ZERO QUANTUM NUCLEAR MAGNETIC RESONANCE EXPERIMENTS
UTILIZING A TOROID CELL and COIL

by

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Over the past ten to fifteen years the area of Nuclear Magnetic Resonance (NMR) Spectroscopy has seen tremendous growth. For example, in conjunction with multiple quantum NMR, molecular structural mapping of a compound can be easily performed in a two dimensional (2D) experiment.

However, only two kinds of detector coils have been typically used in NMR studies. These are the solenoid coil and the Helmholtz coil. The solenoid coil was very popular with the permanent and electromagnet NMR instruments. With the advent of the superconducting magnets the popularity shifted to the Helmholtz coil, which remains the most common coil today for superconducting magnets. The Helmholtz coil, however, has been shown to have lower sensitivity than the solenoid coil. Hoult (1) has pointed out that potentially the Helmholtz coil represents a loss of signal-to-noise (S/N) by a factor of three in comparison to the solenoid coil. Since Hoult’s original work, alternate methods for optimizing S/N have been explored. One of these has been the suggestion of toroid shaped resonators for NMR studies (2). A potential advantage of a toroid cell and coil is the confinement of the $B_1$ field to the torus region. It has been suggested that the toroid has a potential (S/N) advantage of 3.9 - 4.5 in comparison to the conventional Helmholtz cell (3). Since Zero Quantum (ZQ) experiments are independent of $B_0$ homogeneity, 2D ZQ experiments provide a convenient
method of comparing the toroid and Helmholtz coils.

In these zero quantum studies, the toroid and Helmholtz probes will be characterized in terms of several factors: 1) $B_1$ homogeneity, 2) $B_0$ homogeneity, 3) flip angle dependence, and 4) sensitivity. Finally, the two probes will be contrasted using spectral analysis in the spin-spin mapping of a simple molecule (n-butanol) and a complex molecular system (taxol).
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Chapter 1

Introduction

Over the past ten to fifteen years the area of Nuclear Magnetic Resonance (NMR) Spectroscopy has seen a tremendous growth in analytical techniques and equipment. For example, molecular structural mapping of a compound, in conjunction with multiple quantum NMR, can be done easily and in a single two dimensional (2D) experiment.

However, a limitation to obtaining useful information is sensitivity. This is particularly true of nuclei with low natural abundances and low nuclear magnetic moments. Potentially, a means for increasing the NMR sensitivity is the development of a different type of NMR probe utilizing the toroid cell. In the past, only two kinds of detector coils have been typically used in NMR studies. These are the solenoid coil and the Helmholtz coil. The solenoid coil, as shown in figure 1, is a coil of wire that generates a magnetic field \( B_1 \) parallel with the cylinder of the coil. The Helmholtz coil, also known as a saddle coil (Fig. 2), generates a magnetic field \( B_2 \) perpendicular to the cylinder of the saddle shaped coil. These coils can receive and transmit radio frequencies. They can receive signals on the order of milli to microvolts and transmit with power from zero to 5 watts. The solenoid coil was very popular with the permanent and electromagnet NMR instruments. With the advent of the superconducting magnets the popularity shifted to the Helmholtz coil, which remains the most common coil today for superconducting magnets. The Helmholtz coil also allows easy sample loading and withdrawal in the superconducting magnet. Although the solenoid coil can be used in the superconducting magnets in certain applications, such as the sideways spinning probe, user flexibility is a primary reason why the Helmholtz coil is most frequently used today. However, a major compromise was made in switching from the solenoid to the Helmholtz coil. Hoult and Richards (1) point out that the solenoid has a signal-to-noise (S/N) ratio 3 times better than the Helmholtz probe. Thus, a poor compromise was achieved
between S/N and user flexibility. Other types of detectors which have a sample geometry of a shape that allows the sample to be uniformly magnetized have been developed. The toroid cell is one of these detectors that has been developed to regain the sensitivity lost by the Helmholtz coil. Theoretically, the toroid coil configuration could improve the NMR sensitivity due to the improved B_1 field in the torus region. However, a problem that has been a drawback to its use is B_0 inhomogeneity caused by the toroid cell. The shim system of the superconducting magnet is designed to enhance the homogeneity of cylindrical samples about the Z (vertical) axis. Figure 3 shows the axis system normally used in discussing NMR magnetization vectors. The toroid cell lies in the XY plane of the B_0 field of the superconducting magnet. This causes distortions in the B_0 field that cannot be compensated for by the resident shim system in the superconducting magnet. The inhomogeneity problem can be circumvented by observing zero quantum coherences (ZQC) via 2D NMR. Simply stated, a zero quantum coherence occurs when there is a transfer of (nuclear) spins between two energy levels of the equal energy. Normally, these are forbidden transitions where ΔM=0. Single and higher order multiple quantum transitions can be suppressed with a field gradient pulse. These techniques allow analysis of a standard compound by the toroid to be done in one quarter the time needed for the Helmholtz coil. Consequently, the toroid represents a significant increase in signal enhancement.

In this thesis, zero quantum coherences via 2D NMR will be used to evaluate the toroid cell. A main goal will be to demonstrate the potential the toroid cell has for NMR analysis over the currently utilized Helmholtz probe. Further goals will be to characterize the toroid probe and compare it to the Helmholtz probe in terms of B_1 homogeneity, signal suppression, flip angle and τ dependence, sensitivity, and spin-spin mapping of a complex sample using optimized parameters.
Chapter 2

Historical

Characterization of the toroid cell and coil using the 2D zero quantum technique has not been previously described in the literature. Consequently, a short survey of published work related to this study is summarized below:

1. Two dimensional (2D) NMR
2. Zero Quantum NMR
3. The Toroid cell and coil

2.1 Two Dimensional NMR

The concept of 2D NMR spectroscopy began as early as 1962 with double resonance experiments where the resulting spectrum is a function of two frequency parameters. The 2D experiment as it is known today was first proposed by Jeener (2). This is the two dimensional Fourier transformation of two time variables. This yields a spectrum that is a function of two frequency variables. Ernst and co-workers later showed the applicability of 2D NMR to the indirect detection of multiple quantum transitions (4). Since then many new experiments involving 2D Fourier transformation have appeared.

All of these various techniques have a feature that is common to each experiment. The basic 2D experiment can be broken down into four basic periods (5). These are the preparation, evolution, mixing and detection periods. However, the mixing period does not always occur in all 2D experiments (Fig. 4).

The preparation period allows the system to achieve an initial state of thermal equilibrium. This period also consists of a delay time that is 1-5 times the longitudinal ($T_1$) relaxation time of the nuclear spins. Usually, at the end of the preparation time a $90^\circ$ pulse is used to create a non-equilibrium spin system. During the evolution period, also known as the $t_1$ period, the non-equilibrium spin system is allowed to evolve according to the
environment acting upon it. In many experiments the evolution process can be interrupted by a 180° pulse depending on the information desired. The $t_1$ value is systematically varied, or stepped, throughout the experiment to produce a systematic change over time in the resulting spectrum. The mixing period is normally of the form:

$$t_1 \text{— pulse(s) — } t_2$$

where $t_1$ and $t_2$ are time delays in the sequence and the pulses are usually non-selective. A selective pulse is when a single frequency is irradiated. A non-selective pulse is when all of the frequencies within a specified sweep width are irradiated at one time. Usually, NMR experiments are performed with non-selective pulses. It is this period which allows forbidden transitions, such as zero or double quantum frequencies, to evolve in order to be observed in the detection period. Preceeding the final period, known as the detection period, another 90° pulse, or detection pulse, is employed to move the desired transverse magnetization into observable space where it can be detected by the instrument. The resulting information is usually obtained as a function of either amplitude and/or phase modulation depending on the experiment used. The amplitude modulation carries the scalar coupling information, while the phase modulation carries the chemical shift information.

2.2 Zero Quantum NMR

Zero quantum NMR has been observed since the early days of continuous wave (CW) NMR. The main application then was structural connectivity and relative signs of coupling constants (6). With the advent of Fourier transform NMR however, many believed the detection of ZQC was not possible due to the $\Delta M=\pm 1$ selection rule. Also there was the problem that a single pulse experiment does not create any multiple quantum coherence (MQC), nor does MQC induce any physically observable signal. Eventually these problems were circumvented by the use of multiple pulse sequences, where MQC were detected by indirect means (5). Wokaun, Ernst and others (7) have shown that multiple quantum
transitions (MQT) would be useful for several reasons. Of these reasons, zero order MQT were shown to be insensitive to magnetic field inhomogeneity and therefore allow recording of high resolution spectra in inhomogeneous magnetic fields. Another benefit was that zero quantum transitions (ZQT) could be used to directly measure $T_2$ relaxation.

Until recently, there have been relatively few applications of ZQC. Bolton (8) has demonstrated heteronuclear zero quantum coherence as a conformational probe. Magnetization exchange in zero quantum spectra has been analyzed by Blumich and Kaiser (9). Deutrium ($^2$H) and nitrogen ($^{14}$N) quadrupolar coupling constants have been determined by Ruessink, De Kanter, and MacLean (10) through utilizing ZQC techniques to produce high resolution spectra under inhomogeneous magnetic field conditions. Müller has designed a technique for spin–spin mapping of coupled nuclei (11). In his paper, Müller reports a 16 phase cycle sequence and a 4 phase cycle sequence with a homospoil pulse that provides $^1$H–$^1$H and $^{13}$C–$^{13}$C connectivities. The 4 phase cycle demonstrates an economy of time since the homospoil pulse allows the sequence to be compressed into a 4 step cycle. This is in direct contrast to the more commonly used INADEQUATE sequence, which is a double quantum experiment designed to elucidate structural connectivities. The INADEQUATE experiment uses an elaborate 32 step phase cycle sequence. In a more recent paper, Zektzer and Martin (12) compare the zero quantum coherence and the double quantum coherence (DQC) experiments for elucidation of proton coupling pathways. They demonstrate the advantages and disadvantages of zero quantum coherence NMR. Consequently, the simpler phase cycling requirements of Müller’s sequence show an eight-fold savings in time.

2.3 Toroid Cell and Coil

An area that has seen little work in recent years is improvements in the NMR detector. In the permanent or electromagnets the $B_1$ field generated by the solenoid coil (Fig.1) was perpendicular to the magnetic field ($B_0$) generated by the magnet. With the advent
of the superconducting magnets. The $B_0$ field generated by the magnet was now typically parallel to the $B_1$ field generated by the solenoid coil (except in the case of a sideways spinning probe). The Helmholtz coil (Fig. 2) on the other hand, generates a $B_1$ field perpendicular to the $B_0$ field generated by the superconducting magnet. The widespread use of Helmholtz coils in the superconducting magnets has been due to user flexibility (i.e., easy sample placement). However, it has been documented that a poor compromise was reached in regards to optimal signal-to-noise. Hoult has shown that in utilizing the Helmholtz coil there was a loss in $S/N$ by as much as a factor of three in comparison to an optimized solenoid coil (1). One attempt to increase the $S/N$ was the development of the toroid cell as an alternative to the Helmholtz coil (13). The basic design of the toroid cell has several advantages. One potential advantage is that the $B_1$ field is confined to the torus region. This is due to the configuration of the toroid cell and coil. The toroid is a closed structure having symmetry similar to a cylinder. This symmetry allows the lines of force of the $B_1$ field to be more homogeneous and allowing for better sensitivity (Fig. 5). The Helmholtz coil, on the other hand, will have a poorer $B_1$ homogeneity due to the saddle shape of its coil. Figure 6 shows the dispersion of the $B_1$ field due to the saddle shape. In addition, Hoult also suggests in his paper that the $S/N$ is proportional to the $90^\circ$ pulse length for a single coil system under the same conditions (i.e., sample volume, rf power, etc.) (1). The shorter the $90^\circ$ pulse length, the better the $S/N$. Another factor in obtaining good signal-to-noise is $B_1$ homogeneity. In a toroid cell where the $B_1$ homogeneity is uniform over the same sample volume, it has been shown that the $S/N$ is increased 3 to 4.5 times that of a Helmholtz coil (all things kept equal) (14). Furthermore, previous work has shown that the rf field ($B_r$) generated by the toroid coil has an $r^{-1}$ dependence. In solenoid coils the sensitivity has a $B_r/\sqrt{r}$ dependence, where $r$ is the radius of the wire used in the coil (1). In the toroid configuration there is an additional dependence on the width of the toroid cell and the distance from the center of the
"doughnut." Figure 7 shows these distances as \( r_2 \) and \( r_1 \) and their relationship. It was further determined that at a minimum approaching unity for the relationship \( r_2/r_1 \) (Fig. 7), a relatively homogenous \( B_1 \) field can be achieved. A constraint that has arisen utilizing the toroid configuration was \( B_0 \) homogeneity. In previous work with the toroid, the \( B_0 \) inhomogeneity (causing 70 Hz linewidths) was improved by using homemade shim coils. The linewidths in this case were reduced to approximately 10 Hz (14). The severe line broadening of high resolution nuclei (i.e., \(^1\text{H}\)), using the toroid cell and coil, has precluded any work being done via one dimensional NMR experiments. Other work done with a toroid probe has been sensitivity enhancement experiments of nuclei with major line broadening problems, such as \(^{17}\text{O}\).
Chapter 3

Theory for Zero Quantum Coherences

3.1 Zero Quantum Coherences

Several authors have provided theoretical descriptions of zero quantum NMR (5). Consider a simple AX spin system with four energy levels, as in figure 8. Weak coupling is assumed for this system and only the transitions shown are considered. The spin states for a system of spin-1/2 nuclei are usually labeled \( \alpha \) for \( m_i = +1/2 \) and \( \beta \) for \( m_i = -1/2 \). The spin states of an AX spin system can be written as a linear combination of the simple product states \(|\alpha\alpha\rangle, |\alpha\beta\rangle, |\beta\alpha\rangle, \) and \(|\beta\beta\rangle\). An appropriate expression describing the energy levels for a first-order case (i.e. AX systems) is

\[
h^*U = -\Sigma_j \nu_j m_j + \Sigma_{j<k} J_{jk} m_j m_k
\]

where \( \nu_j = |\gamma_j/2\pi|B_0(1-\delta_j) \)

Introducing the AX labels, the above equation becomes

\[
h^*U = -\nu_A m_A - \nu_X m_X + J_{AX} m_A m_X
\]

The energy level \( \alpha\alpha \) and energy levels \( \alpha\beta \) or \( \beta\alpha \) have a quantum spin difference (\( \Delta M=\pm1 \)). The \( \alpha\beta \) and \( \beta\alpha \) energy levels are considered to be equal, although they do differ in energy. If a transition occurs between the \( \alpha\alpha \) and \( \alpha\beta \) transitions, this is normally called a single quantum transition (\( \Delta M=\pm1 \)). If a transition occurs between \( \alpha\beta \) and \( \beta\alpha \), a zero quantum transition has occurred (\( \Delta M=0 \)). However, zero quantum transitions cannot be directly observed by conventional Fourier transform experiments which are governed by the \( \Delta M=\pm1 \) rule.

Most NMR experiments involve single quantum transitions. They can be understood in terms of the effect of pulses on magnetization vectors and subsequent precession of these...
vectors in the rotating frame of reference. However, the zero, double and higher order multiple quantum transitions cannot be visualized as easily since they cannot be directly observed in an FT experiment. The creation and detection of these "forbidden" transitions are best explained through density matrix formalism.

The two spin (1/2) system can be conveniently represented as a 4 x 4 matrix \( \sigma \) written in the eigenbasis of the Zeeman Hamiltonian \( ( \text{and } F, ) \) (6), as in figure 9. Table 1 shows the energy levels and all the possible transition frequencies for the AX spin system. Although there are many methods available to generate zero quantum coherences (or multiple quantum coherences), the pulse sequence shown in figure 10 has proven to be the most convenient and generally applied. Since the AX spin system is a simple one, only the transitions \( \sigma_{11} \) (SQC) and \( \sigma_{22} \) (ZQC) need to be considered. This will be done by starting with the initial (Boltzman) state and following the consequences of each rf pulse and the evolution of these matrix elements at various stages of the pulse sequence (table 2).

The initial state of the density matrix contains only diagonal elements \( (\sigma_{nn}) \) corresponding to the equilibrium populations:

\[
\frac{P_i}{P_j} = \exp \left| \frac{E_j - E_i}{kT} \right| \tag{3}
\]

The initial 90° pulse equalizes all of the diagonal elements and creates only SQC. There are no ZQC created at this time. The density operator can be defined by \( \sigma \) (15), then \( [ \sigma ]_0^* \) is the density matrix elements after a pulse. This can be seen in the \( [\sigma ]_0^\ast \) elements in table 2. There will be non-diagonal SQC and they will precess during the preparation period \( \tau \). During this time, these elements will be of the form

\[
\sigma_{nm}(\tau) = \sigma_{nm}(0^\ast) \exp(-i\omega_{nm}\tau) \exp(-\tau/T_{2nm}) \tag{4}
\]
The signal dampening caused by the transverse relaxation term ($T_{2\text{nm}}$) has been omitted from table 2 for convenience, since it does not contain any frequency information. $\omega_{nm}$ is the angular velocity of the oscillating $\sigma_{nm}$ coherence. As can be seen from table 2, the second 90° pulse does generate ZQC. These elements can be nonzero and can evolve during the evolution period, $t_1$, each at their appropriate ZQT frequencies. Thus, by performing a sequence of experiments where the $t_1$ period is systematically increased, it is possible to trace out the time evolution of the unobservable matrix elements. Although multiple quantum transitions (MQT) can be created using the sequence of figure 10, this discussion will concentrate on the zero quantum transitions. The NMR observable along the $y$ axis of the rotating frame of reference is given by the expression

$$<M_y> = \frac{1}{2}[U_{12} - U_{21} + (0_{13} - 0_{31}) + (U_{za} - U_{az}) + (U_{sa} - U_{as})]$$  \hspace{1cm} (5)$$

This equation shows that the detected signal contains only single quantum coherences of the density matrix. Since the observable signal during the detection period ($t_2$) can arise only from SQC, it is necessary to transfer the ZQC into these elements by a third (mixing) pulse. This can be observed in the final expression for the $[\sigma_{12}]_{2}$ element in table 2. This element contains terms of the type

$$a(r)b(t_1) \exp(-i\omega_{12}t_2)$$  \hspace{1cm} (6)$$

The amplitude of each line in the final 2D spectrum will be governed by the function $a(r)$. The function $b(t_1)$ will contain terms involving zero, single, and higher order multiple quantum transitions. These frequencies will show a complex amplitude modulation of the detected signal as a function of $t_1$ (evolution period). The above expression shows that after a double Fourier transform, the $f_z$ domain will reveal only the observable SQT. The $f_1$
domain, however, will include all of the forbidden transitions. Thus, the \( \tau \) domain (preparation period) and the \( t_1 \) domain (evolution period) respectively influence the amplitude and frequencies of the \( n \)-quantum transitions studied by 2D NMR experiments (6).

One of the primary uses of zero quantum NMR is for nuclear connectivity studies. This is because zero quantum studies have several advantages (6).

1. The zero quantum transitions are independent of the \( B_0 \) homogeneity. This enables high resolution studies for nuclei which are only limited by natural linewidths. The explanation for this can be demonstrated mathematically, as well as by using the AX spin energy level diagram. An elegant illustration was presented by Ad Bax in his dissertation on two dimensional NMR in liquids (5). In this illustration, Bax demonstrates the effects of \( B_0 \) inhomogeneity on zero, single and double quantum signals. The results of figure 11 clearly demonstrate the independence of zero quantum transitions from \( B_0 \) inhomogeneity. In figure 11a, the normal single quantum transitions are shown for an AX spin system. The value \( \Delta \omega \) comes from the inhomogeneity of the \( B_0 \) field. Since the \( B_0 \) field fluctuates slightly, the resulting signals, due to a particular transition (i.e. \( \omega_{u2} \)), will differ slightly in frequencies. These various frequencies cause line broadening in addition to the natural linewidth (\( T_2 \) relaxation in equation 7).

\[
\Delta \nu_{u2} = \frac{1}{\pi T_2} + \frac{\gamma B_0}{2}
\]  

(7)

Figure 11b shows the double quantum transition for the same AX spin system. This figure shows pictorially that the double quantum transition is the sum of the two chemical shift frequencies of the coupled AX nuclei. Consequently, the \( \Delta \omega \) is also additive and is observed
in the line broadening of the double quantum signal. Finally, figure 11c shows the zero quantum signal. This transition is due to the difference between the chemical shift frequencies of the coupled AX nuclei. This spectrum shows that the $\Delta \omega$ value has been eliminated and that the linewidth is dependent only on the $T_2$ relaxation of the coupled nuclei.

2. Since the zero quantum signal is the difference in Larmor frequencies between two coupled nuclei, the resulting spectrum will contain fewer lines and consequently a less complicated spectrum. Furthermore, the difference frequencies may require a smaller window to be observed. This reduces the data matrix which in turn reduces the digitization required for the resulting 2D spectrum. This ultimately reduces the data acquisition and processing time.

3. An advantage related to the first point is the calculation of $T_2$ relaxation time from the linewidth at half—height. Equation 1 shows the relationship between the linewidth at half—height ($\nu_{1/2}$) and $T_2$ relaxation. Since the zero quantum transitions are independent of the $B_0$ inhomogeneity ($\Delta B_0$), the $\Delta B_0$ term goes to zero and the $T_2$ relaxation can be directly measured from the spectrum.

4. Finally, another advantage related to point one is the ability to apply a field gradient pulse. Since the zero quantum transitions are independent of the $B_0$ inhomogeneity, the higher order $p$-quantum transitions can be eliminated by a homospoil (field gradient) pulse at some point during the evolution period. Thus, the need for elaborate phase cycling routines to suppress single and higher order multiple quantum transitions is eliminated.
Chapter 4

Experimental

4.1 Experimental Apparatus

The experimental conditions applied to the analysis of the toroid cell and coil were similar to those performed by Glass and Dorn (14). The difference in these experiments was that a new probe was constructed. An unmodified Bruker 5mm proton probe was used for comparison of a Helmholtz coil. The experiments were conducted on a Bruker WP 200SY superconducting spectrometer, operating at 4.7 T. The toroid cell was constructed of glass by Wilmad Glass, Inc. The toroid was constructed so that the cell was perpendicular to the $B_0$ field of the magnet. The center of the $B_0$ field was determined through field mapping. The probe was built to place the torus region symmetrically in the xy plane (Fig. 12) of the probe and centered in the magnetic field ($B_0$). The cell volume was approximately 130 $\mu l$. The toroid coil was made by soldering three #22 gauge wires in parallel and wrapping them around the toroid cell. The coil was connected across a tank circuit with two tunable picofarad capacitors. These two capacitors served as a tuning and matching circuit. The tank circuit for the toroid coil was singularly tuned to the proton frequency, 200.132 Mhz. The tuning was accomplished by connecting an oscilloscope, a frequency generator, a frequency counter, and a 50 ohm load across a "magic T". The "magic T" is an rf equivalent of a resistive bridge circuit. The frequency generator and the oscilloscope are across one side, while the 50 ohm load and the probe signal are across the other side. When the signals across the bridge are balanced, a minimum signal on the oscilloscope is observed. This means that the probe is tuned to the desired frequency. A deuterium lock channel was not incorporated into the toroid probe. A lock channel would be of little use since the Bruker instrument shims were designed for cylindrical samples. The homospoil coil was made of #22 gauge wire and placed around the outer glass housing of the toroid cell. The homospoil windings were wrapped 5 times clockwise and counterwound 5 times below the toroid coil. The wire configuration was wound
in order to create a strong field gradient parallel with the Z axis of the B₀ field. A variably modulated oscillator (VMOS) switch, controlled by a transistor-to-transistor logic (TTL) signal, was made by the Chemistry Department Electronics Shop at V.P.I. & S.U. (Fig. 13). The VMOS switch was triggered by a TTL pulse from the decoupler channel and delivered a variable dc pulse to the homospoil coil.

4.2 Sample Preparation

The two dimensional zero quantum experiments were optimized on the toroid and the Helmhotlz coils using a 50% (v/v) mixture of n-butanol in d-acetone. The deuterated solvent was used to reduce the proton signal due to the solvent. A proton signal from a protonated solvent would cause dynamic range problems with the peaks of interest. A complex molecule (taxol) was used as a second sample. It was prepared by dissolving 30mg of taxol in 0.5ml of d-chloroform.

4.3 Sample Loading

Sample loading for the toroid probe was performed by first removing the probe from the magnet. Once the outer sleeve was removed, the sample solution was syringed into the inlet of the toroid cell. The sleeve was replaced and the probe reinserted into the magnet. Although this was cumbersome, sample changing took approximately 5-10 minutes. Nominal tuning of the signal could be performed by shimming on the free induction decay (FID) of the sample signal. Sample loading for the Helmholtz probe was done as for any basic superconducting NMR instrument. However, the sample was not spun in the Helmholtz probe so as to reduce the T₁ noise. The experiments for the Helmholtz coil were done with the lock system on and tuned for a non-spinning sample.

4.4 Pulse Sequence and Phasing Routines

The pulse sequences employed in these experiments were designed by Müller (11) and adapted to the Bruker spectrometer. Schemes 1 and 2 show the programs utilized for the 16
phase and the 4 phase cycle sequences, respectively. Scheme 1 shows the pulse program using the 16 phase cycle for signal suppression. This one was used for the Helmholtz probe and does not have homospoil capabilities. Scheme 2 shows the pulse program using the homospoil pulse. This sequence was used for the toroid probe and can be used for any probe with homospoil capabilities. Both programs work out the 2D ZQ pulse sequence shown in scheme 5. The D0 delay in both programs starts at 0 and is systematically increased (usually in milliseconds) to some maximum value that is dictated by the sweep width and number of points used for each experiment. D2 was the r delay in the sequence which allowed the spins to precess between pulses. Also shown are the phase cycles for each sequence (schemes 3 and 4, respectively). The 16 phase cycling routine reduced artifacts via quadrature detection as well as the unwanted single and higher order multiple quantum signals. The 4 phase cycle was only for quadrature detection since the homospoil suppressed the single and higher order multiple quantum signals. The homospoil suppressed the unwanted signals by creating an extremely inhomogeneous field parallel to the Z axis of the spectrometer. This caused the single and higher order signals to dephase randomly instead of coherently. Consequently, the signal intensity dropped to zero. The zero quantum signal, on the other hand, is insensitive to pertubations along the Z axis and thus is not affected by the field gradient pulse. The pulse sequence operates as any other pulse train. The initial 90° pulse sets the single quantum transitions of the coupled nuclei (Fig. 8) into motion (i.e. \( W_1^1, W_1^5 \)). The following 180° pulse refocuses the chemical shifts. The 45° pulse prepares the zero quantum signals for movement into observable space. The final 90° pulse brings the zero quantum signals into observable space where they can be detected by the receiver. The pulses need to be accurate in order to rotate the spins the proper amount so that they may be detected at the end of the pulse train. Another important value is the r period. This period allows the spins to precess the proper amount of time in order to achieve maximum signal intensity.
Chapter 5

Results and Discussion

5.1 Experimental Description

The primary goal for these experiments was to characterize the toroid cell and coil as well as compare it to the Helmholtz coil. However, an initial goal was to see if zero quantum two dimensional NMR could be successfully performed utilizing the homemade toroid probe. Initial experiments had difficulty in suppressing the single and higher order multiple quantum signals created by the 2D experiment. Improvements in the VMOS switch overcame this problem.

Another aspect of the results is the interpretation of the transformed 2D spectra in these experiments. In normal one dimensional (1D) experiments, chemical shift is usually the parameter of interest in what is called the F\textsubscript{2} domain. However, in 2D NMR a second axis, called the F\textsubscript{1} domain, yields additional information depending on the pulse train being used in the experiment. Figure 14 gives a pictorial description of a 2D plot. The information in the F\textsubscript{1} domain arises from a systematically varied delay time in the pulse sequence. This varied delay time allows observation of signal changes (amplitude or phase) over time. Thus, a second variable, or dimension, is created which yields useful information about a molecular system under analysis. This second dimension can project information such as chemical shift, coupling constants, or homonuclear spin-spin coupling interactions between nuclei. In figure 13, each of the transformed spectra represents a single experiment. A single signal, chloroform, is used to demonstrate the basic concept of the 2D experiment. The primary difference between each of these experiments is the systematic increase in the D0 delay. In this case a periodic change in amplitude is observed. Along the F\textsubscript{1} axis only a single signal is observed. However, along the F\textsubscript{2} axis a new function has occurred. If the peak of each signal was connected, going from the first spectrum to the last, a second sinusoidal signal will be observed. This sinusoidal wave carries information with it. If this second FID is transformed,
a second signal will appear. This signal, also known as an interferrogram, is a function of the amplitude modulation. This second function, or parameter, yields additional information about the system under analysis.

In the case of the 2D ZQ NMR experiment, the sinusoidal wave(s) that appears after the first Fourier transformation will be a function of the interaction (or coupling) between the protons that are coupled to each other. The modulation will be a function of the difference in chemical shift frequencies. In the 2D contour plot, the signal from the second Fourier transformation will occur at the difference frequency. This signal will be seen off of the $F_1=0$ Hz line at a distance equal to the difference frequency and at the same value along the $F_2$ axis. Figure 15 shows a simulated spectrum with two sets of homonuclear spin-spin interactions due to coupled nuclei. One set has a chemical shift difference of 400 Hz. Thus, 4 signals will be generated at 400 Hz off of the $F_1=0$ HZ line showing that they are coupled. The same analysis can be applied to the second set of coupled nuclei which have a chemical shift frequency difference of 200 Hz. Furthermore, a residual Z-magnetization, which represents a 1 dimensional spectrum of the sample, will appear along the $F_1 =0$ Hz line of the 2D plot. The spectrum is similar to a COSY (correlated spectroscopy) except instead of the cross peaks occurring off of the diagonal, they appear off of the horizontal line at $F_1=0$ Hz.

5.2 Experiments to Optimize Probe Configurations for Zero Quantum Two Dimensional NMR

5.2.1 Flip Angle Optimization Dependence

In the pulse sequence suggested by Müller, two parameters that have critical values are the flip angles and the $\tau$ delay times. The pulses are vital since the magnetization vectors need to be rotated accurately to develop the desired signals. Since $B_1$ inhomogeneity of the two probes was not known exactly, the optimum value for the flip angles needed to be
determined. Another limiting factor requiring the optimization of the flip angles was the ability of the software and hardware of the spectrometer to deliver an accurate pulse. The programming of the WP 200 spectrometer can deliver a pulse to a tenth of a microsecond. The more recently developed NMR spectrometers can deliver a pulse to a hundreth of a microsecond. Although this seems to be a trivial point, it was important in attaining an accurate 90° pulse on both probes. The values determined for the 90° flip angle was 3.9µs and 4.9µs for the toroid and Helmholtz probes, respectively. The technique for calibrating the 90° flip angle is simple but tedious. The calibration procedure starts by using a small value for the initial pulse (i.e., 1µs) and systematically increasing the pulse width until a null is observed in the spectrum for the transformed signal. A null signal occurs with a 180° or 360° pulse. Once a null is determined, the 90° flip angle is then calculated from this value. However, a null was not observed for either probe. The 90° pulse for the toroid was between 3.9 and 4.0µs. The 90° pulse for the Helmholtz coil was between 4.9 and 5.0µs. The 90° pulse needed calibration in hundreths of a microsecond (i.e., 0.01µs) for both probes. Consequently, a precise 90° pulse could not be determined. Thus, a series of experiments was conducted to ascertain the optimum value for the 90° pulse. The optimum value was determined by comparing the signal intensity of the resulting zero quantum signals in the 2D contour plot.

The normal 1D proton spectrum of n-butanol can be seen in figure 16. Figures 17-20 show the flip angle optimization results for the toroid probe. For the case of n-butanol there will be 4 sets of signals due to the interactions of coupled nuclei occuring off of the horizontal F1=0 Hz. Three interactions can be clearly observed. However, one of the methylene-methylene (-CH2-CH2-) interactions cannot be observed. This is due to the small chemical shift difference frequency (40 Hz) between them and the large inhomogeneity of the residual Z-magnetization along the F1=0 Hz line. The broadness of these peaks overlap the signals of the zero quantum interaction from the two methylene groups. In looking
through these four spectra obtained with the toroid cell one can see maximum signal intensity in the spectrum using an $\alpha$ value of 3.9 $\mu$s. This observation is based on the signal intensities of the (OH--CH$_2$--) and the (–CH$_2$–CH$_2$–) interactions. These have the largest intensity of all of the values used. It may be pointed out that the large (–CH$_2$–CH$_2$–) interaction (occurring at 400 Hz) has approximately the same intensity at 4.0 $\mu$s. This would tend to indicate that the optimum $\alpha$ value for the n-butanol in the toroid probe lies between 3.9 and 4.0 $\mu$s. The 90° pulse was harder to determine for the Helmholtz probe due to the inability of the 16 phase cycle to successfully suppress the single and higher order multiple quantum signals. An advantage though was the increased $B_0$ homogeneity using the Helmholtz probe. The Z-magnetization was less intense along the $F_1=0$ Hz line. This allowed for observation of the zero quantum interaction at 40 Hz that was obliterated by the residual Z-magnetization in the toroid probe. Figures 21–25 show the results of the $\alpha$ optimization of the Helmholtz probe. Although optimization of the pulses gives maximum intensity of the zero quantum signals, it can be seen from these spectra that the variation of the $\alpha$ pulse had no effect in reducing the higher order quantum signals. This was true in the toroid case even though the homospoil was used to suppress single and higher order multiple quantum signals. Consequently, it can be concluded that signal suppression is a function of phase cycling or homospoil pulse and not of the 90° pulse accuracy. The zero quantum 2D spectra of the Helmholtz probe also show that once a 90° pulse is employed all possible transitions among coupled nuclei are in operation even though they are not directly seen in a one dimensional experiment.

Another interesting result obtained in both the toroid cell and the Helmholtz coil was that the pulses could be changed almost 8% beyond the determined 90° pulse and still obtain good zero quantum 2D spectrum. In most spectrometers the $B_1$ field is generated at a fixed power setting. The 90° pulse length is determined by how long (in microseconds) the $B_1$ field is turned on to rotate $M_0$ through an angle of 90°. Some spectrometers have the ability to
adjust the power setting or to switch from high to low power settings. Also, B\textsubscript{1} inhomogeneity can cause a power drop and thus change the 90\textdegree pulse length. The short flip angle was an indication that the B\textsubscript{1} field for both probes was very homogeneous. The ability to change the pulses by almost 8% and still obtain good spectra indicates good B\textsubscript{1} homogeneity in both probes. This is significant when compared to an experiment conducted on a 10mm probe in the WP 200 spectrometer. In this experiment a change of 1% in the 90\textdegree pulse resulted in the loss of the zero quantum signals in the 2D spectrum. The 90\textdegree pulse for the 10mm probe was 26.7\textmu s. The notion of the degree of homogeneity of the B\textsubscript{1} field being related to 90\textdegree pulse arises from Hoult’s work (1). Hoult points out that B\textsubscript{1} homogeneity can be approximated by the amount of time the B\textsubscript{1} field is needed to nutate the magnetization vector (M\textsubscript{o}) 90\textdegree. That is to say, the smaller the pulse length, potentially the greater the B\textsubscript{1} homogeneity. Furthermore, the signal-to-noise (S/N) is inversely proportional to the 90\textdegree pulse length. If the \alpha pulse is short, the S/N will be greater. The principle behind this is the fact that the magnetization vector is not affected so much by the length of time the B\textsubscript{1} field is on but rather by the amount of power the B\textsubscript{1} field radiates. The concentrated B\textsubscript{1} field will nutate more of the vectors in the overall magnetization vector and with greater efficiency. Thus, it can be proposed that the toroid cell and coil has a better B\textsubscript{1} homogeneity than the Helmholtz probe.

5.2.2 Optimization of \(r\) Dependence

It has been shown that the evolution of the zero quantum signal has a dependence on the J, or coupling constant, between two interacting nuclei (4). Consequently, the time delay between pulses in the zero quantum pulse train will be an important value in being able to observe the zero quantum coherences. The problem that arises is not all nuclei have the equal coupling constants. Thus, certain delay times will favor the observance of zero quantum coherences that have that average delay. More specifically, the signals with large coupling
constants will be observed by using shorter $r$ delays. Conversely, the signals with small coupling constants will require a longer delay. The reciprocal relationship is a simple one. The large coupling constants will precess rapidly in the XY plane. They will require a short amount of time to move to the next position for the next pulse. The smaller coupling constants will precess more slowly and thus need more time to evolve. Therefore, if a molecular system has several coupled nuclei with largely different $J$ values, an optimum $r$ delay must be found. This optimum $r$ delay will allow the observance of the most number of nuclear interactions in one experiment. Since n-butanol is not a large molecule, it was expected to see all of the zero quantum interactions at the optimized delay. A series of experiments were done starting at a small value for $r$ and increasing it to some maximum. The optimum $r$ delay was determined by the S/N ratio in the 2D spectrum comparing the height of the zero quantum signal with the S/N of the Z-magnetization. Müller reported a value of 17.5ms (11) as the optimum $r$ delay for n-butanol and used it as a starting point. This value did not work for the toroid probe so more experiments were done increasing the $r$ delay by 10ms each time. Figures 26-31 show these results for the toroid probe. All of the indirect $J$ coupling interactions, except for the (-CH$_3$-CH$_2$-) (40 Hz) interaction, can be observed. The best $r$ delay for the toroid probe was determined to be 60ms. The largest factor in selecting the optimum $r$ value was the relative intensity of the (-CH$_2$-CH$_3$-) (400 Hz) interaction. This same interaction in the 50ms spectrum is more intense than at the 60ms delay. However, the (OH-CH$_2$-) interaction appears more intense at the 60ms delay. In addition, the strongest response for the long range (-CH$_3$-CH$_2$-CH$_3$) interaction was observed at the 60ms delay. Any long range zero quantum interactions were not expected in these experiments. The difference frequency for the long range interaction is comparable to the (OH-CH$_2$-) difference frequency (@ 200 Hz). The 60 ms delay also reduced the amount of $T_1$ noise observed in the spectrum. $T_1$ noise is caused by relaxation processes that allow nuclei to relax faster than the
pulse sequence operates. This causes spurious signals to appear in the spectrum that are not related to the signals of interest generated by the pulse sequence used. These spurious signals are readily apparent in the spectra obtained with the Helmholtz probe. Figures 32-37 show the \( r \) optimization experiments for the Helmholtz probe. The optimum \( r \) value for the Helmholtz probe was 60ms. The optimum \( r \) value did help suppress the \( T_1 \) noise. Another outstanding characteristic was the lack of signal due to the \((-\text{CH}_2-\text{CH}_2-)\) (400 Hz) interaction. In all of the experiments the signals due to this interaction were weak. The smaller frequency interactions, on the other hand, were consistently strong. This was especially true for the \((-\text{CH}_2-\text{CH}_2-)\) (40 Hz) interaction. The reduced inhomogeneity of the residual \( Z \)-magnetization allowed these signals to be observed with less interference. Also, the long range \( ZQ \) interaction of the \((-\text{CH}_2-\text{CH}_2-\text{CH}_3)\) protons was not observed in the Helmholtz spectra as it was in the toroid case.

5.2.3 Homospoil vs. Phase Cycling

Several experiments were performed to determine which method of signal suppression was more efficient. Neither the WP 200 spectrometer nor the Bruker 5mm probe had homospoil capabilities. Consequently, only the 16 phase cycle could be used for signal suppression in the Helmholtz probe. The toroid probe, as mentioned earlier, has homospoil capabilities and the tests were performed on this probe. The results of the 4 phase cycle with the homospoil have already been observed. The first experiment was to use the 16 phase cycle without the homospoil pulse. Figure 38 shows that other than the \( T_1 \) noise, nothing else was eliminated. Zero, single and higher order multiple quantum signals are present. Figure 39 shows the results of using the 4 phase cycle without the homospoil pulse but with 16 scans per experiment. This allows the 4 phase cycle to be repeated 4 times each experiment. The same spectrum is observed as in the previous experiment. A significant difference in this case is that the 4 phase cycle sequence is less efficient in eliminating the \( T_1 \) noise. This is due to
the 4 phase cycle being designed for quadrature detection and not signal suppression. The final experiment (Fig. 40) was the four phase cycle sequence with the homospoil pulse using 16 scans per experiment. The 16 scans per experiment brought about excellent signal to noise. Note on this spectrum there are only signals due to zero quantum interactions. The \( Z \)-magnetization has been significantly reduced, the long range coupling for the \((-\text{CH}_2-\text{CH}_2-\text{CH}_3\) interaction, and the large coupling (400 Hz) interaction of the two \((-\text{CH}_2-\text{CH}_2-)\) methylenes is prominently seen. This technique contains the parameters of choice except that it takes this particular experiment 4 times longer than the original ZQ 2D experiment for the toroid. It is postulated that the \( B_0 \) inhomogeneity of the toroid cell is the cause of the inability of the 16 phase cycle to suppress the single and higher order multiple quantum signals in the toroid cell.

5.2.4 Sensitivity Comparison

The signal sensitivity can be considered two ways in these experiments. The first manner is the signal-to-noise (S/N) comparison. Figure 41 shows the spectra and calculations for this determination. The spectra are \( F_1 \) domain slices taken out of their respective 2D matrices. The top trace comes from the Helmholtz probe and the bottom trace is from the toroid probe. The calculations reveal, in this case, that the toroid probe has a 3:1 S/N advantage over the Helmholtz probe. The calculation was performed by measuring the height of the peak (asterisk) from the bottom of the base line. The average peak-to-peak noise was measured and divide into the peak height. The value for the Helmholtz coil S/N had to be normalized to account for the difference in number of scans and volume. The Helmholtz probe had to be analyzed using 16 scans per experiment as opposed to the 4 scans per experiment for the toroid probe. The normalization factor for the Helmholtz calculation was 7.6.
Further examination of figure 41 reveals other interesting aspects of zero quantum 2D NMR. The compared linewidths of the zero quantum signals are almost identical. This demonstrates the independence of the zero quantum signal due to the $B_0$ inhomogeneity. Measuring these linewidths at half height (in Hz) could give the $T_2$ relaxation time of the hydroxyl proton (Eq. 7). An accurate measurement, however, would be done over a series of 2D ZQ experiments and the resulting $T_2$ values averaged. Another aspect of these spectra is the presence of single and higher order multiple quantum signals in the spectra for the Helmholtz probe. The intensities of these signals affect the S/N of the zero quantum signals. When the $T_1$ noise or higher order multiple quantum signals are large, they can reduce the S/N of the zero quantum signals. The spectra for the toroid, on the other hand, has the signals other than the zero quantum transitions suppressed. Even the $Z$-magnetization is significantly reduced. Hence, the zero quantum signals are much easier to observe. However, the zero quantum signals in the spectrum of the toroid cell have a shoulder peak. Even the $Z$-magnetization signal has a shoulder. Ideally, these shoulder peaks should not be there. As in the spectrum for the Helmholtz probe, the signals should be singlets. It is postulated that these shoulder peaks are due to a ringing effect caused by the proximity of the homospoil coil to the toroid coil.

The second aspect of the signal comparison is the number of off-horizontal interactions in the 2D spectrum. As was mentioned earlier, the toroid cell and coil observed more zero quantum interactions than the Helmholtz probe. The main difference were the large frequency difference (400 Hz) interaction and the long range interaction. The toroid cell observed these consistently, whereas the Helmholtz probe did not.
5.3 Optimized Zero Quantum Experiments for N-Butanol in
the Toroid and the Conventional 5mm Probe

5.3.1 N-Butanol 2D Zero Quantum Coherence in the Conventional 5mm Probe

Figure 42 contains the zero quantum 2D spectrum of n-butanol in the Helmholtz
probe utilizing the optimized \( r \) and \( \alpha \) parameters (60ms and 4.9\( \mu s \), respectively). The 16 phase
cycle sequence was used with 16 scans per experiment. The time for this experiment was 4.21
hours. Signals due to single and higher order multi-quantum transitions are still present. The
\( Z \)-magnetization has been minimized and looks representative of a single dimensional
spectrum. Since the zero quantum signals are more prominent, the correlations of the zero
quantum interactions are more easily made. The proton correlations for n-butanol have been
labeled in figure 42. The hydroxyl-methylene (\( H_{\text{OH}}-H_1 \)) interaction is the strongest. As before,
the methylene (\( H_1-H_2 \)) interaction is weak and barely visible. However, knowing the
difference frequency for these two methylene signals from the one dimensional spectrum, one
can confirm these off-horizontal signals as the correlation frequencies for the interaction of
the coupled \( H_1 \) and the \( H_2 \) protons. Since this is a simple molecule, mapping out the rest of
the spin-spin interactions is straight forward. A final point of interest is the resolution of the
zero quantum signals in both the \( F_1 \) and \( F_2 \) domain. In the \( F_2 \) domain the signals are broad,
"stretched out" along the \( F_2 \) axis. This is due to the \( B_0 \) inhomogeneity. Along the \( F_1 \) axis,
however, the signals are relatively constant in width and not "stretched out" along the \( F_1 \) axis.
The linewidth of these signals in the \( F_1 \) domain is approximately 40 Hz.

5.3.2 Representative Zero Quantum n-Butanol 2D Zero Quantum Coherence in the Toroid Probe

Figure 43 contains the zero quantum 2D spectrum of n-butanol in the toroid probe
using the optimized \( r \) and \( \alpha \) values (60ms and 3.9\( \mu s \), respectively). The 4 phase cycle sequence
with 4 scans per experiment was used for this study. The total time for this experiment was 1.1 hours. In comparison to figure 42 for the Helmholtz probe, the two spectra look relatively the same. However, there are a few differences worth noting. First, the background noise for the toroid is less than the background noise for the Helmholtz coil. This is due to the efficiency of the homospoil pulse to suppress the single and higher order multi-quantum signals. The residual Z-magnetization ($F_1=0$) is still larger in the toroid cell than in the spectrum for the Helmholtz probe. The signals for the ZQ interaction between the $H_2-H_3$ protons are still obscured by the broad Z-magnetization in the toroid probe. However, in comparing the relative intensities of the ZQ transition signals and the Z-magnetization signals, it can be determined that the Z-magnetization has been successfully suppressed in favor of the ZQ signals in the toroid cell. The structural mapping of the spin-spin interactions is the same as in the previous section. A comparison of the zero quantum interactions between the two spectra show the signals in the toroid case are stronger. Another note is the long range interaction of the $H_2-H_4$ protons. These signals occur approximately 200 Hz off the horizontal and correlate with the methyl signal. No other zero quantum interaction is observed that correlates with the off-horizontal signals. However, the $H_2$ and $H_4$ protons have approximately a 200 Hz difference between them. It is logical to assume that these signals are due to a zero quantum interaction between the $H_2$ and $H_2$ protons. It is possible that these signals could be either $T_1$ noise or a single quantum transition. However, since the homospoil pulse is effective in suppressing all other single quantum transitions, it is not likely that these two signals are unaffected.

The difference in $B_0$ homogeneity between the toroid and Helmholtz probes can be appreciated by comparing the 1D spectra inserted into the 2D plots of figures 42 and 43. Yet, the overall resolution of the two 2D plots is not very different. In fact, the linewidths of the zero quantum interactions in the $F_1$ domain for both probes are virtually equal (Fig. 41).
5.4 Application and Comparison of Zero Quantum Results for Taxol

The test case for zero quantum NMR was proven for a simple molecule such as n-butanol. The next step was to test this procedure on a more complicated molecule. Taxol was selected for two reasons. One, taxol was a larger molecule that yielded a complicated one dimensional NMR spectrum. The second reason was that the zero quantum spectrum of taxol would have signals spread out across the 2D matrix. Although there would not be clumping of signals as in a more complicated spectrum, the resulting 2D NMR plot would demonstrate the utility of spin-spin mapping through zero quantum 2D NMR. Figure 44 shows the one dimensional spectrum of taxol. The sample and the assignments of the proton spectrum were donated by Dr. Kingston's group. Using these assignments made it possible to work out the correlations and determine part of the molecular structure. Even if the assignments were not known, partial structural determination could be accomplished based on the principle of frequency differences. For example, in the ZQ 2D spectrum of taxol (Fig.45) in the 5mm probe, two zero quantum interactions stand out. At first it may appear that the central nucleus T is interacting with two other protons. However, on careful inspection it can be seen that there are 4 separate nuclei interacting here. The distance off the F₁=0 line (in Hz) of the ZQ interaction is the difference frequency of the spin-spin coupling of the nuclei involved. The difference frequency can be applied to the one dimensional spectrum (Fig. 44) and the coupled nuclei assigned. The one dimensional spectrum of taxol is inserted to correlate the ZQ interactions. With more correlations the observer can piece together the puzzle of structural connectivity. Figure 46 contains more information of the ZQ 2D spectrum of taxol via the toroid probe. The distance of the correlating signals off of the horizontal (F₁=0) have been measured (in Hz). The assignments have been worked out and labeled on the spectrum.
The difference frequency can be determined from the chemical shift values for the suspected nuclei. The chemical shift of one nucleus is subtracted from the second. The difference is then multiplied by the spectrometer frequency of that particular nucleus. This gives the difference frequency (in Hz) for that set of nuclei. In this case a match was found between the NH and the 3' protons (Fig. 44). This points to the possibility that these two protons are indirectly coupled and are on adjacent nuclei. Another match was found for the H₂ and H₃ protons (Fig. 44). However, it cannot always be assumed that the interactions are caused by indirect coupling from adjacent protons. This is observed in a match where the H₅ and the H₁₀ protons are coupled. The structure (Fig. 44) shows that these protons are not adjacent and yet still show some kind of interaction. Finally, there is a large difference frequency interaction in the lower methyl region. The difference frequency is approximately 600 Hz. A problem arises because there is not a matching set of ZQ interactions to correlate with. However, the difference frequency is known and an educated guess can be made as to which protons are causing this interaction to appear. In this case the postulated assignment was the H₁₀ proton coupled with the H₁₀-OAc proton.
Chapter 6

Conclusion

The 2D zero quantum experiments concerning the toroid cell and coil incorporate some important characteristics regarding NMR analysis and detector design. Important potential differences of the toroid coil and cell and the Helmholtz coil are the following:

1. The $B_1$ fields were shown to be homogeneous for both probes. This is based on the optimization tests, the length of the $\alpha$ pulse, and the relative signal-to-noise ($S/N$). The optimization tests showed the $B_1$ field homogeneous due to the relative zero quantum signal intensity over a wide range of $\alpha$ values. Had the $B_1$ fields not been homogeneous, rapid signal loss would have occurred as the flip angles moved away from the optimum 90° pulse. Also, if the $B_1$ field is inhomogeneous the relative $S/N$ will decrease. Finally, since both probes had such short 90° pulse values it is concluded that both probes have homogenous $B_1$ fields. Moreover, since the toroid had the shorter 90° pulse, it is postulated that the toroid had better $B_1$ homogeneity than the Helmholtz probe.

2. Signal suppression was accomplished either by phase cycling or a gradient pulse. Previous work demonstrates that the 16 phase cycling or gradient pulse does work for the Helmholtz probe (11,12). The 16 phase cycle sequence did not work at all on the toroid probe. It is postulated that the poor $B_1$ homogeneity is the primary problem. However, as demonstrated in Figure 36, the homospoil pulse is effective in suppressing the single and higher order multiple quantum transitions, as well as the $Z$-magnetization.

3. The results of the $r$ dependence studies behaved as expected. The zero quantum two dimensional NMR experiments have been shown to be $J$-dependent. These studies proved to be no different. If a molecule has couplings that are very different in their $J$ values, several experiments may need to be performed in order to map out all of the interactions. The toroid showed more zero quantum interactions at the optimum parameters. Potentially this would reduce the number of overall experiments necessary to characterize the interactions of a
particular molecular system. The dependence studies did not result as predicted. Normally, two dimensional experiments are sensitive to inaccurate pulses. Consequently, accurate 90° and 180° pulses are essential to successful experiments. The dependence studies demonstrated that such was not the case for these two probes. The zero quantum 2D experiments for these two probes were not sensitive to pulse inaccuracy.

4. As was mentioned before, the toroid was shown to have a S/N ratio of 3:1 over the Helmholtz probe. Although this result is better than expected, the nature of the two coils would dictate this type of outcome. A S/N ratio of 3:1 is a considerable difference. The 90° pulse for the toroid was better than the Helmholtz 90° pulse by a microsecond. Consequently, the toroid should have a S/N ratio as good as the Helmholtz and maybe a little better.

5. The n-butanol sample was an easy molecular system to map out. All of the ZQ transition interactions appeared in one experiment. As a test case it is a good sample. We were able to compare the results with those obtained by Müller (11). This allowed us to proceed with the comparison study. The taxol sample was a good case for attempting spin-spin mapping of a "real" molecule. It demonstrated the utility ZQ 2D NMR has for nuclear connectivity studies. It also demonstrated the problems one needs to be aware of in spin-spin mapping. For example, one cannot assume that all of the interactions are indirect coupling of adjacent protons. Another problem the taxol sample demonstrated was that not all of the indirect couplings can be observed at one value.

6. Due to the B₀ inhomogeneity problems associated with the toroid cell and coil, nuclei with small spectral bandwidth (i.e., ¹H) would not be candidates for analysis by the toroid cell. However, these experiments show that there may be potential for high resolution work with low sensitivity nuclei with large spectral bandwidths (i.e., ¹³C, ¹⁵N). In the case of ¹³C-¹³C studies the toroid cell may show distinct advantages. At the present, ¹³C-¹³C studies are performed by the 2D double quantum INADEQUATE sequence. This sequence requires
a 128 step phase cycle sequence per experiment to eliminate the single quantum signals and perform quadrature detection. Using the 4 phase cycle with the homospoil pulse, tremendous savings in time and equipment use are possible.
REFERENCES


   (b) S.W. Roeder, A.A.V. Gibson, and E. Fukushima, 23rd Experimental NMR Conference, Madison, Wis. (1982).


Table 1. Energy level representation and the possible n-quantum transition frequencies for a weakly coupled AX spin 1/2 system.

<table>
<thead>
<tr>
<th>M</th>
<th>level</th>
<th>energy</th>
<th>ΔM</th>
<th>transition</th>
<th>frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>4--</td>
<td>$(f_A+f_B)/2 + J/4$</td>
<td>0</td>
<td>2 → 3</td>
<td>$f_A-f_B$</td>
</tr>
<tr>
<td>0</td>
<td>3++</td>
<td>$(f_A-f_B)/2 - J/4$</td>
<td>1</td>
<td>1 → 2</td>
<td>$f_B-J/2$</td>
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<tr>
<td>0</td>
<td>2+-</td>
<td>$-(f_A-f_B)/2 - J/4$</td>
<td>1</td>
<td>3 → 4</td>
<td>$f_B+J/2$</td>
</tr>
<tr>
<td>+1</td>
<td>1++</td>
<td>$-(f_A+f_B)/2 + J/4$</td>
<td>1</td>
<td>1 → 3</td>
<td>$f_A-J/2$</td>
</tr>
</tbody>
</table>

Table 2. \( \sigma_{12} \) and \( \sigma_{23} \) elements of the (4 x 4) density matrix of an AX system, following the pulse sequence in figure 10.

<table>
<thead>
<tr>
<th>Time</th>
<th>Expressions for the matrix elements, ( \sigma_{12} ) and ( \sigma_{23} )</th>
</tr>
</thead>
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<tr>
<td>0</td>
<td>[ \sigma_{12}]<em>0 = 0 &lt;br&gt;[ \sigma</em>{23}]_0 = 0</td>
</tr>
<tr>
<td>0^+</td>
<td>[ \sigma_{12}]<em>0^+ = i(\sigma</em>{11} - \sigma_{22} + (\sigma_{13} - \sigma_{44})]/4 &lt;br&gt;[ \sigma_{23}]_0^+ = 0</td>
</tr>
<tr>
<td>r</td>
<td>[ \sigma_{12}]<em>r = [\sigma</em>{12}]<em>0^+ \exp(-i\omega</em>{12}r) &lt;br&gt;[ \sigma_{23}]<em>r = [\sigma</em>{23}]<em>0^+ \exp(-i\omega</em>{23}r) = 0</td>
</tr>
<tr>
<td>r^+</td>
<td>[ \sigma_{12}]<em>r^+ = [(\sigma</em>{12} + \sigma_{21}) + (\sigma_{34} + \sigma_{43}) - (\sigma_{13} + \sigma_{31}) + (\sigma_{24} - \sigma_{42})]/4 &lt;br&gt;[ \sigma_{23}]<em>r^+ = i[(\sigma</em>{12} + \sigma_{21}) + (\sigma_{34} + \sigma_{43}) - (\sigma_{13} + \sigma_{31}) + (\sigma_{24} + \sigma_{42})]/4</td>
</tr>
<tr>
<td>t_1</td>
<td>[ \sigma_{12}]<em>{t_1} = [\sigma</em>{12}]<em>r^+ \exp(-i\omega</em>{12}t_1) &lt;br&gt;[ \sigma_{23}]<em>{t_1} = [\sigma</em>{23}]<em>r^+ \exp(-i\omega</em>{23}t_1)</td>
</tr>
<tr>
<td>t_1^+</td>
<td>[ \sigma_{12}]<em>{t_1}^+ = [(\sigma</em>{12} + \sigma_{21}) + (\sigma_{34} + \sigma_{43}) + (\sigma_{13} + \sigma_{31}) + (\sigma_{24} - \sigma_{42})]/4 &lt;br&gt;[ \sigma_{12}]<em>{t_1}^+ = i[(\sigma</em>{14} - \sigma_{41}) + (\sigma_{23} - \sigma_{32})]/4</td>
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<tr>
<td>t_2</td>
<td>[ \sigma_{12}]<em>{t_2} = [\sigma</em>{12}]<em>{t_1}^+ \exp(-i\omega</em>{12}t_2)</td>
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</tbody>
</table>

Scheme 1.

----- FILE: ZEQUANT .AU

1 ZE
2 D1
3 P1 PH1
4 D2
5 P2 PH2
6 D2
7 P3 PH3
10 D0
11 P4 PH4
12 G0=2 PH5
14 WR =1
15 IF =1
16 IN=1
17 EXIT

PH1=A0 A0 A0 A0 A0 A0 A0 A0 A0 A0 A0 A0 A0 A0 A0 A0 A0 A0 A0 A0 A0 A0 A0
PH4=A1 A2 A3 A0 A1 A2 A3 A0 A1 A2 A3 A0 A1 A2 A3 A0 A1 A2 A3 A0
PH5=R0 R1 R2 R3 R0 R1 R2 R3 R2 R3 R0 R1 R2 R3 R0 R1

; D1= RELAXATION DELAY
; P1=90 DEGREE PULSE
; P2=180 DEGREE PULSE
; P3=45 DEGREE DENSITY OPERATOR SPIN CONVERSION PULSE
; P4=90 DEGREE PULSE CONVERTING ZQ COHERENCE TO TRANS. MAG.

; D2=TAU VALUE FOR MIXING & 17 MS
; D0=INCREMENTAL DELAY
; ND0=1 -
Scheme 2.

--- FILE: ZOTORR .AU ---

1 ZE
2 D1
3 P1 PH1
4 D2
5 P2 PH2
6 D2
7 P3 PH3
8 P5=0 PH6
9 D0
10 P4 PH4
11 GO=2 PH5
12 WR #1
13 IF #1
14 IN=1
15 EXIT

PH1=A0 A0 A0 A0
PH2=A1 A3 A1 A3
PH3=A1 A1 A3 A3
PH4=A1 A1 A1 A1
PH5=R0 R0 R2 R2

P4=80 R0 R0 R0 R0
D1=RELAXATION DELAY
P1=30 DEGREE PULSE
P3=130 DEGREE PULSE
P3=45 DEGREE SPIN DENSITY OPERATOR CONVERSION PULSES
P4=90 OR 45 DEGREE DETECTION PULSE
D2=TAU VALUE FOR MIXING 2 17 MS
D0=INCREMENTAL DELAY
ND0=1
### Scheme 3.

**Phase Cycling without Homogeneity**  
Spoiling Pulse at $t_i = 0$

<table>
<thead>
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<th>$\phi_1$</th>
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<th>$\phi_3$</th>
<th>$\phi_4$</th>
<th>$\phi_{\text{meas}}$</th>
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### Scheme 4.

**Phase Cycling with Homogeneity**  
Spoiling Pulse at $t_i = 0$

<table>
<thead>
<tr>
<th>$\phi_1$</th>
<th>$\phi_2$</th>
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</tr>
</tbody>
</table>

Scheme 5.

Zero Quantum 2D Pulse Sequence

Fig. 1. The solenoid coil with $B_0$ and $B_1$ field in configuration for an electromagnetic spectrometer.

Fig. 2. The Helmholtz (or saddle) coil configuration with $B_0$ and $B_1$ field for a superconducting magnet.

Fig. 3. Rotating frame of reference XYZ axis system for routine NMR experiment with magnetic field ($B_0$), rf field ($B_1$), and Magnetization vector ($M_0$).
Fig. 4. The four basic periods of a two dimensional NMR experiment.
Fig. 5. The rf field ($B_1$) distribution due to the proximity effect in a solenoid coil.

Fig. 6. The rf field ($B_1$) distribution due to the proximity effect in a Helmholtz (saddle) coil.

Fig. 7. The toroid cell and coil showing the relative $r_2/r_1$ dependence.

Fig. 8. The energy level diagram for an AX spin system \((m_l = 1/2, m_s = 1/2)\) and the transition probabilities.

- \(W_0\) = Zero Quantum Transition
- \(W_i^\dagger\) = Single Quantum Transition
- \(W_2\) = Double Quantum Transition
Fig. 9. Density matrix for an AX spin system showing zero, single, and double quantum transitions.
Fig. 10. Pulse sequence used to create and detect multiple quantum coherences of all orders via a two dimensional Fourier transform experiment.

Fig. 11. $B_0$ homogeneity dependence with respect to single, double, and zero order transitions of an AX spin system (Fig. 8).

(a) Single quantum spectrum of a sample in an inhomogeneous magnetic ($B_0$) field. The black and shaded areas correspond to two different regions in the sample.
(b) The double quantum spectrum of the same sample.
(c) The zero quantum spectrum of the same sample.

Fig. 12. Relative position of the toroid lying in the XY plane and perpendicular to the magnetic ($B_0$) field of the superconducting magnet.
Fig. 14. Pictorial description of a 2D contour plot showing available information from the $F_2$ and $F_1$ axes.
Fig. 15. Simulated 2D zero quantum spectrum contour plot showing signals resulting from coupling interactions between the proton nuclei (200 MHz spectrometer).
Fig. 16. One dimensional spectrum of n-butanol in deuterated acetone at 200 Mhz.
Fig. 17. 2D zero quantum spectrum contour plot of n-butanol for $\alpha$ optimization of the toroid probe ($\alpha = 3.8\mu s$).
Fig. 18. 2D zero quantum spectrum contour plot of n-butanol for $\alpha$ optimization of the toroid probe ($\alpha = 3.9$ $\mu$s).
Fig. 19. 2D zero quantum spectrum contour plot of n-butanol for α optimization in the toroid probe (α = 4.0μs).
Fig. 20. 2D zero quantum spectrum contour plot of n-butanol for $\alpha$ optimization in the toroid probe ($\alpha = 4.1 \mu s$).
Fig. 21. 2D zero quantum spectrum contour plot of n-butanol for α optimization of the Helmholtz probe (α = 4.8 μs).
Fig. 22. 2D zero quantum spectrum contour plot of n-butanol for \( \alpha \) optimization of the Helmholtz probe \( (\alpha = 4.9\mu s) \).
Fig. 23. 2D zero quantum spectrum contour plot of n-butanol for $\alpha$ optimization of the Helmholtz probe ($\alpha = 5.0\mu$s).
Fig. 24. 2D zero quantum spectrum contour plot of n-butanol for α optimization of the Helmholtz probe (α = 5.1 μs).
Fig. 25. 2D zero quantum spectrum contour plot of n-butanol for α optimization for the Helmholtz probe (α = 5.2μs).
Fig. 26. 2D zero quantum spectrum contour plot of n-butanol for τ optimization of the toroid probe (τ = 17ms).
CH₃ - CH₂ - CH₂ - CH₂ - OH

Fig. 27. 2D zero quantum spectrum contour plot of n-butanol for r optimization of the toroid probe (r = 24ms).
Fig. 28. 2D zero quantum spectrum contour plot of n-butanol for $r$ optimization of the toroid probe ($r = 35.7$ ms).
Fig. 29. 2D zero quantum spectrum contour plot of n-butanol for $r$ optimization of the toroid probe ($r = 50\text{ms}$).
Fig. 30. 2D zero quantum spectrum contour plot of n-butanol for $r$ optimization of the toroid probe ($r = 60$ ms).
Fig. 31. 2D zero quantum spectrum contour plot of n-butanol for $r$ optimization of the toroid probe ($r = 80$ms).
Fig. 32. 2D zero quantum spectrum contour plot of n-butanol for $r$ optimization for the Helmholtz probe ($r = 17\text{ms}$).
Fig. 33. 2D zero quantum spectrum contour plot of n-butanol for $r$ optimization of the Helmholtz probe ($r = 24\text{ms}$).
Fig. 34. 2D zero quantum spectrum contour plot of n-butanol for $r$ optimization of the Helmholtz probe ($r = 35.7$ ms).
Fig. 35. 2D zero quantum spectrum contour plot of n-butanol for $r$ optimization of the Helmholtz probe ($r = 50$ ms).
Fig. 36. 2D zero quantum spectrum contour plot of n-butanol for $r$ optimization of the Helmholtz probe ($r = 60$ ms).
Fig. 37. 2D zero quantum spectrum contour plot of n-butanol for $r$ optimization of the Helmholtz probe ($r = 80$ms).
Fig. 38. 2D zero quantum spectrum contour plot of n-butanol in the toroid probe utilizing the 16 phase cycle sequence without the homospoil pulse.
Fig. 39. 2D zero quantum spectrum contour plot of n-butanol in the toroid probe utilizing the 4 phase cycle sequence with 16 scans per experiment and without the homospoil pulse.
Fig. 40. 2D zero quantum spectrum contour plot of n-butanol in the toroid probe utilizing the 4 phase cycle sequence with 16 scans per experiment and with the homospoil pulse.
Fig. 41. Sensitivity comparison between the toroid and Helmholtz probes by removing a 1D slice from their respective 2D matrices and comparing the signal-to-noise.
Fig. 42. Optimized 2D zero quantum spectrum contour plot of n-butanol using the optimized \( r \) and \( \alpha \) values for the Helmholtz probe.
Fig. 43. Optimized 2D zero quantum spectrum contour plot of n-butanol using the optimized $r$ and $\alpha$ values for the toroid probe.
Fig. 44. 1D proton spectrum of taxol in deuterated chloroform with assignments (270 MHz).
Fig. 45. 2D zero quantum spectrum contour plot of taxol using optimized \( r \) and \( \alpha \) values for the Helmholtz probe.
Fig. 46. 2D zero quantum spectrum contour plot of taxol using optimized \( r \) and \( \alpha \) values in the toroid probe.
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The two page vita has been removed from the scanned document. Page 2 of 2