurement of $T_1$. This experiment is known as the inversion-recovery pulse sequence, and was originally proposed by Carr and Purcell. With pulse methods, a second rf pulse is always necessary in order to measure $T_1$ as the magnetization must be detected in the $x$-$y$ plane. Carr and Purcell observed that after the nuclear magnetization reached its equilibrium value $M_o$, the magnetization could be inverted by the application of a $180^o$ pulse, so that it lies along the $-z$ directions, and $M_z = -M_o$. The particular $T_1$ processes begin to return the $z$ component of the magnetization to its original value. After
a delay time $\tau$, a 90° pulse is applied to rotate the magnetization into the equatorial plane for detection, as the spectrometer receiver coil is sensitive only to the component of the magnetization in the $x$-$y$ plane. The time-dependent value of the magnetization $M(\tau)$ follows the relation

$$M(\tau) = M_0 [1 - 2\exp(-\tau/T_\beta)]$$  \hspace{1cm} (2.11)

The original method of Carr and Purcell\textsuperscript{11} for extracting $T_1$ values from inversion-recovery experiments, which is still employed to get an estimate of $T_1$ values, is the "null" method. When the magnetization recovers to equilibrium after being inverted by the $\pi$ pulse, there will be one particular value of $\tau$, designated $\tau_{\text{null}}$, where there is essentially no observed magnetization. The null method simply involves measuring the time $\tau_{\text{null}}$ required for the magnetization to recover half way after being inverted, for which no signal or a null signal intensity is observed. The value of $T_1$ at the null point can be calculated approximately from

$$T_1 = \tau_{\text{null}}/\ln(2)$$  \hspace{1cm} (2.12)

The use of the null method for measuring $T_1$ values with any degree of certainty is not recommended.\textsuperscript{13}

Vold and co-workers\textsuperscript{10} demonstrated the utility of the inversion-recovery experiment coupled with Fourier transformation of the signal for determining the $T_1$'s of individual lines in complex multi-line high resolution spectra. In this version of the inversion-recovery experiment, an FID is accumulated following the 90° pulse, which is
then Fourier transformed and the resulting signal intensity is measured. A delay time $T_D$, which is much longer than the estimated value of $T_1$ (normally on the order of 5-10 times $T_1$), is inserted after the acquisition in order for re-establishment of the equilibrium Boltzmann distribution, and the two-pulse sequence is then repeated. The pulse sequence is thus

$$[180^\circ - \tau - 90^\circ(FID) - T_D]_N$$

where $N$ is the number of variable delays $\tau$ between the $\pi$ and $\pi/2$ pulses. The time-dependence of the magnetization can be written in the form

$$M_z(\tau) - M_o = [M_z(0) - M_o] \exp(-\tau/T_1)$$

(2.13)

where $M_z(\tau)$ is the magnetization observed at the corresponding variable delay time $\tau$, $M_o$ is the equilibrium magnetization, (also written as $M(\infty)$), and $M_z(0)$ is the magnetization, at $\tau=0$. The value of $T_1$ can thus be found by plotting the logarithm of the observed peak height or signal intensity as a function of $\tau$. In a more accurate approach, $T_1$ can be determined from a 2 or 3-parameter non-linear least-squares fit of the $M_z(\tau)$ data to eq.(2.13) using, for example, $M_o$, $T_1$ and $(M_z(0)-M_o)$ as fitted parameters. This method also provides an estimate of the uncertainty in the value of $T_1$. 1251.52

Other pulse experiments may have certain advantages over the standard inversion-recovery experiment in some applications.13 For example, for nuclei with long $T_1$ values, low receptivity, or natural abundance, the inversion-recovery experiment may be too time
consuming. Most of these experiments employ the same overall basic strategy, however, of monitoring the time dependence of the return to equilibrium of the magnetization after initial displacement by an rf pulse. The $\pi/2-\pi/2$ or saturation-recovery sequence\textsuperscript{13}, for example, has only half the dynamic range of the $\pi-\pi/2$ inversion-recovery experiment, but is more efficient than the inversion-recovery method when for the case when $T_2 < T_1$, since it is possible to perform the saturation-recovery sequence with an arbitrarily short value of the inter-pulse delay $\tau$.

Progressive-saturation or steady-state methods have been developed, mainly by Freeman and co-workers\textsuperscript{13,14,53} for the measurement of spin-lattice relaxation times. These steady state techniques essentially involve omitting the second 90° pulse from the saturation-recovery sequence, or using a small delay time $\tau$ between repeated pulses to create an infinite train of $\pi/2$ pulses. Thus, the pulse repetition rate is such that complete spin relaxation does not occur, and a steady-state will occur whereby there are finite transverse components of the magnetization ($M_x^+$ and $M_y^-$) immediately before each pulse, and a partially saturated z component $M_z^-$ which will be less than $M_0$. The resulting intensity of the magnetization will depend on the pulse repetition rate and on the value of $T_1$. The progressive saturation method has been combined with the inversion-recovery experiment by Canet and colleagues\textsuperscript{54} to facilitate or reduce the time involved in measuring long $T_1$ values (such as in $^{13}$C) by the inversion-recovery method.

Techniques which can measure $T_1$ from a single pulse have been developed to dramatically reduce the overall experiment time\textsuperscript{55}, but these methods do not appear to
have gained any significant popularity despite their claims of a marked reduction in experiment time. The basic inversion-recovery experiment is in general a robust method for determining $T_1^{12}$ and thus remains the method of choice.

2.2.2 Measurement of the Spin-Spin Relaxation Time $T_2$

It was mentioned earlier that, in the presence of extreme narrowing due to rapid molecular motion, as in the case of non-viscous liquids, $T_1 = T_2$. Measuring $T_2$ will in theory yield no information appreciably different from that which can be obtained from $T_1$ measurements in such cases, and additional experiments to measure $T_2$ are thus unnecessary. In media such as solids or viscous liquids or in the presence of chemical exchange or diffusion, $T_1$ and $T_2$ will contain different information, so that it becomes worthwhile to measure $T_2$.

The measurement of the spin-spin or transverse relaxation time $T_2$, however, proves to be considerably more difficult than the measurement of $T_1$. Indeed, there exists no robust analogue of the inversion-recovery experiment for determining $T_2$.

Measurements of the linewidth can be used to obtain $T_2$ values as mentioned earlier, but this can be a highly inaccurate procedure. The lineshape may contain a significant contribution from magnetic field inhomogeneities such that the $T_2$ determined from linewidth measurements ($T_2^*$) is actually the sum of the natural transverse magnetization time $T_2$, the intrinsic relaxation time characteristic of the decay of the transverse relaxation in one of the spin isochromats, as well as a term $\gamma A$ attributed to the
applied magnetic field inhomogeneities, or

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

(2.14)

In the situation where the magnetic field inhomogeneity term \(\gamma \Delta\) is large and dominates \(T_2^*\), little useful information can be obtained from linewidth measurements. If magnetic field inhomogeneity can be minimized, then \(T_2^*\) will increase until it is equal to \(T_2\). Values of \(T_2\) can only be measured directly from linewidths with some degree of confidence for large molecules with relatively long correlation times. Thus, other methods are necessary for the determination of accurate \(T_2\) values.

2.2.2 (a) Pulse Spin Echo Methods for Measuring \(T_2\)

In his pioneering work on pulsed NMR, Hahn discovered a method for measuring \(T_2\) when he first observed the existence of spin echoes. The spin echo method could be used to partially circumvent the problem of magnetic field inhomogeneities encountered when attempting to extract \(T_2\) from linewidths. In his initial work with application of a pair of 90° pulses to a sample of water, Hahn observed the phenomenon of spin echoes. When Hahn applied a second \(\pi/2\) pulse at a time \(\tau\) after an initial \(\pi/2\) pulse, he observed that the initial FID appeared to decay away, but after a certain time on the order of \(2\tau\), the signal again grew in intensity until it reached an amplitude similar to that observed after the initial pulse. Hahn concluded that the individual spin isochromats, which dephased after the first 90° pulse, were refocussed by the second pulse. Hahn showed
from the Bloch equations that the amplitude of the spin echo was dependent upon the value of the delay time \( \tau \) between pulses, with the magnetization decaying exponentially as

\[
M(2\tau) = M_0 \exp(-2\tau/T_2)
\]

(2.15)

where \( T_2 \) is the time constant for the decay process. By plotting the logarithm of the echo amplitude as a function of arbitrary values of \( 2\tau \), Hahn was able to directly measure \( T_2 \) values for protons in an aqueous solutions of ferric ions, which were in good agreement with results obtained by Bloembergen and co-workers.\(^{41}\)

The spin echo method developed by Hahn in principle provides an accurate way of measuring \( T_2 \), a viable alternative to the measurement of linewidths. In liquids and gases, however, diffusion processes exist wherein the molecules move to different parts of the sample where precession rates may differ due to magnetic field inhomogeneities. Such diffusion processes result in a loss of coherence at the echo time, the amplitude of the spin echo being attenuated since the nuclei move from one isochromat to another during the refocussing period. Hahn showed\(^{44}\) that the echo amplitude including the effects of diffusion has the form

\[
M(2\tau) = M_0 \exp \left[ -\frac{2\tau}{T_2} - \frac{k(2\tau)^3}{3} \right]
\]

(2.16)
where \( k \) is a constant containing the diffusion coefficient.

Carr and Purcell\(^{11} \) were able to successfully extend the work of Hahn to develop a more reliable method for measuring \( T_2 \) by minimizing the effects of molecular diffusion. Their procedure\(^{11} \) involved the application of a combination of 90° and 180° pulses. A single \( \pi/2 \) pulse was followed by a series of \( \pi \) pulses applied at times \((2n + 1)\tau\) (\( n=0,1,2,\ldots \)), which induces echo maxima halfway between each pair of \( \pi \) pulses. For the Carr-Purcell sequence, the amplitude of the \( n \)-th echo is given by

\[
M = M_0 \exp(-\gamma B_0 \tau) \exp(-\gamma^2 DG^2 \tau^2 / 3) \tag{2.17}
\]

where \( \tau \) is the total time elapsed corresponding to the \( n \)-th echo, \( D \) is the self-diffusion coefficient, and \( G \) is the magnetic field gradient (\( = dB_z/dz \)).

It may be seen from equations (2.16) and (2.17) that with the Carr-Purcell pulse sequence, the effects of diffusion are reduced compared to the standard Hahn echo experiment. With the Carr-Purcell technique, the delay time \( \tau \) can be made small in comparison to the Hahn echo technique, thereby minimizing diffusion effects, and in the limit of fast pulsing, the echo amplitudes will decay with a time constant exclusively given by \( T_2 \). Carr and Purcell demonstrated the effectiveness of their pulse sequence by comparing the \( T_2 \) values of water determined by their method with that obtained by Hahn's two-pulse procedure. Hahn's method gave a value of 0.2 seconds compared to the Carr and Purcell value of 2.0 seconds.\(^{11} \)

Although steps are undertaken to avoid diffusion effects in careful pulse \( T_2 \)
measurements, the fact that spin echoes can be affected by diffusion to a considerable extent can be taken into account and exploited by using spin echo NMR methods to directly measure molecular self-diffusion coefficients.56-58

While the Carr-Purcell spin echo method for measuring $T_2$ was an improvement over the method of Hahn, the Carr-Purcell method proved to be susceptible to certain sources of error which left room for further improvement. A typical Carr-Purcell sequence may contain many $\pi$ pulses, which may be imperfect due to improper calibration or non-uniform rf field throughout the sample. After a certain period of time, these $\pi$ pulse imperfections may result in cumulative effects which may become rather pronounced. For example, the magnetization may not be perfectly refocussed along an axis in the $x$-$y$ plane, but at some angle of deviation such that the echo amplitude may be artificially altered. Meiboom and Gill59 solved the problem of cumulative $\pi$ pulse imperfections by introducing a 90° phase shift between the $\pi/2$ and $\pi$ pulses. With this phase shift, if $B_1$ for the $\pi/2$ pulse is along the $+x'$ axis, then the rf field for the $\pi$ pulse is applied along the $+y'$ axis such that the echoes form along the $y'$ axis regardless of the exact time angle of the $\pi$ pulse, with the result that the error in the 180° pulses does not accumulate, and the experiment needs to start from equilibrium only once. The formation of a spin echo resulting from a combination of 90° and 180° pulses is shown in Fig.2.3

The Carr-Purcell pulse sequence for measuring $T_2$ used in conjunction with the Meiboom-Gill phase shift modification is accordingly known as the CPMG pulse experiment, and has become the standard experiment for measuring accurate values of the
spin-spin transverse relaxation time $T_2$.\textsuperscript{13,14} The CPMG sequence is however still susceptible to several sources of error, and is not the $T_2$ analogue of the inversion-recovery $T_1$ experiment in terms of robustness and simplicity of implementation. By its very nature as a multiple pulse sequence, there are several experimental parameters and corresponding adjustments and optimizations which are critical to proper implementation of the CPMG sequence, including rf phase, baseline fluctuations and off-resonance effects.\textsuperscript{14-16,60,61} For example, Vold and colleagues\textsuperscript{15} in a detailed examination of CPMG experiments have outlined several sources of systematic error which must be considered in order to minimize errors in $T_2$ measurements. These errors arise from the use of inhomogeneous rf and static magnetic fields, imperfections in pulse shape and phase, and diffusion effects. If the aforementioned sources of error are properly addressed, an accuracy and reproducibility on the order of 1-2\% can be attained.\textsuperscript{15} In addition, spin echoes can be modulated by scalar couplings, even those on the order of 0.05 Hz.\textsuperscript{14,62,63}

A time-saving modification of the CPMG experiment for systems with $T_1 > T_2$ has been reported,\textsuperscript{64} which in many ways is analogous to the fast inversion-recovery method for measuring $T_1$ mentioned previously.\textsuperscript{64}
Figure 2.3. The sequence of events leading to the formation of a spin echo after the application of a 90°-180° pulse sequence. \( \tau \) is the time between the 90° and 180° pulses, and \( t_w \) is the duration of the rf pulse. The spin echo appears at time \( 2\tau \) as depicted in step G of the pulse schematic.
2.2.2 (b) Steady State Methods for Measuring $T_2$

While the difficulties inherent in the measurement of $T_2$ by spin-echo methods have been outlined, the CPMG experiment appears to be the standard method for measuring relatively accurate $T_2$ values,\textsuperscript{13,14} with few viable alternative experiments available. Steady state methods for measuring $T_1$ have been mentioned above, and techniques for measuring $T_2$ based upon the achievement of a steady state of the magnetization have also been reported.\textsuperscript{35,65} A method for measuring $T_2$ based on steady-state free precession (ssfp) has been discussed by Matsui and co-workers\textsuperscript{65}. In this technique, a comparison of steady-state signals immediately prior to and immediately after the application of an rf pulse gives a value of $T_2$, providing $T_1$ is several times longer than $T_2$. The steady-state free precession method is purportedly impervious to rf inhomogeneity effects when the pulse interval is considerably smaller than the spin-lattice relaxation time $T_1$. Although the ssfp method appears to have certain advantages, particularly in the field of NMR imaging, the technique does have limitations especially in terms of applications, as $T_1$ must be several times greater than $T_2$ for successful operation, and as such does not appear to have undergone thorough application.

An alternative method for measuring $T_2$ was reported several years ago in a communication by Bain, Martin and Ho.\textsuperscript{35} This method is also based on the formation of a steady state in the spin system by partial saturation, with subsequent interpretation of the data obtained from the Bloch equations in the presence of saturation. This experiment is discussed in more detail in the following section.
2.2.2 (c) The Steady State Offset-Saturation Experiment

The original method proposed by Bain and co-workers,\textsuperscript{35} or with modifications to the technique which will be discussed shortly, has been called the offset-saturation experiment.\textsuperscript{36} The original form of the experiment\textsuperscript{35} involves saturation of the resonance of a single spin system without coupling, at some offset from resonance ($\omega_0 - \omega$) with an rf field provided by the homodecoupler, for a time period on the order of $5T_1$ to allow the formation of a steady state. The irradiation is then gated off and a 90° observation pulse applied. The FID is acquired and Fourier transformed to give a spectrum which provides a measure of the partially saturated $z$ magnetization. The experiment is repeated for several different values of the irradiation offset, and the value of $T_2$ can be obtained by analysis of the curve of $M_z$ as a function of irradiation offset by considering the Bloch equations\textsuperscript{17} in the presence of a saturating rf field $B_2$ which is generally several orders of magnitude larger than the observing field $B_1$.

In consideration of the presence of the saturating rf field $B_2$ in eq.(2.7c), which gives the $z$ magnetization for a steady state, then if the saturation term $(\gamma B_2)^2 T_1 T_2$ is considerably greater than unity, this term will become dominant and eq.(2.7c) can accordingly be modified to give

$$M_z = M_0 \frac{(\omega_0 - \omega)^2}{(\omega_0 - \omega)^2 + (\gamma B_2)^2 T_1 T_2} \quad \text{(2.18)}$$
It can be shown from eq.(2.18) that the half-width at half-height of the curve of $M_z$ as a function of irradiation resonance offset ($\omega_o - \omega$) is equal to $(\gamma B_2 (T_1/T_2)^{1/2})$. This function has the form shown in Fig.2.4, as shown for an arbitrary set of parameters. By calibrating the strength of the irradiating field $B_2$ and measuring $T_1$ by, for example, an inversion recovery experiment on the same sample under identical conditions, a value of $T_1$ can be obtained. Significantly, provided the value of $B_2$ is sufficiently large, the $T_2$ will be the true spin-spin relaxation time $T_2$, independent of magnetic field inhomogeneities. Using this novel method for measuring $T_2$, Bain et al obtained a $T_1/T_2$ ratio for the proton in a degassed sample of chloroform which compared favourably with a $T_1/T_2$ ratio obtained for the same sample with a $T_1$ value determined using an inversion recovery experiment, and $T_2$ measured by the CPMG pulse experiment.

Experimental pulse schemes similar to the offset-saturation method were employed by James and co-workers who performed off-resonance $T_{1p}$ experiments to study intermediate molecular motions in protein systems and inorganic compounds from low-frequency relaxation time measurements. In these particular experiments, an rf field $B_1$ of approximately 1 G is applied off resonance for a period of time on the order of $5T_1$ so that the spin system is equilibrated along the effective field $B_{\text{eff}}$, where $\gamma B_{\text{eff}} = ((\gamma B_1)^2 + (\omega_o - \omega)^2)^{1/2}$. The rf field is then switched off and the spin system subjected to a homospoil gradient pulse in order to destroy any $x$-$y$ magnetization, and a nonselective 90° observation pulse is applied at the resonance frequency. From the signal intensity of
the resulting Fourier transform spectrum of the FID, and measurement of the spin-lattice relaxation time $T_1$, the desired motional information can be obtained. Caines and Schleich\cite{caines} have recently combined the effects of off-resonance $T_{1\rho}$ measurements and magnetization transfer in analytical expressions from modified Bloch equations, and applied their method to the study of phosphorous metabolites.\cite{schleich}

The groups of Bryant\cite{bryant1,bryant2,bryant3} and Balaban\cite{balaban1,balaban2,balaban3} and others\cite{other} have also used off-resonance irradiation pulse sequences similar to that of the offset-saturation experiment to study cross-relaxation or magnetization transfer contrast effects in heterogeneous biological systems. The term "Z spectroscopy" has been coined by Bryant to describe such experiments which measure the z component of the magnetization.\cite{bryant} In these studies, two separate experiments are normally necessary to determine the relaxation times $T_1$ and $T_2$, typically by inversion-recovery and CPMG experiments respectively. Some approximate use of the original method of Bain and colleagues\cite{bain} to extract the $T_2$ from the width of the $M_z$ curve as a function of irradiation offset through use of the Bloch equations has been attempted.\cite{bryant,bryant2,bryant3}
Figure 2.4. $M_2$ as a function of irradiation resonance offset for a hypothetical offset-saturation experiment. Data were calculated using eq.(2.18) with an arbitrary set of parameters for $T_1/T_2=1$ and $M_0=1$. The irradiation resonance offset values are dimensionless, scaled by the factor $\gamma B_2 (T_1/T_2)^{1/2} = 1$. The half-width at half-maximum height is equal to the quantity $\gamma B_2 (T_1/T_2)^{1/2}$, as indicated on the plot.
2.2.3 Methods for the Simultaneous Measurement of $T_1$ and $T_2$

The importance of the $T_1/T_2$ ratio in studies of molecular motions or dynamics has been mentioned in earlier sections of this thesis. Motivated largely by attempts to reduce the need to perform two or more separate experiments to determine $T_1$ and $T_2$ independently, and to reduce overall total experiment time, several NMR methods have been developed to simultaneously measure both $T_1$ and $T_2$ either absolutely or in their ratio$^{18,19,21,22}$. These techniques may be rather difficult to implement, requiring special hardware considerations, or may involve complicated analysis of the data. Thus, these methods appear to have seen very limited application.

Cosgrove$^{18}$ has reported an interesting spin-echo method to determine both $T_1$ and $T_2$ for single lines in a single scan, in cases where $T_2$ is greater than 10 msec and spin-echo formation is not prohibited. The technique proposed by Cosgrove is essentially a combination of inversion-recovery and CPMG experiments, measuring $T_1$ by sampling and $T_2$ by the CPMG method to measure the complete relaxation decay of single lines. Unlike conventional pulse experiments for measuring relaxation times, there is no need for waiting $5T_1$ before repeating this experiment, as the sequence returns the system to thermal equilibrium. The success of this particular method is dependent on good control of pulse parameters and on the requirement of $B_1$ homogeneity.$^{18}$

The use of steady-state techniques to determine relaxation times has been mentioned earlier. Kronenbitter and Schwenk$^{19}$ have described pulse methods, which are based on the buildup of steady-state magnetization, for measuring the $T_1/T_2$ ratio in
systems where $T_1, T_2 > T_2^*$. They demonstrated the dependence of the steady-state free precession signal amplitude on rf pulse flip angle between regularly repeated pulses spaced by $\tau$, where $\tau < T_1, T_2$. Analysis of the dependence of signal amplitude on pulse flip angle by steady-state treatment of the Bloch equations data gives the $T_1/T_2$ ratio without special modifications to a conventional FT spectrometer. In a complementary experiment requiring considerable memory capacity on the computer of the NMR spectrometer, the exponential buildup to the steady state is monitored. This buildup has a time constant $T^*=(T_1 + T_2)/2$, and by combining both experiments, values for $T_1$ and $T_2$ can be determined. These techniques are somewhat hindered by inhomogeneity of static and rf magnetic fields, but precautions can be taken to avoid such systematic errors.19

Certain multiple-pulse sequences can be used to obtain hybrid relaxation times, which are some combination of $T_1$ and $T_2$.22,23 These hybrid experiments also take advantage of the steady state, with the fundamental tenet being that there is no need to allow for the complete decay of magnetization in the x-y plane when the FID acquisition time is much shorter than the $T_2$ of the system under study. The complete recovery can be sampled in one scan by repetition of the experiment, analogous to the single-scan spin echo method reported by Cosgrove.18 The magnetization decay rates will be independent of the initial and final states if the total magnetization is driven from an initial value to a final steady state value by a repeated sequence of pulses.

Hybrid experiments commonly employ triplet or quadruplet repeated pulse sequences.22,23 A typical example is a repeated $90^\circ-(-180)^\circ-90^\circ$ triplet sequence. For a
simple one-component system, the magnetization in the single-scan recovery hybrid experiment can be described by a simple exponential decay as

\[ M_s(0) - M_w(0) = [M_o(0) - M_w(0)] \exp(-n\tau/T_{12}) \]  

which yields a hybrid relaxation time \( T_{12} \) as the time constant for the decay, which is the weighted average of \( R_1 (=T_1^{-1}) \) and \( R_2 (=T_2^{-1}) \) or

\[ T_{12}^{-1} = pR_2 + qR_1 \]  

where \( p \) and \( q \) are the fractions of time spent in the \( x-y \) plane and the \( z \) direction respectively. Hybrid experiments are quite susceptible to errors in pulse width calibrations and phasing, as with many multiple-pulse methods, and to inhomogeneous static and rf magnetic fields.  

An interesting method capable of measuring both \( T_1 \) and \( T_2 \) in a single rapid experiment has recently been reported by Moore and Metz. This particular technique is rather unique in that is a time-domain method. It can be considered an off-resonance adaptation of the transient nutation experiment, or an off-resonance spin-locking experiment. The relaxation times reported for a variety of simple samples with single-line and two-line spectra were reported to be in good agreement with those obtained by inversion-recovery and CPMG experiments for \( T_1 \) and \( T_2 \) respectively. As the method reported by Moore and Metz does not utilize FT capabilities, it is not generally applicable to multline spectra, and in situations where \( T_1 \) is considerably greater than \( T_2 \), only the latter can be obtained. This method also appears to be subject to errors from static and
rf field inhomogeneities.

A two-dimensional (2D) NMR method has also been reported for the simultaneous measurement of \( T_1 \) and \( T_2 \).\(^{20}\) As with most 2D methods, the need for considerable computer memory as well as experiment time for the acquisition and processing of the data is required, limiting the overall utility of this method.

It has been noted in the previous section that the offset-saturation technique for measuring the spin-lattice relaxation time \( T_1 \) simultaneously determines the ratio \( T_1/T_2 \) from the half-width \( \gamma B_2(T_1/T_2)^{1/2} \) of a plot of the z magnetization as a function of irradiation resonance offset. In a modification of the basic offset-saturation experimental procedure,\(^{35,36}\) absolute values for \( T_1 \) and \( T_2 \) can be measured directly and simultaneously. The insertion of a delay time \( \tau \) which is some fraction of the spin-lattice relaxation time \( T_1 \) after the irradiation is gated off and prior to the 90° observation pulse will allow the z magnetization to partially relax. The minimum intensity in the plot of observed intensity or z magnetization as a function of irradiation offset will then be at some non-zero minimum value. The fact that the z magnetization is partially relaxed can be used to determine the \( T_1 \) by a slight modification to the steady-state solution to the Bloch equations for \( M_z \). The resulting equation for the curve of z magnetization plotted as a function of decoupler irradiation offset, as derived from eq.(2.18), is given by the expression
\[ M_z = M_0 \left( 1 - \frac{(\gamma B_2)^2 T_1/T_2}{(\omega - \omega_0)^2 + (\gamma B_2)^2 T_1/T_2} \right) e^{-\omega T_1} \]  

(2.21)

where all the terms have previously been defined.

The absolute values of T1 and T2 can then be obtained by, for example, non-linear least-squares techniques.\textsuperscript{12,80-83} For a set of offsets and corresponding measured intensities, a non-linear least-squares fit can be performed to eq.(2.21) using the values for the pre-acquisition delay \( \tau \) and the irradiating field \( B_2 \). Absolute values for T1 and T2 may accordingly be obtained simultaneously from a single offset-saturation experiment.\textsuperscript{36} This modified form of the offset-saturation experiment should prove to be a robust and versatile method for determining both T1 and T2 from a single experiment. No special hardware or software considerations are required, as the experiment can be performed on any standard high-resolution NMR spectrometer which is equipped for homodecoupling.
CHAPTER 3

CHEMICAL EXCHANGE AND NMR SPECTROSCOPY

3.1 Introduction

This chapter provides a basic review of the study of chemical exchange by NMR spectroscopy. The effects of chemical exchange on NMR spectra are given by the Bloch equations with incorporation of chemical exchange for a two-site exchanging system, and the various common methodologies for obtaining exchange rate constants over the slow, intermediate, and fast exchange timescales are discussed. The fast exchange regime in particular is characterized by the lack of a suitably convenient method for obtaining rapid rates of exchange.

Many molecules or chemical systems exhibit fluxionality, whereby a molecule changes conformations, or a particular species transfers from one molecule to another in a reaction between two or more species. Chemical exchange may be defined as the exchange of a nucleus between two or more chemically distinct environments in which an NMR-active nucleus precesses at a different Larmor frequency in each of the sites. The exchange mechanism is a time dependent process which may therefore influence the shapes and widths of the NMR signals. The lifetime of the nucleus in a given site is the inverse of the rate constant $k$ when the exchange is unimolecular and of first order. From NMR studies of exchanging systems, it is possible to determine rate constants and
thermodynamic parameters for the exchange processes which are present in many chemical systems.

The study of chemical exchange or dynamic NMR (DNMR) has often provided the impetus for innovations in NMR techniques. New developments in NMR methodology are often promptly applied to DNMR problems. The earliest continuous-wave NMR methods, pulse and Fourier-transform methods and multi-dimensional experiments as well as advents in computer and spectrometer technologies have all been incorporated into NMR studies of chemical exchange. An extensive literature of both fundamental and applied topics has now been compiled, and numerous books and review articles have been written on the topic of chemical exchange in its 40 years of existence,\textsuperscript{24,26,54,69} while at least a single chapter is devoted to the subject in most texts on NMR spectroscopy.

The examination of chemical exchange kinetics by NMR methods has several advantages over the use of other kinetic techniques. DNMR methods can encompass timescales over several orders of magnitude, with rate constants measurable in the range from about $10^1$ to $10^5$ or $10^6$ s\(^{-1}\). NMR is in general a non-destructive technique, and has the unique advantage of giving direct information about the specific part or parts of a molecule involved in the exchange process, which cannot readily be achieved by the majority of other techniques for kinetic measurement. DNMR studies are also in general performed on systems in thermodynamic equilibrium, with the advantage that degenerate systems in which the exchange process renders the species in different sites chemically indistinguishable from one another can be studied by DNMR techniques.\textsuperscript{24,26,54}
The theory of nuclear transfer effects in NMR was originally developed by Gutowsky, McCall and Slichter⁹⁰ who observed the interruption of spin-spin coupling in the \(^1\text{H}, \text{ }^{19}\text{F}\text{ and }^{31}\text{P}\) spectra of inorganic phosphorous and boron compounds in the liquid state, and surmized that a nuclear exchange process was occurring. They accordingly modified the Bloch equations to include the effects of chemical exchange between two equally populated sites. The desired kinetic information in the majority of DNMR studies has been obtained from an analysis exchange-broadened spectra using a lineshape or bandshape analysis.²⁴⁻²⁶,⁸⁹

The first detailed application of lineshape analysis to an uncoupled exchanging system was the classic study of Gutowsky and Holm²⁷ for the hindered rotation hindered C-N bond rotation of the methyl groups in the simple amides \(N,N\)-dimethylformamide (Fig.3.1) and \(N,N\)-dimethylacetamide. Gutowsky and Holm²⁷ observed that at low temperatures, a symmetrical doublet was observed in the proton spectra, with one resonance for each of the \(N\)-methyl groups. As the temperature was raised, each component of the doublet broadened until the spectra appeared to coalesce, and above the point of coalescence, a single resonance was observed which sharpened or decreased in linewidth as the temperature increased.

Subsequent to the first theoretical descriptions of exchange undertaken by Gutowsky and colleagues,²⁷,⁸⁰,⁹¹ other formalisms considered the exchange between unequally populated sites,⁹² and also the effects of spin-spin coupling with exchange⁹³.
Hahn and Maxwell\textsuperscript{93} and McConnell\textsuperscript{92} simultaneously generalized the modified Bloch equations into a more useful formalism, directly incorporating exchange into the Bloch equations. McConnell additionally considered the case of a nucleus being transferred between two sites having different relaxation properties.\textsuperscript{92}

As chemical exchange is merely a special case of general relaxation theories, it may be comprehensively treated by quantum mechanical formulations. The exchange theory of nuclear spins in magnetic resonance in quantum mechanical terms has been developed, based largely on the early work of Kaplan\textsuperscript{94} and Alexander,\textsuperscript{95,96} who derived a complete solution for the lineshape of the steady state spectrum using density matrix techniques. Further extensions of the quantum mechanical formulation of exchange using Liouville-space superoperator techniques have been made.\textsuperscript{25,89,97} Sophisticated quantum mechanical formalisms of exchange have since been refined and form the basis for computer programs for which generate lineshapes to fit NMR spectra with chemical exchange.\textsuperscript{24,25,28,89,98,99} These theories will not be discussed here but are significant for their
accurate description of exchanging lineshapes, especially for complicated fluxional systems such as those in the presence of scalar coupling.\textsuperscript{24-26,89}

The NMR chemical exchange timescale may be considered to consist of three distinct regions; namely, the slow exchange, intermediate exchange, and fast exchange regimes. These timescales are determined by the ratio of the exchange rate to, in the simplest context of exchange between two sites, the chemical shift separation between the two distinct sites in the absence of exchange. A detailed discussion of the current state of DNMR techniques and accompanying theories would be exhaustive, but an abbreviated overview of the characteristic NMR spectra exhibited in each of the three distinct regions of exchange, and the major techniques which have been applied in each of the three cases, will be presented together with a description of the lineshapes encountered in a two-site exchange process. The abbreviated description of the calculation exchange lineshapes described herein will follow the earliest formalisms based on the Bloch equations\textsuperscript{37,50-92} as they are an extension of the phenomenological description of NMR presented previously. Such a formulation serves sufficiently to describe a typical exchange process.
3.2 The Bloch Equations With Incorporation of Chemical Exchange

The modification of the Bloch equations for treatment of chemical exchange\(^{24,27,90-92}\) between two uncoupled sites A and B for the two-site exchange process A ⇄ B with spin-1/2 nuclei commences by considering that for each distinct site or environment there is a complex resultant moment of magnetization

\[
G_n = u_n + iv_n
\]  

(3.1)

where \(n\) is A or B, and the imaginary component of the magnetization is proportional to the absorption mode signal. The Bloch equations (2.6a) and (2.6b) can accordingly be rearranged to accommodate the two Larmor precession frequencies in site A and site B, \((\omega_0 + \delta \omega/2)\) and \((\omega_0 - \delta \omega/2)\) respectively, where \(\delta \omega = \omega_A - \omega_B\) is the chemical shift separation between the two sites A and B, to obtain two equations, one for each site, which can be written as

\[
\frac{dG_A}{dt} + \left[ \frac{1}{T_{2A}} - i(\Delta \omega + \delta \omega/2) \right] G_A = -i\omega_1 M_0
\]  

(3.2a)

\[
\frac{dG_B}{dt} + \left[ \frac{1}{T_{2B}} - i(\Delta \omega - \delta \omega/2) \right] G_B = -i\omega_1 M_0
\]  

(3.2b)
The two equations for site A and site B can now be modified for exchange by inclusion of defined lifetime parameters \( \tau_A \) and \( \tau_B \), which represent the lifetimes for nuclei in sites A and B, and are the reciprocals of the first-order rate constants for the transfer of the nuclei out of each of the respective sites (\( k_A = 1/\tau_A \), \( k_B = 1/\tau_B \)). By considering that \( \tau_A^{-1} \) is the probability per unit time of a nucleus flipping from A to B, \( \tau_A^{-1} G_A \) will be the rate of decrease of \( G_A \) due to the transfer \( A \rightarrow B \). The additional assumptions are made that the time spent between sites is considered negligible compared to the lifetime in a particular site, \( \tau_A \) and \( \tau_B \), and that the natural characteristic relaxation times \( T_1 \) and \( T_2 \) for nuclei in sites A and B are independent of the lifetime \( \tau_A \) or \( \tau_B \) in each site. It is also assumed that the transverse relaxation time for each site in the absence of exchange are equal, or \( T_{2A} = T_{2B} = T_2 \), although McConnell has analytically treated the case when this equality is not assumed.\(^{92}\)

The terminology of the exchange-modified Bloch equations can be somewhat simplified by introducing the symbol \( \alpha \) to replace the constant multiplying \( G \), which includes the \( T_2^{-1} \) term, in equations (3.2a) and (3.2b) above. When exchange is occurring, it is necessary to take account of the time-dependent first order depopulation of those nuclei from each site with initial magnetizations \( G_A^0 \) and \( G_B^0 \) which are the values of \( G_A \) and \( G_B \) respectively at time \( t=0 \). The two modified equations thus become
\[
\frac{dG_B}{dt} + \alpha_B G_B = -i\gamma B_1 M_{ob} + \tau_A^{-1} G_A - \tau_B^{-1} G_B
\]  

(3.3b)

where \(M_{oA}\) and \(M_{ob}\) are the equilibrium magnetizations for site A and B respectively.

Equations (3.3a) and (3.3b) can be solved by equating the time derivatives to zero as in the normal Bloch equations, and solving for \(G_A\) and \(G_B\). McConnell\(^{100}\) has considered the case when the steady-state solution does not apply. From the solutions for \(G_A\) and \(G_B\), a total complex magnetic moment \(G\) may be defined, where

\[
G = G_A + G_B
\]

\[
= -i \gamma B_1 M_o \frac{\tau_A + \tau_B + \tau_A \tau_B (\alpha_A p_A + \alpha_B p_B)}{(1 + \alpha_A \tau_A)(1 + \alpha_B \tau_B) - 1}
\]  

(3.4)

The total or observable equilibrium magnetization \(M_o\) is related to the individual site equilibrium magnetizations through the relations

\[
M_{oA} = p_A M_o; \quad M_{oB} = p_B M_o
\]  

(3.5)

where \(p_A\) and \(p_B\) are the fractional populations of the sites such that \(p_A + p_B = 1\), which are related to the individual site lifetimes \(\tau_A\) and \(\tau_B\) through considerations of detailed balancing for simple two-site exchange by

\[
p_A = \frac{\tau_A}{\tau_A + \tau_B}; \quad p_B = \frac{\tau_B}{\tau_A + \tau_B}
\]  

(3.6)
Since the $V$ component of the total complex magnetic moment $G$ is proportional to the absorption intensity, the exchange modified signal is given by the imaginary part of $G$ in eq.(3.4). It is therefore necessary to extract the imaginary part of $G$, since $\alpha_A$ and $\alpha_B$ contain imaginary terms, in order to describe the absorption mode signal, and a complicated expression results. Simplifications to eq.(3.4) are possible, however, primarily for the two limiting cases when the lifetimes $\tau_A$ and $\tau_B$ are long and short, or in the regions of slow and fast exchange respectively.

A series of NMR lineshapes for a two-site equally-populated exchanging system characteristic of the varying relationship between exchange lifetime and chemical shift difference is shown in Fig.3.2.

3.2.1 Slow Exchange Regime

In the slow exchange region, the rate of exchange is generally considerably smaller than the chemical shift separation $\delta \omega$ between the exchanging sites. In terms of a timescale, the rate constants are normally on the order of $10^{-1} - 10^{2}$ s$^{-1}$. If the lifetimes $\tau_A$ and $\tau_B$ are sufficiently large compared with the inverse of the separation of the exchanging sites, the resulting NMR spectrum will consist of distinct signals for each site, $\omega_A=(\omega_0+\delta \omega/2)$ and $\omega_B=(\omega_0-\delta \omega/2)$, where $\omega_0$ is the weighted mean frequency of the two resonances. Thus, the variation in linewidth of separated resonances may be studied in the slow exchange limit. At low temperatures, however, the rates may become too slow to cause appreciable line broadening, but are comparable to spin-lattice relaxation rates.
Figure 3.2. Hypothetical exchange lineshapes calculated for a two-site equally-populated system at various exchange rates. The relationship between the exchange lifetime \( \tau \) and the chemical shift separation between sites, \( (\omega_A - \omega_B) \) for various characteristic spectra in the fast, intermediate, and slow exchange regimes is given below each spectrum.
The solution to equation (3.4) for the computation of lineshapes in the slow exchange limit can be simplified by effectively considering the component of the signal for each site separately. For example, if the radiofrequency is near $\omega_A$, and accordingly far from $\omega_B$, then $G_B$ in eq.(3.4) will be effectively zero. Thus, the solution is the imaginary part of $G$ near resonance frequency $\omega_A$, or

$$G = G_A = -i\gamma B_1 M_a \left( \frac{p_A \tau_A}{1 + \alpha_A \tau_A} \right)$$

(3.7)

The imaginary part is

$$V = -\gamma B_1 M_a p_A \left[ \frac{T'_{2A}}{1 + (T'_{2A})^2(\omega_A - \omega_0)^2} \right]$$

(3.8)

which gives a signal centered at $\omega_A$ with linewidth which may be given by the introduction of a new relaxation parameter $T'_{2A}$, which includes the exchange contribution to the overall linewidth, where

$$(T'_{2A})^{-1} = (T_{2A})^{-1} + \tau_A^{-1}$$

(3.9)
with $T'_{2A}$ the spin-spin relaxation time in site A and $\tau_A$ the lifetime in site A as previously defined.

The formulation presented above shows that the exchange process leads to an additional broadening of the individual signals. The line will thus be Lorentzian in shape with a width $W = 2/T_{2A}$ in radians per second at half height. If $T_{2A}$ is known, and providing the exchange broadening is not large enough to result in overlap of the signals, then measurement of the widths of the exchange broadened signals provides a means of estimating $\tau_A$ and thus the rate constant $k$. The value of $T_{2A}$ can be determined at lower temperatures or in cases where measurement of the natural linewidth is limited by magnetic field inhomogeneity, the linewidth from a sharp signal from an included sample such as tetramethyldisilane may be used as a linewidth reference standard. Similarly, there will be a corresponding signal centered about $\omega_b$. It may be observed that eq.(3.8) is analogous to the normal $V$ mode solution obtained for the unmodified Bloch equations, the bracketed term being formally identical with the ordinary $v$ mode solution as in eq.(2.7b) with the simplification $\omega_1^2 T_1 T_2 \ll 1$. An example of lineshapes in the slow exchange regime for a two-site equally populated ($p_A = p_B = 0.5$) is shown in Fig.3.2(a).

The fact that the exchange rates in the slow exchange region may be of the same order as spin-lattice relaxation times has been exploited. Double-resonance techniques and generalizations of the inversion-recovery experiment have been used to study slow chemical exchange. Hoffman and Forsen\textsuperscript{101-104} introduced a method which proved to be capable of extending the range of rate processes not accessible by conventional single
resonance techniques, particularly for exchange lifetimes in the range 1-10 s. is a transient or time-dependent NMR method. Their technique was a transient or time-dependent double-resonance saturation-transfer method which could be implemented on continuous-wave NMR spectrometers. Essentially this method involves a study of the slow exchange limit for two sites A and B by strongly irradiating and subsequently saturating the B resonance with a second (decoupling) rf field while the observing the A resonance with the rf field B. The time dependence of the z magnetization in site A, M$_{zA}$, is accordingly given by the Bloch equation for M$_{z}$ incorporating chemical exchange, from which the lifetimes or exchange rates can ultimately be determined.

One application of the saturation transfer method is the classic study of the ring inversion in deuterated (d$_{11}$) cyclohexane by Anet and Bourne, who combined computerized lineshape fits for determining rates in the fast and intermediate exchange regimes with saturation transfer experiments to obtain rate constants over a range of several orders of magnitude. Excellent correlation of results was observed for the combination of methods.

The advent of pulse FT NMR spectroscopy and the relative ease with which spin-lattice relaxation time measurements can be performed on modern spectrometers has seen an increased use of pulse FT techniques analogous to the saturation transfer method for the study of slow chemical exchange. Many related techniques have been developed, including two-dimensional NMR experiments in which cross peaks arise in sites that are in slow chemical exchange, thus creating qualitative maps of exchange pathways which
are useful in complicated multisite exchange processes.\textsuperscript{106-108} Generalizations of the inversion-recovery experiment for $T_1$ measurement\textsuperscript{109-111} have proven to be particularly suitable for studying slow exchange processes, as modern pulse methods permit facile and versatile manipulation and subsequent observation of a spin system undergoing exchange.

Selective inversion experiments in particular exploit the advantage of pulse FT NMR and have proven to be of extreme utility in the determination of rates in systems undergoing slow exchange, largely supplanting the classic saturation-transfer method. Selective inversion experiments for the determination of slow exchange rates are analogous to the saturation-transfer method in that they involve selective manipulation of one of the exchanging sites, while observing the subsequent effect on the second site as a function of time.\textsuperscript{109-111} The presence of chemical exchange provides an alternative route to normal spin relaxation processes which a spin system undergoes if perturbed at the start of an experiment, and the rate of relaxation will depend on both the exchange rate $k$ and the relaxation rate ($T_1$)$^{-1}$. The initial non-equilibrium state largely determines the relative contributions of exchange and normal spin-lattice relaxation to the re-establishment of equilibrium. If the magnetizations in both sites are inverted, the return to equilibrium will be dominated by spin relaxation providing the relaxation rates for both sites are similar. For a simple two-site system undergoing slow exchange, the application of a non-selective inversion pulse sequence such as the common $180^\circ - \tau - 90^\circ$ experiment results in the inversion of the initial $z$ magnetization of both sites after the $\pi$ pulse, which will then return to equilibrium along the $z$ axis at a rate determined by both the relaxation
rate \((T_1)^{-1}\) and the exchange rate, \(k\).

For a selective inversion recovery experiment in a two-site exchanging system, the initial conditions differ for the two sites upon the initial manipulation of the spin system, unlike the case in a standard or non-selective inversion recovery experiment. Relaxation for both sites can occur through both spin-lattice relaxation mechanisms and chemical exchange with the other site. For a two-site system where site B is inverted and the time-dependence of the magnetization in site A is monitored, the initial conditions for A and B will be \(M_A(0) = M_{0A}\) and \(M_B(0) = -M_{0B}\), where \(M_i(0)\) is the magnetization at time \(t=0\) for \(i=\)A or B, and \(M_{0i}\) where \(i=\)A or B is the equilibrium magnetization. The return to equilibrium for the magnetization in site A can be solved analytically\(^{112}\) as given by eq.(3.10) where

\[
M_o-M_A(t) = \frac{1}{2} \left[ e^{-r \cdot 2k t} + e^{-r} \right] [ M_o-M_A(0) ]
+ \frac{1}{2} \left[ -e^{-(r \cdot 2k)t} + e^{-r} \right] [ M_o-M_B(0) ]
\]  

In eq.(3.10), \(M_A(t)\) and \(M_B(t)\) are the magnetizations at time \(t\) for sites A and B respectively, \(M_o\) is the equilibrium value of the magnetization in either site, \(r\) is the spin-lattice relaxation rate \((=1/T_1)\) for the two sites, which are assumed to be equal, and \(k\) is the exchange rate. The exchange rate \(k\) can be determined from eq.(3.10) by standard non-linear least-squares fitting methods, from which reasonable estimates of the errors in the rates can be determined.\(^{110,111}\)
The initial states for the spin system in a selective-inversion experiment can be created using various selective excitation pulse sequences such as the DANTE experiment\textsuperscript{113,114} or a form of the $\pi/2 - \tau - \pi/2$ sequence,\textsuperscript{111,115} which is similar to the NOESY experiment and offers the advantage of offering two parameters, namely the rf transmitter offset and the delay time $\tau$, which can be varied independently to create a variety of initial selective excitation conditions, which is particularly useful in the study of multi-site exchanging systems.\textsuperscript{111} The initial $\pi$ pulse in the standard non-selective inversion-recovery experiment can be replaced by a 90° - $t_d$ - 90° pulse sequence for the selective excitation or preparation of the spin system,\textsuperscript{111} followed by a 90° observation pulse, for a pulse sequence 90° - $t_d$ - 90° - $\tau$ - 90°. The delay $t_d$ between the initial $\pi/2$ pulses is set fixed to half the inverse frequency difference between the two sites to allow the signals to get 180° out of phase, while the following 90° pulse flips the magnetization back along the +z and -z directions. After the variable delay time $\tau$ the final $\pi/2$ pulse of the sequence allows detection of the signal.

3.2.2 Intermediate Exchange Regime

In the intermediate exchange regime for two sites, the rate constants are comparable to the difference in Larmor frequency between the two sites ($\omega_A-\omega_B$), and are generally in the range of 10 - 10⁴ s⁻¹. In this region, the spectra may be observed as broad overlapping peaks which coalesce to a broad singlet at higher temperatures or faster exchange rates, as shown in Fig.3.2(c),(d). It is difficult to make approximations in the
lineshape equations to obtain a simple closed form of the exchange equations for evaluating the lifetimes $\tau_a$ and $\tau_b$ in the intermediate exchange region. Early procedures attempted for determining intermediate exchange rates using closed form solutions of the lineshape equation proved very susceptible to systematic errors and thus no longer find use\textsuperscript{24,54}. The most accurate method for obtaining rates in the intermediate region and which is applicable over the largest range of rates is the generation of the lineshape and direct comparison with experiment,\textsuperscript{24-26,116} calculating the imaginary part of $G$ in eq. (3.4) to compute a lineshape which can be compared with experiment to determine the lifetimes. Such comparisons of observed and calculated spectra are accomplished most preferably by a computerized complete lineshape fitting method using a least-squares comparison of the observed and computed normalized absorption intensities over an appropriate frequency range at suitable frequency intervals.\textsuperscript{24,28,29,54} Reeves and Shaw,\textsuperscript{29} in a formulation of the Bloch equations modified for chemical exchange, showed that for two sites, the lineshapes even at coalescence can be described by two Lorentzian components.

One important relationship which has survived from the earliest days of DNMR\textsuperscript{27} and which is still used to estimate exchange rates in the intermediate regime is the relationship between the exchange rate $k_c$ which is the rate that just results in coalescence of the A and B signals into a broad, single, flat-topped absorption, as in Fig.3.2(d). From differentiation of the lineshape equation, it is possible to obtain the simple expression\textsuperscript{27} which is applicable only for equally populated systems, and when the signal separation
\[ k_c = \pi (v_A - v_B) / \sqrt{2} \]  

\( (v_A - v_B) \) in Hz is large compared with the linewidths at half-height for A and B. The accurate use of eq.(3.11) to obtain rate constants and values of the corresponding thermodynamic activation parameters at the coalescence temperature depends largely on the ability to adequately control the temperature and obtain the coalescence point accurately.\textsuperscript{24,84}

3.2.3 Fast Exchange Regime

In the region of fast exchange, the lifetimes \( \tau_A \) and \( \tau_B \) are small such that \( k > (\omega_A - \omega_B) \), and the spectrum for a two-site exchanging system is essentially a single averaged line, broadened primarily by the exchange process, as shown in Fig.3.2(f). Rate constants in the fast exchange regime are generally on the order of \( 10^2 - 10^6 \) \( \text{s}^{-1} \). As the rate increases in the fast exchange regime, the line will decrease in half width until the value \( (2/T_A) \) in radians \( \text{sec}^{-1} \) is achieved in the limit of very fast exchange, \( (\tau \delta \omega) < 1 \). Simplications to the general lineshape expression of eq.(3.4) can be used to facilitate the determination of exchange rates in the fast exchange region. Since the lifetimes \( \tau_A \) and \( \tau_B \) are very short in the region of fast exchange, terms involving the products of \( \tau_A \) and \( \tau_B \) may be omitted so that eq.(3.4) may then be simplified to

\[ G = -i\omega_M \frac{\tau_A^{1/2} + \tau_B^{1/2}}{\alpha_A \tau_A^{1/2} + \alpha_B \tau_B^{1/2}} = - \frac{i\omega_M}{P_A \alpha_A + P_B \alpha_B} \]  

(3.12)
for which the imaginary part may be written

\[ V = -\omega_l M_x \frac{T_2'}{1 + (T_2')^2 (p_A \omega_A + p_B \omega_B - \omega)^2} \]  \hspace{1cm} (3.13)

where the symbols have been defined previously. Equation (3.13) represents an exchange-broadened averaged signal centered on a mean frequency \( \omega_{\text{mean}} \) which is defined as

\[ \omega_{\text{mean}} = p_A \omega_A + p_B \omega_B \]  \hspace{1cm} (3.14)

and a width as given by

\[ \frac{1}{T_2'} = \frac{p_A}{T_{2A}} + \frac{p_B}{T_{2B}} \]  \hspace{1cm} (3.15)

in the limit of very fast exchange.

It may be seen that eq.(3.13) merely gives the \( V \) mode signal in the absence of exchange, as in eq.(2.7b), when \( \omega_i^2 T_1 T_2 < 1 \). In the region where significant exchange broadening occurs, the exchange will not be rapid enough to collapse the exchange contribution to the linewidth and thus eq.(3.15) will not correctly define the linewidth. In this instance, the terms involving products of the lifetimes \( \tau_A \) and \( \tau_B \) are too large to be omitted in eq.(3.4), and a more correct expression to describe the exchange-broadened linewidth can be obtained using the modification of the equation of Gutowsky and Holm\(^{27}\) by Meiboom and co-workers\(^{117}\) from their study of the proton exchange in water. This
modification consists of substituting $\omega_{\text{mean}}$ as given by eq.(3.14) for $\omega$ in eq.(3.4) and expanding in powers of $\tau$, to give

$$\frac{1}{T'_2} = \frac{P_A}{T_{2A}} + \frac{P_B}{T_{2B}} + P_A^2 P_B^2 (\delta \omega)^2 (\tau_A + \tau_B)$$

(3.16)

where $\delta \omega = (\omega_A - \omega_B)$. The lifetimes or exchange rates can thus be obtained from eq.(3.16) by measuring the total linewidth and the corresponding exchange contribution to the linewidth. In the simple case of an equally-populated two-site system for which $T_{2A} = T_{2B}$ and $\tau_A = \tau_B = 2\tau$, eq.(3.16) then reduces to

$$\frac{1}{T'_2} = \frac{1}{T_2} + \left( \frac{\tau (\delta \omega)^2}{2} \right)$$

(3.17)

where line broadening due to magnetic field inhomogeneity is included in the term $(T_2)^{-1}$. As the linewidth in radians sec$^{-1}$ is $(2/T'_2)$, the exchange contribution to the line broadening is $(\tau(\delta \omega)^2)$. In the instances where $\delta \omega$ is large and $T_2$ is long then it can be seen that the first term on the right-hand side of eq.(3.17) is small and can be neglected.

Similarly, Piette and Anderson$^{118}$ derived approximate expressions for obtaining the rate constant from measurement of the linewidth, which are applicable to multi-site and unequally-populated exchange problems. These equations relate the observed linewidth $W^*$ ($=1/\pi T'_2$) in frequency units, which includes the exchange contribution to the linewidth, to the rate constants (reciprocal lifetimes) for each site $k_A$ and $k_B$ as$^{24,118}$
\[ k_A = 4\pi p_A p_B^2 (\delta\nu)^2 \left( W^* - \frac{p_A T_{2A} + p_B T_{2B}}{\pi T_{2A} T_{2B}} \right)^{-1} \] (3.18a)

\[ k_B = 4\pi p_A^2 p_B (\delta\nu)^2 \left( W^* - \frac{p_A T_{2A} + p_B T_{2B}}{\pi T_{2A} T_{2B}} \right)^{-1} \] (3.18b)

In the simplest case where \( p_A = p_B = 0.5 \), \( T_{2A} = T_{2B} = T_2 \) and \( k_A = k_B = k \), the Piette and Anderson equations above reduce to

\[ k = \frac{\pi (\delta\nu)^2}{2} (W^* - W)^{-1} \] (3.19)

where \( W = (\pi T_2)^{-1} \) is the effective linewidth in the absence of exchange. The Piette and Anderson equations have been scrutinized by calculations\(^\text{32}\) and shown to be accurate for the determination of fast exchange rates, but are subject to serious systematic errors if used to obtain rates of the same order as \( k_c \).\(^\text{32}\) Swift and Connick\(^\text{119}\) have also derived similar approximate expressions for the fast exchange limit from the McConnell equations.

It may be noted that the validity of the averaging of NMR parameters in the fast exchange limit has recently been questioned, which has, not surprisingly, resulted in some controversy.\(^\text{120,121}\)

The measurement of accurate exchange rates in the fast exchange regime is thus dependent on the ability to measure linewidths and thus values of the spin-spin relaxation
time $T_2$ with a minimum degree of error. The linewidth of resonances in fast exchange is in practice limited by magnetic field homogeneity, with the true linewidth approaching the natural linewidth $2/T_2$ in angular units. The problems inherent in the measurement of accurate $T_2$ values have previously been discussed, and the measurement of exchange lifetimes by extracting $T_2$ directly from linewidth measurements is subject to these inherent errors. The complete lineshape analysis method accordingly has limitations imposed on it, largely by a combination of timescales and instrumental or magnetic field inhomogeneity broadening of the lines. The lineshape analysis method has an associated upper limit of applicability on the order of $10^3$ s$^{-1}$.\textsuperscript{24,26,122} Thus, alternative methods for accurately measuring faster rates of exchange are necessary in order to perform a full kinetic study encompassing as wide a range of rates as possible.

### 3.2.3(a) Spin-Echo Studies of Fast Chemical Exchange

The merits of the spin echo method for removing the contribution of instrumental broadening or magnetic field inhomogeneities ($T_2^{inhom}$)\textsuperscript{-1} to the observed $T_2$ have been outlined in an earlier section of this chapter. It has also been shown that spin echo techniques can be used to measure self-diffusion coefficients by the imposition of external magnetic field gradients or gradient pulses. In a similar fashion, chemical exchange results in a modulation of the Larmor frequency of the exchanging nuclei, leading to a random dephasing of the spins with subsequent attenuation of the echo amplitudes, somewhat analogous to the diffusion process. The exchange should therefore be affected by
changing the pulse repetition rate, $2\tau$. It is thus reasonable to assume that chemical exchange can be investigated by the Carr-Purcell or CPMG experiment, and this is indeed the case, with spin-echo studies of chemical exchange among the first applications of pulsed NMR by chemists.  

Woessner\textsuperscript{123} emphasized the analogy between diffusion and chemical exchange in liquids, and that its effect could be reduced by a rapid pulse repetition rate in the Carr-Purcell experiment. Woessner analytically predicted the effects of two-site exchange on the FID following the application of a pulse using the Bloch equations, and calculated the amplitude of spin echoes following a $\pi/2-\pi$ sequence of pulses in the presence of chemical exchange, neglecting diffusion effect. The predictions of Woessner\textsuperscript{123} were experimentally verified by Reeves and Wells\textsuperscript{124} for Carr-Purcell experiments at varied temperatures for the two-site exchange observed in the O-N bond hindered rotation in methyl nitrite.

Theoretical treatments describing the effects of exchange on the decay of spin echo amplitudes from CPMG-type experiments have been made by various research groups in order to extract rate constants or exchange lifetimes from the observed dependence of $T_2$ upon the pulse repetition frequency for two-site systems undergoing fast exchange.\textsuperscript{30,57,123,126} Luz and Meiboom\textsuperscript{125} derived an approximate analytical expression from the McConnell equations.\textsuperscript{92} The expression for the two-site case is

where $(T_2(g))^{-1}$ is the observed echo decay constant with a pulse repetition rate of $g$ pulses per second, $T_o$ is the true or natural relaxation time in the absence of exchange observed
\[
\left( \frac{1}{T_2(g)} - \frac{1}{T_o} \right) = p_s p_b (\delta \omega)^2 \tau \left[ 1 - 2\tau g \tanh(2\tau g)^{-1} \right]
\] (3.20)

at the fastest pulse repetition rates, \( \tau \) is the mean lifetime between exchanges, and the other symbols are as defined previously. Allerhand and Gutowsky\textsuperscript{127} numerically tested the equation of Luz and Meiboom\textsuperscript{125}, and also applied the method to determine exchange rates and barriers for the hindered internal rotation in \( \text{N,N-dimethyltrichloroacetamide} \) and \( \text{dimethylcarbamyl chloride} \). The apparent relaxation times as a function of pulse repetition rate were measured to \( \pm 10\% \).\textsuperscript{127}

Allerhand and Gutowsky\textsuperscript{30} further derived closed-form expressions for the echo amplitude decay by modification of the Hahn-Maxwell\textsuperscript{97}/McConnell\textsuperscript{92} equations for the time dependence of site magnetizations, which proved to be applicable to both slow and fast exchange.\textsuperscript{30} Allerhand and Gutowsky were able to show that at fast pulse repetition rates, the exchange contribution to the observed \( T_2 \) decreases, and \( (T_2)^{-1} \) reaches the limiting value of \( (T_2^0)^{-1} \), which is independent of the exchange rate.\textsuperscript{30} For very fast exchange, \( (1/\tau) \ll \delta \omega \), the expression

\[
(1/T_2) = (1/T_2^0) + 1/(4(\delta \omega)^2 \tau)
\] (3.21)
is obtained, where $\delta \omega$ is the chemical shift in radians/sec between the sites, $\tau$ is the exchange lifetime, and $T_2^\circ$ is the spin-spin relaxation time in the absence of exchange. This expression shows that the instrumental line broadening effects can be eliminated using a Carr-Purcell sequence as a function of pulse repetition rates. An iterative computer procedure was utilized to obtain values for $1/2\tau$, $T_2^\circ$, and $\delta \omega$, so that the need to obtain the chemical shift difference $\delta \omega$ by freezing out the exchange at low temperatures was unnecessary. Allerhand and Gutowsky applied their formulation to obtain exchange rates and the barrier for the chair-chair isomerization in d$_{11}$-cyclohexane, and were able to obtain rate constants over approximately five orders of magnitude, from 0.5 s$^{-1}$ to $4 \times 10^4$ s$^{-1}$, in the temperature range from -100 to +25 °C, with errors in the exchange rates on the order of ± 15% reported.$^{128}$ The advantage of the spin-echo method in increasing the upper limit of rate measurement was thus demonstrated.

Bloom, Reeves and Wells$^{126}$ derived a theory for the dependence of spin echo amplitudes in exchanging systems on pulse spacing using probability theory for phase angle distribution of the spin isochromats, and a matrix operator technique to generate the amplitude of alternate echoes. This formulation could be generalized to include extension to multi-site and unequally-populated exchange problems. For the simple two-site case, it was shown that in the Carr-Purcell sequence, the echo amplitudes for two sites in the absence of spin coupling can in general be expressed as the sum of two exponentials for two Larmor frequencies $\omega_A$ and $\omega_B$. An iterative computer procedure was used to determine the rate constant and chemical shift, similar to the method of Allerhand and
Gutowsky\textsuperscript{30} Errors in the T\textsubscript{2} values and rate constants determined with this method were on the order of ± 10% and ± 15% respectively for experimental application of the theory of Bloom \textit{et al.}\textsuperscript{31}

In the determination of exchange rates from spin echo experiments using the theories of Allerhand and Gutowsky\textsuperscript{30} or Bloom, Reeves and Wells,\textsuperscript{126} values of the spin-spin relaxation time in the absence of exchange, T\textsubscript{2}°, were obtained either from the limiting value of the observed T\textsubscript{2} in the limit of fast pulsing, or from curve-fitting procedures. Restrictions in the ability of these methods for obtaining T\textsubscript{2}° were noted, however, especially at the high-temperature extremes of the dependence of the observed T\textsubscript{2} on pulse spacing. In these instances it was necessary to assume that T\textsubscript{2}°=T\textsubscript{1}, which entailed separate measurements of T\textsubscript{1}.\textsuperscript{31,128}

A density matrix formulation of the relationship between spin echoes and chemical exchange which was applicable to more complex spin systems was developed.\textsuperscript{129} Application of the spin-echo method to more complicated cases such as multisite exchange and systems with spin-spin coupling proved to be somewhat cumbersome, however, especially in terms of numerical analysis of the data.\textsuperscript{57,130,131}

The general difficulties in performing Carr-Purcell or CPMG experiments, such as careful control of pulse width calibrations, rf field inhomogeneity effects and baseline drift, have previously been mentioned. These problems will of course be paramount in the determination of accurate exchange rates, and in application of the spin-echo method to chemical exchange several sources of systematic and random errors have been
encountered, and are discussed in reviews on the technique.\textsuperscript{31,32,57} A primary disadvantage of the spin-echo method is its lack of selectivity, with all nuclei of one type, for example protons, on or near resonance during pulses, so that non-exchanging protons should in theory be specifically deuterated. Other additional \( T_2 \) relaxation mechanisms such as coupling to quadrupolar nuclei such as the \(^{14}\text{N} \) in amides, or unresolved long range couplings, need to be considered.\textsuperscript{31,57}

In cases where exchange problems were studied over a wide range of temperatures by both the spin-echo and total lineshape analysis methods, the latter was deemed to be more reliable\textsuperscript{31,32}. The advantage of the spin-echo method in extending the upper limit of accessible exchange rates remains, however, and these early studies actually represented the first use of pulsed NMR by chemists.

\subsection{3.2.3(b) Measurement of Fast Chemical Exchange Rates by \( T_{1p} \) Methods}

The spin-lattice relaxation time in the rotating frame \( T_{1p} \), the time constant for the rate of decay of the magnetization spin-locked along the rf field \( B_1 \), has been discussed as a parameter which can give useful information about low-frequency motions of molecules. Measurements of \( T_{1p} \) can also be used to give exchange rates, as first demonstrated by Meiboom\textsuperscript{132}. Deverell and co-workers\textsuperscript{33} subsequently elaborated the technique and showed that it could be used as an alternative experiment for the measurement of fast exchange rates, applying the method to the chair-chair isomerization in cyclohexane.
Using the density matrix formalism for the simple equally-populated and uncoupled two-site case, the expression\textsuperscript{33}

$$\frac{1}{T_{1p}(ex)} = \frac{1}{T_{1p}(obs)} - \frac{1}{T_1} - \frac{(\delta \omega)^2}{4} \left[ \frac{\tau_c}{1 + \omega_1^2 \tau_c^2} \right]$$

(3.22)

was derived, where $\delta \omega$ is the chemical shift separation in radians s\textsuperscript{-1}, $\omega_1$ is the frequency of the rf field in radians per second, and $\tau_c$ is the mean lifetime of the nuclei in each site, related to the rate constant $k$ as $(1/2 \tau_c) = k$. As in the case spin-echo determinations of exchange rates, other contributing relaxation mechanisms must be subtracted from the observed $T_{1p}$ ($T_{1p}^{(obs)}$) to obtain the exchange contribution, $T_{1p}(ex)$. It can be seen from eq.(3.22) that $T_{1p}$ will have a linear dependence on $\omega_1$ with a slope of $[4 \tau_c/(\delta \omega)^2]$ and an intercept given as $T_{1p} = [4/(\delta \omega)^2 \tau_c]$ in the limit $\omega_1 \rightarrow 0$. Thus, both the exchange rate or lifetime and the chemical shift $\delta \omega$ can both be measured from a plot of $(T_{1p})^{-1}$ as a function of $\omega_1$, with the important implication that the chemical shift can be determined in cases where actual measurement of $\delta \omega$ is not possible, such as in systems with very low barriers to rotation or inversion.

Deverell et al\textsuperscript{33} determined rates for cyclobexane ring inversion in the temperature range 215 K to 250 K, with rate constants up to $2 \times 10^3$ s\textsuperscript{-1} measured by the $T_{1p}$ method. The capability of the $T_{1p}$ method for measuring exchange rates on the order of $10^4$ s\textsuperscript{-1}, up to two orders of magnitude greater than the upper limit of the spin-echo method, was postulated, providing the spectrometer is capable of producing rf of the sufficient required
strength.\textsuperscript{33} Despite the claims of considerably extending the upper range of accessible rate constants, the \( T_{1p} \) method appears to have undergone limited application,\textsuperscript{34,57,133} probably due to the somewhat specialized nature of the technique.

Thus, the need for a relatively convenient and accurate method for obtaining rate constants in the fast exchange regime is apparent. The offset-saturation method described previously\textsuperscript{35,36} lends itself naturally to such an application, with its ability to simultaneously determine \( T_1 \) and \( T_2 \), and especially to determine \( T_2 \) without any contribution from magnetic field inhomogeneities and with no special hardware requirements. A preliminary attempt was recently made to study fast exchange of protons in water-ethanol solutions by a form of the offset-saturation technique\textsuperscript{78} in which \( T_1 \) and \( T_2 \) were determined separately, but no critical evaluation of this method or serious analysis of the data were undertaken.
CHAPTER 4

THE OFFSET-SATURATION EXPERIMENT: DATA AND ERROR ANALYSIS, EXPERIMENT DESIGN AND OPTIMIZATION

4.1 Introduction

The steady-state offset-saturation method\textsuperscript{35} introduced in Chapter 2 offers a convenient alternative to linewidth measurements or the CPMG spin echo pulse experiment for measuring the spin-spin relaxation time $T_2$, and also offers the capability of simultaneous determination of $T_1$ and $T_2$. In the present chapter, the offset-saturation method is investigated in detail. Several fundamental aspects of the offset-saturation technique are examined, in order to provide a basic understanding of the experimental methodology, and to facilitate the application and analysis of the experiment. Methods for the analysis of experimental data, the determination of errors, and the accurate calibration of the saturating rf field $B_2$, are addressed. The design and optimization of offset-saturation experiments pertinent to the location of sampling points (irradiation resonance offsets) and the selection of experiment parameters such as pre-acquisition delay times are examined. The offset-saturation method is then applied to determine $T_1$ and $T_2$ for a simple single-spin system, namely, distilled water at low pH.
4.2 Methodology of the Offset-Saturation Experiment

As described in Chapter 2, the basic steady-state offset-saturation experiment is relatively uncomplicated, involving irradiation of a spectral line with an rf field $B_2$ provided by the spectrometer homodecoupler, at an offset from resonance ($\omega_o - \omega$) for a period of time long enough for the $z$ magnetization to reach a steady state. A 90° pulse is then applied to measure the $z$ magnetization, which is subsequently plotted as a function of resonance offset. A plot of the $z$ magnetization (intensity) as a function of resonance offset is characterized by the existence of a dip or minimum occurring at the resonance frequency $\omega_o$. The half-width of this curve at half-maximum height is equal to $(\gamma B_2)(T_1/T_2)^{1/2}$.

In order to obtain absolute values for the relaxation times $T_1$ and $T_2$, the basic offset-saturation experiment can be modified by the insertion of a delay time $\tau$ after gating off the decoupler irradiation and prior to acquisition. This delay time is selected to be some fraction of the estimated spin-lattice relaxation time $T_1$ of the particular nucleus under examination, to allow partial longitudinal relaxation of the spin system. The experimental pulse scheme for the offset-saturation experiment with the aforementioned modification is thus as depicted in the pulse sequence schematic of Fig.4.1.

The measured $z$ magnetization as a function of resonance offset with the partial relaxation delay $\tau$ is given by $^{35,36}$
Figure 4.1. Offset-saturation experiment pulse sequence. Delay time $\tau$ is inserted prior to acquisition to allow determination of the absolute values for relaxation times $T_1$ and $T_2$. 
Figure 4.2. Plot of $z$ magnetization as a function of irradiation resonance offset for hypothetical offset-saturation experiment with partial relaxation delay. Data points were calculated using eq.(4.1) with values of $\gamma B_2/2\pi = 100$ Hz, $T_1 = T_2 = 1$ s, $\tau = 0.25$ s, $M_s = 1$. Resonance offset values are dimensionless, with absolute values of offsets divided by the quantity $\gamma B_2(T_1/T_2)^{1/2}$. 
\[ M_z = M_o \left( 1 - \frac{(\gamma B_2)^2 T_1/T_2}{(\omega_0 - \omega)^2 + (\gamma B_2)^2 T_1/T_2} e^{-\nu \tau} \right) \]  \hspace{1cm} (4.1)

A plot of the observed signal intensity or \( z \) magnetization as a function of irradiation offset from resonance has the typical appearance as shown in Fig.4.2, with the minimum of the curve at a non-zero intensity, due to the relaxation delay \( \tau \).

The irradiation may also create transverse components of the magnetization. In the presence of a saturating rf field \( B_2 \) under the condition that the saturation factor \((\gamma B_2)^2 \) \( T_1T_2 < 1 \), the \( U \) and \( V \) components of the magnetization may be obtained from the Bloch equations (2.7a) and (2.7b) respectively in the same manner that eq.(2.18) is obtained from eq.(2.7c). The transverse \( U \) and \( V \) components of the magnetization may thus accordingly be described by the equations:

\[ U = M_o \frac{(\omega_0 - \omega)\gamma B_2}{(\gamma B_2)^2 T_1/T_2 + (\omega_0 - \omega)^2} \]  \hspace{1cm} (4.2)

\[ V = M_o \frac{\gamma B_2}{T_2(\omega_0 - \omega)^2 + (\gamma B_2)^2 T_1} \]  \hspace{1cm} (4.3)
The components of the magnetization as given by equations (4.2) and (4.3) as a function of dimensionless resonance offset (absolute offset value divided by the value of $\gamma B_2 (T_1/T_2)^{1/2}$) are illustrated in Fig.4.3 together with the $z$ component $M_z$ given by eq.(2.18). It is apparent from Fig.4.3 that there may be a rather substantial $U$ or dispersion component of the magnetization near the vicinity of $\pm 1$ resonance offset units, or at the value of the half width of the $z$ magnetization ($M_z$) as a function of offset. The $V$ or absorption component of the magnetization is essentially very small over the entire resonance offset range, for the chosen value of $B_2$, and is therefore negligible under such circumstances. The widths of these components of the magnetization are determined by the saturation factor, containing $B_2$ and the relaxation times, which will dominate any contributions from magnetic field inhomogeneities.

The fact that there may be a finite transverse component of the magnetization implies that certain precautions must be taken so that only pure $z$ magnetization is measured in offset-saturation experiments. To promote suppression of the transverse components of the irradiation so that only the $z$ magnetization is detected, as well as to validate the irradiation resonance offset behaviour of the $z$ magnetization given be eq.(2.18), a nominal value, on the order of a few Hz, is sufficient for the magnitude of the saturating rf field $B_2$ for high resolution applications of the offset-saturation method in liquids. Such rf field strength requirements are readily provided by standard homodecouplers equipping high resolution NMR spectrometers.
Figure 4.3. Dispersion ($U$), absorption ($V$) and $M_z$ components of the nuclear magnetization as a function of dimensionless irradiation offset (offset divided by $\gamma B_0(T_1/T_2)^{1/2}$) calculated for $\gamma B_0/2\pi = 100$, $T_1 = T_2 = 1$, with $M_0 = 1$. The $U$ component is represented by the dashed line, the solid line of low intensity is the $V$ component, and the dotted line represents the $z$ magnetization.
The use of homospoil or gradient pulses to promote dissipation of the transverse components of the magnetization prior to acquisition\textsuperscript{66,69,74,78} in experimental schemes similar to those of the offset-saturation experiment has been mentioned in Chapter 2. The partial relaxation delay $\tau$ employed in the offset-saturation pulse sequence, however, provides the additional role of permitting decay of the transverse magnetization.\textsuperscript{35,36} The use of receiver phase cycling further serves to safeguard against the mixing of longitudinal and transverse components of the magnetization, as the latter should be phase-incoherent with the receiver. The use of a $90^\circ$ observation pulse, in addition, reduces the possibility of mixing the longitudinal and transverse components of the magnetization. Thus, any residual transverse magnetization resulting from the irradiation of the spin system in offset-saturation experiments can essentially be removed by a combination of phase cycling of the receiver, $90^\circ$ acquisition pulse, and the preacquisition delay time $\tau$.\textsuperscript{35,36}

In order to avoid the possibility of detrimental interference effects from the irradiation of spinning sidebands,\textsuperscript{134,135} offset-saturation experiments should be performed without spinning of the sample tube.\textsuperscript{36} While there may be some additional line broadening as a result of not spinning the sample, the $T_2$ obtained from offset-saturation effects should nonetheless be independent of static field inhomogeneities provided the value of $B_2$ is sufficiently large.\textsuperscript{17,35,36}
4.3 Calibration of the Saturating RF Field $B_2$

4.3.1 Methods for the Calibration of Pulse Widths/RF Field Strengths

The value of $T_2$ obtained from an offset-saturation experiment is extracted from the width at half-maximum height ($\gamma B_2 (T_1/T_2)^{1/2}$) of the curve of $M_z$ as a function of irradiation resonance offset. It is therefore essential for the determination of accurate $T_2$ values that the irradiating field $B_2$ be calibrated as accurately and precisely as possible. For example, in order to measure a value of $T_2$ with an error on the order of 5% when the error in $T_1$ is 1%, then it is necessary to calibrate $B_2$ to approximately 2% or better. This fact has been overlooked in applications of experiments similar to that of the offset-saturation method.\textsuperscript{74,78} It is also desirable to perform such calibrations as quickly as possible. The single-spin double resonance experiment\textsuperscript{37,28,136,137} best satisfies the preceding criteria for calibrating the saturating rf field.

Several methods have been used for the calibration of radiofrequency field strengths. The most common method for determining rf field strengths is undoubtedly the measurement of a 90° pulse width, or some multiple thereof. The calibration of rf field strengths from the measurement of pulse widths can be inaccurate, however, attributable primarily to the difficulties of observing an adequate null signal. Due to the effects of rf or static magnetic field inhomogeneities, or the possibility of rf transmitter leakage to the receiver, the signal observed with a 180° or 360° pulse may be 5 or 10% of the maximum signal when calibrating pulse widths. Thus, the use of pulse width measurements to
determine rf field strengths is not sufficiently accurate for the present application to offset-saturation experiments.

Haupt\textsuperscript{138} has proposed a relatively simple method for determining the width of a 90° pulse which saves time compared to the standard method of searching for the null for a multiple of a 90° pulse. The method of Haupt uses only the ratio of two intensity measurements, with the requirement that the pulse width of the second experiment is twice that of the first experiment. This method, while simple, has errors claimed to be on the order of 5%, relying on accurate intensity measurements, and therefore may not be of sufficient accuracy to calibrate $B_2$ for use associated with offset-saturation experiments.

Relatively sophisticated pulse sequences have been used for the calibration of decoupler rf field strength.\textsuperscript{139,140} Heteronuclear (typically $^{13}$C-$^1$H) experiments can yield a measurement of the decoupler field strength through the effects of decoupling on the intensities of the observed spectra. Such methods, however, require relatively intensive analysis of the data, which can be time-consuming, and these methods are not necessarily of optimal accuracy. Two-dimensional methods have been devised to calibrate decoupler rf field strengths.\textsuperscript{141-145} In these 2D-methods, the duration of the rf pulse width to be determined is treated as an evolution time ($t_1$) by incrementing the pulse width. The resulting amplitude modulation is detected in $t_2$, which is Fourier transformed to yield the rf field strength and corresponding pulse width. A high degree of accuracy of the order of 1% is claimed for these methods\textsuperscript{141,146} but the use of spectral processing software is required, with the accompanying time factor accordingly involved, in addition to the time
and computer memory requirements for acquiring 2-D spectra.

The phenomenon of Bloch-Siegert shifts\textsuperscript{17,116,147} can be used to calibrate relatively weak decoupling fields in an indirect fashion through its influence on observed spectra.\textsuperscript{6,7} In the Bloch-Siegert effect, the influence of a second, counter-rotating rf field component as investigated in the original work of Bloch and Siegert\textsuperscript{116} or the analogous case of the application of a second rf field such as decoupler irradiation, as described by Ramsey\textsuperscript{147} may cause a shift in the observed resonance position of a line. In a co-ordinate frame rotating at the frequency of the second rf field $B_2$, ($\omega_2$) the nuclear spins will experience an effective rf field $B_{\text{eff}}$, which is the resultant of $B_2$ and the resonance offset ($\omega_\text{a}$-$\omega_2$). The magnitude of the observed shift away from the Larmor frequency $\omega_\text{a}$ is directly related to the strength of the decoupler field $B_2$ through the expression

$$\Delta f = \frac{(\gamma B_2)^2}{2(\omega_\text{a}-\omega_2)}$$

(4.4)

where $\Delta f$ is the shift in the resonance position ($\omega$-$\omega_\text{a}$) resulting from the irradiation.

Measuring the change in frequency of a line before and after the application of the second rf field therefore allows a calibration of $B_2$ in the optimal case where the irradiation is far from resonance.
4.3.2 The Single-Spin Double-Resonance Experiment for the Calibration of $B_2$

A method for calibrating homodecoupler rf field strengths which is more suitable for use in conjunction with offset-saturation experiments, is the classic single-spin double-resonance experiment.\textsuperscript{37,38,136,137} The single-spin double-resonance method involves the irradiation of a single spin with a second rf field.\textsuperscript{37,38} The application of the second rf field causes a perturbation in the resonance frequency of the irradiated nucleus, which experiences an effective magnetic field from the resultant of the irradiating field $B_2$ and the resonance offset. The nuclear spins become polarized along a direction different from the laboratory $z$-axis; in this instance, they become polarized along the effective field so that in the rotating frame, the magnetization $M$ is aligned along the effective field $B_{\text{eff}}$, which is oriented at some angle with respect to the $z$ axis. When the total magnetization $M$ is perturbed from its steady-state orientation along the effective field, it will precess about the effective field as it relaxes back to its equilibrium position. The signal detected in the $x$-$y$ plane as a result of the projection of the ellipsoidal trajectory of $M$ onto the $x$-$y$ plane results in two signals or satellite peaks.\textsuperscript{37,104,146} These satellite peaks are symmetrically displaced about the irradiation frequency, and occur at frequencies $\omega_A$ and $\omega_B$ given by

$$\omega_{A,B} = \omega_z \pm \left[(\omega_0-\omega_z)^2 + (\gamma B_2)^2\right]^{1/2}$$

(4.5)
where \( \omega_0 \) is the Larmor frequency of the spin in the absence of the irradiation, and \( \omega_2 \) is the rf irradiation frequency.

The magnitude of the irradiating field manifests itself in a rather straightforward manner in single-spin double-resonance spectra, resulting in a facile method for the calibration of \( B_2 \). From eq.(4.5) it may be seen that the magnitude of the irradiating field \( B_2 \) can be determined from the distance between the satellites \( \omega_\alpha \) and \( \omega_\beta \) as

\[
\gamma B_2 = \left( \frac{\omega_\alpha - \omega_B}{4} \right)^2 - \left( \omega_\alpha - \omega_\beta \right)^2
\]

(4.6)

It is possible to calibrate \( B_2 \) quickly and with good precision by performing the single-spin double-resonance experiment with several different values of the irradiation offset \( (\omega_\alpha - \omega_\beta) \), determining an average value for \( B_2 \) together with a standard deviation. It is not necessary to employ a 90° observation pulse in the single-spin double-resonance experiment\(^{137} \) so that the effects observed in the resulting spectrum are due almost exclusively to the irradiating field. For spectrometers employing receiver phase-cycling, it is recommended that only a single scan be performed, unless the proper receiver phase synchronization is employed\(^{36,136,137,148-150} \). For the irradiation of protons, acquiring a single scan provides sufficient signal strength. Calibration should ideally be performed on a single-spin species present in the same sample tube as the species on which the offset-saturation experiment is to be performed, and in many cases both experiments can be performed on the same spin system. Spinning of the sample should also be avoided during
single-spin double resonance experiments to avoid the possibility of irradiating spinning sidebands.\textsuperscript{134}

An example of a single-spin double-resonance experiment of a sample of CHCl\textsubscript{3} is shown in Fig. 4.4. The homodecoupler irradiation was placed on resonance in this instance, and the two satellites of opposite phase can be seen flanking the intense signal at the resonance frequency. This experiment was recorded after a single scan. By performing the single-spin double-resonance experiment for several values of the decoupler irradiation frequency $\omega_2$, $B_2$ can be calibrated with a precision on the order of 1%. In addition, the shape of the satellite resonances is an indicator of the homogeneity of both the static magnetic field $B_0$ and the irradiating rf field, $B_2$, as in other double-resonance experiments so that the single-spin double-resonance experiment provides the additional purpose of monitoring rf field homogeneity.\textsuperscript{36,151}

The satellite resonances observed in the single-spin double resonance experiment are inverted in phase with respect to each other when the irradiation is not far removed from resonance. The precession in one case will be of the same phase as the Larmor precession around the effective field, while the other spin transition of opposite phase results from precession in the opposite sense around the effective field.\textsuperscript{37,104,148} The relative intensities of the two satellites vary with offset, and has been the object of some previous study, with single-spin double-resonance spectra used to compare steady-state and continuous-wave spectra in the presence of a second relatively strong rf field.\textsuperscript{38,136,148-150} The intensity ratio of the two satellite features observed in the Fourier transform mode is given by\textsuperscript{148}
Figure 4.4. A 200 MHz $^1$H NMR spectrum of a single-spin double-resonance experiment performed on a sample of CHCl$_3$ in CDCl$_3$. The spectrum was recorded after a single scan, non-spinning, on a Bruker AC-200 spectrometer equipped with a $^1$H/$^{13}$C dual probe using a 5mm sample tube. Irradiation was placed on resonance. The value of $\gamma B_z / 2\pi$ in this instance is 79.4 Hz.
\[ \frac{S_+}{S_-} = - \frac{(1 + \cos \theta)}{(1 - \cos \theta)} \]  \hspace{1cm} (4.7)

where \( S_+ \) and \( S_- \) refer to the signals of positive and negative phase respectively, and \( \theta \) is the angle between the effective and static magnetic fields, such that

\[ \tan \theta = \frac{\gamma B_2}{(\omega_0 - \omega_2)} \]  \hspace{1cm} (4.8)

It is to be noted that there is an irradiation offset dependence of the phase difference between the two satellites, with simulations performed indicating that this phase difference varies from the extremes of 180° when the irradiation is on resonance, and essentially 0° when irradiating very far from resonance. This fact may become important when calibrating relatively large irradiating rf fields, and when there are a number of resonances in a particular sample, in which the satellites from the irradiation may overlap or be partially obscured by other lines. The offset-saturation experiment is therefore best performed on an isolated resonance in the sample, such as TMS if present, in complicated samples with many NMR signals present.

The central intense resonance observed at the irradiation frequency appears to be a superposition of a spin transition at the irradiating frequency and decoupler where \( B_2^{obs} \) is the observed or calibrated value of the homodecoupler field, and \( B_2^{inst} \) is the instantaneous value of the homodecoupler field for each individual pulse. This equivalence, however, appears to be restricted to small duty cycles, on the order of 20% or less, due to the approximations or simplifications made in determining the analytical expression describing the effects of a series of repeated soft pulses. It is also
breakthrough\textsuperscript{136} although it has been suggested by Tomlinson\textsuperscript{137} that this signal is exclusively due to a spin transition.

Single-spin double-resonance experiments have largely been undertaken with cw homodecoupling, and relatively few such experiments of the pulsed homodecoupler analogue have been attempted\textsuperscript{36,137}. Pulsed homodecoupling, which is commonly employed with the majority of modern high resolution FT NMR spectrometers, involves a time sharing of the irradiation and acquisition so that the irradiation is actually on for a short period of time, appearing as a series of regularly repeated identical pulses, interrupted by signal acquisition. The equivalence of cw radiation to a such a series of soft rf pulses is important to establish in order that the pulsed single-spin double resonance experiment can be used to calibrate the homodecoupler field \( B_2 \) which is ultimately used as the irradiating field in offset-saturation experiments.

In the next chapter, the equivalence between cw rf and the rf produced using a series of identical soft pulses is addressed theoretically. The relationship or equivalence between the observed or calibrated value of \( B_2 \) determined by the single-spin double-resonance experiment and the instantaneous value of \( B_2 \) (the value per individual pulse) and the is shown analytically to be

\[
B_{2}^{\text{obs}} = B_{2}^{\text{inst}} \times \text{duty cycle} \quad (4.9)
\]
shown in the next chapter that when performing offset-saturation experiments or calibrating the irradiating field by the single-spin double-resonance experiment on NMR spectrometers equipped with pulsed homodecouplers, the individual decoupler flip angles should be kept small to ensure the equivalence of pulsed and cw rf.

It may be shown\textsuperscript{36,147} that the Bloch-Siegert shift mentioned previously is a first-order approximation of the basic single-spin double-resonance experiment. From equations (4.5) and (4.6), the observed frequency \( \omega_i \) for a line such as the satellites \( \omega_a \) or \( \omega_b \) can be expressed as

\[
(\omega_i - \omega_o)^2 = (\omega_o - \omega_a)^2 + (\gamma B_2)^2
\]

(4.10)

Upon rearrangement and expanding using the binomial theorem with the condition \( \omega_o - \omega_a \gg \gamma B_2 \), eq. (4.10) becomes\textsuperscript{36,147}

\[
\omega_i - \omega_o = \frac{(\gamma B_2)^2}{2(\omega_o - \omega_a)}
\]

(4.11)

which is the formula for the Bloch-Siegert shift presented in eq. (4.4).\textsuperscript{36,147} The shifted line in the Bloch-Siegert effectively corresponds to one of the satellites \( \omega_a \) or \( \omega_b \) of the single-spin double-resonance experiment, while the other satellite has very low intensity. The relationship between the single-spin double-resonance experiment and the Bloch-Siegert shift is thus apparent. While use of the full single-spin double-resonance experiment is preferred, Bloch-Siegert shifts may be used to calibrate \( B_2 \) when the sidebands may prove difficult to observe, such as in a complex spectrum or when irradiating far off resonance.
4.4 Data Analysis: Determination of $T_1$ and $T_2$ Relaxation Times 
From Offset-Saturation Experiments

The absolute values of the relaxation times $T_1$ and $T_2$ are obtained from offset-saturation experiments as based upon equation (4.1). In order to determine these relaxation times accordingly with minimal error, such a problem lends itself to solution by least-squares or regression analysis methods.\textsuperscript{80-83,152} As mentioned in the previous chapter, non-linear least-squares routines have become routine in fitting inversion-recovery experimental data to determine values of $T_1$,\textsuperscript{12,51,52,24} and allow a more rigorous calculation of errors in the parameters.\textsuperscript{12} For the present work, relaxation times are determined from offset-saturation experiments by means of a three-parameter least-squares fit to the experimental data set consisting of irradiation offsets and the corresponding intensities, using $(\gamma B_2)T_1/T_2$, $M_o$ and $e^{i\nu_1}$ as fitted parameters.\textsuperscript{12,35,36} The data are fitted to a model based on eq.(4.1), which can be written in the simplified form

$$M(\Delta \omega) = m \left(1 - \frac{br^{-e}}{(\Delta \omega)^2 + b}\right)$$  \hspace{1cm} (4.12)

where $\Delta \omega$ is the irradiation offset from resonance, $m$ corresponds to $M_o$, $b$ corresponds to $(\gamma B_2)^2 T_1/T_2$, and $r$ corresponds to $e^{i\nu_1}$ in eq.(4.1).

The nature of eq.(4.1) or eq.(4.12) necessitates the use of a non-linear least-squares regression.\textsuperscript{12,35} For problems requiring non-linear regression treatments, the data cannot
be fitted explicitly to a general linear model, but the same approach used for linear problems can be followed. In such cases, a $\chi^2$ function is defined and minimized to determined the best-fit parameters is employed, with the requirement that the minimization must proceed iteratively. Initial or trial values are given for the parameters to be fitted, and a procedure is used to improve the trial solution which is repeated until $\chi^2$ is minimized or stops decreasing in value. Several methods are available for performing the $\chi^2$ minimization in non-linear regression, among the most common traditionally being linearization, Marquardt, and steepest-descent methods.\textsuperscript{80-82}

The linearization method, which is used in the present application, assumes that the model behaves linearly in a local sense,\textsuperscript{81,82} and that for small changes in the observables $y_i$, the changes in the parameters that minimize $\chi^2$ locally can be calculated by a treatment of the normal equations described above for the general linear case, while iterating on the solution using calculated increments to the parameters until the $\chi^2$ function is minimized. For the non-linear case, the effects of a small change in the observable $y_i$ can be expressed as

$$dy_i = \sum_{k=1}^{M} \left( \frac{\partial \chi^2}{\partial a_k} \right) da_k \quad (4.13)$$
where $dy_i = y_{\text{obs}} - y_{\text{calc}}$ and $da_k$ is the change in parameter $a_k$ for a model with $M$ adjustable parameters. The minimization solution to the $\chi^2$ function can be written in terms of the first and second derivatives of the $\chi^2$ function with respect to the fitted parameters as:

$$
\frac{\partial^2 \chi^2}{\partial^2 a_k} = -2 \sum_{i=1}^N \frac{[y_i - y(x_i;a)]}{\sigma_i^2} \frac{\partial y(x_i;a)}{\partial a_k}
$$

(4.14)

where $N$ is the number of data points, and

$$
\frac{\partial^2 \chi^2}{\partial a_k \partial a_l} = 2 \sum_{i=1}^N \frac{1}{\sigma_i^2} \left[ \frac{\partial y(x_i;a)}{\partial a_k} \frac{\partial y(x_i;a)}{\partial a_l} - [y_i - y(x_i;a)] \frac{\partial^2 y(x_i;a)}{\partial a_k \partial a_l} \right]
$$

(4.15)

where $k = 1,2,...,M$.

The solution to eq.(4.13) proceeds such that $da_k$ satisfies the matrix equation

$$
\sum_{l=1}^M \alpha_{kl} da_l = \beta_k
$$

(4.16)

where the matrix elements $\alpha_{kl}$ are defined as

$$
\alpha_{kl} = \sum_{i=1}^N \frac{1}{\sigma_i^2} \left[ \frac{\partial y(x_i;a)}{\partial a_k} \frac{\partial y(x_i;a)}{\partial a_l} \right]
$$

(4.17)
In the general non-linear regression procedure, the general linear model for the set of equations defined by eq.(4.13) is solved for \( da_n \), the changes in that particular parameter necessary to minimize \( dy_n \), the difference between the observed and calculated measurable quantity. The incremental change is then added to the current approximate value for \( a_n \), and this iteration procedure is repeated until \( \chi^2 \) is minimized.\(^{50-52}\)

Data from offset-saturation experiments were analyzed using a computer program called DIPPER, which is written in Turbo Pascal and can be run on most personal computers. The irradiation offsets and corresponding intensities, resonance frequency, calibrated value of \( B_2 \) and the decoupler gating delay time \( \tau \) are used as input data for the program. The linearization technique is used to determined the best-fit values for parameters \( m, b \) and \( r \) in eq.(4.12). From the best-fit determinations of \( b \) and \( r \), the value of \( T_2 \) is then obtained using the calibrated value of \( B_2 \).

4.5 Error Estimation for Offset-Saturation Experiments

There will be errors associated with the estimation of the parameters and ultimately, the relaxation times \( T_1 \) and \( T_2 \) obtained from the offset-saturation experiments by the least-squares fitting algorithm. These errors, which may be of both a random and systematic nature, can be estimated from the fit to the data by two distinct methods. The first of these methods involves calculating the standard deviation for each of the parameters \( m, b \) and \( r \) from the variance-covariance matrix of the fit. One consequence
of the non-linear least-squares regression routine used to obtain the relaxation times $T_1$ and $T_2$ from offset-saturation data is that estimates of the standard deviations for each of the fitted parameters can be obtained as a byproduct of the data fit. From the set of equations for the non-linear regression as defined by eq.(4.16), the elements of the inverse of the matrix $\alpha$, $[\alpha]^{-1}$, are a close approximation to the uncertainties of the fitted parameters $a^{12,31}$, which may be given as

$$a_j = \sum_{k=1}^{M} [\alpha]^{-1}_{jk} \beta_k = \sum_{k=1}^{M} C_{jk} \left[ \sum_{i=1}^{N} \frac{y_i X_i(x_j)}{\sigma_i^2} \right]$$

(4.18)

where $X_i(x_j)$ are basis functions of $x$. The variance associated with the estimate of each parameter, $\sigma^2(a_j)$, can be obtained from

$$\sigma^2(a_j) = \sum_{i=1}^{N} \sigma_i^2 \left( \frac{\partial a_j}{\partial y_i} \right)^2$$

(4.19)

where

$$\frac{\partial a_j}{\partial y_i} = \sum_{k=1}^{M} C_{jk} X_i(x_j)/\sigma_i^2$$

(4.20)

to ultimately give

$$\sigma^2(a_j) = \sum_{i=1}^{N} \sum_{j=1}^{M} C_{ij} C_{jk} \left[ \sum_{i=1}^{N} \frac{X_i(x_j)X_i(x_j)}{\sigma_i^2} \right]$$

(4.21)
The term in square brackets in eq.(4.21) is the matrix \([\alpha]\), which is the inverse of matrix \([C]\), so that eq.(4.21) simplifies to

\[ \sigma^2(a_j) = C_{jj} \]  \hspace{1cm} (4.22)

From eq.(4.22), it is apparent that the diagonal elements of the matrix \([C]\) are the variances of the fitted parameters. Similarly, the off-diagonal elements \(C_{jk}\) represent the covariances between parameters \(a_j\) and \(a_k\). The matrix \([C]\) is accordingly referred to as the variance-covariance matrix for the regression,\(^{12,31,52}\) and can be directly obtained from the non-linear regression routine, to give an estimate of the standard deviation \((\sigma)^{1/2}\) for each of the parameters involved in the regression.

The variances in the parameters \(m, b\) and \(r\) from fits to offset-saturation experiments can thus be approximated from the non-linear least-squares fit as the inverses of the elements of the diagonal matrix elements of \([\alpha]^{-1}\) corresponding to the appropriate parameters. This method of error analysis, as mentioned, involves certain basic assumptions, namely the absence of correlation amongst the fitted parameters. The standard deviations as determined may thus be considered as approximate or non-rigorous estimates of the errors in the parameters.

A more realistic representation of the errors in the fitted parameters can be obtained through a calculation of the confidence intervals for the parameters. There will be a probability distribution of the errors in the estimation of the parameters. This probability distribution, which is a function defined in the \(M\)-dimensional space of the parameters,
can in effect be assigned boundaries in the form of confidence limits. These confidence limits will thus define a confidence interval or region of the M-dimensional space containing a certain percentage of the total probability distribution at a specified confidence level.\textsuperscript{50-52}

The $\chi^2$ function evaluated in the non-linear regression represents a surface, which, when calculated at the desired confidence level, (commonly the 95\% confidence limit) provides more realistic boundaries or confidence limits for the errors in the fitted parameters.\textsuperscript{12,35,80-82} The value of $\chi^2$ will be at a minimum, $\chi_{\text{min}}^2$, for the best-fit values of the calculated parameters. The value of $\chi^2$ will accordingly increase by some amount $\Delta \chi^2$ for values of the chosen parameter which are different from the optimal value of that parameter defining the minimum for $\chi^2$, so that the region in which $\chi^2$ increases by no more than a specific amount $\Delta \chi^2$ will define a confidence region around that particular parameter.\textsuperscript{81,82} These confidence regions are thus defined surfaces, and the $\chi^2$ surface can be described by the general expression

$$\frac{\chi^2}{\chi_{\text{min}}^2} = 1 + \frac{M}{N-M} F(M,N)$$

(4.23)
where \( M \) is the number of parameters, \( N \) is the number of data points, and \( F(M,N) \) is the \( F \)-statistic\(^{81,82} \) obtained from tables of \( F \)-statistics, chosen for the desired confidence level.

A hypothetical confidence interval surface for the case of two fitted parameters which forms an ellipsoidal surface, is illustrated in Fig.4.5. The confidence limits can be determined from the intersection of the tangent planes of the \( \chi^2 \) surface with the appropriate parameter axis.\(^{12,81} \) and generally represent a more rigorous error estimate than the first method presented for calculating the standard deviations.\(^{12,80-82} \) The shape and orientation of the major axis of the error ellipsoid are both important indicators of the extent of correlation or covariance between parameters, and reflect the general quality of the data or the experimental design.\(^{80,82} \) Confidence limit error ellipsoids or \( \chi^2 \) surface contours have previously been used in the NMR field for the determination of confidence limit errors for such applications as the calculation or simulation of NMR spectra by computer methods,\(^{143} \) and in non-linear least-squares regression fits to inversion-recovery data for the measurement of \( T_1. \)^{12,51}

The second method of error analysis for the offset-saturation experiments presented in this work is accordingly a calculation of the confidence intervals at the nominal 95% confidence level for each of the fitted parameters from the intersection of the 95% confidence limit error ellipsoid tangent planes with the appropriate parameter axes. This method of error determination is also essentially a natural extension of the non-linear least-squares regression routine, with the \( \chi^2 \) values determined in the \( \chi^2 \) minimization routine of the fitting algorithm to determine the best-fit values of the parameters.
Figure 4.5. A hypothetical $\chi^2$ confidence ellipsoid for the estimate of two parameters $\beta_1$ and $\beta_2$. The confidence limit errors are determined from the intersection of the ellipsoid tangent planes with the appropriate parameter axes. The highlighted contour represents the 90% confidence region for $\beta_1$ and $\beta_2$. The individual 90% confidence intervals for each of the parameters are also shown. (Reproduced from reference [83])
While three parameters are fitted in the regression analysis, a simpler two-dimensional $\chi^2$ surface can be calculated by holding one of the parameters constant while calculating the $\chi^2$ surface for the other two parameters, computing sum of squares values at increments corresponding to specified fractions of the standard deviation for each parameter. Essentially a grid of the $\chi^2$ surface is then traversed to compute the tangent planes of the $\chi^2$ contour, and the intersection of these tangent planes with the parameter axes to determine the errors from these confidence intervals.

4.6 Design and Optimization of Offset-Saturation Experiments

The relaxation times are obtained from offset-saturation experiments by a three-parameter non-linear regression fit to eq.(4.12) which involves an iterative correction to the parameters $m$, $b$ and $r$ from the initial or "first guess" values of the parameters, as described previously. For the three-parameter fit to offset-saturation data, the procedure accordingly utilizes a 3x3 matrix, with matrix elements consisting of the partial derivatives of the data with respect to the parameters, as given for the general case by eq.(4.17),\textsuperscript{12,81} where the $y(x_i; \alpha)$ correspond to the $z$-magnetizations, and $a_x$, $a_t$ correspond to the various fitted parameters $m$, $b$, and $r$. It was shown from equations (4.18)-(4.22) that the inverse of the matrix $[\alpha]$ evaluated with the best-fit values of the fitted parameters gives the variance-covariance matrix $[C]$ for the general least-squares regression, with the elements of the variance-covariance matrix given by eq.(4.22). The
determination of the relaxation times $T_1$ and $T_2$ from offset-saturation experiments by means of the non-linear least-squares fit thus involves a calculation of the partial derivatives of the observed $z$ magnetizations or intensities with respect to each of the fitted parameters.

The partial derivatives of the magnetizations with respect to the fitted parameters can be used for optimizing offset-saturation experiments in terms of the selection or location of experimental parameters such as irradiation offsets and delay times. The criterion for utilizing partial derivatives of the observable quantity with respect to fitted parameters is based on the premise that in order to minimize the error or variance in the fitting of that parameter, the sampling points (in this case irradiation offsets) should be chosen that maximize the partial derivative of that parameter. This is evident from eq.(4.24), where the estimate of the variance $\sigma^2$ for a parameter $a_i$ can be given approximately by

$$\sigma^2(a_i) = 1 / \sum_{k=1}^{N} \left( \frac{\partial M_k}{\partial a_i} \right)$$

This hypothesis was tested and demonstrated experimentally for the somewhat simpler case of the inversion-recovery experiment for measuring $T_1$. The partial derivatives calculated from eq.(4.12) for the general offset-saturation experiment are accordingly
\[
\frac{\partial M_z}{\partial m} = 1 - \frac{br^{-\tau}}{(\Delta\omega)^2 + b}
\]  
(4.25)

\[
\frac{\partial M_z}{\partial b} = -mr^{-\tau}\left(\frac{(\Delta\omega)^2}{((\Delta\omega)^2 + b)^2}\right)
\]  
(4.26)

\[
\frac{\partial M_z}{\partial r} = \frac{mb^{-\tau(r-1)}}{(\Delta\omega)^2 + b}
\]  
(4.27)

The partial derivatives of the observed z-magnetization \(M_z\) with respect to each of the fitted parameters \(m\), \(b\), and \(r\), have been calculated for an arbitrary set of relaxation times \(T_1\) and \(T_2\), irradiating field \(B_2\), and delay time \(\tau\), using equations (4.25)-(4.27). Normalized and absolute-valued partial derivatives, plotted as a function of dimensionless irradiation resonance offset (absolute irradiation offset \(\omega_o - \omega\) divided by \(\gamma B_2 (T_1 / T_2)^{1/2}\)) are shown in Figure 4.6.

It may be observed from Fig.4.6 that \(\partial M_z/\partial m\) has minimum value at resonance (zero offset), and is maximized at \(M_o\). \(\partial M_z/\partial b\) is maximized at \(\pm 1\) resonance offset unit, and decreases rapidly in value away from this maximum value, while \(\partial M_z/\partial r\) is at a maximum on resonance and decreases at large irradiation offsets. It is apparent from the somewhat contradictory behaviour of the three partial derivatives as a function of relative irradiation offset, that the optimal offset-saturation technique inherently involves some degree of compromise. A certain amount of bias is thus necessary when performing an offset-saturation experiment, with one or more of the fitted parameters inevitably being favoured. As an extreme example, locating the majority of the resonance offsets far off
Figure 4.6. The absolute-valued, normalized partial derivatives of the $z$-magnetization with respect to each of the fitted parameters $m$, $b$, and $r$ as given by equations (4.25)-(4.27), as a function of dimensionless irradiation resonance offset (absolute irradiation offset $\omega_0 - \omega$ divided by $\gamma B_0 (T_1/T_2)^{1/2}$). The solid line represents $\partial M_z/\partial r$, the dotted line indicates $\partial M_z/\partial b$, and the dashed line shows $\partial M_z/\partial r$. Derivatives are calculated using values $\gamma B_0/2\pi = 100$ Hz, $T_1 = T_2 = 1$ s, $\tau = 1$ s, and $m = 1$ s.
resonance may result in a good estimation of $m$, but a poor estimation of $b$ and $r$. Hence, in this instance, the relaxation times $T_1$ and $T_2$ will be poorly estimated.

In order to exploit the offset-saturation experiment as a convenient method for measuring the transverse relaxation time $T_2$, the majority of sampling points or resonance offsets should be located in the vicinity close to the expected value of $\gamma B_0 (T_1/T_2)^{1/2}$ in order to reduce error in $T_2$ measurement. The partial derivative with respect to $b$, the parameter containing $T_2$, is quite sensitive, decreasing rapidly away from its maximum or optimal value, as evident in Fig.4.6, therefore justifying the biasing of the sampling procedure towards the parameter $b$.

Figures 4.7, 4.8 and 4.9 show the partial derivatives with respect to $m$, $b$ and $r$ respectively as a function of relative irradiation offset computed for several different values of the pre-acquisition delay $\tau$ ranging from 0.1 to 0.9 seconds, for a hypothetical $T_1$ of 1 second. It is evident from these figures that the choice of $\tau$ value for an offset-saturation experiment also appears to bias the experiment in favour of one or more of the parameters. For example, at a given irradiation offset value, Fig.4.7 appears to indicate that the partial derivative of the magnetization with respect to $m$ (corresponding to the equilibrium magnetization) increases with increasing value of $\tau$, which is most apparent close to resonance. The best estimate of $r$, which contains $T_1$, also occurs at the longest delay time for a given resonance offset, as shown in Fig.4.9. Conversely, Fig.4.8 suggests that the parameter $b$, containing the transverse relaxation time $T_2$, will be optimized at the shortest value of $\tau$. Again, the set-up of offset-saturation experiments is chosen to favour
Figure 4.7. Plot of normalized partial derivative $\partial M/\partial m$ as given by eq.(4.25), as a function of dimensionless irradiation offset (absolute irradiation offset $\omega_o-\omega$ divided by $\gamma B_2 (T_1/T_s)^{1/2}$), for various values of the offset-saturation preacquisition delay time $\tau$. The solid line represents points calculated using $\tau = 0.1$ s, the data represented by the dotted line were calculated using $\tau = 0.5$ s, and the dashed line is data calculated for $\tau = 0.9$ s. Data are calculated with values of $(\gamma B_2 / 2\pi) = 100$ Hz, $T_1 = T_2 = 1$ s, and $m = 1$ s.
Figure 4.8. Plot of normalized partial derivative $\partial M_\omega/\partial b$ as given by eq.(4.26), as a function of dimensionless irradiation offset (absolute irradiation offset $\omega_\omega - \omega$ divided by $\gamma B_2 (T_1/T_2)^{1/2}$), for various values of the offset-saturation preacquisition delay time $\tau$. The solid line represents points calculated using $\tau=0.1 \text{ s}$, the data represented by the dotted line were calculated with $\tau=0.5 \text{ s}$, and the dashed line is data calculated for $\tau = 0.9 \text{ s}$. Data are calculated for values of $(\gamma B_2/2\pi) = 100 \text{ Hz}$, $T_1 = T_2 = 1 \text{ s}$, and $m = 1 \text{ s}$. 
Figure 4.9. Plot of normalized partial derivative $\partial M/\partial r$ given by eq.(4.27) as a function of relative irradiation offset (absolute irradiation offset $\omega_x - \omega$ divided by $\gamma B_z(T_1/T_2)^{1/2}$) for various values of the offset-saturation experiment preacquisition delay time $\tau$. The solid line represents points calculated for $\tau = 0.1$ s, the data represented by the dotted line were calculated with $\tau = 0.5$ s, and the dashed line is data calculated for $\tau = 0.9$ s. Data are calculated with values of $\gamma B_z/2\pi = 100$ Hz, $T_1 = T_2 = 1$ s, and $m = 1$s.
the estimation of the parameter \( b \), so that the most precise value of the transverse relaxation time \( T_2 \) will be obtained.

The choice of a relatively short value of \( \tau \) biases only one of the three fitted parameters, but the parameter \( b \) appears experimentally to be the parameter most difficult to estimate, as will be demonstrated shortly. The experimental design is thus direct towards minimizing the error incurred in fitting the parameter \( b \). Accordingly, a relatively short \( \tau \) value, (approximately 10\% of \( T_1 \)) is employed in the offset-saturation experiments undertaken in the present work. By selecting a relatively short value for \( \tau \), we also gain the advantage of minimizing the total experiment time. In addition, the delay \( \tau \) may serve to allow for the decay of any transverse magnetization, as discussed previously.

4.7 Experimental Application of the Offset-Saturation Method

4.7.1 Introduction

As an example for trial application of the offset-saturation method, a system with predictable behaviour of the relaxation times \( T_1 \) and \( T_2 \), is desirable. Such an ideal situation would be the simple case in which \( T_1 \) is approximately equal to \( T_2 \). Meiboom,\textsuperscript{132} in his study of proton exchange in water molecules mentioned earlier, demonstrated that the proton exchange rate in water is pH dependent, and that for the approximate pH ranges \( 10 \leq \text{pH} \leq 4 \), \( T_1 \) and \( T_2 \) are essentially equal. Offset-saturation experiments were accordingly performed on a sample of distilled and deionized water with pH adjusted to
110

\( T_1 \) to inhibit proton exchange effects on the NMR spectra.

For the acidified water sample, the resulting relaxation times determined from an offset-saturation experiment were compared with a value of \( T_1 \) determined from a conventional inversion-recovery experiment, and the spin-spin relaxation time \( T_2 \) was compared with the corresponding value measured by a CPMG spin-echo experiment. Excellent agreement between the relaxation times was observed in both cases. Errors are determined for the relaxation times using the two methods discussed previously, namely, the standard deviations determined from the variance-covariance matrix for the fit, and from the \( \chi^2 \) confidence limits calculated at the 95% confidence level. Offset-saturation experiments were also performed with the water sample using different values of the pre-acquisition delay \( \tau \), and different strengths of the saturating rf field.

4.7.2 Experimental

A sample of distilled, deionized and filtered water obtained courtesy of the laboratory of Prof. A. Corsini was acidified to a pH of 1.5 by dropwise addition of 1M HCl. The pH was monitored by means of a Fisher Accumet digital pH meter. Glassware was previously washed with detergent, then with a solution of 0.1M EDTA to remove any paramagnetic ions which may be present, rinsed with distilled water, and dried. The water sample was then injected by disposable syringe into a Wilmad 2mm diameter spherical sample bulb and sealed. The sample bulb insert was coaxial with a 5mm NMR tube containing \( \text{CDCl}_3 \) (purchased from MSD isotopes) to provide a lock signal. The NMR
experiments were run on a Bruker MSL-100 spectrometer equipped with a 5mm dual
$^1\text{H}/^9\text{F}$ high-resolution probe with a $^1\text{H}$ 90° acquisition pulse of 4.25 μsec duration. The
temperature was controlled by a Bruker variable temperature control unit, at a stable room
temperature of 295 ± 1 K, as checked by an external copper/constantan thermocouple.

For the initial offset-saturation experimental results presented, for which the results
are compared with the inversion-recovery and CPMG experiments, a 30% decoupler duty
cycle was used. The irradiating field, expressed in units of Hz as $\gamma B_2/2\pi$, was calibrated
by the single-spin double-resonance method to be $247.5 ± 2.44$ Hz. The reported value
of $\gamma B_2/2\pi$ and the error are the average and standard deviation respectively as determined
from eight separate single-spin double resonance experiments performed on the water
resonance, with irradiation resonance offsets ranging from on resonance to approximately
± 50 Hz off resonance, as indicated in Table 4.1. The decoupler irradiation time for the
offset-saturation experiments was 12.3 seconds, and a delay time of 0.1 s was used
between gating the decoupler off and acquiring the spectrum, with an acquisition time of
0.82 seconds and a dwell time of 100 μs. Spectra were acquired non-spinning in order to
avoid interference from the possible irradiation of spinning side bands. A total of 20
separate irradiation offsets was employed, and these were entered in the frequency list
used by the spectrometer software in a random fashion. Approximately half of the total
number of irradiation resonance offsets were located approximately 100 Hz from the
predicted value of the half-width of the $M_z$ vs. irradiation offset curve $(\gamma B_2)(T_1/T_2)^{1/2}$,
while the remaining offsets were distributed between sampling the equilibrium
Table 4.1

Results of $B_2$ Calibration by Single-Spin Double Resonance Method for Acidified Water Sample

<table>
<thead>
<tr>
<th>Decoupler Irradiation Frequency (Hz)</th>
<th>Satellite Frequency (Hz)</th>
<th>$\gamma B_2/2\pi$ (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1850</td>
<td>2097.8</td>
<td>245.7</td>
</tr>
<tr>
<td></td>
<td>1603.4</td>
<td>244.5</td>
</tr>
<tr>
<td>1750</td>
<td>2010</td>
<td>251.0</td>
</tr>
<tr>
<td></td>
<td>1490</td>
<td>251.0</td>
</tr>
<tr>
<td>1800</td>
<td>2046.6</td>
<td>245.9</td>
</tr>
<tr>
<td></td>
<td>1548.5</td>
<td>250.83</td>
</tr>
<tr>
<td>1825</td>
<td>2071.6</td>
<td>246.5</td>
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<td></td>
<td>1577.2</td>
<td>247.7</td>
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<td>1775</td>
<td>1519.9</td>
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<tr>
<td></td>
<td>2026.5</td>
<td>247.8</td>
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<tr>
<td>1820</td>
<td>1573.4</td>
<td>246.6</td>
</tr>
<tr>
<td></td>
<td>2066.6</td>
<td>246.6</td>
</tr>
<tr>
<td>1875</td>
<td>1624.8</td>
<td>243.6</td>
</tr>
<tr>
<td></td>
<td>2126.5</td>
<td>244.9</td>
</tr>
<tr>
<td>1815</td>
<td>1568.4</td>
<td>246.6</td>
</tr>
<tr>
<td></td>
<td>2064.0</td>
<td>249.0</td>
</tr>
</tbody>
</table>

Resonance frequency ($H_2O$ signal) = 1817.8 Hz

$(\gamma B_2/2\pi)_{\text{avg}} = 247.5 \pm 2.4$ Hz
magnetization (far from resonance) and $T_1$ (close to resonance). The individual 
spectralsize was 8K data points, with an average of 8 scans acquired for each irradiation 
resonance offset. Spectra were processed using the standard spectrometer computer 
software. The spectra were Fourier transformed using a line-broadening factor of 10 Hz 
to ensure symmetrical lineshapes, and a baseline fit was performed in each case to 
minimize errors in intensity measurements arising from baseline fluctuation. The intensitiy 
of $M_z$ value corresponding to the irradiation offset value furthest from resonance was set 
to an absolute intensity value of 100. The intensities of the spectra corresponding to the 
remaining irradiation resonance offset values for the total experiment were then processed 
relative to this standard value.

The standard ($\pi$-variable delay-$\pi/2$-acquire) inversion recovery experiment was 
employed to independently determine a value of $T_1$ for the water protons at the same 
sample temperature at which the offset-saturation experiment was performed. Twenty 
variable delay values ranging from 0.1 s to 25 s were entered randomly in the variable-
delay list used in the inversion-recovery program of the spectrometer software. A recycle 
delay of 25 s between scans was employed, with 32 scans acquired per variable delay 
value with a dwell time of 100 $\mu$s. As an independent check on the value of $T_2$ for the 
water sample, a CPMG spin-echo experiment was performed. A $[(\pi/2)_x-(-\tau-(\pi)_y-\tau-\tau-(\pi)_y-\tau-\tau-\pi/2)_n$-acquire] pulse sequence was used, where $n$ is the number of repetitions of the $\pi$ pulse 
train. Twenty-five echo sampling times ranging from 0.02 to 10 s were recorded in 
increments of 0.4 s. The spectra were obtained without sample spinning.
4.7.3 Results

4.7.3(a) Application of the Offset-Saturation Experiment to Determine $T_1$ and $T_2$ for Water at Low pH; Comparison of Results with Inversion-Recovery and CPMG Experiments

The offset-saturation experiment on the water sample resulted in a $T_1$ value of 1.92 ± 0.01 s, and a $T_2$ of 1.52 ± 0.04 seconds, where the errors represent the 95% confidence limits and will be discussed in further detail shortly. The observed and fitted curve of $M_z$ versus irradiation offset for the offset-saturation experiment as determined from the non-linear least-squares fit to eq.(4.12) is presented in Fig.4.10, and demonstrates the excellent agreement between the observed and calculated or best-fit data. A $T_1$ value of 1.82 ± 0.04 % was determined by inversion-recovery experiment. The inversion-recovery data were analyzed by the computer program T1CALS\textsuperscript{12} which performs a non-linear least squares three-parameter fit to eq.(2.13) in the form

$$M(\tau) = a - be^{-\tau}$$

(4.28)

where $M(\tau)$ is the magnetization at time $\tau$, parameter $a$ corresponds to $M_0$, $b$ is $M(0)-M_0$, and $r$ represents $1/T_1$. The computer program T1CALS bears many similarities to the program DIPPER used for the analysis of offset-saturation experiment data, and calculates errors both as the standard deviations of the fitted parameters, and as the 95% confidence limits. The error quoted is the average of the latter. The corresponding inversion-recovery data curve for the water sample is given in Fig.4.11.
Figure 4.10. $M_z/M_0$ as a function of irradiation resonance offset for offset-saturation experiment performed on sample of acidified, distilled water. $M_z$ is the partially-saturated $z$-magnetization and $M_0$ is the best-fit value of the equilibrium magnetization. The experimental data are denoted by the circles, and the solid line represents the calculated or best-fit data. The value of $\gamma B_z/2\pi$ for this experiment was 247.5 Hz, the pre-acquisition delay time $\tau$ was 0.1 s, and the irradiation time was 12.3 s. The non-linear least-squares fit gave a value of $T_1 = 1.92 \pm 0.01$ s, and $T_2 = 1.52 \pm 0.04$ s.
The CPMG experiment used to check the value of $T_2$ resulted in a value of 1.58 ± 0.02 seconds, where the error represents two standard deviations at the 95% confidence level. The CPMG data curve of echo amplitude as a function of echo time is shown in Fig.4.12. The value of $T_2$ was obtained from the reciprocal slope of a plot of the natural logarithm of the observed intensity of the magnetization as a function of echo time, as given by eq.(2.15), where the echo time is given by $2\tau$ in eq.(2.15). The observed and fitted plot of $\ln[M(t)]$ as a function of echo time $t$ is shown in Fig.2.12. As an interesting addendum to the spin-echo measurement of $T_2$ for the water sample, a simple [90°-τ-180°-τ-acquire] two-pulse experiment was initially attempted to obtain an estimate of the $T_2$ for the water sample. The resulting $T_2$ for the water sample as obtained by the two-pulse echo method was 0.4 s, compared to 1.58 s obtained with the full CPMG experiment. The large discrepancy between the $T_2$ values obtained by the two spin-echo experiments can be primarily attributed to the effects of diffusion of the water molecules. While this result is not unexpected, it serves to verify the classic work of Carr and Purcell. 11

The results for the determination of $T_1$ and $T_2$ for the water sample obtained by the offset-saturation experiment, and by inversion-recovery and CPMG methods for $T_1$ and $T_2$ respectively, are summarized in Table 4.2. It should be noted that although the water sample for this particular set of experiments was contained in a spherical sample bulb which would help maximize rf homogeneity, such apparatus is not a necessity for successful offset-saturation experiments. Rather, the spherical sample cell was found to
Figure 4.11. Magnetization as a function of time for inversion-recovery experiment on sample of acidified, distilled water. The symbols (x) represent observed data, while the solid line represents the calculated or best-fit line. A value of $T_1 = 1.82 \pm 0.04$ s was obtained from the non-linear least-squares fit.
Figure 4.12. Magnetization or echo amplitude as a function of echo time for CPMG experiment on sample of acidified, distilled water.
Figure 4.13. Plot of $\ln[M(t)]$ as a function of echo time for CPMG experiment on sample of distilled, acidified water. Symbols (x) represent observed data points, the solid line represents the best-fit data. Regression analysis gives a value of $T_2 = 1.58 \pm 0.02$ s.
found to facilitate the CPMG experiment, presumably by minimizing rf inhomogeneity.

The errors associated with each of the three fitted parameters, calculated by the two methods discussed previously, are shown in Table 4.3. It may be observed in Table 4.2 that the errors calculated as the 95% confidence intervals are approximately a factor of three greater than the error given in terms of the standard deviation from the fit, for each of the three fitted parameters, rather than the expected difference of $2\sigma$ at the 95% confidence limit.\textsuperscript{81,82} In fact, this factor of three difference observed between the two types of error calculation appears to hold for the offset-saturation experiment in general, regardless of the sample or experimental parameters. This may be indicative of some correlation between the fitted parameters.\textsuperscript{80-82} In such instances, the standard deviations do not serve as accurate estimates of the parameter errors.\textsuperscript{80-82} A similar observation has been made for an analysis of errors in the inversion-recovery experiment.\textsuperscript{12} It is evident that the parameter $b$, containing $T_2$, has the largest associated error, being a factor of 4 to 6 times larger than the error for either of the other two parameters.

Two-dimensional $\chi^2$ confidence limit ellipsoids for the parameters $b$ and $r$ from the offset-saturation experiment calculated using eq.(4.23), are shown in Fig.4.14. Parameter $b$ is represented on the horizontal axis, and the parameter $r$ given on the vertical axis. The innermost ellipsoid is calculated at the 75% confidence level, the middle ellipsoid at the 90% confidence level, the outermost ellipsoid represents the 95% confidence ellipsoid. The point at the center of the ellipsoid (0,0) indicates the point of best fit, with zero error, for the two parameters. The errors for each of the parameters are determined from
Table 4.2

$T_1$ and $T_2$ Relaxation Times for Acidified Water
Determined by Offset-Saturation, Inversion-Recovery, and CPMG Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>$T_1$ (s)</th>
<th>$T_2$ (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Offset-Saturation</td>
<td>1.92 ± 0.01</td>
<td>1.52 ± 0.04</td>
</tr>
<tr>
<td>Inversion-Recovery</td>
<td>1.82 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>CPMG</td>
<td></td>
<td>1.58 ± 0.02</td>
</tr>
</tbody>
</table>

Table 4.3

Parameter Errors for Fit to
Offset-Saturation Experimental Data for Acidified Water

<table>
<thead>
<tr>
<th>Error</th>
<th>Fitted Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$m$</td>
</tr>
<tr>
<td>% Error as Standard Deviation</td>
<td>0.22</td>
</tr>
<tr>
<td>% Error from intersection of 95% confidence interval ellipsoid with parameter axis</td>
<td>0.69</td>
</tr>
</tbody>
</table>
Figure 4.14. Confidence interval error ellipsoids calculated for parameters $b$ (abscissa) and $r$ (ordinate) evaluated at the best-fit value of parameter $m$ (equilibrium magnetization). The single point indicated represents the best-fit point for parameters $b$ and $r$ (0,0). The axis scales represent the percent error of the appropriate parameter from the best-fit value. The innermost contour was calculated at the 75% confidence level, the middle ellipsoid was calculated at the 90% confidence level, and the outermost ellipsoid was evaluated at the 95% confidence level.
intersection of the $\chi^2$ surface ellipsoid tangent planes with the appropriate parameter axes. The shape of the contours indicates that parameter $b$ is less well-determined than parameter $r$. The orientation or tilt of the major axis of the ellipsoid indicates the existence of a correlation between these two parameters.\textsuperscript{80-82} The partial derivative method for optimizing the offset-saturation experiment assumes no correlation amongst the fitted parameters and it is apparent from real experimental data that correlation does exist. The use of partial derivative method does appear to have merit in the design and optimization of offset-saturation experiments in a qualitative and approximate manner.\textsuperscript{12,36}

4.7.3(b) Offset-Saturation Experiments on Acidified Water with Varied Pre-Acquisition Delay $\tau$

In order to experimentally observe possible effects of the choice of the partial relaxation or pre-acquisition delay time $\tau$ on the observed values and associated errors for the spin-lattice and spin-spin relaxation times $T_1$ and $T_2$ respectively, several offset-saturation experiments, each having a different value of $\tau$, were performed on a sample of distilled, acidified water. All experimental variables such as decoupler power or strength and duty cycle, irradiation time, dwell time and temperature were held constant for each experiment with the exception of the delay time $\tau$, which was varied from 0.10 to 1.0 s. Five separate offset-saturation experiments were performed, with $\tau$ values of 0.1, 0.25, 0.50, 0.75, and 1.0 s. In each case, 20 sampling points or resonance offsets were
used. A 30% decoupler duty cycle was employed, and the saturating rf field $B_2$ was calibrated to be $74.9 \pm 1.2$ Hz by the single-spin double-resonance method. A dwell time of 100 $\mu$s was used, and the 90° acquisition pulse width was 4.25 $\mu$s. The irradiation time for each experiment was 12.3 s. An average of 32 scans was recorded in each case, and the resulting spectra were processed in the same manner as the offset-saturation experiment of the previous section, with a line broadening of 10 Hz used in each case prior to Fourier transformation of the spectra. The data were analyzed with the program DIPPER. A non-spinning linewidth on the order of 3 Hz was observed.

The results of the variable-$\tau$ offset-saturation experiments are summarized in Table 4.4. The precision of the offset-saturation technique is indicated by the consistency of the $T_1$ and $T_2$ determined for this series of experiments, which are essentially independent of the value of $\tau$, with $T_1$ in the range 1.94-1.97 s, while $T_2$ varies only from 1.40 to 1.48 s. The errors calculated for the fitted parameters or relaxation times are essentially seen to be independent of the value of $\tau$, as indicated in Fig.4.15. The errors determined for the parameter $b$, containing $T_2$, calculated as the 95% confidence limits, range randomly with respect to $\tau$ from approximately 2 to 5%, with the standard deviations for this parameter ranging from approximately 1.0 to 1.6%, again a factor of three less than the errors calculated as the 95% confidence limits. The error in the parameter $r$, containing the spin-lattice relaxation time $T_1$, also behaved randomly with respect to $\tau$, with the 95% confidence interval errors ranging from approximately 0.50% to 1.0%, with the corresponding standard deviations ranging from 0.17 to 0.3%. The parameter $b$ is again
Table 4.4

Relaxation Times and Parameter Errors for Acidified Distilled Water from Offset-Saturation Experiments with Varied Delay Time τ

<table>
<thead>
<tr>
<th>τ(s)</th>
<th>T₁(s)</th>
<th>T₂(s)</th>
<th>%σ(m)</th>
<th>95%CI (m)</th>
<th>%σ(b)</th>
<th>95%CI (b)</th>
<th>%σ(r)</th>
<th>95%CI (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>1.94</td>
<td>1.48</td>
<td>0.30</td>
<td>0.95</td>
<td>1.38</td>
<td>4.28</td>
<td>0.23</td>
<td>0.72</td>
</tr>
<tr>
<td>0.25</td>
<td>1.94</td>
<td>1.46</td>
<td>0.21</td>
<td>0.66</td>
<td>1.01</td>
<td>3.14</td>
<td>0.17</td>
<td>0.52</td>
</tr>
<tr>
<td>0.50</td>
<td>1.96</td>
<td>1.43</td>
<td>0.29</td>
<td>0.89</td>
<td>1.56</td>
<td>4.85</td>
<td>0.28</td>
<td>0.88</td>
</tr>
<tr>
<td>0.75</td>
<td>1.97</td>
<td>1.45</td>
<td>0.25</td>
<td>0.77</td>
<td>1.55</td>
<td>4.81</td>
<td>0.28</td>
<td>0.86</td>
</tr>
<tr>
<td>1.0</td>
<td>1.95</td>
<td>1.40</td>
<td>0.23</td>
<td>0.73</td>
<td>1.70</td>
<td>5.26</td>
<td>0.30</td>
<td>0.93</td>
</tr>
</tbody>
</table>

In Table 4.4, %σ refers to the error expressed as a standard deviation in terms of percentage for a particular parameter, while 95%CI refers to the error in the parameters calculated as the average of the 95% confidence interval from the error contour tangent plane intersection with the parameter axis.
Figure 4.15. Errors calculated as the 95% confidence limits for the parameters $m$, $b$ and $r$ as a function of delay time $\tau$, for offset-saturation experiments on sample of acidified, distilled water. The symbol $x$ represents the parameter $m$, the parameter $b$ is represented by the squares, and the triangles represent the parameter $r$. 
the least well-determined of the three parameters, as in the previous experiment. The 95% confidence limit errors for each of the three parameters are plotted as a function of $\tau$ to illustrate more clearly the independence of each of the errors of these parameters on the value of $\tau$.

The variable-$\tau$ experimental results may seem contradictory to the predictions of the partial derivatives of the magnetizations with respect to the parameters, which indicate in figures 4.7-4.9 that the parameter $b$, for example, is optimized with shorter values of $\tau$, while the error in parameter $r$ should be minimized with longer values of $\tau$. As indicated earlier, the theoretical partial derivatives assume no correlation of errors between the parameters, which has been shown to exist in the previous experimental example. The experimental results presented in this section do not contradict the partial derivative criteria, but serve to indicate that the use of partial derivatives in experimental design and optimization is restricted to a qualitative role unless the partial derivatives based on the off-diagonal elements of the variance-covariance matrix are considered. The results of the variable $\tau$ offset-saturation experiments do appear to indicate that the resulting values of $T_1$ and $T_2$ and the errors associated with the three-parameter fit are essentially independent of the value of $\tau$. The use of a relatively short value of $\tau$, on the order of 10% or less of the value of $T_1$, which would reduce experiment time, while having no apparent detrimental effects on the resulting relaxation times and errors, is accordingly justified. The robustness of the offset-saturation method is also demonstrated with this set of experiments with varied pre-acquisition delay.
The observed and best-fit values of the curves of the magnetizations as a function of irradiation resonance offset for three of the variable $\tau$ offset-saturation experiments are shown in Fig. 4.16. The minimum of the curves is shown to increase with increasing value of the delay $\tau$, as predicted by eq. (4.1). The apparent well-behaved nature of these curves near resonance also illustrates no significant mixing of the $U$ or $V$ components with the $z$ component of the magnetization, so that the short values of $\tau$ selected here are sufficient to permit decay of any extraneous transverse components of the magnetization.

4.7.3(c) Offset-Saturation Experiments as a Function of Strength of Saturating RF Field $B_2$

In the present section of this thesis, a series of offset-saturation experiments was undertaken with differing values of the irradiating field $B_2$, thereby allowing examination of the possible effects of the value of $B_2$ on the resulting relaxation times and corresponding errors. In addition, this series of experiments serves to examine the overall precision and robustness of the offset-saturation method. From the results of the previous section, it is apparent that the choice of the pre-acquisition delay time $\tau$ has little apparent effect on the relaxation times $T_1$ and $T_2$ or the errors in the parameters determined from the nonlinear regression to the offset-saturation data. The experimenter thus has some freedom in terms of the choice of a delay time $\tau$ when implementing an offset-saturation experiment. In a similar manner, a value for the saturating or irradiating rf field $B_2$ must be chosen to perform a particular offset-saturation experiment. The value of $B_2$ can be
Figure 4.16. $M_z/M_0$ as a function of irradiation resonance offset for offset-saturation experiments on sample of acidified, distilled water for various values of the pre-acquisition delay $\tau$. The symbol (X) represents data for $\tau = 0.25$ s, squares represent data for $\tau = 0.5$ s, and $\tau = 1.0$ s for the data represented by the triangle symbols. The solid lines represent the best-fit lines for each set of data. The value of the saturating rf field in each case was 74.9 Hz, and the irradiation time was 12.3 s in each case.
selected most readily by the setting of a specific decoupler power, or if the spectrometer has the capability of altering the decoupler duty cycle, B₂ can be varied at a constant decoupler power by changing the value of the duty cycle.

Five separate experiments were performed, with values of B₂ (expressed in Hz as γB₂/2π) ranging from a low value approximately 100 Hz to a high value of nearly 2 kHz, as calibrated by the single-spin double resonance experiment. The value of B₂ was changed by altering the decoupler power setting, which is controlled by the internal microprocessor of the NMR spectrometer. The decoupler duty cycle was fixed at 30% in each case, and the experiments were performed at a constant probe temperature of 295K, with a dwell time of 100 μs or sweep width of 5000 Hz, an irradiation time of 12.3 s, and a prequisition delay time of 0.1s utilized. An average of 20 irradiation offsets was employed in each experiment, with 32 scans per irradiation offset obtained.

The results of the offset-saturation experiments using different values of the saturating rf field are summarized in Table 4.5. It is firstly apparent that the value of B₂ is calibrated to a good degree of precision, less than 2 % error in the worst case, by the single-spin double resonance experiment. It is evident that there is more scatter in the data than was present in the data for the variable τ experiments of the previous section, with the T₁ values ranging from 1.77 to 2.06 s, while the values obtained for T₂ vary from 1.35 to 1.65 s. The absolute values of the relaxation times do not appear to be dependent in any discernible fashion on the value of B₂. The overall T₁/T₂ ratio for the set of experiments with varied B₂ is approximately constant over the range of B₂ values.
Table 4.5

Relaxation Times and Parameter Errors for Acidified Water from Offset-Saturation Experiments with Varied Value of $B_2$

<table>
<thead>
<tr>
<th>$\gamma B_2/2\pi$ (Hz)</th>
<th>$T_1$(s)</th>
<th>$T_2$(s)</th>
<th>%σ(m)</th>
<th>95%CI (m)</th>
<th>%σ(b)</th>
<th>95%CI (b)</th>
<th>%σ(r)</th>
<th>95%CI (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>127.6 ± 2.2</td>
<td>1.77</td>
<td>1.35</td>
<td>0.43</td>
<td>1.33</td>
<td>1.71</td>
<td>5.3</td>
<td>0.31</td>
<td>0.96</td>
</tr>
<tr>
<td>247.5 ± 2.4</td>
<td>1.92</td>
<td>1.52</td>
<td>0.22</td>
<td>0.69</td>
<td>0.81</td>
<td>2.54</td>
<td>0.13</td>
<td>0.40</td>
</tr>
<tr>
<td>515.6 ± 4.7</td>
<td>2.06</td>
<td>1.61</td>
<td>0.45</td>
<td>1.38</td>
<td>1.52</td>
<td>4.74</td>
<td>0.20</td>
<td>0.62</td>
</tr>
<tr>
<td>995.3 ± 3.6</td>
<td>2.03</td>
<td>1.53</td>
<td>0.97</td>
<td>3.01</td>
<td>2.57</td>
<td>8.26</td>
<td>0.22</td>
<td>0.69</td>
</tr>
<tr>
<td>1909 ± 13</td>
<td>1.91</td>
<td>1.65</td>
<td>0.74</td>
<td>2.32</td>
<td>2.34</td>
<td>6.88</td>
<td>0.34</td>
<td>1.07</td>
</tr>
</tbody>
</table>

In Table 4.5, %σ refers to the error expressed as a standard deviation in terms of percentage for a particular parameter, while 95%CI refers to the error in the parameters calculated as the average of the 95% confidence interval from the error contour tangent plane intersection with the parameter axis.
Figure 4.17. $T_1/T_2$ ratio as a function of strength of saturating rf field $\gamma B_z/2\pi$ determined from offset-saturation experiments on sample of acidified water.
employed, as shown in Fig. 4.17. Thus, the value of $B_2$ used has no apparent
distinguishable trend with respect to the absolute values or ratios of the relaxation times
determined by offset-saturation experiments on the sample of acidified water. The offset-
saturation experiment thus appears overall to be somewhat less robust in terms of the
choice of $B_2$ than for the choice of the pre-acquisition delay $\tau$. The method is, in general,
relatively insensitive to the value of $B_2$ in terms of quantifiable trends on the results
obtained.

The values of the errors in the fitted parameters follow the general trend of the data
presented in the previous section, with the parameter $r$, containing the spin-lattice
relaxation time $T_1$, being the best-determined parameter, with errors calculated as the 95% confidence limits on the order of 1% or less, while the parameter $b$, containing the spin-
spin relaxation time $T_2$, is the least well-determined and most sensitive parameter, with
95% confidence interval errors ranging from 2.5% to over 8%.

The same number of sampling points or resonance offsets was used for each
experiment. Since a larger value of $B_2$ employed results in a larger value for the dip width
$\gamma B_2(T_1/T_2)^{1/2}$, the parameter $b$ may then be less well estimated at higher values of $\gamma B_2$, as
the sampling points have to be distributed over a wider frequency range. The

The corresponding larger errors observed in the parameter $b$ at higher $B_2$ values could be
decreased to a certain degree by refining the experiment such that the sampling points are
relocated once a value of the dip width has been estimated by an initial experiment, or
more sampling points could be used if distributed by sensible means, following the partial
derivative criteria for the location of sampling points.

The apparent increase in errors for the parameters with higher values of $\gamma B_2$ does demonstrate that judicious choice of sampling points must be made if relatively large values of $B_2$ are used. For this reason, the use of a relatively modest value of the irradiating field strength is recommended. The value of $B_2$ must of course be sufficient to satisfy the necessary requirement that the saturation factor $(\gamma B_2)^2 T_1 T_2 > 1$, and to aid in suppressing residual transverse components of the steady-state magnetization. The possibility of rf heating of the sample may occur at higher values of $\gamma B_2$, especially if the irradiation is occurs over a prolonged long period of time, although there was no evidence of such heating in the present experiments.

4.8 Discussion

Various aspects of the offset-saturation experiment for the determination of $T_1$ and $T_2$ relaxation times have been investigated in the present chapter. The offset-saturation experiment appears to be a relatively robust experiment for the simultaneous measurement of $T_1$ and $T_2$ relaxation times, and is in particular, a facile method for the determination of $T_2$ spin-spin relaxation times. The partial derivative method provides a good qualitative criterion for the design and optimization of offset-saturation experiments in terms of the selection of sampling points and delay times, and for observing correlations amongst the parameters involved in the non-linear least-squares fit used to extract the relaxation times. It is stressed that the partial derivative method is a qualitative guideline
only, since it does not consider covariance or correlation between the fitted parameters.

The single-spin double resonance method used to calibrate the saturating rf field $B_2$ used for the offset-saturation experiments has been shown to be a simple, rapid and precise technique. A precision on the order of 1% or less can be obtained with the single-spin double resonance experiment. Difficulties may arise when attempting to use the single-spin double resonance experiment to calibrate decoupling fields when irradiating nuclei of low sensitivity, or with complicated spectra, but appears to be the ideal experiment to calibrate the homodecoupler strength for use in conjunction with $^{1}$H offset-saturation experiments.

The $T_1$ and $T_2$ values for sample of distilled, acidified water obtained by the offset-saturation method were in excellent agreement with those obtained by the accepted standard inversion-recovery and CPMG techniques for measuring $T_1$ and $T_2$ respectively, demonstrating the good accuracy of the offset-saturation technique.

The errors in the fitted parameters calculated as the 95% confidence limits are approximately a factor of three times greater than the standard deviation errors calculated from the variance-covariance matrix for the non-linear regression, which, together with the appearance of the two-dimensional $\chi^2$ surface ellipsoid, indicates the existence of correlation between parameters in offset-saturation experiments. Thus, the covariance has an appreciable effect on the true error estimate.

The series of offset-saturation experiments performed on the sample of acidified, distilled water with variable value of the pre-acquisition delay time $\tau$ demonstrates the
precision of the offset-saturation method, and shows that the method is relatively robust with respect to the choice of \( \tau \), with both the values of \( T_1 \) and \( T_2 \) as well as the errors in the fitted parameters independent of the choice of \( \tau \). A relatively short value of \( \tau \) can thus be chosen to help minimize the overall experiment time.

From the set of offset-saturation experiments performed on the same sample of acidified water using various values of the irradiating field \( B_2 \), a relative insensitivity of the resulting \( T_1 \) and \( T_2 \) relaxation times to the value of \( B_2 \) was observed, although the results show more variation than for the experiments based on the choice of \( \tau \). The errors in the fitted parameters were demonstrably larger at higher values of \( B_2 \), which illustrates that a judicious choice or distribution of sampling points becomes more apparent with higher values of \( B_2 \). In the two series experiments with varied delay \( \tau \) and \( B_2 \), it is evident that the parameter \( r \), containing the spin-lattice relaxation time, is the most robust and best-determined parameter, while the parameter \( b \), containing the spin-spin relaxation time \( T_2 \), is the parameter consistently determined with the highest error. These observations support the experimental design and optimization criteria dictated by a calculation of the partial derivatives of the observed magnetizations with respect to the fitted parameters, for biasing the experiment in favour of determining the dip width \( \gamma B_2 (T_1/T_2)^{1/2} \) as accurately as possible.

In general, accuracy and precision on the order of 2% for \( T_1 \) and 5% for \( T_2 \) may be expected from an offset saturation experiment, providing good calibration of the saturating rf field, and proper location of the sampling points (irradiation resonance
offsets). Although the water sample on which the offset saturation experiments were performed in this chapter was contained in a small spherical sample cell which would help optimize rf field homogeneity, such apparatus is not necessary for high quality offset-saturation experiments, as mentioned earlier. Inhomogeneity of the rf field would be expected to have a detrimental effect on offset-saturation experiments, but the width of the satellites in the single-spin double-resonance sample spectrum in Fig.4.4 shows that the rf field homogeneity is quite good even for a standard 5mm NMR tube. In addition, simulations of offset-saturation experiments in which a distribution of rf fields was used showed that the experiment is quite robust and insensitive to rf field inhomogeneity. The determination of the T₁ for a particular sample by an inversion-recovery experiment, for example, prior to performing an offset-saturation experiment on that sample serves as a guideline to setting up the offset-saturation experiment in terms of the choice of irradiation time and pre-acquisition delay τ, but is also not a necessity for successful offset-saturation experiments.

An examination of the offset-saturation experiment has been undertaken in the present chapter. The offset-saturation technique is a relatively robust and convenient method for the simultaneous determination of T₁ and T₂ relaxation times, and offers a facile alternative method for measuring the spin-spin relaxation time T₂. The values of T₁ and T₂ for a sample of acidified, distilled water determined by a non-linear least-squares fit to a set of offset-saturation data are in excellent agreement with the relaxation times measured by inversion-recovery and CPMG methods respectively. Errors determined as
the 95% confidence limits are in general less than 5%, and the confidence limit errors for each of the parameters are approximately a factor of three greater than the errors determined as standard deviations. The single-spin double-resonance method provides a simple and precise method for calibration of the irradiating field $B_2$. 
CHAPTER 5

THE EQUVALENCE OF PULSED AND CONTINUOUS WAVE RADIATION IN NMR

5.1 Introduction

The use of a pulsed rf source to provide the saturating field $B_2$ for the single-spin double-resonance method of calibrating the rf field strength, and for the offset-saturation experiments undertaken in this thesis has been mentioned in the previous chapter. As the strong rf fields necessary for the majority of homodecoupling experiments can often result in problems of leakage between the transmitter and receiver in homodecoupling experiments, the use of time-shared decoupling has become standardized in high-resolution NMR spectrometers. In time-shared decoupling, as depicted in Fig.5.1, the decoupling rf field is not applied in continuous-wave fashion, rather it is accomplished using a series of short rf pulses of some duration $t_p$ between sampling intervals $\tau_s$.

The decoupling pulse duration is often fixed at a specific duty cycle in high-resolution NMR spectrometers, normally in the range of 10-20% of the dwell time. As the homodecoupler may be left on during acquisition, the irradiation field strength may then be calibrated using the single-spin double-resonance method, which has traditionally utilized a cw rf source. In addition, the Bloch equations which form the basis of the offset-saturation method were derived assuming a continuous-wave (cw) rf
source. These facts thus invoke the question of equivalence between an infinite series of pulses and cw irradiation. The confirmation of this equivalence would thus permit the use of a pulsed homodecoupler in single-spin double-resonance experiments, and in offset-saturation experiments, as eq.(2.18) essentially assumes a cw source of irradiation, and additional complications or limitations may arise in the technique with the use of pulsed rf irradiation.

For decoupling purposes, the effective decoupler power is usually considered to be the product of the instantaneous power of the decoupler, and the duty cycle, the fraction of the dwell time that the rf occupies. The extent of this equivalence is, however, uncertain. The offset-saturation experiment involves a balance between relaxation and irradiation, so a question which arises is the possibility of detrimental effects arising from regular interruption of the irradiation. Furthermore, the pulses provided by the decoupling
irradiation are soft such that the rf field strength and the resonance offset are comparable in magnitude, so that the effective field of the instantaneous irradiation is not the same as the equivalent average power. In general, it is not clear that cw and pulsed radiation are simply related.

In this chapter, the question of the equivalence between pulsed and cw rf radiation is addressed theoretically. It is shown that a series of identical pulses, under certain assumptions, leads to behaviour of the \( z \) magnetizations analogous to that predicted by the Bloch equations, and that the effective rf magnetic field is the product of the instantaneous field and the duty cycle.

The effects of a series of identical rf pulses, as occurring in homodecoupling, for example, have been examined for some time, and were addressed in original work on FT NMR by Ernst and Anderson\textsuperscript{46} as well as in studies of phase anomalies in FT spectra\textsuperscript{39,155} and in multiple-pulse experiments on solids,\textsuperscript{156-158} and are still of relevance at the present time. For example, in their recent work on an experiment to simultaneously measure \( T_1 \) and \( T_2 \), Moore and Metz\textsuperscript{21} have examined the equivalence of pulsed and cw rf, as their technique was based on the original assumptions of a cw rf source but spectrometer hardware considerations necessitates the application of the rf as a series of regularly spaced 90\(^\circ\) repeated pulses. Numerical computations were performed by integrating the Bloch equations to show that their data were identical in form to that of the cw experiment, but no results or sample computations of this work were presented.

When a spin system is irradiated with a series of pulses, a steady state may be
achieved. Carr\textsuperscript{159} in his pioneering early work on pulsed NMR steady-state free precession showed that a steady state is established in a spin system in a liquid when an intense rf pulse is applied repeatedly at a regular time interval which is shorter than the relaxation times for the particular spin system in question. Carr described the effects of a series of a series of identical short pulses on the $x$, $y$, and $z$ components of the magnetization before and after the pulses were applied, using a classical approach based on the Bloch equations.

Several pulsed NMR techniques have been developed or adapted to take advantage of the existence or formation of a steady state, and a number of these methods have been mentioned earlier in connection with the measurement of spin-lattice and spin-spin relaxation times.\textsuperscript{15.22,23,53,65} The steady-state approach has been used as the basis for experiments to facilitate the detection of problematic nuclei, such as those which are insensitive due to low gyromagnetic ratios or low natural abundance, and are very slow or very fast with respect to their relaxation rates.\textsuperscript{160-162}

The first detailed studies of the effects of a series of identical pulses on a single-spin system in a high resolution spectrum were undertaken in the aforementioned work of Ernst and Anderson.\textsuperscript{46} They considered the effects of a series of repeated short rf pulses followed by Fourier transformation of the acquired free induction decay. Ernst and Anderson considered the motions of the magnetization vector at various times during the pulse sequence, namely at the beginning and at the end of the pulse, and at the end of the FID, using a Bloch equations formalism similar to that used by Carr.\textsuperscript{159} Freeman and
Hill\(^{46}\) subsequently investigated irregularities observed in signal amplitude and phase in \(^{13}\text{C}\) FT NMR spectra occurring when the pulse repetition interval was short in comparison to the relaxation times of the nuclei, although only hard pulses were considered. Waldstein and Wallace\(^{39}\) generalized the treatment of Ernst and Anderson\(^{46}\) to include off-resonance components, or soft pulses, to investigate signal enhancement methods using rapidly repeated pulses. These early studies were primarily concerned with observing the effects of the transverse \(x\) and \(y\) components of the magnetization after the pulse.

In the present chapter, the original work of Waldstein and Wallace is extended to derive a general expression for the \(z\) magnetization during an infinite series of soft pulses. It is shown numerically that this expression obtained for the \(z\) magnetization exhibits a dip at resonance, analogous to the Bloch equations form for the \(z\) magnetization as a function of irradiation resonance offset for the offset-saturation experiment, as given by eq.(2.18). Based on the full form expression obtained for the \(z\) magnetization, an approximate analytical expression is derived which is equivalent to the Bloch equation form of eq.(2.18), while containing decoupler pulse parameters such as flip angles and evolution times, and establishes that a series of identical pulses is, in the limit of small duty cycles, equivalent to cw irradiation with the same average magnetic field. The relationship between the calibrated or ensemble average of the irradiating field \(B_2\) as determined from a single-spin double-resonance experiment, and the instantaneous value of \(B_2\) (the value per individual pulse) results from the analytical expression obtained for the \(z\) magnetization. Decoupler flip angle effects are also accessible to investigated using
the full form for the $z$ magnetization by performing simulations with appropriate values of the pulse parameters.

5.2 Results

5.2.1 The Equivalence of Pulse and CW RF Irradiation

5.2.1(a) Steady-State Solution of the $z$-Component of the Steady-State Magnetization for a Series of Identical Soft Pulses

In order to demonstrate the equivalence of a series of repeated soft pulses (as provided by a homodecoupler of a spectrometer) to continuous wave (cw) rf irradiation, the solution presented by Waldstein and Wallace\textsuperscript{39} is augmented here to include the off-resonance components of the $z$ magnetization for a series of soft pulses. In the present case the pertinent information is exclusively the form of the $z$ magnetization during the series of pulses the pulse, $M^z$, although the full expressions obtained for the $x$, $y$ and $z$ components of the magnetization are presented in this work as a means of validating the present results.

Following the method of Waldstein and Wallace,\textsuperscript{39} conditions are restricted to the case of a single spin with resonance frequency $\omega$, relative to the decoupler irradiation. A series of soft pulses from a decoupler rf field $B_2$ is applied along the -$y$ axis in the present work, where the standard rf field $B_1$ was used in the earlier work.\textsuperscript{39} An effective field
given by \( \gamma B_{\text{eff}} = (\gamma B_2^2 + \omega^2)^{1/2} \) will exist at some angle \( \phi \) from the \( z \) axis, or \( \cos \phi = \gamma B_2 / \gamma B_{\text{eff}} \). The rf pulse will flip the magnetization some angle \( \alpha \) about the effective field, as given by

\[
\alpha = (\gamma B_{\text{eff}})^{-1} t_p
\]  \hspace{1cm} (5.1)

where \( t_p \) is the pulse width as given by

\[
t_p = \text{duty cycle} \cdot \text{dwell time}
\]  \hspace{1cm} (5.2)

The magnetization will then precess between pulses at some angle \( \theta = \omega \tau \) around the static magnetic field \( B_0 \), where \( \tau \), the evolution time, is

\[
\tau = (1 - \text{duty cycle}) \cdot \text{dwell time}
\]  \hspace{1cm} (5.3)

Relaxation during the pulse is neglected in order to simplify the calculations, but is included during the evolution period.

The motion of the magnetization vector during a regular series of identical pulses \( M \) can be described by a classical formulation, as in previous related studies.\(^{39,46,155}\) The effect of a pulse applied along the \(-y\) axis can be represented as a transformation relating the magnetization immediately before the pulse, \( M^- \), to the magnetization before the pulse, \( M^\circ \), through a rotation operator, as

\[
M^- = R_{-y}(\alpha, \phi) M^\circ
\]  \hspace{1cm} (5.4)
where

\[
R_y(\alpha, \phi) = \begin{bmatrix}
\cos \alpha & \sin \alpha \sin \phi & \sin \alpha \cos \phi \\
-\sin \alpha \sin \phi & \cos^2 \phi + \cos \alpha \sin^2 \phi & (\cos \alpha - 1) \sin \phi \cos \phi \\
-\sin \alpha \cos \phi & (\cos \alpha - 1) \sin \phi \cos \phi & \sin^2 \phi + \cos \alpha \cos^2 \phi \\
\end{bmatrix}
\]  

(5.5)

Between the repeated pulses, the spins will precess freely through an angle \( \theta \) about the \( z \) axis as previously defined. Spin-lattice and spin-spin relaxation will occur during this interval. Thus, the magnetization at time \( \tau \) after the pulse is turned off, \( M(\tau) \), is

\[
M(\tau) = R_z(\theta)S(\tau)M_o + S'M_o
\]  

(5.6)

where \( R_z(\theta) \) is the operator for rotation around the \( z \) axis, and \( S(\tau) \) is the operator for relaxation during time \( \tau \) such that

\[
R_z(\theta)S(\tau) = \begin{bmatrix}
E_z \cos \theta & E_z \sin \theta & 0 \\
-E_z \sin \theta & E_z \cos \theta & 0 \\
0 & 0 & E_1
\end{bmatrix}
\]  

(5.7)

and

\[
S' = \begin{bmatrix}
0 & 0 & 0 \\
0 & 0 & 0 \\
0 & 0 & (1 - E_1)
\end{bmatrix}
\]  

(5.8)
where \( E_1 = \exp(-\tau T_1) \) and \( E_2 = \exp(-\tau T_2) \) are the terms containing the relaxation times.

For a steady-state solution, \( M(\tau) = M^* \) and thus eq.(5.6) can be solved for \( M(0) = M^* \) in terms of \( M_e \), the equilibrium magnetization. The three components of the magnetization before the pulse are thus accordingly found to be\(^{154} \) (Appendix 1)

\[
M_x^* = \frac{(1 - E_1)M_e \cos \phi [- E_2^2 \sin \alpha - (E_2^2 - E_2) \sin \theta \sin \phi (1 - \cos \alpha) + E_2 \cos \alpha \cos \theta]}{D(\alpha \theta \phi)}
\]

(5.9)

\[
M_y^* = \frac{(1 - E_1)M_e \cos \phi [1 - \cos \alpha] \sin \phi (E_2^2 + E_2 \cos \theta) + E_2 \sin \alpha \sin \theta]}{D(\alpha \theta \phi)}
\]

(5.10)

\[
M_z^* = (1 - E_1)M_e \cos \phi [1 + E_2 (2 \sin \alpha \sin \theta \sin \phi - \cos \theta (\cos^2 \phi + \cos \alpha \sin^2 \phi + \cos \alpha))] + E_2^2 (\cos \alpha \cos^2 \phi + \sin^2 \phi) / D(\alpha \theta \phi)
\]

(5.11)

where

\[
D(\alpha \theta \phi) = 1 - E_1 E_2^3 + 2 E_2 (1 - E_1) \sin \alpha \sin \theta \sin \phi - E_2 (1 - E_1) (\cos^2 \phi + \cos \alpha + \cos \alpha \sin^2 \phi) \cos \theta + (E_2^2 - E_1) (\sin^2 \phi + \cos \alpha \cos^2 \phi)
\]

(5.12)
Equation (5.12) is equivalent to the denominator term for the magnetization expressions derived by Waldstein and Wallace\textsuperscript{39}. In the hard pulse limit ($\phi=0$) equations (5.9), (5.10) and (5.11) collapse to the appropriate forms as presented by Freeman and Hill\textsuperscript{155}, the $x$ and $y$ magnetizations for the present case being the reverse of those derived by Freeman and Hill, who used the case of rotation about the $x$-axis, while for the present case as well as in the work of Waldstein and Wallace\textsuperscript{39} the rotation operates about the $y$-axis.

A plot of $M_z^*$ (the superscript will henceforth be omitted) as a function of resonance offset $\omega$ for fixed values of $T_1$ and $T_2$, $B_2$, duty cycle and dwell time, is shown in Fig.5.2, together with data calculated using the Bloch equation form of eq.(2.18) plotted as a function of the same resonance offsets and relaxation times. It must be noted that the value of $B_2$ employed in eq.(5.11) is the instantaneous value (per individual pulse), while that in eq.(2.18) is the observable average or calibrated value of $B_2$. Thus, to make the two values of $B_2$ equivalent in each of the two sets of calculations, it is assumed at present that the ensemble average $B_2$ in eq.(2.18) is equivalent to the product of the instantaneous value of $B_2$ and the duty cycle employed in eq.(5.11). This assumption is to be proven valid in the subsequent derivation of the analytical form of $M_z$. The equivalence of the two methods of calculating $M_z$ is clearly demonstrated in Fig.5.2.
Figure 5.2. $M_z$ as a function of dimensionless irradiation resonance offset (absolute offset divided by $\gamma B_z (T_1/T_2)^{1/2}$) calculated using continuous-wave (eq.(2.18)) (solid line) and pulsed rf (eq.(5.11)) (symbol X) for the parameters $T_1 = T_2 = 1$ s, $\gamma B_z / 2\pi$ (instantaneous) = 1 kHz, duty cycle = 20%, dwell time = 50 μs, $M_o = 1$. 
5.2.1(b) Derivation of an Analytical Form for the $z$-Magnetization as a Function of Irradiation Resonance Offset Equivalent to the Bloch Equation for $M_z$

For the equivalence of pulsed and cw rf to hold for a series of repeated soft pulses, it is reasonable to assume that the $z$-magnetization as a function of offset given by eq.(5.11) for repeated pulses should have a simplified form analogous to the cw Bloch equations, as given by eq.(2.18). Equation (2.18) can be expressed in a more simplistic manner by dividing the numerator and denominator by $(\omega_0-\omega)^2$ to give

$$M_z = M_0 \left[ \frac{1}{1 + \frac{(\gamma B_2)^2 T_1 T_2}{(\Delta \omega)^2}} \right]$$

(5.13)

where $\Delta \omega = \omega_0 - \omega$.

In order to derive an analytical or algebraic form demonstrating the equivalence between cw and pulsed rf $B_2$ sources from eq.(5.11) which bears resemblance to eq.(5.13), it is accordingly necessary to make simplifications or approximations to certain terms in the full form for the $z$-magnetization given by eq.(5.11). The following second-order approximations

$$E_1 = 1 - \frac{\tau}{T_1}, \quad E_2 = 1 - \frac{\tau}{T_2}$$

$$\cos \alpha = 1 - \frac{\alpha^2}{2}, \quad \sin \alpha = \alpha$$

$$\cos \theta = 1 - \frac{\theta^2}{2}, \quad \sin \theta = \theta$$
for the appropriate terms in eq.(5.11), while retaining the full expressions for \( \cos \phi \) and \( \sin \phi \), were found numerically to be consistent with the full form of eq.(5.11) at relatively low duty cycles. The numerical solutions above were compared with the solution obtained using the full form solution of eq.(5.11), using identical sets of parameters and resonance offsets in both cases. The chosen approximations faithfully reproduced the data calculated using the full form of eq.(5.11), as demonstrated by the example of the simulations presented in Fig.5.3, when the duty cycle was on the order of 20% or less. These approximations can thus be used to simplify eq.(5.11).

Thus, making substitutions for the appropriate approximations in eq.(5.11) and expanding, the numerator of eq.(5.11), \( M_2^{\text{num}} \), becomes

\[
M_2^{\text{num}} = \frac{M_o \tau}{T_1}[1+(1-\tau/T_2)(2\alpha \theta(1-A)^{1/2} -(1-\theta^2/2)
(A+(1-\alpha^2/2)(1-A)+(1-\alpha^2/2)) + ((1-\tau/2)((1-\alpha^2/2)(A)+(1-A)))
\]

(5.14)

where

\[
A = \left( \frac{(\gamma B_z)^2}{(\gamma B_z)^2 + \omega^2} \right)
\]

(5.15)

As \( \tau \) is normally much smaller than the relaxation times \( T_1 \) and \( T_2 \), terms of order greater than 1 in \( \tau \) may be neglected. Thus, eq.(5.14) may be simplified to yield
Figure 5.3. $M_z$ as a function of relative irradiation resonance offset (absolute frequency $\omega$, divided by $\gamma B_z (T_1/T_2)^{1/2}$) calculated using the approximations for $E_1$, $E_2$, $\cos \alpha$, $\sin \alpha$, $\cos \theta$ and $\sin \theta$ in eq.(5.11), (symbol X) and using the full form of eq.(5.11) (solid line). The values $T_1 = T_2 = 1$ s, $\gamma B_z/2\pi$ (instantaneous) = 1 kHz, duty cycle = 10%, dwell time = 50 $\mu$s, $M_0 = 1$ were used in both cases.
\[ M_{z}^{num} = \frac{M_{o} \tau}{T_{1}} \left[ \left( \frac{2\alpha \theta \omega}{((\gamma B_{2})^{2} + \omega^2)^{1/2}} + \frac{2\alpha^2 + 2\theta^2 - (\alpha \theta)^2}{2} \right) \frac{((\alpha \gamma B_{2})^{2} - 4(\alpha \gamma B_{2})^{2}}{4((\gamma B_{2})^{2} + \omega^2)} (1 - \frac{\tau}{T_{2}}) \right] \]  

(5.16)

Making similar substitutions and approximations in eq.(5.12), the expression for the denominator of \( M_{z} \), \( M_{z}^{den} \) is then

\[ M_{z}^{den} = \frac{\tau}{T_{1}} \left[ \left( \frac{(\gamma B_{2})^{2}}{(\gamma B_{2})^{2} + \omega^2} \right)^{1/2} + \frac{2\alpha^2 + 2\theta^2 - (\alpha \theta)^2}{2} + \frac{(\alpha \gamma B_{2})^{2} - 4(\alpha \gamma B_{2})^{2}}{4((\gamma B_{2})^{2} + \omega^2)} \right] \frac{(\alpha \gamma B_{2})^{2} T_{1}}{((\gamma B_{2})^{2} + \omega^2) T_{2}} \]

(5.17)

The analytical expression for \( M_{z} \) can now be obtained from the quotient of the numerator and denominator terms, eq.(5.16) and eq.(5.17) respectively, which can be expressed as

\[ M_{z} = M_{o} \left[ \frac{1 - \frac{\tau}{T_{2}}}{1 + \chi \left( \frac{T_{1}}{T_{2}} \right)} \right] \]

(5.18)
where the substitution
\[
\chi = \frac{4(\alpha \gamma B_2)^2}{[8\alpha \theta \omega (\gamma B_2)^2 + (\omega)^2]^{1/2} + 4(\theta \gamma B_2)^2 + 4(\alpha \omega)^2 + 4(\theta \omega)^2 - (\alpha \theta \gamma B_2)^2}
\] (5.19)
has been used for simplicity. The substitutions \( \alpha = \gamma B_{\text{eff}} \delta \eta \) and \( \theta = \omega(1-\delta)\eta \) where \( \delta \) is the duty cycle and \( \eta \) is the dwell time may now be made in the appropriate terms in eq.(5.18) and (5.19) above.

Using the aforementioned relation \( \gamma B_{\text{eff}} = (\gamma B_2^2 + \omega^2)^{1/2} \) for the effective magnetic field created by the off-resonance irradiation, and neglecting terms of order greater than 1 in \( \eta \), then in the limit of small duty cycles, eq.(5.18) simplifies to
\[
M_z = M_o \left( \frac{1 - \tau / T_2}{(\delta \gamma B_2)^2 T_1} \right) \left( 1 + \frac{1}{\omega^2(1+2\delta)T_2} \right)
\] (5.20)

For the condition \( \tau \ll T_2 \), as normally encountered in high-resolution NMR in liquids and in the limit of small duty cycles, eq.(5.20) further reduces to eq.(5.21):
\[
M_z = M_o \left( \frac{1}{(\delta \gamma B_2)^2 T_1} \right) \left( 1 + \frac{1}{\omega^2 T_2} \right)
\] (5.21)

It is evident that eq.(5.21) is identical in form to eq.(5.13) for the dependence of
the \( z \) magnetization as a function of irradiation resonance offset. In comparing equations (5.21) and (5.13), it is apparent that the product of the duty cycle and the instantaneous value of \( B_2 \) in eq.(5.21) is equal to the ensemble average or calibrated value of \( B_2 \) in eq.(5.13).

The equivalence of pulsed and \( \text{cw rf} \) has thus been demonstrated. The validity of this equivalence is restricted to small duty cycles. A plot of observed dip width \( (\gamma B_2 (T_1/T_2)^{1/2}) \) determined by eq.(5.20) as a function of duty cycle in Fig.5.4 shows that the dip width starts to deviate noticeably from the expected value based on eq.(5.13) when the duty cycle exceeds approximately 10\%. This deviation is possibly due to the neglect of relaxation during the pulse in the simplified model used for the \( z \) magnetization expression. Numerical calculations performed using a more sophisticated model indicated that relaxation during a pulse may be pronounced during a long series of identical pulses. Such relaxation effects may accordingly become cumulative at higher duty cycles.

5.2.2 Pulse Flip Angle Dependence of Equivalence Between Pulsed and CW RF

When examining the equivalence of pulsed and \( \text{cw rf} \) in terms of the single-spin double resonance and offset-saturation experiments, it is worthwhile to address the possible effects of flip angles of the individual homodecoupler pulses on the observed magnetization. An examination of such flip angle effects is accessible through the use of eq.(5.11), which explicitly contains the pertinent pulse parameters. Simulations were
Figure 5.4. Per cent relative dip width (absolute dip width $\gamma B_2 (T_1/T_2)^{1/2}$) divided by instantaneous $B_2$) as a function of duty cycle calculated by eq.(5.20) (X) and by eq.(5.13) (solid line) for $B_2$ (instantaneous) = 1 kHz, $T_1 = T_2 = 1$ s, dwell time = 50 $\mu$s.
therefore performed by solving eq.(5.11) numerically for $M_z$ as a function of varied irradiation offset values, while varying flip angle parameters. As the flip angle is the product of three parameters, namely, the effective rf field, the duty cycle and the dwell time, three separate sets of simulations were performed. In each set, two of the three flip angle parameters were held constant while the third parameter was varied. The effect of the varied flip angle parameter on the observed half-width $(\gamma B_2(T_1/T_2)^{1/2})$ of the curve of $M_z$ as a function of irradiation offset could therefore be examined. Values of the parameters were chosen such that the flip angles were equivalent in each of the three sets, for a given irradiation offset value.

The results of the simulations are illustrated in Fig.5.5. The differences between the half-width calculated by means of eq.(5.11) and the "predicted" value from the Bloch equations (eq.(5.23) with $B_2 = B_2(\text{instantaneous}) \times \text{duty cycle}$) are plotted as a function of flip angle for each of the three sets of data in Fig.5.5. In one set of calculations (points denoted by $x$) the duty cycle was fixed at 5% and the dwell time fixed at 1 ms, while the instantaneous value of $\gamma B_2/2\pi$ was varied between 1 and 7 kHz. In a second set of calculations (points denoted by an asterisk symbol), the instantaneous value of $\gamma B_2/2\pi$ was held constant at 1 kHz with a 1 ms dwell time, and the duty cycle was varied between 5 and 35%. In the third set of calculations (points denoted by empty squares), a 1 kHz $\gamma B_2$ was employed together with a 5% duty cycle, while the dwell time was varied between 1000 and 7000 $\mu$sec.

It is apparent from Fig.5.5 that flip angle effects may become significant (10% or
greater difference between observed and "predicted" half-widths in $M_z$ vs. irradiation offset curve) when the flip angle becomes large ($\alpha \geq 1$ radian). This observation appears intuitive, when considering, for example, the effects of a 360° pulse. The results obtained in the present section are therefore not entirely unexpected, but serve to substantiate the basis of the calculations employed in this chapter. Early work on the equivalence of FT pulsed and cw NMR experiments by Ernst and co-workers,\textsuperscript{150} Bain and Martin\textsuperscript{163,164} and others\textsuperscript{136} using a density matrix description showed that for general equivalence between the two types of NMR to hold, rf pulses of small flip angles should be employed.

It is also apparent from Fig.5.5 that over the range of flip angles examined, the flip angle dependence of the difference between observed and "predicted" half-widths produced by varying the instantaneous value of $B_2$ and the dwell time are virtually the same, while the flip angle dependence of the half-width calculated by varying the duty cycle is somewhat less pronounced. This could be a result of the cumulative effects of neglecting relaxation during the pulse in the model employed as in eq.(5.11), the effects of which may become more pronounced at higher duty cycles. General theoretical treatments of relaxation in the presence of an rf field have recently been presented,\textsuperscript{165,166} and have been included in the theoretical description of relaxation effects occurring during shaped pulses using a Bloch equations formalism.\textsuperscript{167}
Figure 5.5. Per cent deviation of half-width ($\gamma B_z (T_1/T_2)^1/2$) of simulated $M_z$ vs. irradiation resonance offset curve from value predicted by Bloch equations as a function of individual decoupler flip angle for the three individual sets of calculations, described in the text.
5.3 Discussion

The equivalence of a series of identical, closely-spaced repeated soft rf pulses to cw radiation has been demonstrated in this chapter. A mathematical expression for the dependence of the $z$ magnetization on irradiation resonance offset during a series of soft pulses which contains explicit pulse parameters such as dwell time, duty cycle, flip angle, as well as the instantaneous value of the irradiating field $B_2$, has been obtained. Numerical simulations show that this expression is equivalent to the Bloch equations form for the $z$ magnetization based on cw radiation, giving identical results. From the full expression for the $z$ magnetization, a simplified analytical form for $M_z$ as a function of irradiation offset is obtained using certain approximations, such as truncated forms for the relaxation matrix elements, and flip and precession angles, as well as the neglect of relaxation during the pulses. This equation is shown to be equivalent to the expression obtained from the Bloch equations for the dependence of the $z$ magnetization on irradiation resonance offset, as employed in the basic offset-saturation experiment.

One important consequence of the equivalence of the pulsed and cw forms of irradiation for soft pulses undertaken in this chapter is the demonstration that the ensemble average of the saturating rf field $B_2$, as calibrated by the single-spin double resonance experiment, for example, is equal to the product of the instantaneous rf field strength, the value per individual pulse, and the decoupler duty cycle. This equivalence appears to be limited to small duty cycles, on the order of 20% or less, which is most probably due to the neglect of relaxation effects during the pulses in the derivation of the
model describing the irradiation frequency behaviour of the z magnetization during a series of identical, soft pulses.

The full expression for the irradiation offset dependence of the z magnetization as given by eq.(5.11) has been used to investigate the possible flip angle dependence of the magnetization. The individual decoupler pulse flip angles were varied by changing one of the flip angle parameters, either the decoupler field strength B₂, dwell time, or duty cycle, while holding the other two parameters constant. The effect of varying the single flip angle parameter on the width of the curve of the z magnetization as a function of irradiation resonance offset is then observable as a function of decoupler pulse flip angle. From these calculations, it is apparent that flip angle effects on the observed z magnetization become significant when the rf pulse flip angle size approaches one radian, with the calculated z magnetization deviating noticeably from that predicted by the simple Bloch equations expression for Mₜ with the assumption of cw radiation. These calculations, although not proven experimentally, support quantitatively the premise that the equivalence of pulsed and cw rf radiation holds only for small pulse flip angles.

Thus, when performing offset-saturation experiments or calibrating the irradiating field by the single-spin double-resonance experiment on NMR spectrometers equipped with pulsed homodecouplers, the individual decoupler flip angles should be kept small to ensure the equivalence of pulsed and cw rf. With many conventional high-resolution NMR spectrometers, the duty cycle is set at some fixed value and cannot be altered. Under such circumstances, the decoupler flip angle may be altered by changing decoupler
field strength or the dwell time, both parameters being readily accessible. The experimenter should use a judicious choice of the combination of these two parameters, and be aware of the possible consequences when large flip angles are employed in individual decoupler pulses. As in the case of the general equivalence between pulsed and cw rf, the differing flip angle effects produced by changing the duty cycle are likely the result of the omission of the effects of relaxation during the individual pulses.

Approximations for terms containing flip and precession angles and relaxation matrix elements in the original complete expression for the steady-state $z$-magnetization allow the determination of an analytical form equivalent to the Bloch equation for $M_z$ in the presence of saturation which serves as the basis for the offset-saturation experiment, with the product of the instantaneous value of the irradiating field $B_2$ and the duty cycle equal to the ensemble average $B_2$ in the Bloch equation which assumes cw rf radiation. This theoretical equivalence between pulsed and cw rf is limited to small duty cycles as a result of the simplified nature of the model equation, and is limited to small decoupler pulse flip angles. The limitation of small decoupler pulse flip angles should be borne in mind when undertaking offset-saturation or single-spin double resonance experiments, in order to ensure that the condition of equivalence between pulsed and cw rf is not violated.
CHAPTER 6

APPLICATION OF THE OFFSET-SATURATION EXPERIMENT
TO THE STUDY OF FAST CHEMICAL EXCHANGE

6.1(a) Introduction

This chapter is concerned with application of the offset-saturation method to
determine exchange rates for relatively simple two-site systems undergoing fast chemical
exchange. Application is restricted to systems in which both sites are equally populated,
such as ring inversions and hindered bond rotation. The need for a relatively robust and
facile method for measuring reliable fast exchange rates which are beyond the upper limit
of the lineshape method, has been discussed in the Chapter 3. The use of spin-echo and
T$_1p$ methods which can measure rates on the order of $10^4$ s$^{-1}$ or greater has been restricted,
as mentioned earlier, largely due to a combination of inherent errors, difficulties in
implementation, and the need for specialized hardware requirements. As the offset-
saturation method can be implemented with minimal difficulty on standard high resolution
NMR spectrometers equipped for homodecoupling, with both relaxation times T$_1$ and T$_2$
obtained from a single experiment with relatively straightforward data analysis, the
potential of the offset-saturation technique as a tool for the study fast chemical exchange
becomes apparent. The attributes of the offset-saturation experiment thus offer the
possibility of extending the upper limit of accessible exchange rates.
After an initial cursory examination of the viability of the use of the offset-saturation method as a tool for studying fast chemical exchange using the classic chair-chair interconversion of cyclohexane as a test case, a thorough application of the offset-saturation method to the two-site case of the hindered N-C bond rotation in the heterocycle N-acetylpyrrole is undertaken. In the study of the latter system, the offset-saturation method is used in combination with other NMR rate measurement techniques, such as lineshape analysis and selective-inversion methods for the intermediate and slow-exchange regions, to perform a thorough kinetic analysis over a wide range of rates, covering several orders of magnitude. Protons are the nuclei of interest in both cases.

6.1(b) The Determination of Activation Parameters from Rate Constants

The activation parameters for the kinetic processes involving the species investigated in the present work are determined from the rate constants using the standard formulations for first-order kinetics. The activation energy \( E_a \) for a particular kinetic process has traditionally been obtained from the Arrhenius activation theory by the well-known empirical relationship

\[
k = A \exp(-E_a / RT)
\]  
(6.1)
where the pre-exponential factor $A$, also known as the frequency factor, is usually interpreted as the number of effective collisions per unit time, $R$ is the gas constant, and $T$ is the temperature. The activation energy $E_a$ and the frequency factor $A$ are normally obtained from the slope and intercept respectively from a plot of $\ln(k)$ as a function of $T^{-1}$, provided a suitable range of rate constants have been measured.

Values of the enthalpy of activation, $\Delta H^*$, and $\Delta S^*$, the entropy of activation, can then be determined, assuming temperature independence of $E_a$ and $A$ at a single temperature, using the equations

$$\Delta H^* = E_a - RT$$ \hspace{1cm} (6.2)

$$\Delta S^* = R \left( \ln A - \ln \frac{k_B T}{h} \right)$$ \hspace{1cm} (6.3)

A more complete kinetic formulation relating the rate constant to the thermodynamic activation parameters for the unimolecular processes such as those examined in the present work is the absolute or transition state rate theory which remains the standard way of interpreting kinetics by NMR methods. Transition state theory can be summarized by the Eyring equation which gives the relationship of the rate constant and the temperature dependence of the corresponding thermodynamic parameters as
\[ k = \kappa \frac{k_B T}{\hbar} \exp(-\Delta G^*/RT) \]
\[ - \kappa \frac{k_B T}{\hbar} \exp(-\Delta H^*/RT) \exp(\Delta S^*/R) \]

where \( \kappa \) is the transmission coefficient, the fraction of all reacting molecules reaching the transition state which then proceed to product molecules, which is normally assumed to be unity for the types of processes investigated by NMR.\(^{24,26} \) \( k_B \) is the Boltzmann constant, \( \hbar \) is Planck's constant, \( T \) is the absolute temperature, and \( \Delta G^* \), \( \Delta H^* \) and \( \Delta S^* \) are the free energy, enthalpy and entropy of activation respectively.

The enthalpy of activation \( \Delta H^* \), can be obtained from the slope of the Eyring plot of \( \ln(k/T) \) as a function of \( (T)^{-1} \), while \( \Delta S^* \), the entropy of activation \( \Delta S^* \) is calculated from extrapolation of the Eyring plot to the intercept where \( (T)^{-1} = 0 \). This extrapolation may occur over a considerable temperature range. A degree of error will be introduced into the determination of the activation parameters, particularly in the entropy, if there is any bias in the rate measurements.\(^{24,26,31,32} \) For this reason, it is necessary to obtain rates over as wide a range of temperatures as possible. It is thus apparent that any method which can adequately extend the upper limit of rate measurement will assist in reducing the extent of error in the determination of the activation parameters, and specifically, the entropy of activation.
6.2 Application of the Offset-Saturation Method to the Chair-Chair Ring Inversion of Cyclohexane

6.2.1 Introduction

The classic chair-chair ring inversion of cyclohexane is depicted in Fig. 6.1. In this dynamic process, the ring protons oscillate between axial and equatorial positions at rates accessible by NMR methods. Cyclohexane presents a dynamic system that has been well studied since the early days of investigation of chemical exchange by NMR. Cyclohexane has accordingly served a test case for the development of NMR methods suitable for studies of chemical exchange, including lineshape analysis,\textsuperscript{31,105,170} spin echo,\textsuperscript{128} $T_{1p}$,\textsuperscript{33} and saturation-transfer\textsuperscript{105} techniques. These studies have resulted in a range of values for the activation parameters for the ring-inversion process, with values of the activation energy $E_a$ for example, encompassing the range of 9-12 kcal/mol. Relatively few of these studies have provided rate information for temperatures above approximately 260K.\textsuperscript{128} Cyclohexane has also served as a model subject for various hypotheses concerning the significance of entropies and enthalpies of activation,\textsuperscript{24,170} proving to be a source of some controversy among various research groups.

The observation of chemical exchange in cyclohexane was first reported by Jensen and co-workers in 1960\textsuperscript{171} when they noticed line broadening in the proton NMR spectra of cyclohexane in CS$_2$ solvent occurred when the sample was cooled to approximately
Figure 6.1. The chair-chair ring interconversion of cyclohexane.

-100°C, but a detailed investigation of the exchange process was not undertaken until Jensen and colleagues\textsuperscript{172} used the method of Gutowsky and Holm\textsuperscript{27} at coalescence to obtain a value of $\Delta G^\ddagger = 10.0$ kcal mol\textsuperscript{-1}.

It is important to note that cyclohexane in the chair form is actually a 12-spin system, with two possible chemical shifts (axial and equatorial) and four distinct types of scalar coupling constant, such that with slow rates of exchange, a very complicated $A_2B_6$ \textsuperscript{1}H spectrum\textsuperscript{9} results. The majority of research has contracted the exchange problem of cyclohexane into a simplified two-site process, however. Harris and Sheppard\textsuperscript{173} used linewidth measurements in the fast exchange limit to obtain exchange rates for cyclohexane. The equation of Piette and Alexander\textsuperscript{118} described previously was used to obtain rates, and the corresponding activation parameters were in satisfactory agreement with those of Jensen and colleagues\textsuperscript{172}.

In order to avoid the inherent complications of cyclohexane with 12 spins, deuteration of all but one of the ring protons, to give C\textsubscript{6}D\textsubscript{11}H, in which the single proton is axial in one chair conformation and equatorial in the other form, has been
used.\textsuperscript{105,128,174,175} All coupling constants are then first order H-D coupling which can be removed by decoupling of the deuterium. This procedure was employed by Anet and Bourne\textsuperscript{105} in their seminal study of cyclohexane (C\textsubscript{6}D\textsubscript{11}H) noted in Chapter 3, whereby a combination of lineshape analysis for fast and intermediate rates and saturation transfer in the slow exchange region, to probe exchange rates over a wide range of temperatures. The work of Anet and Bourne\textsuperscript{105} stands as the most thorough study of exchange in cyclohexane, albeit in the form with deuterium substitution for all except one of the protons. Binsch and co-workers\textsuperscript{170} performed a detailed \textsuperscript{1}H bandshape analysis of the ring inversion in 1,1,2,2,3,3,4,4-d\textsubscript{4} cyclohexane, with deuterium decoupling, to investigate in detail the entropies and transition states of the ring inversion of cyclohexane and related monosubstituted derivatives.

In the present context, cyclohexane was selected as a test specimen on which a preliminary evaluation regarding the feasibility of the offset saturation method for measuring exchange rates in the fast exchange regime. Several \textsuperscript{1}H offset-saturation experiments were performed at a spectrometer frequency of 100 MHz on a sample of cyclohexane (without deuteration of any of the proton sites on the ring) in the temperature range where the exchange was fast in the NMR timescale. The offset-saturation experiments were combined with a coalescence rate determination, and with some exchanges rates determined from direct linewidth measurements in the region where significant line broadening due to exchange occurred.
6.2.2 Experimental

A sample containing approximately 1 wt.% cyclohexane in CDCl$_3$ solvent together with a small amount of TTM ([(CH$_3$)$_3$Si]$_4$C) standard in a degassed and sealed 5 mm NMR tube was used for the offset-saturation experiments on cyclohexane. NMR experiments were performed on an Bruker MSL 100 spectrometer, with $^1$H being the observed nucleus. Offset-saturation experiments were performed over a range of temperatures of approximately 60 degrees, from a low of 260 K to nearly 320 K. In this temperature range, the system was in fast exchange, with a single resonance observed in the $^1$H spectrum of cyclohexane. Temperatures were controlled by means of a Bruker VT variable temperature control unit, with temperatures monitored by an external copper/constantan thermocouple. Cooling of the sample when required was accomplished by the use of liquid N$_2$. Temperatures were controlled to an accuracy of ± 1 °C. After changing temperatures, the sample was left to equilibrate for at least half an hour before experiments were performed.

The value of the saturating field $B_2$ employed in the offset-saturation experiments was in the range of 250 Hz, using a decoupler duty cycle of 30%. Decoupler calibrations were performed by the single-spin double-resonance experiment at each of the temperatures studied. The calibrations of $B_2$ were generally performed on the narrow resonance of the internal TTM standard. Irradiation times for the offset-saturation experiments varied according to temperature, with longer irradiation times on the order of 100 s, required at higher temperatures in accordance with the increased $T_1$ relaxation.
time. An inversion-recovery experiment performed at an ambient probe temperature of 295 K on the MSL-100 spectrometer gave a value for the cyclohexane spin-lattice relaxation time $T_1$ of $14.2 \pm 0.4$ s.

In accordance with the relatively long $T_1$ value observed for cyclohexane, a preacquisition delay time on the order of 1 s was used for the offset-saturation experiments. Dwell times on the order of 50 $\mu$s were utilized, with an average spectral size of 8 K. Twenty irradiation resonance offset values were typically employed in each offset-saturation experiment, with eight scans per offset value sufficient to provide adequate signal strength. The offset-saturation data were processed as described previously for the experiment on the water sample, using a linebroadening factor of 10 Hz. The $T_1$ and $T_2$ relaxation times were obtained using the computer program DIPPER.

6.2.3 Results

The $^1$H spectrum of cyclohexane at ambient temperature appeared as a single resonance line with some apparent exchange broadening compared to that of the TTM standard present in the sample. The exchange at such temperatures was accordingly in the timescale regime suitable for offset-saturation experiments. At a temperature of approximately 280 K, the linewidth of the cyclohexane signal was approximately 1.2 Hz compared to 0.5 Hz for the linewidth of the internal TTM standard. As the temperature was lowered, pronounced exchanging broadening became evident. For example, at a temperature of 233 K, the linewidth at half maximum height of the TTM internal standard
was 0.5 Hz, and the linewidth of the cyclohexane resonance was 15.3 Hz. Coalescence was observed to occur in the vicinity of 213 K. At temperatures below 213 K, two distinct resonances were observed, indicating that the system was undergoing slow exchange. The chemical shift separation between sites was on the order of 46 Hz at the spectrometer operating frequency of 100 MHz at low temperatures where the effect of exchange was not apparent in the NMR spectra.

The results of the offset-saturation experiments for cyclohexane are summarized in Table 6.1. The errors given in Table 6.1 represent the 95% confidence limits for the quantity \((\gamma B_2)^{2}T_1/T_2\) and accordingly for the observed \(T_2\) value, from the non-linear least-squares fit to the data. These errors are seen to vary from around 5% to nearly 20% at the highest temperature studied, but are generally in the region of 10%. The value of \(T_1\) obtained by the offset-saturation method at a temperature of 295 K is in good agreement with the value of 14.2 s obtained by the inversion-recovery experiment, and the errors for \(T_1\) in each case are less than those for \(T_2\), varying from approximately 1% to less than 5%. An example of an offset-saturation experiment performed on cyclohexane at a temperature of 303 K is given in Fig.6.2, which shows a plot of \(M_2/M_0\) as a function of irradiation resonance offset.

The values of \(T_2\) measured in the presence of an exchange process will contain a contribution from the exchange to the observed \(T_2\), as discussed in Chapter 3. In order to determine rate constants from the offset-saturation data for cyclohexane in the fast exchange limit, it is accordingly necessary to remove the contributions from other
Table 6.1

Relaxation Times for Cyclohexane from Offset-Saturation Experiments at Varied Temperatures

<table>
<thead>
<tr>
<th>T(K)</th>
<th>$T_1$(s)</th>
<th>$T_2$ (s)</th>
<th>%Error ($T_2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>262.0</td>
<td>7.87</td>
<td>1.56</td>
<td>11.2</td>
</tr>
<tr>
<td>272.5</td>
<td>8.77</td>
<td>3.29</td>
<td>10.9</td>
</tr>
<tr>
<td>279.5</td>
<td>9.25</td>
<td>5.11</td>
<td>13.7</td>
</tr>
<tr>
<td>295.5</td>
<td>14.19</td>
<td>7.28</td>
<td>5.3</td>
</tr>
<tr>
<td>302.9</td>
<td>15.09</td>
<td>8.42</td>
<td>9.4</td>
</tr>
<tr>
<td>310.4</td>
<td>16.75</td>
<td>11.63</td>
<td>10.5</td>
</tr>
<tr>
<td>315.7</td>
<td>17.87</td>
<td>13.41</td>
<td>18.2</td>
</tr>
</tbody>
</table>

In Table 6.1, the error quoted is the percentage error for the observed $T_2$ as determined from the average of the 95% confidence limit errors for the quantity $(\gamma B_2)^5T_1/T_2$ from the non-linear least-squares regression to the offset-saturation data.
relaxation processes to the observed value of T₂ to obtain the exchange broadening. With the offset-saturation method, the magnetic field inhomogeneity contribution is excluded from T₂*. Thus, in the limit of neglect of scalar coupling, the other major contribution to linebroadening and to T₂* will be from the dipole-dipole relaxation mechanism between neighbouring protons, as concluded in previous studies of exchange in cyclohexane. ¹⁰⁵,¹²⁸

For relaxation by a dipole-dipole mechanism for small molecules in a non-viscous liquid, as discussed in Chapter 2, the relaxation times T₁ and T₂ can be considered to be equal, where T₂ is the natural transverse relaxation time. The rate constant k in the fast exchange limit can thus be determined by eq.(3.19) which can explicitly be expressed in terms of the relaxation times as

\[
k = \frac{(\pi \delta v)^2}{2} T_2^{\text{exch}} - \frac{(\pi \delta v)^2}{2} (1/T_2^{\text{obs}}) - (1/T_1)
\]

(6.5)

where T₂^{obs} is the observed T₂ as obtained from the offset-saturation experiment, δv is the chemical shift separation (in Hz) between protons in axial and equatorial sites, and T₂^{exch} is the exchange contribution to T₂^{obs}. The ability of the offset-saturation method to simultaneously measure both T₁ and T₂ is thus especially advantageous for the application to the study of chemical exchange, when the exchange contribution to T₂^{obs} can obtained simply by subtracting contributions from other T₂ relaxation mechanisms in the form of T₁, as in the present case.

The value of δv used in eq.(6.5) for calculating the exchange rate constants was
45.4 Hz as determined from low temperature spectra. Previous studies of cyclohexane have concluded that the axial-equatorial chemical shift difference is essentially independent of temperature.\textsuperscript{33,105,128} The exchange rates calculated accordingly from the $T_2$ values obtained by the offset-saturation method are summarized in Table 6.2. It may be observed that exchanges rates on the order of $10^5$ s$^{-1}$ for the chair-chair inversion process in cyclohexane have been determined by the offset-saturation method. The study of exchange in cyclohexane by Deverell and co-workers\textsuperscript{33} using the rotating frame $T_1p$ technique measured rates on the order of $10^3$ s$^{-1}$ at a maximum temperature of 243.5 K, and the offset-saturation experiments have been performed at temperatures up to 75 K beyond the upper limit of this previous $T_1p$ study of exchange in cyclohexane.

In order to supplement the offset-saturation data, some rate constants were determined at temperatures in the region near coalescence by approximate methods. The rate constant at coalescence was calculated using eq.(3.11). Rate constants were estimated in the slow exchange region from exchange broadening of the linewidth of either resonance using eq.(3.9), at temperatures of approximately 210 K and 205 K, where the exchange lifetime is of sufficient duration to result in observable exchange broadening of both of the resonances. A value of the natural linewidth in the absence of exchange was determined from the linewidth of the internal TTM standard, which was then accordingly subtracted from the measured linewidth, to obtain a value of the exchange contribution to the linewidth.\textsuperscript{92,4,34,86,87,105} In a similar manner, some rate constants were estimated in the fast exchange region above coalescence where substantial exchange broadening was
Figure 6.2. $M_z/M_0$ as a function of irradiation resonance offset for offset-saturation experiment on cyclohexane at a temperature of 302.9 K. The symbols (X) represent the observed steady-state $z$ magnetization, and the solid line represents the best fit to the data. The irradiation time was 102.5 s, and the strength of the rf field $\gamma B_2/2\pi$ was 243.6 Hz. A pre-acquisition delay of 1.0 s was used. The value of $T_1$ obtained from the non-linear least-squares fit to the data was 15.1 s, and the $T_2$ was 8.4 s.
observed. These rate constants were calculated using eq. (6.5), with the linewidth of the internal TTM standard again used to provide a value of $T_1$ (or the natural $T_2$ in the absence of exchange), and the chemical shift difference $\delta v$ of 45.4 Hz as used previously in the calculation of exchange rates determined from the offset-saturation experiments is again employed. The rate constants determined from the slow exchange, coalescence and fast exchange measurements are summarized in Table 6.2, together with the rates determined from the offset-saturation experiments.

6.2.4 Determination of Activation Parameters for Chair-Chair Interconversion of Cyclohexane

Values of the activation energy, enthalpy and entropy for the chair-chair isomerization of cyclohexane can be determined from the exchange rates given in Table 6.2, using the methods outlined in section 6.1 of this chapter. Using the standard Arrhenius rate theory of eq. (6.1), as value of the activation energy $E_a$ can be obtained from the slope of a plot of the natural logarithm of the exchange rates as a function of reciprocal temperature. In the present work, the value of $E_a$ was determined from a standard linear least-squares fit to the experimental data. The corresponding Arrhenius plot for the cyclohexane data is shown in Fig. 6.3. The experimental data are represented by the points, while the solid line represents the best-fit value determined from the linear regression fit to the data. Some scatter in the data is apparent in the lower end of the region where the offset-saturation method was employed in the region where exchange
broadening is more pronounced, but the offset-saturation data overall appear to be collinear with the data determined by the approximate methods in the low-temperature limit.

The resulting value of $E_v$ obtained is $11.3 \pm 0.7$ kcal/mol. The error in $E_v$ represents two standard deviations, as determined from the error in the estimation of the slope by the linear regression routine. This value for the activation energy agrees extremely well with that of by Anet and Bourne\textsuperscript{105} who obtained a value of 11.25 kcal/mol using a combination of lineshape fits and selective inversion experiments on cyclohexane-$d_{11}$. The enthalpy and entropy of activation can be determined using the more complete transition state theory treatment outlined earlier. A plot of $ln(k/T)$ as a function of reciprocal temperature in accordance with the Eyring expression as given by eq.(6.4), was constructed using the rate data in Table 6.2, and a linear regression performed on the data. The resulting plot is shown in Fig.6.4. The enthalpy $\Delta H^*$ as calculated from the intercept of the Arrhenius plot has a value of $10.7 \pm 0.7$ kcal mol$^{-1}$, and the corresponding value of the entropy of activation $\Delta S^*$ calculated from the value of the slope of the Eyring plot as given by eq.(6.4) is $1.8 \pm 0.75$ cal deg$^{-1}$ mol$^{-1}$, with the error representing two standard deviations as determined from the least-squares fit to the $y$-intercept of the Eyring plot. This relatively small value for the entropy of activation lies in the wide range of values obtained from previous studies of cyclohexane.\textsuperscript{24,105,128} The thermodynamic parameters determined for the ring inversion process in cyclohexane from the rate data of Table 6.2 are summarized in Table 6.3, along with the value of the Arrhenius frequency factor $A$. 
Table 6.2

Rate Constants ($k$) for Chair-Chair Isomerization in Cyclohexane as a Function of Temperature

<table>
<thead>
<tr>
<th>$T$ (K)</th>
<th>$k$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>204.7</td>
<td>45.2</td>
</tr>
<tr>
<td>209.2</td>
<td>53.9</td>
</tr>
<tr>
<td>213.2</td>
<td>$1.01 \times 10^2$</td>
</tr>
<tr>
<td>230.2</td>
<td>$5.44 \times 10^2$</td>
</tr>
<tr>
<td>238.0</td>
<td>$1.06 \times 10^3$</td>
</tr>
<tr>
<td>262.0</td>
<td>$1.98 \times 10^4$</td>
</tr>
<tr>
<td>272.5</td>
<td>$5.36 \times 10^4$</td>
</tr>
<tr>
<td>279.5</td>
<td>$1.16 \times 10^5$</td>
</tr>
<tr>
<td>295.5</td>
<td>$1.52 \times 10^5$</td>
</tr>
<tr>
<td>302.9</td>
<td>$1.94 \times 10^5$</td>
</tr>
<tr>
<td>310.4</td>
<td>$3.87 \times 10^5$</td>
</tr>
<tr>
<td>318.7</td>
<td>$5.17 \times 10^5$</td>
</tr>
</tbody>
</table>
Figure 6.3. Arrhenius plot of the natural logarithm of the exchange rate constant \( k \) as a function of reciprocal temperature for the chair-chair interconversion process of cyclohexane. The symbols (X) represent the experimental data of Table 6.2, and the solid line represents the line of best fit through the data as determined by the linear regression. The experimental data between \( 1000/T = 3.1 - 3.8 \) were obtained from offset-saturation experiments, while the remainder were determined from linewidth and coalescence temperature (\( 1000/T = 4.65 \)) measurements.
Figure 6.4. Eyring plot of ln (k/T) as a function of reciprocal temperature, for the chair-chair interconversion process of cyclohexane. The symbols (X) represent the experimental data from Table 6.2, and the solid line represents the line of best fit through the data as determined by the linear regression.
Table 6.3

Activation Parameters for Chair-Chair Isomerization of Cyclohexane

<table>
<thead>
<tr>
<th>$E_a$ (kcal/mol)</th>
<th>$\Delta H^*$ (kcal/mol)</th>
<th>$\Delta S^*$ (eu)</th>
<th>$A$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.3 ± 0.7</td>
<td>10.7 ± 0.7</td>
<td>1.8 ± 0.75</td>
<td>3.5 x 10$^{13}$</td>
</tr>
</tbody>
</table>

6.2.5 Discussion

The present study of cyclohexane in which the offset-saturation experiment was used to obtain rates in the fast exchange regime for the chair-chair inversion process in cyclohexane has indicated the potential applicability of the offset-saturation method to study fast chemical exchange in simple two-site systems. Combined with rate data from coalescence point and linewidth measurements, reasonable values of the thermodynamic activation parameters for the ring inversion process are obtained. The offset-saturation method has enabled exchange rates on the order of $10^5$ s$^{-1}$ to be measured, thereby extending the upper limits of previous studies of cyclohexane by techniques such as the $T_{1p}^{33}$ spin echo$^{128}$ and lineshape/saturation transfer$^{105}$ methods.

The value of 11.3 ± 0.7 kcal/mol for the activation energy agrees well with the value of 11.24 kcal/mol obtained by Anet and Bourne in their seminal study of cyclohexane.$^{105}$ The enthalpy of activation, $\Delta H^*$, determined to be 10.7 ± 0.7 kcal/mol, agrees with the range of approximately 9 to 11.5 kcal/mol obtained in previous studies.$^{105}$
The entropy of activation and the exact nature of the transition state or activated complex for the ring inversion process in cyclohexane has been the source of some controversy over the years, resulting in various values for the entropy in the general range of -6 to +5 eu.\textsuperscript{24,31,105,128,170} The small positive value of 1.8 ± 0.75 eu obtained in the present study thus falls within the range of the previous values quoted for this quantity.

From equations (6.1) and (6.4), the frequency factor $A$ obtained from the $y$-intercept of the Arrhenius plot for unimolecular processes should be equal to $(k_B T/h)$, which for rate processes where the entropy of activation is expected to be small or near zero, should be on the order of $10^{13}$ s$^{-1}$. Pronounced deviations from the value of $10^{13}$ for the frequency factor may be indicative of the presence of systematic error in the methods used to obtain the rate data.\textsuperscript{57,84,128} The value of $3.5 \times 10^{13}$ s$^{-1}$ for the frequency factor obtained for the present study of cyclohexane is therefore encouraging.

Some scatter or deviation from linearity in the temperature dependence of the cyclohexane offset-saturation data is evident, as mentioned previously, particularly at lower temperatures where the exchange broadening is more pronounced. The observed $T_2$ will accordingly be shorter at these lower temperatures, resulting in larger values of $(\gamma B_2)^2 T_1/T_2$, the width of the curve of $M_x$ as a function of irradiation offset at half-maximum height. Some reduction in the errors incurred for the $T_2$ from the offset-saturation experiments may be possible by more thorough location of the sampling points, as described in the previous chapter for the experiments on the water sample. Errors in some instances exceeded 10% which will of course be translated into uncertainties in the
measured rate constants.

A source of error in the offset-saturation experiments may be the neglect of spin-spin coupling which will be present in the cyclohexane sample used in these experiments which was not deuterated. The purpose of the present study is to probe the feasibility of applying the offset-saturation method to the study of fast chemical exchange, rather than performing the definitive study of the chair-chair interconversion of cyclohexane. If the latter were to be accomplished, cyclohexane-$d_{11}$ should be used to remove all possibilities of scalar coupling amongst the ring protons, as in the study by Anet and Bourne.\(^{105}\)

An overall appraisal of the results obtained for the present study of cyclohexane by the offset-saturation method in conjunction with rates obtained by approximate methods such as linewidth and coalescence point measurements indicates the applicability of the offset-saturation technique for studies of fast chemical exchange in such relatively simple two-site systems. The inherent advantage of the offset-saturation method in simultaneously providing values of both relaxation times $T_1$ and $T_2$ from a single experiment under identical conditions permits a facile determination of the exchange contribution to the observed $T_2$. The rates can thus be readily be calculated. As the $T_2$ determined from offset-saturation experiments is void of any contribution from magnetic field inhomogeneity, exchange rates can be determined at relatively high temperatures where exchange broadening effects are less pronounced. The offset-saturation method can thus be used to extend the upper limits of rate studies, as in the present case where rates on the order of $10^3$ s$^{-1}$ were obtained for cyclohexane.
6.3 Application of the Offset-Saturation Method to the Study of Hindered Internal Rotation in N-Acetylpyrrole

6.3.1 Introduction

The preliminary study of cyclohexane presented in the previous section has demonstrated the potential of the offset-saturation method for studying fast chemical exchange and ultimately for extending the upper limit of accessibility of rate measurements in two-site systems. In order to appraise more thoroughly the offset-saturation method as a potential tool for the study of dynamic systems, a more intensive application of the technique is warranted. In this section, the offset-saturation method is therefore applied to another two-site equally-populated system, the five-membered heterocyclic ring species N-acetylpyrrole, illustrated in Fig.6.5, in which the two ring protons labelled H₂ and H₃ interchange as a result of the hindered rotation process about the C-N bond.

The present study of N-acetylpyrrole is aimed at demonstrating the utility of the offset-saturation method as a relatively facile and reliable method for studying fast chemical exchange, with the ability to extend the upper boundary of accessibility of rates which are beyond the limitation of the conventional lineshape method. The offset-saturation technique is used in combination with lineshape analysis, using a time-domain method for calculating the lineshapes which has certain advantages over the usual approaches for calculating lineshapes, to obtain intermediate exchange rates, while selective inversion experiments were used to obtain slow (0.1 - 10 s⁻¹) rates of exchange.
for N-acetylpyrrole. This combination of NMR methods is used to study the exchange in the proton spectra of the protons H₂ and H₄ adjacent to the nitrogen atom on the pyrrole ring of N-acetylpyrrole. As discussed earlier in this chapter, rate data should be obtained over as wide a range of temperatures as possible in order to obtain accurate values for the activation parameters for a kinetic process. Any one method of rate measurement is valid only over a restricted temperature range, so a variety of techniques is useful to extend the range.24,33,84,105,181,188 The present combination of methods accordingly facilitates the measurement of a very wide range of rate constants, and permits the accurate determination of values of the enthalpy, ΔH*, and the entropy, ΔS*, of activation from an Eyring plot of log (k/T) as a function of (1/T). Realistic values of the errors in the activation parameters can also be obtained when rate constants are determined over a wide range of temperatures. The methods chosen to obtain the rates of exchange for N-acetylpyrrole over the appropriate range of timescales are discussed in more detail below, including the range of temperatures over which the methods were employed.

The study of hindered bond rotation by NMR methods, especially typified by rotation about partial double bonds in amide [C(O)-N] type systems, has been the subject of an extensive body of research, representing the earliest applications of NMR to the study of dynamic systems, as mentioned in Chapter 3, and has been reviewed in detail by several authors.84,176-178 The interaction of the nitrogen lone pair with the carbonyl π bond has attracted much interest, both of a theoretical and experimental nature. In addition, models of the amide bond contribute to the understanding of the biologically
important peptide link, and are needed to study the protein folding problem.\textsuperscript{179} The energy barriers for the hindered rotation in the majority of cases are generally in the range of 15-25 kcal/mol,\textsuperscript{84,176-178} high enough to be readily accessible by NMR methods. As a result, such systems have also served as test subjects for the application of new NMR methods over the years.\textsuperscript{31,118,127,180-182}

N-acetylpyrrole has the additional interesting feature that the \( \pi \) system in the ring appears to interact with the amide group, with the result that the carbonyl and the ring \( \pi \) systems compete for the nitrogen lone pair, the nitrogen lone pair of electrons thus forming part of the aromatic sextet of the pyrrole ring. This additional interaction results in a lowering of the barrier, compared to the barriers in other amide systems.\textsuperscript{84,98,183-186} Dahlqvist and Forsen\textsuperscript{98} performed a detailed study of the exchange in solutions of N-acetylpyrrole in CD\(_2\)Cl\(_2\) solvent, using lineshape analysis based on density matrix theory, over the temperature range -70 to + 50 °C. A value of \( E_a = 12.6 \pm 0.5 \) kcal/mol was obtained for the activation energy, while \( \Delta H^* = 12.0 \pm 0.5 \) kcal/mol and \( \Delta S = -0.6 \pm 2.3 \) eu were the resulting values for the enthalpy and entropy of activation respectively for the hindered rotation.

\[
\begin{align*}
\text{CH}_3 & \quad \text{CH}_3 \\
\text{H} & \quad \text{H} \\
\text{C} & \quad \text{C} \\
\text{N} & \quad \text{N} \\
\text{C} & \quad \text{C} \\
\text{O} & \quad \text{O}
\end{align*}
\]

Figure 6.5. Hindered C-N bond rotation in N-acetylpyrrole.
The results obtained by Dahlqvist and Forsen were subsequently disputed by Pinder\textsuperscript{184} who claimed that the data of the original study were misinterpreted. Matsuo and co-workers\textsuperscript{185,186} performed coalescence temperature and lineshape analysis studies of the hindered rotation in 1-Acypyrroles, and obtained a value of 12.6 ± 0.1 kcal/mol for the free energy ΔG\textsuperscript{*} at coalescence for the barrier to rotation in N-acetylpyrrole in CDCl\textsubscript{3}. McClung and Muhandiram\textsuperscript{187} used N-acetylpyrrole as a test system for application of \textsuperscript{1}H-\textsuperscript{13}C DEPT magnetization transfer theory and experiments to the study of chemical exchange, calculating rates of exchange with uncertainties on the order of 15\%, with no activation parameters for the hindered rotation reported. Thus, some discrepancy remains with respect to the values for the energy barrier, enthalpy and entropy of activation for the hindered C-N bond rotation in N-acetylpyrrole.

**Fast Exchange**

In the fast exchange regime, where the spectrum is a single broad line, the offset-saturation method of measuring the spin-spin relaxation time, T\textsubscript{2}, was used, in the manner discussed in the previous section for cyclohexane. The offset-saturation method was used over a temperature range of some fifty degrees, from room temperature (297 K) to an upper limit of 347 K. The exchange rates were calculated using eq.(6.5) similar to the previous example of cyclohexane, and it is also assumed that the value of T\textsubscript{2} in the absence of exchange is equal to T\textsubscript{1}. A value of 114.6 Hz obtained from the low temperature NMR spectrum was used as the chemical shift difference δν in eq.(6.5) at
each temperature. This chemical shift difference in N-acetylpyrrole was observed to be independent of temperature in the slow exchange region.

**Intermediate Exchange**

In the intermediate exchange regime, the rates are comparable to the difference in Larmor frequency between the two sites, and significant line broadening followed by coalescence occurs. As discussed in Chapter 3, the use of approximate methods to calculate reliable rates of exchange in the intermediate exchange regime is inappropriate, and therefore a complete lineshape analysis should be undertaken in such cases. The complete formulae for the lineshapes are well known from the earliest work on exchange\textsuperscript{27,92}, but Reeves and Shaw\textsuperscript{29} showed that even near coalescence the lineshapes can be described as two Lorentzian lines, and consequently developed a procedure to calculate lineshapes which was extendable to multi-site exchange problems.\textsuperscript{189-191} In the formulation of Reeves and Shaw,\textsuperscript{27} the position, phase, intensity and width of the Lorentzians are strong functions of the exchange rate, but the spectrum is always described by just two Lorentzians. In the frequency domain, these results simply lead to the standard results such as those obtained by Gutowsky and Holm.\textsuperscript{27} In the time domain however, the fact that there are just two Lorentzians simplifies the calculations dramatically. A calculated FID can readily be constructed, and processed in parallel with the experimental data, which allows the use of powerful NMR processing packages, ultimately simplifying comparisons of calculated and observed spectra. Furthermore,
contributions from the natural linewidth can be included in the time domain as a simple multiplication, rather than the mathematical convolution that is required in the frequency domain.

In the formulation of Reeves and Shaw\textsuperscript{29} for the two lines in a two-site, equally populated exchanging system, a frequency difference $2\Omega$ (in radians/sec) between the two sites is defined, while $k$ is the rate at which magnetization leaves one site, and $T_2$ is the natural spin-spin relaxation time for each site. Three further parameters can be defined in equations (6.6)-(6.8) inclusive:

\begin{align*}
  r &= \frac{1}{T_2} + k \quad (6.6) \\
  \varepsilon &= (\Omega^2 - k^2)^{1/2} \quad (6.7) \\
  \alpha &= (k^2 - \Omega^2)^{1/2} \quad (6.8)
\end{align*}

For the case of $k < \Omega$ (below coalescence), the spectrum consists of two symmetrical lines at $\pm \varepsilon$, one of which is described by equation (6.9) where

\begin{equation}
  V(x) = A \left[ \frac{r}{r^2 + (x-\varepsilon)^2} - \frac{k}{\varepsilon} \frac{x-\varepsilon}{r^2 + (x-\varepsilon)^2} \right] \quad (6.9)
\end{equation}
In eq.(6.9), \( x \) is the frequency variable, \( V(x) \) is the lineshape function, and \( A \) is a normalization constant. The first term represents an absorption mode which is mixed, to the extent of \( k\varepsilon \), with the dispersion mode in the second term, and a similar term involving \((x + \varepsilon)\) which describes the other half of the spectrum also exists.

Above the point of coalescence, when \( k > \Omega \), the spectrum can be described as two absorption-mode lines at \( x = 0 \), one positive and relatively narrow, and the other negative in phase, broader, and vanishing in intensity in the limit of fast exchange. These two lines are described by equations (6.10) and (6.11):

\[
V_+(x) = - A(1 - \frac{k}{\alpha} \left[ \frac{r + \alpha}{(r + \alpha) + x^2} \right]) \tag{6.10}
\]

\[
V_-(x) = A(1 + \frac{k}{\alpha} \left[ \frac{r - \alpha}{(r - \alpha) + x^2} \right]) \tag{6.11}
\]

In the limit of fast exchange, when \( k >> \Omega \), \( \alpha \) approaches \( k \) and thus only the \( V_+(x) \) term will exist under such circumstances.

In order to calculate the lineshapes for the two-site exchange, free induction decays were calculated using the intensity, real parts, imaginary parts, and decay rates from the above equations. These calculated FID's were then processed like the real data using the NMR data processing package NMR286. The real and calculated spectra could then be compared using a dual display function in the software. The fitting was done visually as
opposed to performing iterations of the spectra.

**Slow Exchange**

In the slow exchange regime, the rates may be too slow to cause significant line-broadening, but are comparable to spin relaxation rates. In such instances, therefore, generalizations of the inversion-recovery experiment, in the form of selective-inversion experiments, are advantageous, as discussed previously in Chapter 3. In such cases, both non-selective and selective inversions can be used to prepare the spin system, which is then observed during the relaxation back to equilibrium.

For two equally-populated sites in chemical exchange, the return to equilibrium of the magnetization at site A following a perturbation is given by eq.(3.10), where it may be recalled that in this equation, $M_A(t)$ and $M_B(t)$ are the magnetizations at time $t$ for sites A and B respectively, $M_e$ is the equilibrium value of the magnetization at either site, $r$ is the spin-lattice relaxation rate of the two sites (which are assumed to be equal) and $k$ is the rate at which a spin leaves one site to go to the other.

It is apparent that there are five parameters, namely, $r$, $k$, $M_A(0)$, $M_B(0)$ and $M_e$ in eq.(3.10). Using standard non-linear least squares methods, best fit values of these parameters were obtained for the experimental data, using a version of a computer program based on the program SIFIT. In addition, the non-linear least-squares fit allows at least an estimate of the errors in the parameters, as discussed previously. In any non-linear problem, the parameters will not be statistically independent, so that the error
associated with a single parameter is not well defined. Further details regarding the selective inversion experiments performed on N-acetylpyrrole in the slow exchange limit are given the following section.

6.3.2 Experimental

The sample of N-acetylpyrrole was prepared courtesy of Ms. Sabine Ternieden according to the method of Reddy. Samples containing a few drops of N-acetylpyrrole in CDCl₃ (MSD Isotopes, 99.8 atom % D) were prepared in 5 mm NMR tubes. The samples were not degassed.

NMR spectra were run on a Bruker AC-300 spectrometer at a magnetic field of 7 T, using a 5 mm four nucleus probe, with ¹H being the observed nucleus in all the experiments reported herein. The proton π/2 pulse width was 7.8 μs. Temperatures were controlled with a BVT 2000 temperature controller on the spectrometer and checked with a copper-constantan thermocouple in a 5 mm NMR tube inserted into the probe. Temperature readings between the thermocouple in the probe, and the thermocouple used by the temperature controller agreed to within 0.5 °K, and were stable over the course of an experiment to within ± 0.3 °K.

The offset-saturation experiments were performed as described previously, using the proton homodecoupler on the spectrometer console in the high power range (10H to 20H) to provide the saturating rf. Typical values of the irradiating field γB₂/2π were 100 Hz, which was calibrated using the single-spin double-resonance experiment, as described
in previous sections. The single-spin double resonance experiments were performed on
the isolated upfield methyl (-CH₃) resonance of the N-aceyl moiety, and satellite signals
adequate for decoupler calibration could be observed in a single scan. The T₁ for the spins
of interest was approximately in the range of 7-15 s, so the irradiation times ranged from
approximately 50 s at the lower temperatures to 80 s at the higher temperatures. An
average delay of 1.0 s to allow partial T₁ relaxation was used prior to acquisition which
was accomplished using a π/2 pulse with a sweep width of 10 kHz, or a dwell time of
50 μs.

The offset-saturation experiments were performed without spinning of the samples,
and an average of sixteen scans was accumulated for each spectrum. An average of thirty
irradiation resonance offset values was used in each offset-saturation experiment, and
distributed around the resonance position according to the partial derivative sampling
criteria described in Chapter 4, with the majority of the offsets located close to the
expected value of the half-width at half-height of the curve of Mₑ as a function of
irradiation offset. The spectra were processed in the manner described previously, with
a line broadening factor of 10 Hz used prior to Fourier transformation of the spectra. The
computer program DIPPER was again used to extract the T₁ and T₂ values from the
offset-saturation data.

The free induction decays for the spectra used for the lineshape analysis in the
intermediate exchange region, were transferred from the spectrometer to an IBM PS/2
microcomputer using the program NMRLINK, and then processed off-line with the
spectral processing computer program NMR286 on a personal computer. The selective inversion experiments were perform by using a $\pi/2 - \tau - \pi/2$ excitation sequence. For two-site exchange, the transmitter was placed on one of the lines, and the value of $\tau$ was set to $1/(2\Delta v)$, where $\Delta v$ is the difference in frequency between the two lines, measured in Hz. Following the excitation, the system was allowed to relax for a series of variable delays, and then the spectrum was acquired with a $\pi/2$ pulse.

6.3.3 Results

A 300 MHz $^1$H NMR spectrum recorded at a temperature of 216 K is shown in Fig.6.6. The doublet with components of equal intensity centred at approximately 7.4 ppm can be assigned to the individual sites for the $H_2$ and $H_3$ protons. Several variable temperature $^1$H NMR spectra for the $H_2$ and $H_3$ protons are shown in Fig.6.7, and are typical of the spectra of a two-site equally populated system as discussed in Chapter 3. It is evident that the system is in the slow exchange region at temperatures on the order of 260 K or lower, while in the fast exchange regime at temperatures above approximately 265 K. At room temperature, the exchange is well into the fast exchange regime, and a single narrow line is observed for protons $H_2$ and $H_3$.

The offset-saturation experiments were performed over a fifty degree temperature range. At the lowest temperature of 297 K, the linewidth of the exchange-averaged resonance for protons $H_2$ and $H_3$ which was irradiated in the offset-saturation experiments was approximately 10.4 Hz at half-maximum height, compared to a linewidth of 0.5 Hz
Figure 6.6. 300 MHz $^1$H NMR spectrum of N-acetylpyrrole at a temperature of 216K.
Figure 6.7. Variable temperature 300 MHz $^1$H NMR spectra of ring protons H$_2$ and H$_3$, adjacent to the nitrogen atom in N-acetylpyrrole.
for the resonance assigned to the methyl group of the N-acetyl moiety. Considerable exchange broadening was therefore present at room temperature. The results of the offset-saturation experiments are summarized in Table 6.4, which gives the $T_1$ and $T_2$ values obtained directly from the offset-saturation experiments at each temperature. The errors in the observed $T_2$ ($T_2^{obs}$) values as determined from the non-linear least-squares fit to the parameter $(\gamma B_2) T_1/T_2$ are also included in Table 6.4. These errors are seen to vary from approximately 2% to a maximum of 13%, but are on the average around 7%. In all instances, the error in the determination of $T_1$ from the non-linear regression was less than the error in $T_2$, and were generally on the order of 1.5 %.

Some scatter and inconsistency is evident in the $T_1$ data, which is somewhat disconcerting. For the higher temperatures studied ($> 320$ K), the values of $T_1$ obtained from the offset-saturation experiment deviated significantly from the expected values of the $T_1$. For the lowest temperatures, the $T_1$ values obtained from inversion-recovery and offset-saturation experiments were generally in the range of 7 - 8 seconds. At higher temperature, e.g. 335 K, the value obtained from the offset-saturation experiment was 14 seconds. This phenomenon will be discussed in the subsequent section of this chapter. The values of $T_2$ obtained from the offset-saturation experiments appear to be self-consistent, as indicated in Table 6.4, following the expected trend with temperature. A plot of the observed half-width of the curve of $M_2$ at half-maximum height $(\gamma B_2(T_1/T_2)^{1/2})$ as a function of temperature is shown in Fig.6.8, and shows the expected trend for narrowing of the linewidth as the exchange rate increases.
Table 6.4
Relaxation Times for N-Acetylpyrrole from Offset-Saturation Experiments on at Varied Temperatures

<table>
<thead>
<tr>
<th>T(K)</th>
<th>T₁ (s)</th>
<th>T₂ (s)</th>
<th>% Error (T₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>297.2</td>
<td>8.74</td>
<td>0.08</td>
<td>10.0</td>
</tr>
<tr>
<td>300.4</td>
<td>9.14</td>
<td>0.11</td>
<td>2.6</td>
</tr>
<tr>
<td>305.0</td>
<td>12.06</td>
<td>0.14</td>
<td>2.9</td>
</tr>
<tr>
<td>308.7</td>
<td>7.12</td>
<td>0.18</td>
<td>12.5</td>
</tr>
<tr>
<td>310.7</td>
<td>9.21</td>
<td>0.19</td>
<td>8.9</td>
</tr>
<tr>
<td>315.0</td>
<td>15.25</td>
<td>0.31</td>
<td>8.4</td>
</tr>
<tr>
<td>319.8</td>
<td>8.59</td>
<td>0.39</td>
<td>2.0</td>
</tr>
<tr>
<td>324.2</td>
<td>7.48</td>
<td>0.48</td>
<td>6.6</td>
</tr>
<tr>
<td>329.6</td>
<td>14.63</td>
<td>0.7</td>
<td>6.1</td>
</tr>
<tr>
<td>335.0</td>
<td>13.95</td>
<td>0.97</td>
<td>5.2</td>
</tr>
<tr>
<td>337.3</td>
<td>18.14</td>
<td>1.29</td>
<td>7</td>
</tr>
<tr>
<td>342.0</td>
<td>24.85</td>
<td>1.87</td>
<td>4.9</td>
</tr>
<tr>
<td>347.0</td>
<td>57.93</td>
<td>4.18</td>
<td>5.9</td>
</tr>
</tbody>
</table>

In Table 6.4, the error quoted is the percentage error for the observed T₂ as determined from the average of the 95% confidence limit errors for the quantity $(\gamma B_2)^2 T_1 / T_2$ from the non-linear least-squares regression to the offset-saturation data.
The fast exchange rate constants determined using the relaxation times obtained from the offset-saturation experiments using eq.(6.5) are given in Table 6.5. An example of an offset-saturation experiment on N-acetylpyrrole is given in Fig.6.9, with the observed and best-fit data obtained at a temperature of 335 K. The intermediate exchange rates are also summarized in Table 6.5. Some examples of the observed and corresponding calculated spectra are shown in Fig.6.10.

An additional example of fitted lineshapes is presented in Fig.6.11, which shows the two Lorentzian components that comprise the overall observed lineshape. The results for the selective inversion experiments in the slow exchange region are given in Table 6.5. The errors quoted in Table 6.5 are two standard deviations, where as determined from the variances of the variance-covariance matrix for the fit. Errors in the lineshape fits are estimated to be on the order of 10%. Figure 6.12 shows an example of a typical agreement between the fit and the experimental data for a selective inversion experiment.

6.3.4 Determination of Activation Parameters for Hindered Rotation in N-Acetylpyrrole

The rate data for N-acetylpyrrole summarized in Table 6.5 can be used to determined values of the thermodynamic activation parameters for the hindered C-N bond rotation using the standard Arrhenius and Eyring theories outlined earlier in this chapter. The rate constants obtained for the exchange in N-acetylpyrrole cover several orders of magnitude, from approximately 0.5 s to $3 \times 10^5$ s$^{-1}$, and thus should provide the means
Figure 6.8. Plot of $\gamma B_2 (T/T_2)^{1/2}$ as a function of temperature from offset-saturation experiments on N-acetylpyrrole. The values of $\gamma B_2 (T/T_2)^{1/2}$ were determined from the non-linear least-squares fit to the observed intensities ($z$-magnetizations) as a function of irradiation resonance offset for each temperature. The value of $B_2$ was approximately equal in each case.
Figure 6.9. $M_z/M_o$ as a function of irradiation resonance offset for an offset-saturation experiment on N-acetylpyrrole at a temperature of 335 K. The circles represent the steady-state z magnetization after irradiation, as a function of irradiation frequency offset from resonance. The irradiation time was 80 seconds, and the strength of the rf field, $\gamma B_2/2\pi$, was 102 Hz. The solid line represents the best fit to the data, giving a value for $T_2$, the spin-spin relaxation time of 0.97 s.
Figure 6.10. Calculated (left) and observed (right) 300 MHz $^1$H NMR spectra at various temperatures for N-acetylpyrrole. The calculated exchange rates (k) are given for each spectra, together with the corresponding temperature. The feature in the observed spectra at approximately 6.7 ppm appears to be an impurity.
Figure 6.11. Observed and calculated 300 MHz $^1$H NMR spectra of the ring protons next to the nitrogen in N-acetylpurrole at temperature of 256.7 K. The upper spectrum is the experimental spectrum. The small peaks on top of the lineshape are due to impurities. The bottom spectrum represents the best fit calculated spectrum showing the full lineshape (heavy line) and its two Lorentzian components, calculated according to equation (6.9).
Figure 6.12. Results of a selective inversion experiment on exchanging protons 2 and 5 in N-acetylpyrrole at 212 K. The points show the recovery of the magnetization following the selective inversion of one line: the circles represent the line that was inverted and the triangles show the magnetization of the other site. The solid line represents the best fit to the data.
Table 6.5

Rate Constants (k) for Hindered Rotation in N-Acetylpyrrole
as a Function of Temperature

<table>
<thead>
<tr>
<th>Temperature (K°)</th>
<th>k (s⁻¹)</th>
<th>% Error</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>209.9</td>
<td>0.48</td>
<td>8</td>
<td>Selective Inversion</td>
</tr>
<tr>
<td>212.9</td>
<td>0.77</td>
<td>5</td>
<td>Selective Inversion</td>
</tr>
<tr>
<td>219.9</td>
<td>1.80</td>
<td>4</td>
<td>Selective Inversion</td>
</tr>
<tr>
<td>231.7</td>
<td>20*</td>
<td>10</td>
<td>Lineshape</td>
</tr>
<tr>
<td>235.6</td>
<td>27*</td>
<td>10</td>
<td>Lineshape</td>
</tr>
<tr>
<td>241.2</td>
<td>37</td>
<td>10</td>
<td>Lineshape</td>
</tr>
<tr>
<td>245.8</td>
<td>58</td>
<td>10</td>
<td>Lineshape</td>
</tr>
<tr>
<td>252.0</td>
<td>95</td>
<td>10</td>
<td>Lineshape</td>
</tr>
<tr>
<td>256.7</td>
<td>1.44 x 10²</td>
<td>10</td>
<td>Lineshape</td>
</tr>
<tr>
<td>259.7</td>
<td>1.95 x 10²</td>
<td>10</td>
<td>Lineshape</td>
</tr>
<tr>
<td>268.8</td>
<td>4.45 x 10²</td>
<td>10</td>
<td>Lineshape</td>
</tr>
<tr>
<td>279.6</td>
<td>1.00 x 10³*</td>
<td>10</td>
<td>Lineshape</td>
</tr>
<tr>
<td>290.8</td>
<td>1.80 x 10³*</td>
<td>10</td>
<td>Lineshape</td>
</tr>
<tr>
<td>297.0</td>
<td>5.23 x 10³</td>
<td>10</td>
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</tr>
<tr>
<td>300.0</td>
<td>7.21 x 10³</td>
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</tr>
<tr>
<td>305.0</td>
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</tr>
<tr>
<td>307.5</td>
<td>1.19 x 10⁴</td>
<td>13</td>
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</tr>
<tr>
<td>310.0</td>
<td>1.25 x 10⁴</td>
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</tr>
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<td>315.0</td>
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</tr>
<tr>
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<td>2.64 x 10⁴</td>
<td>2</td>
<td>Offset-Saturation</td>
</tr>
<tr>
<td>325.0</td>
<td>3.32 x 10⁴</td>
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</tr>
<tr>
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<td>Offset-Saturation</td>
</tr>
<tr>
<td>335.0</td>
<td>6.75 x 10⁴</td>
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<td>337.3</td>
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</tr>
<tr>
<td>342.0</td>
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</tr>
<tr>
<td>347.0</td>
<td>2.92 x 10⁵*</td>
<td>6</td>
<td>Offset-Saturation</td>
</tr>
</tbody>
</table>

Data points marked with an asterisk were excluded from the determination of the activation parameters.
for obtaining accurate values for the energy, enthalpy and entropy of activation for the hindered rotation process. The Arrhenius plot of $ln(k)$ as a function of reciprocal temperature is shown in Fig.6.13. The results for the three separate NMR methods of rate measurement employed in this study are seen to correlate very well, as demonstrated by the collinearity of the data over the majority of the temperature range in Fig.6.12.

It may be noted that at the ends of the range of lineshape measurements, the tabulated rates deviate from the straight line, and apparently give a slightly lower slope. This effect is most likely due to the neglect of the small proton-proton couplings that occur in this molecule (reported values: $J_{23} = 3.00$ Hz, $J_{24} = 1.42$ Hz, $J_{25} = 2.06$ Hz, $J_{34} = 3.00$ Hz, $J_{35} = 1.47$ Hz, $J_{45} = 3.15$ Hz at 50°C). In treating the N-acetylpyrrole molecule as a simple two-site exchange without scalar coupling, the linewidth was assumed to be due to exchange, when in fact there is a contribution from the coupling so that the rate that corresponded to the experimental lineshape at low temperature would be overestimated. Likewise, the rate from the fit in the upper bounds of fast exchange limit would be underestimated. In this case, the incorrect treatment of the lineshape in the appears to bias the slope in favour of value lower than the true one.

Certain rate data were obtained when the particular method employed was beginning to break down, and therefore provide erroneous or inconsistent results. These points are marked with an asterisk in Table 6.5, and were omitted in the determination of the thermodynamic parameters. The regression analysis for the Arrhenius plot of Fig.6.13 results in a value of $13.2 \pm 0.2$ kcal/mol for the energy of activation $E_a$ for the
Figure 6.13. Arrhenius plot of ln(k) as a function of reciprocal temperature for the rate data for N-acetylpyrrole given in Table 6.5. The points (X) represent the experimental data indicated in Table 6.5, while the solid line represents the best-fit line obtained by linear regression. Points in the range (1000/T = 4.45 - 4.25) were determined using selective-inversion experiments, the data in the middle range centered around 1000/T = 3.9 were obtained from lineshape analysis, while the points in the region 1000/T = 2.85 - 3.3 were determined from offset-saturation experiments.
hindered rotation.

As mentioned earlier, there is good overall linearity amongst the temperature dependence of the rates determined by the three methods. The Arrhenius plot representative of the rate data determined exclusively by the offset-saturation method is shown in Fig. 6.14, and a standard least-squares regression on the data, with the exception of the deviant point at the highest temperature, yields a value of $14.0 \pm 0.8$ kcal/mol for the activation energy from the slope of the Arrhenius plot, where the error is approximately two standard deviations. A value of $9.3 \times 10^{13}$ was obtained for the frequency factor $A$. Thus, the offset-saturation experiments exclusively yield reasonable values for the activation energy for the hindered rotation process, and the frequency factor.

The linear regression methods used to extract the enthalpy and entropy of activation from an Eyring plot will, in addition to giving values for the slope and intercept of the line, often provide errors for these parameters, and these errors are often quoted in the literature. Particularly for entropies of activation, which may involve a considerable extrapolation of the data, reasonable estimates of the errors are crucial in understanding whether values are significant or not. However, the calculated errors must be interpreted carefully, since many software packages do not emphasize the possibility of correlations of the errors. There will be an error associated with the determination of the $y$-intercept. The error in the slope, which is required for the extrapolation, may also contribute significantly to the uncertainty in the intercept. Under such circumstances, quoting the
variance of the intercept without including the covariance may result in inaccurate
determination of the error associated with the intercept.

The problem of the existence of covariance between the slope and the intercept can
be treated by moving the value of the zero of the independent variable (1/T in this case)
so that the covariance vanishes. The covariance between the slope and intercept is
proportional to the sum of the x values (in this case, the sum of the values of 1/T). If the
average of the values of 1/T is used as the origin about which to perform the regression,
then the errors in the slope and intercept will be uncorrelated. The slope always remains
the same, but the intercept has been changed by shifting the data. It is then possible to
estimate the error of the intercept with the best-fit line running through the true (1/T=0)
point. Details of this procedure for removing the covariance between slope and intercept
are given in Appendix 2. The composite Eyring plot is shown in Figure 6.15, with the
best-fit linear regression line through the data, which is extrapolated back to 1/T = 0.

The linear regression on the data for the Eyring plot, using the method of shifting
the zero point on the set of data in Table 6.5, gives a value for the enthalpy of activation
$\Delta H^*$, of 12.7 ± 0.2 kcal/mol and the entropy of activation, $\Delta S^*$, is 1.2 ± 0.7 eu, where the
errors are two standard deviations. A value of 1.2 ± 0.2 eu was obtained for the entropy
from the standard linear regression without consideration of covariance between slope and
intercept, with the error quoted being the standard deviation in the intercept as provided
by the linear least-squares program. The activation parameters for the hindered rotation
in N-acetylpyrrole are summarized in Table 6.6. These values compare well with
Figure 6.14. Arrhenius plot of the natural logarithm of the exchange rate constant $k$ as a function of reciprocal temperature for N-acetylpyrrole as determined by the offset-saturation method.
Figure 6.15. Eyring plot of ln(k/T) versus reciprocal temperature for hindered internal rotation in N-acetylpyrrole. The rate constants have been reduced by the pre-exponential factor in eq.(6.4), assuming \( \kappa = 1 \). The symbol \( k_b \) represents Boltzmann’s constant, and \( h \) is Planck’s constant. The circles represent the data from Table 6.5 determined using the particular method indicated in the table, and the straight line represents the linear regression performed about the average of the values of 1000/T, which is indicated by the dotted vertical line.
Table 6.6

Activation Parameters for Hindered Rotation in N-Acetylpyrrole

<table>
<thead>
<tr>
<th>$E_a$ (kcal/mol)</th>
<th>$\Delta H^\ast$ (kcal/mol)</th>
<th>$\Delta S^\ast$ (e.u.)</th>
<th>$A$ ($s^{-1}$)</th>
</tr>
</thead>
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<tr>
<td>13.2 ± 0.2</td>
<td>12.7 ± 0.2</td>
<td>1.2 ± 0.7</td>
<td>3.5 x 10^{13}</td>
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</tbody>
</table>

Dahlqvist and Forsen's values\(^{98}\) of 12.6 ± 0.5 kcal/mol for $E_a$, $\Delta H^\ast = 12.0 ± 0.5$ kcal/mol, and $\Delta S^\ast = -0.6 ± 2.3$ eu, which were obtained in methylene chloride over a narrower range of temperatures, but disagree with Pinder's re-interpretation of their data,\(^{184}\) as mentioned previously.

An *ab initio* calculation performed at the HF/6-31G** level of theory with the assumptions of a planar ground state and a transition state in which the acetyl group is rotated 90° with respect to the pyrrole ring resulted in a value of 12.6 kcal/mol for the barrier to hindered rotation in N-acetylpyrrole, which corresponds to $\Delta H^\ast$ in the gas phase.\(^{195}\) Provided that solvent effects are not strong, such calculations can be useful in modelling the electronic structure of amides and other systems with conjugated $\pi$ systems.
6.3.5 Discussion

The combination of three complementary NMR techniques employed in the present study of hindered rotation in N-acetylpyrrole provides a novel and powerful way to investigate such rate processes with relatively small energy barriers separating molecular conformations. The use of the offset-saturation method for $T_2$ measurement in the fast exchange region, and selective inversion methods in the slow exchange NMR regime, extend the normal NMR lineshape methods greatly. In particular, the fast exchange results are important, since they extend the measurements toward $(1/T) = 0$. This combination of methods for rate measurement results in an Eyring plot over one of the widest temperature ranges studied using NMR methods, and provides excellent values for the enthalpy and entropy of activation, along with good estimates of the errors.\textsuperscript{195} The values for the energy, enthalpy and entropy of activation obtained from the present study are in general agreement with the previous work of Dahlqvist and Forsen,\textsuperscript{98} and the upper range of reliable rate determination has been extended nearly fifty degrees beyond that achieved in the earlier study.\textsuperscript{98}

The value of $1.2 \pm 0.7$ eu obtained for the entropy is consistent with the anticipated small or essentially zero values of entropies of activation expected for hindered C-N bond rotation in small amido-type compounds.\textsuperscript{31,84,176-178} Entropies of activation are sensitive to errors in rate determinations,\textsuperscript{24,31,32,84} and deviations from the value anticipated for the entropy for a particular rate process may be indicative of the presence of systematic error, similar to frequency factors.\textsuperscript{31,32,84,196} The small entropy obtained for the present study of
N-acetylpyrrole suggests negligible systematic error overall, with the possibility existing that the combined systematic errors for the three separate methods of rate measurement average to zero.

The technique of shifting the origin of the Eyring plot to remove covariance between slope and intercept provides a more realistic value of the error in the entropy of activation compared to the common practice of simply quoting the error or standard deviation in the determination of the intercept provided by the particular least-squares regression routine utilized. In the present case of N-acetylpyrrole, the difference in errors calculated by the two different procedures is not dramatic, with an error of ± 0.2 eu provided directly by the regression, compared to an error of ± 0.7 eu at the 95% confidence limit determined by shifting the origin of the Eyring plot. In such cases, however, the difference may be significant enough to distinguish whether or not an entropy value obtained is statistically meaningful, especially for entropies expected to be near zero.²⁴,⁴²,⁸⁴

From the temperature dependence of the rate data for N-acetylpyrrole, as evident either in the form of the Arrhenius plot of Fig.6.12 or the Eyring plot of Figure 6.14, the possible detrimental effects in the use lineshape data alone without including the effects of scalar coupling²⁸,⁹⁹ are illustrated. Particularly in the extremes of fast and slow exchange, the data at temperature extremes are essential to give good values for the regression through the points. Unless the natural linewidths are well established, the errors at either end of the Eyring or Arrhenius plot may result in an incorrect value for the
slope, and ultimately the enthalpy and activation energy will be erroneously determined.

The offset-saturation experiments performed over a temperature range of some fifty degrees have yielded rates of exchange up to $10^5 \text{ s}^{-1}$, with an average associated error for the measured rates on the order of $\pm 10\%$ or less. As noted earlier, there is a discrepancy in the $T_1$ values obtained from the offset-saturation experiment, and those expected on the basis of inversion-recovery experiments. This discrepancy is more pronounced at higher temperatures. At some temperatures where these $T_1$ anomalies were observed, some repetitions of offset-saturation experiments were performed in which experimental parameters such as the irradiation time, pre-acquisition delay time $\tau$ and the value of the irradiating field $B_2$ were variously changed, in an attempt to observe whether one or other of these parameters were improperly selected. These experiments proved inconclusive, and the $T_1$ values did not noticeably change in any systematic manner. The reason for the observed $T_1$ discrepancy is thus not understood, and does not appear to be readily interpreted as an experimental artifact, or an idiosyncrasy of the particular system being studied. As noted in an earlier section of this chapter, the spin-lattice relaxation time $T_1$ values obtained by the offset-saturation method for cyclohexane were in agreement with those expected on the basis of inversion-recovery experiments.

The possible errors in rate constants resulting from the $T_1$ discrepancy fortunately does not significantly affect the value of the exchange rate determined using eq. (6.5) at temperatures where the exchange broadening still dominates the linewidth. Undoubtedly this observed $T_1$ effect will provide an upper restriction on the accessible rate limit in
such cases, with an associated systematic error which cannot be quantified to any degree
of certainty. It is assumed that the $T_1$ values, and the natural transverse relaxation times
$T_2$ in the absence of exchange are equal for the protons in each of the two sites involved
in the exchange process for the ring protons. The low-temperature $T_1$ measurements did
not violate this assumption.

The presence of scalar coupling is a possible source of systematic error which
must be considered in the offset-saturation measurements, as with the lineshape fits
discussed previously. The couplings between ring protons as quoted above are small in
magnitude compared to the chemical shift difference between sites at the spectrometer
frequency of 300 MHz used in the present studies so that the spectra are first order.
Alexander$^{197}$ postulated that spin-spin coupling does not affect the exchange-averaged
spectrum in the fast exchange limit, provided that second order spectra at low
temperatures collapse to a single sharp line at high temperatures in the fast exchange
limit. The neglect of spin-spin couplings has been shown to be a source of error in
lineshape analysis.$^{31,32,167,188,194,198}$ Unresolved long-range spin-spin couplings, even five-
bond proton-proton ($^5J_{H_5,H}$) couplings, have been shown to influence the linewidths in
hindered N-C bond rotation in amides.$^{194,198}$ The effects of neglecting weak spin-spin
couplings will be most acute when the relative chemical shift difference between
exchanging sites is small.$^{89}$

The neglect of the spin-spin couplings in N-acetpyrrole and in cyclohexane
studied in the previous section, may contribute to errors in the determination of the
exchange contribution to $T_2$ and hence to the exchange rates most seriously when, as mentioned previously, the effects of exchange broadening of the lineshape are minimal and the observed linewidth approaches the natural linewidth in the absence of exchange. This may be manifest in some departure in linearity of the temperature dependence of the exchange rates, as evident for the rates at the highest limits of application of the offset-saturation technique in the Arrhenius or Eyring plots of figures 6.12 and 6.14 respectively. Such rates were accordingly omitted in the determination of the activation parameters.

A dipole-dipole relaxation mechanism has been assumed to be the dominant process for the spin-lattice relaxation of the ring protons $H_2$ and $H_5$ in $N$-acetylpyrrole, as assumed in the previous example of cyclohexane. Aside from the contribution to linewidth from the neglected scalar coupling, there may be a contribution to the proton linewidth resulting from scalar relaxation due to coupling quadrupolar relaxation of the $^{14}N$ nucleus, which is a function of the $^{14}N T_1$.

These possible scalar relaxation effects were determined negligible in the lineshape analysis of $N$-acetylpyrrole by Dahlqvist and Forsen. The $T_1$ values for $^{14}N$ in amide systems are generally sufficiently short that the neighbouring protons are effectively decoupled from the $^{14}N$ by the $^{14}N$ spin-lattice relaxation, thereby making negligible contributions to the observed linewidths.

The upper limit of rates accessible in the fast exchange region is in principle limited by the ability to measure the exchange contribution to the observed $T_2$, when the exchange broadening is comparable to the experimental error in measuring the total linewidth. Based on the capabilities of high resolution NMR spectrometers at that time,
Allerhand and colleagues\textsuperscript{32} estimated that a practical upper limit to which exchange rates could be determined accurately for protons was of the order of $\delta \nu 2\tau \geq 0.05$, where $\delta \nu$ is the chemical shift difference in Hertz, and $\tau$ is the exchange lifetime. The capability of measuring $T_2$ without any contribution from magnetic field inhomogeneity accordingly endows the offset-saturation method with the potential to extend the upper limit of rate accessibility, and this has been demonstrated both for N-acetylpyrrole and for cyclohexane, with rates on the order of $10^5$ s\textsuperscript{-1} determined in both cases.

In addition, the extension of the offset-saturation method to determine relaxation times and corresponding rates of exchange for unequally populated two-site systems appears to be relatively straightforward extension of the treatment of equally populated systems presented in this chapter, as indicated by preliminary studies of the \textit{cis-trans} isomerization in 2-furfuraldehyde.\textsuperscript{200-202} Forward and reverse rates can be determined using, for example, the equations of Piette and Anderson,\textsuperscript{24,118} presented previously as equations (3.18a) and (3.18b).

Neglect of scalar couplings and the anomalous $T_1$ values for N-acetylpyrrole measured by the offset-saturation method appear to have imposed restrictions on the upper boundary of rate measurement in the present applications to exchange, but it is feasible that rate constants on the order of $10^6$ s\textsuperscript{-1} could be measured with the offset-saturation method under more ideal circumstances. Knuttel and Balaban\textsuperscript{78} have made a similar postulation based on a study of hydroxyl proton exchange in water-ethanol solutions using a more basic form of offset-saturation experiment. The offset-saturation method thus
compares favourably with the rotating frame T₁p method for fast exchange, for which it has been hypothesized, based on an analytical form for the upper rate limit of k ≤ 1/4 (Δω)²T₁, would provide rates up to 10⁶ s⁻¹ ³³ but has to date proven difficult to obtain experimentally. The spin-echo method for measuring rates in fast exchange, which has a closed form for the upper limit similar to that of the T₁p method, has been shown to be capable of measuring rates to 10⁴ s⁻¹, as mentioned in Chapter 3.

The use of the offset-saturation technique to obtain rate data for systems in fast exchange requires the use of the chemical shift value between exchanging sites as determined from low temperature spectra when resonances for the individual sites can be observed. It is also assumed that this chemical shift is independent of temperature. As noted earlier, the CPMG spin-echo and T₁p methods can be used to determine absolute values of the rate constant, rather than the rate relative to the frequency difference, by imposing a timescale on the exchange such as the effects of changing the pulse spacing on the observed T₂ in the former method, and from the dependence of T₁p on the magnitude of the rf field B₁ in the latter technique. In a similar manner, the possibility of determining the absolute exchange rates from offset-saturation experiments by observing the dependence of the exchange contribution to T₂ on the strength of the saturating field B₂ arises. Some preliminary studies of such dependence were undertaken with both cyclohexane and N-acetylpyrrole, but no quantifiable effects could be determined.

The present approach employed for applying the offset-saturation method to
studying fast exchange should be used with some caution at lower temperatures. Allerhand *et al.* have indicated that inaccurate rates may result when eq.(6.5) (or eq.(3.19)) is used to determine rates at temperatures close to coalescence, and in order to keep the errors due to the approximate nature of these equations below 5%, and these equations should only be used for rates such that \(\delta v 2\tau \leq 0.2\). Alternative expressions for calculating fast exchange rates have been proposed, but are more cumbersome in nature and as a result, have seen only limited use. The offset-saturation method is therefore optimally employed when the temperature is safely above the coalescence point, and rate data in the region of coalescence is best obtained using lineshape methods. In addition, the severe exchange broadening close to coalescence, accompanied by small values for the observed \(T_2\), may result in a large value for the half-width at half-height \(((\gamma B_z) (T_1/T_2)^{1/2})\) of the curve of \(z\) magnetization as a function of irradiation offset, accordingly increasing the error in the measured \(T_2\) and exchange rates at such temperature.

The rate constants for the exchange in N-acetylpyrrole determined in the present study by the offset-saturation method appear to be consistent with those determined at lower temperatures by the lineshape calculation and selective-inversion methods, and this combination of methods provides a good approach to the study of chemical exchange in relatively simple systems. A complete lineshape analysis remains the most practical or efficient and accurate way of determining rates of exchange over the fast, intermediate and slow regimes of exchange, while the offset-saturation and selective-inversion
techniques have been shown to be valuable for extending the upper and lower regions of rate measurement respectively.

The lower activation energy determined for N-acetylpyrrolo compared to those of other amides suggests that the barrier to the hindered C-N rotation in N-acetylpyrrolo is primarily due to the loss of partial double-bond character in the transition state. *Ab initio* calculations have indicated that the C-N bond in N-acetylpyrrolo increases in length from 1.39 Å in the planar ground state to 1.434 Å in the transition state. The interaction of the ring π system with the nitrogen lone pair, which may be largely localized in the pyrrolo ring in this case, does appear to result in a lower barrier to rotation about the C-N bond, compared to typical amide systems in which the nitrogen lone pair interacts exclusively with the carbonyl group. This effect is also observed in many cases where there is an N-vinyl substituent on the amide group.

In this chapter, the offset-saturation method has been applied to systems undergoing fast chemical exchange. The ability of the offset-saturation method to conveniently determine *T*₂ values without contribution from magnetic field inhomogeneities has enabled exchange rates on the order of 10⁵ s⁻¹ to be measured, as demonstrated both for the chair-chair interconversion of cyclohexane, and for the hindered C-N bond rotation in N-acetylpyrrolo. The advantage of determining rates over as wide a range of temperatures as possible in order to obtain accurate values of the entropy of activation is illustrated with N-acetylpyrrolo. Shifting the origin of the Eyring plot to the average of the reciprocal temperature values facilitates determination of a reasonable value of the error
for the entropy as obtained from the $y$-intercept of the Eyring plot by linear regression methods. Values of the thermodynamic activation parameters obtained for cyclohexane and for N-acetylpiprro were in general agreement with the corresponding values determined from previous studies of these species.

The offset-saturation technique possesses the capability of extending the upper limit of rate measurement. The use of the offset-saturation method, in combination with other methods such as lineshape analysis and selective-inversion methods for accessing the intermediate and slow exchange regimes respectively, affords the possibility of obtaining rate data over a wide range of temperatures, thereby proving to be a useful method for studying chemical exchange in two-site systems by NMR.
CHAPTER 7
SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

7.1 Summary and Conclusions

The difficulties inherent in the measurement of the spin-spin relaxation time \( T_2 \), and the need for a convenient method for measuring \( T_2 \) for application to measure rates of exchange in the fast exchange regime have been discussed in previous chapters of this thesis. The potential utility of a method capable of simultaneously measuring the spin-lattice (\( T_1 \)) and spin-spin relaxation times in studies of molecular motions is also apparent. The offset-saturation method which has been investigated in this thesis has been shown to be a facile technique for measuring \( T_2 \) and for determining fast rates of exchange. In addition, the offset-saturation technique has the capability of measuring both \( T_1 \) and \( T_2 \) from a single experiment. Several aspects of the offset-saturation experiment for simultaneously measuring \( T_2 \) and \( T_1 \) relaxation times, and for measuring rate constants for systems exhibiting fast chemical exchange rates have accordingly been examined in this thesis.

The offset-saturation method examined in the present work involves the irradiation of a single-spin system with an rf field \( B_2 \) provided by the standard homodecoupler of an NMR spectrometer at a series of offsets from the resonance position of the line results in partial saturation of the \( z \) magnetization. A plot of the resulting partially-saturated \( z \)
magnetization as a function of irradiation resonance offset shows a functional form described by the Bloch equations, with the significant feature that the width of the curve at half-maximum height is equal to the quantity $\gamma B_z (T_1/T_2)^{1/2}$. The ratio $T_1/T_2$ can thus be obtained if the value of $B_z$ is known. The insertion of a pre-acquisition delay time which is some fraction of the spin-lattice relaxation time $T_1$ after gating off the decoupler allows partial relaxation of the spin system, and results in a non-zero minimum of the curve of $M_z$ as a function of irradiation resonance offset. A three-parameter non-linear least-squares fit to a set of irradiation offsets and corresponding intensities is used to obtain absolute values of $T_1$ and $T_2$.

The relative simplicity of the offset-saturation technique, together with the fact that it can be implemented on modern high resolution NMR spectrometers without modifications to existing spectrometer hardware, gives this method unique advantages over other existing methods for the simultaneous determination of $T_1$ and $T_2$, and especially for the determination of the spin-spin relaxation time $T_2$. Several distinct facets pertinent to the overall methodology have been explored during the course of this work. The calculation of errors for parameters and relaxation times for offset-saturation experiments, experimental design and optimization in terms of the choice of sampling points and experimental parameters, methods for the calibration of the irradiating field $B_z$, and the equivalence between pulsed and cw rf radiation have been addressed in the course of this work.

The results presented for measurements of $T_1$ and $T_2$ for a sample of distilled,
acidified water demonstrated that the $T_1$ and $T_2$ values obtained by the offset-saturation method were in excellent agreement with those obtained from standard inversion-recovery and CPMG experiments respectively. Errors for the parameters and corresponding relaxation times measured by offset-saturation experiments through the non-linear least-squares analysis have been determined by two separate methods. The first method of error determination is the commonly-used standard deviation. The standard deviations can be calculated from the variance-covariance matrix of the non-linear least-squares fit to the data from offset-saturation experiments. The second method of error determination is a calculation of the 95% confidence intervals from the intersection of the $\chi^2$ confidence surface contour tangent planes with the appropriate parameter axis.

The errors determined as the 95% confidence limits for the fitted parameters for offset-saturation experimental data were consistently approximately three times the value of the standard deviations. The confidence limits accordingly represent a more realistic error evaluation, and these errors are therefore the error values given for the relaxation times determined by offset-saturation experiments in the present study. The existence of covariance amongst the parameters, which is neglected in the determination of the standard deviations, is evident in the off-axis orientation of the $\chi^2$ confidence region contours, as well as in the consistent observation of the confidence limit errors being a factor of three greater than the standard deviations, rather than the expected value of two standard deviations at the 95% confidence level. The overall quality of the experimental data is also reflected in the shape of the confidence limit contours. With errors calculated
as the 95% confidence limits, the offset-saturation experiments performed on acidified distilled water illustrate that $T_1$ can be determined with an error of less than $\pm 0.5\%$, while $T_2$ can be measured with a precision of $\pm 2.5\%$. These small errors in the measurement of the relaxation times compare favourably with the errors obtained by other methods, such as the inversion-recovery method for $T_1$ measurement, and the CPMG technique for $T_2$ determination. In general, errors for $T_1$ and $T_2$ as determined from offset-saturation experiments are on the order of $\pm 5\%$. Sets of offset-saturation experiments were performed in which the irradiating field $B_2$ and the pre-acquisition delay $\tau$ were separately varied, using the water sample. These sets of experiments demonstrated the overall robustness of the offset-saturation method, with an overall insensitivity of the experimental results with respect to changes in these parameters evident. The offset-saturation method thus appears to be a relatively robust and facile method for the simultaneous determination of $T_1$ and $T_2$. Importantly, provided the value of $B_2$ is sufficiently large, the value of $T_2$ obtained from offset-saturation experiments is essentially free from contributions due to magnetic field inhomogeneities, a factor which hinders the measurement of the spin-spin relaxation time by linewidth measurements. It may be concluded that the major attribute of the offset-saturation method is accordingly the offering of a relatively simple alternative for measurement of the spin-spin relaxation time $T_2$. The offset-saturation method offers the advantages of convenience and relative ease of implementation and robustness compared to the standard CPMG spin-echo method for measuring $T_2$ values, and therefore should prove to be a viable alternative to the
CPMG method for measurements of $T_2$.

For straightforward measurements of the spin-lattice relaxation time $T_1$, the classic inversion-recovery experiment remains the most simple and robust method for measuring $T_1$ exclusively. The discrepancy observed for the $T_1$ values obtained by offset-saturation and inversion-recovery methods for the exchanging system N-acetylpiprrrole indicates that the $T_1$ obtained by the offset-saturation method may in some instances be suspect, and this phenomenon should be investigated in further detail by experimental applications of the offset-saturation method to a variety of systems. The $T_1$ values obtained by the two separate methods for both water and cyclohexane were however in good agreement.

The potential of the offset-saturation method for use as a dynamic NMR technique has been explored in Chapter 6 of this thesis. The need for a facile method for measuring fast rates of exchange for which accurate measurement of $T_2$ are often difficult represents an area potentially suitable for investigation by the offset-saturation method. Although the CPMG and rotating frame $T_{1p}$ methods have been employed to studies of chemical exchange, and have the advantage of measuring rates of exchange exceeding the upper limit accessible by lineshape analysis methods, these techniques suffer from difficulties in implementation or the need for modifications to spectrometer hardware. The relative ease of operation and versatility of the offset-saturation technique accordingly compares favourably to the spin-echo and $T_{1p}$ methods for studies of fast chemical exchange.

The ability to obtain $T_2$ values with minimal error from offset-saturation experiments relies largely on accurate calibration of the irradiating field $B_2$. Methods for
determining rf field strengths such as the measurement of the width of a 180° or 360°
degree pulse width, are not generally of the required accuracy. In the present study, the
single-spin double resonance experiment has been used for calibration of B₂. In the single-
spin double resonance experiment, the irradiation of a single-spin system with the
homodecoupling field B₂ results in satellite resonances symmetrically placed about the
resonance position which are observed when the irradiation is gated on during acquisition.
The distance between satellite peaks is proportional to the value of B₂ employed. With
the single-spin double resonance experiment, it is possible to determine a value of B₂ to
a precision on the order of ± 1 %. The single-spin double resonance method thus provides
a relatively simple and precise method of decoupler calibration to use in conjunction with
offset-saturation experiments. In addition, the linewidth of the single-spin double-
resonance satellites serves as a qualitative indicator of magnetic field inhomogeneities.

The partial derivative criteria for design of offset-saturation methods described in
Chapter 4 provides an adequate qualitative guideline for the optimal choice of sampling
points of irradiation resonance offsets, and pre-acquisition delay times. The three-
parameter non-linear fit used to extract the T₁ and T₂ values utilizes a step-wise correction
to the parameters using a matrix of the partial derivatives of the observable (z-
magnetizations) with respect to the fitted parameters. The inverse of this matrix evaluated
with the best-fit values of the parameters gives the variance-covariance matrix for the fit.
The partial derivative criteria is based on the premise that maximizing the partial
derivatives of the magnetizations with respect to the fitted parameters should in theory
minimize the variance or standard deviation of that particular parameter.

The partial derivatives calculated for simulated offset-saturation experiments illustrate the difficulties in performing or designing a perfect offset-saturation experiment. With three parameters involved in the non-linear regression analysis, the behaviour of the three partial derivatives as a function of irradiation resonance offset, for example, is sometimes conflicting. This fact thus necessitates biasing the experimental design in favour of one or two of the parameters at the expense of another parameter. As the true virtue of the offset-saturation method is undoubtedly the relative facility with which \( T_2 \) values can be obtained, the experiments are therefore designed in such as manner as to attempt to optimize the particular parameter which contains \( T_2 \). The majority of the sampling points or irradiation resonance offsets are therefore located in the vicinity of the estimated value of the width of the curve of \( M_2 \) as a function of irradiation offset ant half-maximum height. Experimental applications of the offset-saturation method, as demonstrated by the results of the acidified water sample, have verified that the quantity \( (\gamma B_2)^2 \frac{T_1}{T_2} \) is the most difficult parameter to determine.

The partial derivative criteria also predicts that for optimal estimate of \( T_2 \), the pre-acquisition delay \( \tau \) should be minimized. The series of offset-saturation experiments performed on the water sample with varied values of \( \tau \) demonstrates that in fact, the offset-saturation method was relatively robust in terms of the choice of \( \tau \). The omission of covariance between parameters in the partial derivative optimization criteria may be somewhat responsible for the failure of the experiments to verify the theoretical
predictions with respect to the delay $\tau$. The partial derivative method should at best be considered a qualitative guideline for optimizing the choice of sampling points and experimental parameters in techniques such as the offset-saturation method.

The offset-saturation technique has been demonstrated to be a useful dynamic NMR technique for studying fast exchange from applications to relatively simple two-site exchanging systems such as the chair-chair ring interconversion process in cyclohexane, and the hindered C-N bond rotation in N-acetylpyrrole. Rate constants on the order of $10^5$ s$^{-1}$ are shown to be readily obtainable using the offset-saturation method to measure $T_2$. It may be concluded that the offset-saturation method provides a viable alternative to the CPMG and $T_{1p}$ methods for fast rate measurement. The advantages of the offset-saturation method over the difficult CPMG technique, are apparent, and are highlighted by the fact that spin-echo methods are limited to studies of rates with an upper limit of $10^4$ s$^{-1}$.$^{32,57}$ The convenience of the offset-saturation method endows it with a unique advantage compared to the $T_{1p}$ method which is not readily implemented on many high resolution NMR spectrometers. The additional advantage of simultaneous measurement of $T_1$ and $T_2$ by the offset-saturation method is also apparent in applications to exchange, where the contribution to the observed $T_2$ from the natural $T_2$, which is equal to $T_1$ for a dipole-dipole relaxation mechanism, is obtained along with $T_2$, from a single experiment.

The fact that exchange rates on the order of $10^5$ s$^{-1}$ have been measured for the systems studied by the offset-saturation method presents the potential applicability of this method to systems such as molecules with low energy barriers to hindered bond rotation
or conformational interconversions. In the present work, the systems studied were not truly ideal test subjects in terms of suitability for establishing the upper limit of applicability of the offset-saturation method. The presence of scalar coupling which cannot be accounted for theoretically in the Bloch equations basis of the offset-saturation method hinders a clear investigation of the true limitations of the offset-saturation method in terms of studies of fast chemical exchange. The additional complication observed in N-acetylpyrrole whereby the $T_1$ values obtained by the offset-saturation method were, especially at higher temperatures, in disagreement with the values expected on the basis of inversion-recovery experiments, presents a dilemma concerning applications of the offset-saturation method to exchanging systems, although correct $T_1$ behaviour was observed in the study of cyclohexane. A thorough study of a system such as cyclohexane-$d_{11}$ would therefore be beneficial in testing the durability and robustness of the offset-saturation method in applications to dynamic NMR problems. Full extension of the offset-saturation experiment to exchanging systems with stronger coupling would undoubtedly entail a complicated analysis of the data, as encountered in spin-echo studies of such systems.$^{57,129-131}$

The use of the offset-saturation method for determining fast rates of exchange, in conjunction with other methods such as lineshape analysis for determining intermediate rates of exchange, and selective-inversion methods for studying slow exchange, provides a powerful combination of methods for obtaining rates of exchange over several orders of magnitude. The measurement of exchange rates over a wide range of temperatures
facilitates the determination of accurate values of the thermodynamic activation parameters for a fluxional process, as demonstrated in the study of hindered bond rotation in N-acetylpipyrrole. The ability of the offset-saturation experiment to extend the upper limit of rate measurement is apparent in such applications is valuable, especially for the determination of entropies of activation, where considerable extrapolation of the Eyring plot of \( \ln(k/T) \) as a function of reciprocal temperature may be necessary. The values of the thermodynamic activation parameters for both cyclohexane and N-acetylpipyrrole were in general agreement with those obtained in previous studies of these systems. The N-acetylpipyrrole results, in particular, represent a comprehensive study of rates spanning several orders of magnitude, allowing calculation of accurate values of the thermodynamic activation parameters, together with reasonable estimates of the errors for the activation parameters, and specifically for the entropy of activation.

The technique of shifting the origin of the x-axis of the Eyring plot to the average of the x-values as the revised zero point effectively removes correlation of errors between slope and intercept, thereby permitting accurate determination of the error in the entropy of activation. For the study of N-acetylpipyrrole in the present work, a value of 1.0 ± 0.7 eu was obtained for the entropy of activation. This procedure should be used to determine errors in entropies of activation in subsequent studies if any significance is to be placed on the value of the entropy. In light of the controversies often surrounding entropies of activation, this procedure could also be applied to rate data previously obtained for particular fluxional systems, in order to re-evaluate the entropies of activation from these
earlier studies in order to draw comparisons with future studies on the same or similar systems.

Pulsed homodecouplers are used as the rf source for the saturating field $B_2$ in the offset-saturation and single-spin double resonance experiments in the present work. The irradiation under such circumstances is essentially a series of repeated, equally spaced soft pulses. The classic single-spin double resonance experiment for calibrating $B_2$ utilized a cw irradiating source, and the effects of regular interruption of the irradiation the time-shared decoupling procedure on the overall effective strength of the rf field has entailed an investigation regarding the equivalence of pulsed and continuous-wave rf radiation. The equivalence of pulsed and cw rf sources is established theoretically by solving for the $z$ component of the steady-state magnetization observed during a series of repeated pulses. Numerical solutions of the resulting expression for the $z$ magnetization containing pulse parameters were in good agreement with the classic Bloch equations form for the $z$ magnetization as a function of irradiation resonance offset calculated with the assumption of a cw rf source.

With approximations in the expanded forms for decoupler pulse flip and precession angles and relaxation matrix elements, an analytical expression for the dependence of the $z$ magnetization as a function of irradiation offset can be obtained from the full form solution of the $z$ magnetization containing pulse parameters. This expression is shown to be equivalent to the Bloch equations form in the limit of small duty cycles. Thus, it may be concluded that pulsed and cw rf radiation sources are equivalent, in the limit of small
duty cycles for the pulsed rf radiation. The limitation of this equivalence to small duty cycles is likely due to the neglect of spin relaxation during pulses in the steady-state formalism used to obtain the analytical form for the irradiation frequency dependence of the z magnetization. This hypothesis could be substantiated most readily using numerical solutions for the z magnetization in which relaxation effects are included explicitly in the calculations. In establishing the equivalence between pulsed and cw rf forms, it is verified that the observed rf field, as calibrated by the single-spin double resonance method, is equal to the product of the instantaneous value rf field per individual pulse, and the decoupler duty cycle, or \( B_{2}^{\text{obs}} = B_{2}^{\text{inst}} \times \text{duty cycle} \). Thus, the use of a pulsed rf source of irradiation, as commonly employed with modern high resolution NMR spectrometers, is suitable for the calibration of the saturating field \( B_{2} \) by the single-spin double resonance experiment, and for the irradiating source in offset-saturation experiments.

The dependence of the equivalence of pulsed and cw rf radiation on the size of the flip angles of individual decoupler pulses has also been examined. Numerical solutions of the full expression for the z magnetization as a function of irradiation resonance offset obtained for a series of repeated soft pulses verify that the equivalence of pulse and cw rf radiation is maintained only when the flip angles of the individual decoupler pulses are kept relatively small, on the order of 1 radian or less. Thus, the strength of the decoupler field \( B_{2} \), the duty cycle and the dwell time should be chosen in such a manner that the decoupler pulse flip angles are not excessively large in applications such as the offset-saturation experiment.
7.2 Recommendations for Future Work

Many possibilities exist regarding future studies involving the offset-saturation experiment, both in terms of direct applications to a wide variety of species to measure relaxation times, and for modifications to the existing methodology. It is apparent that this thesis has been limited to protons as the observed nucleus. The offset-saturation method should in principle be capable of extension to other nuclei, such as $^{13}$C, $^{31}$P and $^{19}$F. Measurements of $T_1$ and $T_2$ for compounds containing these other nuclei by the offset-saturation method could accordingly provide dynamic or motional information for species of concern in the inorganic, organometallic and biological/biochemical areas. One drawback to the use of the offset-saturation technique to study nuclei other than protons is the fact that many high-resolution NMR spectrometers do not have the capability of decoupling nuclei other than protons. Additional hardware modifications, such as the use of a frequency synthesizer, may be required under such circumstances, in order to irradiate other nuclei. The endowment of the offset-saturation method with facile multinuclear capability would enhance the versatility of the technique, and undoubtedly encourage its use in a variety of applications.

The application of the offset-saturation method has been restricted to the study of molecules with no scalar coupling, or neglecting small couplings which may be presented. The Bloch equations which form the theoretical basis of the offset-saturation method are by nature exclusive of the effects of spin coupling, which formally require the more rigorous theoretical description provided by the density matrix formalism. The extension
of the offset-saturation technique to a proper study of systems with scalar coupling would be a benefit to the problem of the measurement of T₂ relaxation times in complex spin systems. Such an extension to coupled systems would accordingly require a revision of the Bloch equations description of the offset-saturation method, and could be undertaken using the Superspin formalism¹⁶³,¹⁶⁴,²⁰⁵-²⁰⁷ for NMR.

The successful use of the offset-saturation experiment for studies of fast chemical exchange has been demonstrated in the present body of work. As noted in Chapter 6, the use of the offset-saturation method to determine rate constants for fast rates of exchange requires prior knowledge of the chemical shift difference between sites as determined from low-temperature spectra. In rate studies of certain systems with low energy barriers to rotation, it is not always possible to measure this chemical shift separation directly. In addition, the chemical shift difference between sites in certain systems may exhibit a temperature dependence. Observations of this chemical shift difference at low temperatures may be extracted into the region of fast exchange in order to predict the chemical shift at certain temperatures, if the temperature dependence of the chemical shift is linear. As discussed previously, the offset-saturation method could, in theory, be used to obtain absolute exchange rates from the dependence of the exchange T₂ on the value of the saturating rf field B₂ when the exchange rate and B₂ become comparable in magnitude, similar to the CPMG spin-echo and rotating frame T₁p methods for studying fast chemical exchange. The endowment of the offset-saturation technique with the capability of measuring absolute rates of exchange without requiring knowledge of the
chemical shift from low-temperature spectra would greatly enhance the versatility and applicability of the offset-saturation experiment as a method for measuring fast rates of exchange. As noted previously, some preliminary studies of the $B_2$ dependence of $T_2$ were undertaken on both cyclohexane and N-acetylpyrrole, but these cursory studies proved inconclusive. Further detailed experimental and theoretical studies should accordingly be directed toward the goal of obtaining the chemical shifts in the application of the offset-saturation method to chemical exchange. As opposed to the $T_{1p}$ and CPMG methods, however, the timescale imposition required for offset-saturation experiments may be more difficult to obtain, as homodecoupling field strengths of a magnitude on the order of $10^3$ Hz or greater may not be attainable on many high resolution NMR spectrometers.

It was also noted in Chapter 6 that preliminary studies which were not presented in this thesis indicate that the offset-saturation technique is applicable to two-site exchanging systems with unequal populations in fast exchange. The offset-saturation method could also be extended to rate studies of multi-site exchanging systems. Such an extension would require a modification of the Bloch equations basis of the offset-saturation method to include decomposition of the observed $z$ magnetization into the relative contributions from the various exchanging sites, which could be accomplished through construction of the appropriate rate matrix for the exchange problem under consideration.

In the studies of exchanging systems presented in Chapter 6, the offset-saturation method has been applied exclusively in the fast exchange regime. An extension of the
methodology to determine rates in the slow exchange regime when the distinct resonances for both sites in a two-site exchanging system exhibit exchange broadening is feasible. Off-resonance partial saturation of slow-exchanging systems has been reported, primarily as an off-resonance analogue of the Hoffman-Forsen saturation transfer or $T_{1p}$ methods. The extension of the offset-saturation method to determine rates of exchange in the upper limit of the slow exchange regime would render the offset-saturation technique a more versatile dynamic NMR method, capable of spanning exchange rates over several orders of magnitude. The slow exchange regime, is however undoubtedly best served by the selective-inversion method when the exchange rates are comparable to the spin-lattice relaxation time $T_1$. The main attributes of the offset-saturation method should enhance this method as an alternative dynamic NMR technique to the spin-echo, $T_{1p}$ or complete lineshape analysis methods for determining rates in the fast exchange regime.

Many possibilities thus exist for use of the offset-saturation experiment for the simultaneous determination of $T_1$ and $T_2$ NMR spin times. Applications of the method presented herein to measure relaxation times in a variety of compounds, or extensions or modifications of the offset-saturation methodology await further attention. The offset-saturation method should be of considerable utility of the elucidation of the motional or dynamic properties of a variety of chemical or molecular systems by the measurement of relaxation times, especially as a facile and robust method for the measurement of $T_2$. The offset-saturation technique should prove to be a viable alternative to accepted methods.
such as the CPMG spin-echo experiment for measuring the spin-spin relaxation time, and as a method for obtaining both $T_1$ and $T_2$ from a single experiment for applications requiring knowledge of both spin relaxation times $T_1$ and $T_2$. The merits of the offset-saturation technique as a dynamic NMR method for studying fast chemical exchange should prove to be a useful complement to the existing standard dynamic NMR methodology.
REFERENCES


APPENDIX 1

STEADY-STATE SOLUTION OF THE COMPONENTS OF THE MAGNETIZATION FOR A SERIES OF REPEATED PULSES

The $x$, $y$, and $z$ components of the steady-state magnetization for a series of repeated and equally-spaced soft pulses can be obtained from eq.(5.6) for the conditions $M(t) = M^*$ and $M(0) = M^*$. Under these conditions, eq.(5.6) then becomes

$$M^* - R_{\zeta}(\theta)S(\tau)R_{\varphi}(\alpha,\theta)M^* + S'M(\infty) \quad (A1.1)$$

which can be rearranged to give

$$M^* \left[ 1 - R_{\zeta}(\theta)S(\tau)R_{\varphi}(\alpha,\theta) \right] = S'M(\infty) \quad (A1.2)$$

where

$$1 - \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}$$
The appropriate terms of eq. (A1.2) in complete matrix form are

\[
M^* = \begin{bmatrix}
M_x^- \\
M_y^- \\
M_z^-
\end{bmatrix}
\]

\[
R_y(\alpha, \phi) = \begin{bmatrix}
\cos \alpha & \sin \alpha \sin \phi & \sin \alpha \cos \phi \\
-\sin \alpha \sin \phi & \cos^2 \phi + \cos \alpha \sin^2 \phi & (\cos \alpha - 1) \sin \phi \cos \phi \\
(\sin \alpha \cos \phi) & (\cos \alpha - 1) \sin \phi \cos \phi & \sin^2 \phi + \cos \alpha \cos^2 \phi
\end{bmatrix}
\]

\[
R_z(\theta)S(\tau) = \begin{bmatrix}
E_z \cos \theta & E_z \sin \theta & 0 \\
-E_z \sin \theta & E_z \cos \theta & 0 \\
0 & 0 & E_i
\end{bmatrix}
\]

and

\[
S' = \begin{bmatrix}
0 & 0 & 0 \\
0 & 0 & 0 \\
0 & 0 & (1 - E_i)
\end{bmatrix}
\]

Upon substitution of the appropriate matrix terms above, equation (A1.2) can be solved as a matrix equation for the unknowns \( M_x^-, M_y^-, \) and \( M_z^- \) using Cramer’s rule for the solution of three equations in three unknowns, such that
\[ M_k^* = \frac{D_k}{D} \] (A1.3)

where \( k = x, y \) or \( z \), \( D_k \) is the determinant evaluated with the terms on the right-hand side of eq. (A1.2) in the \( k \)th column, and \( D \) is the determinant of the coefficients of the system of the equations on the left-hand side of equation (A1.2), and corresponds to the term \( D(\alpha, \theta, \phi) \) as given by eq. (5.12).
APPENDIX 2

CONSIDERATION OF COVARIANCE BETWEEN SLOPE AND INTERCEPT
IN LINEAR REGRESSION

In many instances, scientific data can be described by a simple linear model, where the measurement at some independent variable $x_i$ gives a result $y_i$ given by the following equation.

$$y_i = m \cdot x_i + b$$  \hspace{1cm} (A2.1)

where $m$ is the slope and $b$ is the intercept.

If there are $N$ such independent measurements, then the following quantities can be defined in terms of $x_i$ and $y_i$:

$$S_x = \sum_{i=1}^{N} x_i$$  \hspace{1cm} (A2.2a)

$$S_y = \sum_{i=1}^{N} y_i$$  \hspace{1cm} (A2.2b)
\[ S_{xx} = \sum_{i=1}^{N} x_i^2 \]  
(A2.2c)

\[ S_{xy} = \sum_{i=1}^{N} x_i y_i \]  
(A2.2d)

If the measurements have equal variances, then the best-fit values of the slope and intercept can be given by the following equations:

\[ m = \frac{N S_{xy} - S_x S_y}{\Delta} \]  
(A2.3)

\[ b = \frac{S_{xx} S_y - S_x S_{xy}}{\Delta} \]  
(A2.4)

where

\[ \Delta = N S_{xx} - S_x^2 \]  
(A2.5)

\[ S_R = \frac{\sum (y_i - x_i - b)^2}{N-2} \]  
(A2.6)
where $S_R$ is the variance about the regression.

The variances of $m$ and $b$, and the covariance between $m$ and $b$ are given by

$$\text{var} \ (m) = S_R \frac{N}{\Delta} \quad (A2.7)$$

$$\text{var} \ (b) = S_R \frac{S_{mx}}{\Delta} \quad (A2.8)$$

$$\text{cov} \ (m,b) = - S_R \frac{S_x}{\Delta} \quad (A2.9)$$

If the $x$ values are chosen so that $S_x = 0$, it may be seen that the covariance in eq.(A2.9) vanishes, and the variance of any value $y_k$ on the best-fit or regression line at a corresponding value $x_k$ is then given by the following expression$^{81,83,192}$

$$\text{var} \ (y_k) = x_k^2 \text{var} \ (m) + \text{var} \ (b)$$

$$= S_R \left( \frac{N x_k^2}{\Delta} + \frac{S_{mx}}{\Delta} \right) \quad (A2.10)$$

As $S_x$ was assumed to be zero, $x_k$ is measured from the value $x = 0$, which is the average of all the $x$ values.

Thus, the covariance between slope and intercept in a linear regression can be removed by the procedure outlined above.