Solid-State Nuclear Magnetic Resonance of Quadrupolar Nuclei with Applications to Biological Solids

by

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This doctoral thesis has been examined by a committee of the Department of Chemistry as follows:

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Sylvia T. Ceyer...............................................................
To my parents, James and Virginia Rovnyak.
Acknowledgements

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Now to wrap this up by going back to the beginning. I have never forgotten Mr. Curry’s ability to describe chemistry with satisfying clarity and the high expectations he placed on us. Hopefully, he has forgotten the grade I got in his class. And I would like to end by thanking Dr. Raymond Dominey. I have pursued research and NMR because of Dr. D, who projects to all around him the excitement of trying to solve a problem and gave generously of his time to introduce undergraduates to research.
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with

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Submitted to the Department of Chemistry on May 21, 1999, in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Abstract:

This thesis reports on the development of new methods for studying spin > 1/2 nuclei in solids using nuclear magnetic resonance (NMR), and the application of these and other solid-state NMR techniques to probing local structure in biological solids.

The need to improve solid state NMR methods for quadrupolar nuclei, with emphasis on applications to biopolymers, is motivated in Chapter 1. The requisite theory for describing nuclear spin systems under NMR experiments is extensively described in Chapter 2. There are four important derivations in Chapter 2, with supporting calculations in the appendices, which do not appear together in any single source in the literature. Some are straightforward, such as the perturbation treatment in Section 2.2.2.; however the tensor expansion in Section 2.2.4 is more complex. It is hoped that this may assist others who undertake to develop a background in quadrupolar theory.

Chapter 3 presents several new methods for single-resonance NMR spectroscopy of half-integer quadrupolar nuclei, while Chapter 4 presents new double-resonance methods which provide multi-spin correlations involving quadrupolar nuclei.

Chapter 5 begins with an instructive demonstration of the utility of $^{11}$B NMR in an α-lytic protease-inhibitor complex, and presents an in-depth $^{23}$Na NMR study of guanine rich oligonucleotides which model human chromosomai telomeres. Chapter 6 will briefly summarize this work and emphasize important outstanding problems.
In the appendices, several supplemental calculations to support Chapter 2 are provided. Also, a software program which provides a complete environment for the design, execution, display and analysis of NMR simulations of quadrupolar nuclei is presented. Finally, a collaboration conducted during this thesis work which does not directly compliment the title of this thesis is presented.

Thesis Supervisor: Robert G. Griffin
Title: Professor of Chemistry
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Foreword

One of my standing concerns for a while was that, by focusing on quadrupoles, I had narrowed my focus too much. Over some time I appreciated that this was not a concern but a luxury, and I have enjoyed this sincerely.

Behold the fool saith, “Put not all thine eggs in the one basket” - which is but a manner of saying, “Scatter your money and attention;” but the wise man saith, “Put all your eggs in the one basket and – WATCH THAT BASKET.”

-Mark Twain
1. Introduction

1.1 Quadrupolar Nuclei in Nature

A quadrupolar nucleus has a spin quantum number greater than 1/2, and transitions among the possible spin energy levels (S, S-1, ...-S) may be detected by nuclear magnetic resonance (NMR) spectroscopy. The most common nuclei studied by solid-state and solution NMR are spin-1/2 such as $^{13}\text{C}, ^{15}\text{N}$ and $^1\text{H}$. The challenge introduced by quadrupolar nuclei is that the interaction of a nuclear electric quadrupole moment with a surrounding electric field gradient can significantly perturb the nuclear spin energy levels away from the Zeeman splittings.

However, it is well known that quadrupolar nuclei play key roles in the structure and function of proteins and nucleic acids[1, 2]. A compelling demonstration of this is to consider the periodic table and note all elements for which an accessible, spin-active quadrupolar nucleus is known (i.e. S=1, 3/2, 2, 5/2, 7/2 and 9/2). This is illustrated in Figure 1.1. Approximately 70% of the periodic table offers quadrupolar nuclei for NMR observation. And in many cases, for example $^{17}\text{O}$ (S=5/2), $^{11}\text{B}$ (S=3/2), $^{67}\text{Zn}$ (S=5/2) and $^{23}\text{Na}$ (S=3/2), the only spin-active isotopes available are half-integer quadrupoles.
Figure 1.1. Periodic table of the elements with entries shaded for which an isotope exists which exhibits a nuclear electric quadrupole moment and is accessible by NMR.

The significance of the quadrupole coupling is its extreme sensitivity to local symmetry and structure about the quadrupolar nucleus. In half-integer quadrupolar nuclei - S=3/2, 5/2, etc.- quadrupole parameters may be extracted from appropriate solid-state NMR spectra, from which structural information can often be deduced. The information obtained from the quadrupole coupling has been applied in at least one case to substantially refine the crystal structure of an inorganic salt which had previously been solved by x-ray crystallography [3].

Solid-State NMR (SSNMR) of half-integer quadrupolar nuclei is complicated by the influence of the quadrupole coupling on the nuclear spin states, which can be quite profound. Consider, for example, the magic-angle spinning (MAS) cross-polarization experiment which is used for generating substantial signal enhancements for rare spin-1/2 nuclei and is a fundamental building block in spin-1/2 SSNMR experiments. Due to complex spin-locking dynamics, the MAS-CP experiment fails for half-integer quadrupolar nuclei. This behavior will be considered in detail in this thesis.
Recently, the feasibility of observing quadrupoles such as $^{23}$Na, $^{67}$Zn, $^{17}$O, and $^{59}$Co has been demonstrated by several authors [4-11]. In parallel with this progress, several powerful methods for obtaining NMR spectra of quadrupolar nuclei in solids have been introduced which significantly extend the information available from quadrupolar NMR in solids. The most visible examples of this recent work are isotropic methods based on the novel multiple-quantum magic-angle spinning (MQMAS) spectroscopy method [12, 13]. Also, since the magnitude of the quadrupole coupling is inversely proportional to the applied static field, the steady increase in available static fields (e.g. 17.6 Tesla = 750 MHz for $^1$H) for solid-state NMR has made it possible to study increasingly challenging samples with lower spin densities and larger quadrupole couplings. One of the principle goals of the work reported here has been to develop new quadrupolar NMR experiments for studying challenging biological systems. Contributions reported here include methods for measuring crystallographic site populations, performing hetero- and homo-nuclear correlations, studying sample motions in amorphous solids, and solving spectral assignments for multi-spin systems.

The motivations for directly observing quadrupolar nuclei with solid-state NMR are as follows. First, many quadrupolar nuclei have high natural abundances, e.g. $^{23}$Na (S=3/2) (100%) and $^{11}$B (S=3/2) (%)80. Even $^{67}$Zn (S=5/2) (4.0) is four-fold more abundant than $^{13}$C(1.0). Also, the Larmor frequencies of many quadrupoles are similar or higher than those of spin-1/2 nuclei. Thus, quadrupolar nuclei are no less sensitive than typical spin-1/2 nuclei. Second, quadrupolar nuclei are frequently invisible to x-ray and solution NMR methods. In the former, quadrupolar nuclei have been mis-assigned to water molecules or may not give well-resolved electron densities. In solution NMR, quadrupolar induced relaxation often broadens NMR lines and in extreme cases prevents the observation of quadrupolar nuclei. Solid-state NMR is one of the few means to directly observe quadrupolar nuclei in bulk solids. The final and most significant advantage of studying quadrupolar nuclei is that they tend to be localized to the active or functional sites of biopolymers. They offer the promise of directly observing structural features in the most interesting regions of proteins and nucleic acids without having to resort to site-specific isotopic enrichment procedures. This report justifies these goals with a $^{11}$B study of a protein/inhibitor complex and a $^{23}$Na study of guanine-rich DNA.
1.2. References

2. Theory & Background

2.1. General Concepts and Theory

2.1.1. Nuclear Spin Angular Momentum

The measurement of nuclear spin level transitions is the basis of nuclear magnetic resonance (NMR) spectroscopy. Nuclei may possess a non-zero spin angular momentum, represented by a quantum number $S$ which may take values $S=\frac{1}{2}$, $1$, $\frac{3}{2}$, $\frac{5}{2}$, $\frac{7}{2}$, and even $\frac{9}{2}$. Nuclei with non-zero $S$ are termed 'spin active' to indicate that transitions and coherences involving nuclear spin states may be stimulated and observed with an appropriate experimental design. A nucleus with quantum number $S$ may have $2S+1$ projections $S_z = [S, S-1, ..., -S]$ and $2S$ possible single-quantum transitions between these levels. In the absence of any fields, the two energy levels of an $S=\frac{1}{2}$ nucleus are degenerate. For $S>\frac{1}{2}$, the quadrupole interaction may lift this degeneracy, and this is the foundation for nuclear quadrupole resonance (NQR) experiments that are performed in the absence of a magnetic field[1]. If we consider a ‘bare’ $S=\frac{1}{2}$ nucleus in the presence of a static, externally supplied magnetic field, the energy levels are separated proportional to the magnitude of the applied field. This is the nuclear Zeeman effect and, for magnetic fields of several Tesla (1 Tesla = 10,000 gauss), the induced energy differences between nuclear spin levels occur in the radio frequency regime, which is on the order of $10^2$ MHz.
2.1.2. Pulsed Fourier Transform NMR (FT-NMR)

Modern NMR spectrometers apply short, high strength, radio-frequency (RF) fields to stimulate nuclear spin transitions in a sample which is placed inside an appropriately tuned coil. The decay of the excited spin states can be recorded since the NMR observable is a coherence (satisfying certain selection rules) that is associated with an oscillating net magnetic moment of the sample. The frequency of oscillation corresponds to spin energy level differences. This oscillating magnetic moment induces an RF current in the surrounding coil, which decays as the spin system relaxes to thermal Boltzmann populations. Such a signal, the free induction decay (FID), may be detected and digitized with a suitable receiver, which typically must be able to record for 10-100 milliseconds (ms). A frequency spectrum is obtained by applying a complex Fourier transform (realized computationally through the fast-Fourier-transform algorithm). Pulsed NMR can measure the complex spectrum and thus record pure-phase spectra which are free of the line shape distortions of continuous wave (CW) NMR [2]. Of particular importance, the remarkable ability to control and manipulate nuclear spin states and coherences with RF pulses has allowed FT-NMR to become a powerful tool for the determination of structure and dynamics in locally ordered samples ranging from glasses, plastics and zeolites to lipids, proteins and nucleic acids[2, 3]. It is beyond the scope of this report to give a general description of pulsed NMR methodology, and the reader is referred to several excellent texts for more background [2-6].

2.1.3. The Solid-State NMR Hamiltonian

The fields and interactions which determine nuclear spin energy levels and the evolution of coherences in NMR spectroscopy are introduced here. The convention of dividing these interactions into "external" and "internal" is used to distinguish between fields introduced from the experimental apparatus and fields which are inherent to local effects of the nuclei and electrons themselves. It will be shown in section 2.3 that NMR experiments can be accurately simulated without having to solve the time dependent Schrödinger equation.

External Hamiltonians. A nuclear spin which is 'aligned' parallel to an external magnetic field has a lower energy than a spin which is anti-parallel to the static field. This is the nuclear Zeeman effect and can be assumed to cause the largest splitting of nuclear energy
levels in NMR experiments. The Zeeman term is denoted $H_Z$. The application of pulsed radio frequency fields to the sample, generally via a tuned coil in the probe, is the means by which nuclear spin coherences are created and manipulated. This term is denoted $H_{RF}$.

**Internal Hamiltonians.** The local electronic environment about a nucleus in a static magnetic field may react in ways to oppose or reinforce the strength of the magnetic field at the nuclear position. The magnetic field experienced by a nucleus due to surrounding electrons gives the chemical shift effect, denoted $H_{CS}$. The local electronic environment of a molecule will stimulate fields of different magnitudes on a given nucleus depending on how the molecule is oriented in the static external field. The chemical shift is therefore anisotropic, and more generally is termed the chemical shift anisotropy (CSA).

Every nuclear spin is associated with a very small magnetic moment. When two spins are close in space, the magnetic field due to one spin modifies the total field experienced by the other. This through-space interaction is the nuclear dipole-dipole interaction and is denoted $H_D$. Again, as in the case with the CSA interaction, the dipole-dipole coupling is also sensitive to the orientation of the internuclear vector in the static magnetic field.

When two spins are covalently bonded, whether through a single, double or triple bond, the electrons couple the spin states of the two nuclei. This through-bond, electron-mediated interaction is termed the J-coupling, and denoted $H_J$. Although in principle the J-coupling can be anisotropic, the anisotropy is generally negligible and, for a given spin pair, the J-coupling will be a fixed constant independent of the orientation as well as the applied field. The J-coupling is of critical importance to solution NMR experiments[2, 3, 6], however it will not be considered further.

For the case of a $S>1/2$ nucleus, the interaction of the nuclear charge distribution (quadrupole moment) with a local electric field gradient (efg) can cause very large splittings in the nuclear spin states. The quadrupolar coupling is a particularly sensitive function of the symmetry about the nucleus as well as the orientation of the chemical moiety in the static field and is denoted $H_Q$.

And for completeness it is mentioned that, in rare cases, the nuclear spin states may couple to the rotational angular momentum of the molecule in which the spin is located. This is the spin-rotation interaction which will not be considered further.

We will assume the following hierarchy in the magnitudes of these interactions:
These Hamiltonians are described in Table 2.1, where the quadrupolar interaction will be treated in detail in Section 2.2. The external Zeeman and radio-frequency interactions are simply the vector products of the spin angular momentum and a magnetic field vector. This is a good basis for understanding the forms of the interaction Hamiltonians which can be thought of as the spin angular momentum interacting with an effective local field.

\[
H_z > H_Q >> H_{RF} > H_{CS} > H_D > H_J .
\]

(1)

<table>
<thead>
<tr>
<th>External</th>
<th>Zeeman</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radio Frequency</td>
<td>[-γI \cdot B_0 = -ω_0 I_z ; ; ω_0 = γ B_0]</td>
</tr>
<tr>
<td>Internal</td>
<td>Chemical Shift</td>
</tr>
<tr>
<td></td>
<td>[I \cdot  \hat{σ}_{cz} \cdot B_0]</td>
</tr>
<tr>
<td></td>
<td>Dipolar</td>
</tr>
<tr>
<td></td>
<td>[I^I \cdot  \hat{D}_q \cdot I^I]</td>
</tr>
<tr>
<td></td>
<td>Indirect Spin-Spin</td>
</tr>
<tr>
<td></td>
<td>[I \cdot  \hat{J}_{IS} \cdot S]</td>
</tr>
</tbody>
</table>

Table 2.1. List of NMR interaction Hamiltonians with second-rank interaction tensors for the internal fields. See references [7-9] for more details.

Specifically, \(\hat{σ}_{cz} \cdot B_0\) represents the field induced at spin I by the local electronic environment, \(\hat{D}_q \cdot I^I\) is an effective field at spin i due to nucleus j, and \(\hat{J}_{IS} \cdot S\) is an effective field at spin I due to electron-mediated coupling from the S spin. At this stage the concept of a tensor is introduced as a mathematical construct which is simply a linear transformation. A tensor may be a constant (i.e. a scalar), a vector, or matrix of any dimension, which is also known as the ‘rank’. In Table 1, \((\hat{σ}_{cz}, \hat{D}_q, \hat{J}_{IS})\) are second rank interaction tensors (i.e. 3x3 two-dimensional matrices) which transform a given vector \((B_0, I^I, S)\) into a vector which represents an effective local field at the nucleus of interest. In this sense, a tensor is a \textit{rule} for transforming an input vector into an output vector\(^1\).

\(^1\) An example of the physical interpretation of a tensor can be seen by considering some mechanical properties of solid materials. By applying a force against an object (i.e. a stress), the response of the object can then be measured (i.e. the strain). The stress is applied with a given magnitude and direction with respect to crystallographic axes and thus is a vector. The material responds by deforming in another direction with some magnitude and is also a vector. The stress-strain behaviour of a material can be completely described with a tensor which operates on a vector, which is an arbitrary applied stress, and returns another vector which is the induced strain. (Thanks to Dr. D. Blom for his insights here.)
The tensor notation of Table 2.1 helps to provide an intuitive understanding of the internal interactions but in fact this form will not be used here. Instead, all internal interactions may be rewritten in a special type of notation employing irreducible spherical tensor components \( \lambda = Q, CSA, D, J \) \([7, 9]\):

\[
H^\lambda = \sum_{l=0}^{2} \sum_{m=-l}^{l} (-1)^m R_{lm}^\lambda m_{l,m} ,
\]

where the geometric dependence of the interaction is described by the \( R_{lm} \) tensor components, and the spin operators are contained in the \( T_{lm} \) tensor components. This is an extremely powerful representation of the NMR Hamiltonian. Transformations of Equation 2 to account for mechanical manipulations of the sample and RF pulses are described next.

### 2.1.4. Irreducible Spherical Tensors, Rotations, and Euler Angles

A second rank tensor is equivalent to an ellipsoid. There must exist some frame, the principal axes system (PAS), in which a second rank tensor is diagonal. The three diagonal principal components in such a Cartesian frame are given by

\[
\hat{R}_{\text{cartesian,PAS}} = \begin{bmatrix}
R_{XX} & 0 & 0 \\
0 & R_{YY} & 0 \\
0 & 0 & R_{ZZ}
\end{bmatrix},
\]

and these may be thought of as the three axes of an ellipsoid. An immediate observation is that the shape of an ellipsoid is invariant to rotations. A powerful way to take advantage of this is to form a basis of irreducible spherical tensor components such that, if a rotation is applied to a tensor that is expressed as a sum of these irreducible components, the resulting tensor will be some linear combination of these irreducible components. In other words, the set of irreducible spherical tensor components forms a group under the operation of rotation. The spherical tensors do not form a group under multiplication however. The basis must be expanded to represent the product of two tensors. This is a very important aspect of tensors which is directly related to the addition of angular momenta and is the basis for understanding the higher order terms of the quadrupole coupling.

The irreducible spherical tensor components are constructed from the Cartesian principal components in the PAS frame. Any Cartesian tensor may be expressed as the sum
of a scalar component \( (\rho_{00} + \rho_{20}) \), an antisymmetric component \( (\rho_{21} + \rho_{2-1}) \) and a symmetric component \( (\rho_{22} + \rho_{2-2}) \):

\[
\rho_{00} = \frac{1}{2} \text{Tr}(R), \quad \rho_{2,\pm 1} = 0, \\
\rho_{20} = \sqrt{\frac{1}{2}} (R_{ZZ} - \frac{1}{3} \text{Tr}(R)), \quad \rho_{2,\pm 2} = \frac{1}{2} (R_{YY} - R_{XX}), \tag{4}
\]

where it is common to introduce a set of parameters \( (R, \delta, \eta) \) to define the \( \rho_{m} \)'s,

\[
R = \frac{1}{3} \text{Tr}(R), \quad \eta = \frac{R_{YY} - R_{XX}}{R_{ZZ} - R}, \quad \delta = R_{ZZ} - R, \\
\rho_{00} = R, \quad \rho_{2,\pm 1} = 0, \\
\rho_{20} = \sqrt{\frac{1}{2}} \delta, \quad \rho_{2,\pm 2} = -\frac{1}{2} \eta \delta. \tag{5}
\]

This follows the convention of Spiess, who also provides a derivation of these correspondences in his monograph[9]. As emphasized previously, the internal NMR interactions are anisotropic. Since the spin operators, represented by the \( T_{m} \)'s in Equation 2, are quantized with the static external field (i.e. the laboratory frame), the space tensors must be expressed in this frame, not in the unique frame (PAS) in which they are diagonal. This is done by applying an Euler transformation, which can be thought of as a rotation of the tensor through a set of angles \( (\alpha, \beta, \gamma) \).

\[
R_{0m}^\lambda = \sum_{m'} D_{m',m}^{(i)} (\alpha, \beta, \gamma) \rho_{l,m}. \tag{6}
\]

The rotation can be completely described in the original axes of the PAS (X,Y,Z). Following Spiess, \( (\alpha, \beta, \gamma) \) represent a rotation of the tensor by gamma about its principal Z axis, followed by a rotation about the Y axis by beta, and finally by another rotation by alpha about the original Z axis. This is shown in Figure 2.1, where it must be remembered that the gamma rotation is applied first. The \( D_{m',m}^{(i)} \) are elements of Wigner rotation matrices. There are several different conventions for specifying the Euler angles, which in turn lead to inconsistencies in the Wigner matrices, and we will continue to follow Spiess for self-consistency. While a great number of texts give Wigner rotation matrices, the rotation matrices reported in appendix C of Spiess[9] are consistent with the definitions used in this report.
Of equal importance are the influence of r.f. fields on the spin tensor components in Equation 2. These terms are quantized in the LAB frame along the external, static field, but may be experimentally manipulated by applying r.f. fields which act as rotations on the spin angular momentum operators that comprise the $T_{2m}$’s. It is also necessary to perform rotations of the spin tensors when transforming into a Zeeman rotating frame, or any other interaction frame. These transformations are briefly summarized in appendix 7.1.4, which provides a general framework for all basic spin rotations.

### 2.2. Theoretical Treatments of the Quadrupole Coupling

In this section we present many of the key derivations in the theory of quadrupolar NMR. While this is not a complete review of quadrupolar theory, it does collect in one location several derivations of varying ‘hardness’ in the hopes that it may be helpful to others.

The physical basis and exact expressions for the coupling of a nuclear quadrupole moment to a surrounding electric field gradient are stated in section 2.2.1. Since the
magnitude of the quadrupole coupling is typically on the order of several MHz for half-integer quadrupolar nuclei, expressions which are approximations of the quadrupolar Hamiltonian must be taken to two orders. These approximations can be useful for deriving expressions that govern the spin dynamics of quadrupolar nuclei, as in section 2.2.5. Sections 2.2.2. and 2.2.3. present these approximations by perturbative and coherent averaging approaches, respectively. In section 2.2.4. an important reformulation of the high order term is summarized which will later provide a great deal of insight for designing high resolution experiments. The derivation for the result in section 2.2.4. is not entirely trivial and is given in detail in the Appendix 7.1.1. Finally, a novel treatment of the quadrupolar coupling during r.f. irradiation is given in 2.2.5.

### 2.2.1. Classical and Quantum Expressions for the Quadrupolar Interaction

Spin-1/2 nuclei possess no electric dipole or quadrupole moments and such nuclei can be considered to have a spherical distribution of the proton charge. Nuclei with spin>1/2 possess no electric dipole moment but do exhibit a quadrupole moment which may interact with a surrounding electric field gradient[10]. Nuclei with spin>1/2 may be visualized as having a non-spherical distribution of charge. These concepts are illustrated in Figure 2.2.

![Diagram of spin-1/2 and spin>1/2 nuclei to illustrate the distribution of positive charge which is spherical for S=1/2 nuclei, and ellipsoidal for S>1/2 nuclei. For a non-spherical nucleus, its orientation in the surrounding electric-field-gradient influences the nuclear spin energy levels.](image-url)
The quadrupole/electric-field-gradient (efg) interaction can significantly influence the nuclear energy levels and lead to nuclear spin level splittings, in the absence of an applied magnetic field, on the order of several MHz. This magnitude can be comparable to the Zeeman splitting induced by currently accessible static fields, however NMR can be used only in the case where the Zeeman effect at least partially truncates the quadrupolar interaction as represented by the relative magnitudes in Equation 1.

The form of the quadrupole coupling is obtained by writing a classical expression for the energy of a nuclear charge distribution interacting with an electron cloud,[10]

\[ E^{(Q)} = \int \frac{\rho_e(r_e)\rho_n(r_n)dr_e dr_n}{|r_n - r_e|} , \]  

where the center of the nucleus is taken as the origin for the two vectors \( r_e \) and \( r_n \) and the denominator in Equation 7 may be expanded to give the classical energy[10]

\[ E^{(Q)} = \sum_{l,m} A_{ln} B_{lm} \]

\[ A_{lm} = \sqrt{\frac{4\pi}{2l+1}} \int \rho_n(r_n) r_n^l Y^m_l(\theta_n, \phi_n) dr_n \]

\[ B_{lm} = \sqrt{\frac{4\pi}{2l+1}} \int \rho_e(r_e) r_e^{-l-1} Y^m_l(\theta_e, \phi_e) dr_e . \]

By application of the correspondence principle, a Hamiltonian may be derived from Equation 8. The details are given by Abragam (Chapter 6[10]), and the result is stated here for a quadrupolar Hamiltonian in the principal axes of the electric field gradient tensor,

\[ H^{(Q)}(PAS) = \frac{e^2 qQ}{4I(2I-1)} \left[ 3I_2^2 - I(I + 1) + \frac{1}{2} \eta(I_2^2 + I_2^2) \right] \]

\[ = \omega_Q \sum_m R_2^{(PAS)} T_{2,-m} , \]

where \( \omega_Q = \frac{e^2 qQ}{\hbar^2 (2I-1)} \), \( R_2^{(PAS)} \) is the electric field gradient tensor in the principle axes (i.e. \( R_2^{(PAS)} = R_2^{(2m)} \); see Equation 5) and the spin tensor, \( T_2 \), governs the evolution of the spin dynamics. There is little consistency in the research literature as to the distribution of constants through the space and spin tensors for quadrupoles. We will use a form that is reasonably widely reported[7, 11-14]. Although different from Abragam’s notation as well as

---

Note that the most general form of Equation 9 sums over rank 0 through 2 tensor components. The quadrupole coupling is represented by a traceless, symmetric cartesian tensor and so the rank 0 and 1 terms, respectively, vanish in the irreducible representation.
others, this form is convenient and a good candidate for wider acceptance. To express
Equation 9 in the LAB frame, the components are obtained from Equation 6

\[ R_{20} = \sqrt{\frac{3}{8}} \left[ 3\cos^2 \beta - 1 + \eta \cos^2 \beta \cos 2\alpha \right], \]

\[ R_{2\pm 1} = \frac{1}{2} \sin \beta e^{\pm i\eta} \left[ \pm (3 - \eta \cos 2\alpha) \cos \beta - i\eta \sin 2\alpha \right], \quad (10) \]

\[ R_{2\pm 2} = \frac{1}{2} e^{\pm i\eta} \left[ \frac{3}{2} \sin^2 \beta + \frac{1}{2} (1 + \cos^2 \beta) \cos 2\alpha \pm i\eta \cos \beta \sin 2\alpha \right]. \]

And the \( T_{2m} \) tensor basis is:[15, 16]

\[ T_{20} = \frac{1}{\sqrt{6}} (3I_Z^2 - I(I + 1)), \]

\[ T_{2,\pm 1} = \mp \frac{1}{2} (I_Z I_\pm + I_\pm I_Z), \]

\[ T_{2,\pm 2} = \frac{1}{2} I_Z^2. \quad (11) \]

It is not uncommon to incorporate the \( \omega_Q \) as a fore factor in each tensor component of
Equation 10 to further simplify Equation 9. To summarize, we have the Lab-frame Hamiltonian

\[ H^{(Q)} = \omega_Q \sum_m R_{2m} T_{2,-m}. \quad (12) \]

The energy levels of a half-integer quadrupolar nucleus are best understood by
 picturing successive corrections to the Hamiltonian, as shown for a S=3/2 nucleus in Figure
2.3. These corrections are derived in the following two sections, however we highlight here the two important observations that should be made about Figure 2.3. The first is that the lowest order term, which is proportional to \( I_Z^2 \), does not affect the symmetric transitions, \( (\frac{1}{2}, \frac{1}{2}) \) and \( (\frac{1}{2}, \frac{3}{2}) \). The next order term is proportional to \( I_Z \) and thus perturbs the symmetric transitions. Since \( H_Q^{(0)} \) is typically on the order of MHz, and \( H_Q^{(1)} \) is in the kHz regime, the side or satellite transitions, \( (\frac{3}{2}, \frac{1}{2}) \) and \( (\frac{3}{2}, \frac{3}{2}) \), are usually unobservable in MAS NMR. The second point is that \( H_Q^{(0)} \) transforms under sample rotation as a second-order Legendre polynomial and this allows the anisotropy to be averaged by magic-angle spinning.3 The high-order term, \( H_Q^{(1)} \), transforms as both second- and fourth-order Legendre polynomials under rotation, and cannot be fully averaged by rotation about any one angle.

3 Although the time average of \( H_Q^{(0)} \) over one rotor period during MAS is 0, the MAS frequency must be on the MHz regime in order to collapse the sideband manifold to an isotropic peak. The implications for the spin dynamics of an oscillating MHz-regime component in the Hamiltonian turn out to be astonishing, as shown in Section 3.5.
2.2.2. Approximations by perturbation theory

The derivation of diagonal first and second order approximations to the quadrupolar Hamiltonian are given using static perturbation theory, following Goldman et al. who considered truncation effects in quadrupolar Hamiltonians in some detail [17]. The general approach is based on writing a full Hamiltonian

\[ H = H^{(0)} + H^{(1)} \]  

(13)

where \( H^{(0)} \) and \( H^{(1)} \) represent a large Hamiltonian and a smaller perturbing Hamiltonian, respectively. In our case, \( H^{(0)} \) and \( H^{(1)} \) are the Zeeman and quadrupolar Hamiltonians. It is assumed that the energies and eigenstates of \( H^{(0)} \) are exactly known:

\[ H^{(0)} | j \rangle = E^{(0)}_j | j \rangle \]  

(14)

One would like to know the true energies and eigenstates of the full Hamiltonian,

\[ H | v_j \rangle = E_j | v_j \rangle \]  

(15)

however, we are usually forced to approximate these in the eigenbasis of \( H^{(0)} \). We will focus now just on the energies,

\[ E_j = E^{(0)}_j + E^{(1)}_j + E^{(2)}_j + ..., \]  

(16)

where

Figure 2.3. Energy-level diagram of a S=3/2 nucleus. The Hamiltonians represent the zero'th and first order-AHT, or the first and second-order Perturbation approximations, which are identical. Note that the central transition is not affected to lowest order.


\[ E_j^{(1)} = \langle j | H^{(1)} | j \rangle, \]  

\[ E_j^{(2)} = \sum_{k \neq j} \frac{\langle j | H^{(1)} | k \rangle \langle k | H^{(1)} | j \rangle}{E_j^{(0)} - E_k^{(0)}}. \]  

After evaluating equations 17 and 18, the results will be used to determine diagonal corrections to \( H^{(0)} \) in the Zeeman eigenbasis:

\[ D^{(n)} = \sum_j |j\rangle E_j^{(n)} \langle j|, \]

\[ (H^{(0)} + D^{(1)} + D^{(2)}) |j\rangle = (E_j^{(0)} + E_j^{(1)} + E_j^{(2)}) |j\rangle. \]

According to perturbation theory, any higher order treatments would require a change out of the Zeeman basis. In general we hope to be able to find a reasonably simple expression for the quadrupole coupling and, in addition, it turns out to be important for the purpose of computing spin dynamics to be able to express interactions in the Zeeman basis. Therefore, we will not attempt approximations to any higher order. We will first find \( D^{(1)} \), and can write the expression for \( E_j^{(1)} \),

\[ E_j^{(1)} = \omega_0 \sum_m (-1)^m R_{z,-m} \langle j | T_{2m} | j \rangle. \]

In analyzing Equation 20, we draw on the spin tensor definitions of Equation 11. For \( m \neq 0 \), the raising and lowering operators which comprise the \( T_{2m} \)'s will collapse the bra-ket to zero due to the orthogonality of the state vectors. Thus, only the \( m = 0 \) case survives and so we substitute Equation 20 into 19 to get

\[ D^{(1)} = \omega_0 R_{20} \sum_j |j\rangle \langle j| T_{20} |j\rangle \]

\[ = \omega_0 R_{20} \sum_j |j\rangle \langle j| T_{20} \]

\[ = \omega_0 R_{20} T_{20}, \]

where we used the fact that the diagonal operator \( |j\rangle \langle j| \) commutes with \( T_{20} \) and finished by invoking closure. Next we write an expression for \( E_j^{(2)} \),

\[ E_j^{(2)} = \sum_{k \neq j} \frac{\langle j| \omega_Q \sum_m (-1)^m R_{z,-m} T_{2m} |k\rangle \langle k| \omega_Q \sum_m (-1)^m R_{z,-m} T_{2m} |j\rangle}{\omega_0 (j - k)} \]

\[ = \omega_0^2 \sum_{m'} \sum_m (-1)^{m'+m} R_{z,-m'} R_{z,-m} \sum_{k \neq j} \frac{\langle j| T_{2m'} |k\rangle \langle k| T_{2m} |j\rangle}{(j - k)}. \]
Similar to the treatment of $E_j^{(1)}$ we wish to identify which terms survive the bra-kets. From Equation 11 it can be seen that $T_{2m}$ can raise or lower a given state by $m$. Using this observation we can form two rules which must be simultaneously satisfied,

$$
\langle j | T_{2m} | k \rangle \neq 0 \iff j = k + m',
$$

$$
\langle k | T_{2m} | j \rangle \neq 0 \iff k = j + m,
$$

(23)
to give the constraint $m' = -m$. We enforce these constraints by substituting $(j + m)$ for $k$ to eliminate the sum over $k$, and substituting $-m$ for $m'$ to eliminate the sum over $m'$. The result is

$$
E_j^{(2)} = \frac{\omega^2}{\omega_0} \sum_{m \neq 0} R_{2,m} R_{2,-m} \frac{\langle j | T_{2,-m} | j + m \rangle \langle j + m | T_{2,m} | j \rangle}{(-m)}.
$$

(24)

We next insert an expanded closure relation into Equation 24.

$$
1 - \sum_{k \neq j + m} |k\rangle\langle k| = |j + m\rangle\langle j + m|,
$$

(25)

$$
E_j^{(2)} = \frac{-\omega^2}{\omega_0} \sum_{m \neq 0} \left( \frac{1}{m} R_{2,m} R_{2,-m} \langle j | T_{2,-m} T_{2,m} | j \rangle - \sum_{k \neq j + m} \langle j | T_{2,-m} | k \rangle \langle k | T_{2,m} | j \rangle \right).
$$

(26)

The second term in Equation 26 always violates the $k = j + m$ rule and can be discarded. Inserting the first term into Equation 19 and using the same procedure as for $D^{(1)}$ gives

$$
D^{(2)} = \frac{-\omega^2}{2\omega_0} \sum_{m \neq 0} \frac{1}{m} R_{2,m} R_{2,-m} T_{2,-m} T_{2,m}.
$$

(27)

Although this is a concise form, the more common representation is given by applying the identity $\frac{1}{2} [T_{2,-m}, T_{2,m}] = T_{2,-m} T_{2,m}$, giving

$$
D^{(2)} = \frac{\omega^2}{2\omega_0} \sum_{m \neq 0} \frac{1}{m} R_{2,m} R_{2,-m} [T_{2,m}, T_{2,-m}].
$$

(28)
2.2.3. **Quadrupolar Average Hamiltonian**

Average Hamiltonian theory (AHT) is a powerful technique for describing spin systems in which some periodic modulation of the Hamiltonian is used to create a time-averaged Hamiltonian which describes the net behavior of a spin system after a given cycle. For quadrupolar nuclei, this can be done in a straightforward manner using standard tensor notation[14]. The general strategy is to take a laboratory frame Hamiltonian, accelerate it into a frame rotating according to the Zeeman interaction and, based on this periodicity of the Hamiltonian in the rotating frame, form an approximation to the average Hamiltonian.

Beginning with the Hamiltonian in the normal lab frame, we have

\[ H_{\text{lab}} = H_Z + H_Q \]

\[ = \omega_0 I_Z + \omega_Q \sum_{m=-2}^{2} (-1)^m R_{2,-m} T_{2m} , \]

which has already been described in section 2.2.1. We recognize the form of this as

\[ H = H_0 + H_1 , \]

and transform to an interaction representation,

\[ \tilde{H}(t) = e^{i\omega_0 t I_Z} H e^{-i\omega_0 t I_Z} \]

\[ = \omega_Q \sum_{m=-2}^{2} (-1)^m R_{2,-m} T_{2m} e^{i\omega_Q t} . \]

We can now calculate the zero and first order average Hamiltonians based on the Zeeman periodicity, \( \tau_c = 2\pi / \omega_0 \), which now governs \( \tilde{H}(t) \) in Equation 30.

\[ H_Q^{(0)} = \frac{1}{\tau_c} \int_0^{\tau_c} \tilde{H}(t) dt = \omega_Q R_{20} T_{20} . \]

\[ H_Q^{(1)} = \frac{-i}{2\tau_c} \int_0^{\tau_c} dt_1 \int_0^{t_1} dt_2 \left[ \tilde{H}(t_1), \tilde{H}(t_2) \right] \]

\[ = \frac{-i\omega_Q^2}{2\tau_c} \int_0^{\tau_c} dt_1 \int_0^{t_1} dt_2 \left[ \sum_{m=-2}^{2} (-1)^m R_{2,-m} T_{2m} e^{i\omega_Q t_1}, \sum_{m=-2}^{2} (-1)^m R_{2,-m} T_{2m} e^{i\omega_Q t_2} \right] \]

\[ = \frac{-i\omega_Q^2}{2\tau_c} \int_0^{\tau_c} dt_1 \int_0^{t_1} dt_2 \sum_{m=-2}^{2} \sum_{m'=-2}^{2} (-1)^{m+m'} R_{2,-m} R_{2,-m'} e^{i\omega_Q t_1} e^{i\omega_Q t_2} \left[ T_{2m'}, T_{2m} \right] . \]

Before carrying out the double integration in Equation 32, a few special cases must be checked first. Note that the commutator vanishes for \( m = m' = 0 \), and the summation is assumed to exclude this case. What about \( m=0 \neq m' \)? This case may also be neglected as it will leave a
term proportional to $e^{im'\omega_0 t_1}$, with $m'\neq 0$, which will not survive the final integration. The first integration proceeds as follows:

$$H^{(1)}_Q = \frac{-\omega_0^2}{2\omega_0 \tau_C} \left[ dt \sum_{m'=-2}^{2} \sum_{m=-2}^{2} (-1)^{m'+m} R_{z,-m} R_{z,-m} e^{im'\omega_0 t_1} \left[ T_{z,m'}, T_{z,m} \right] (e^{im\omega_0 t_1} - 1) \right]$$

$$= \frac{-\omega_0^2}{2\omega_0 \tau_C} \left[ dt \sum_{m'=-2}^{2} \sum_{m=-2}^{2} (-1)^{m'+m} R_{z,-m} R_{z,-m} \left[ e^{i(m'+m)\omega_0 t_1} \left[ T_{z,m'}, T_{z,m} \right] - e^{im\omega_0 t_1} \right] \right].$$

(33)

For the second integration, it is again advantageous to analyze the integrand to identify the time-independent parts that will survive the integration over one period of the Zeeman frequency. This is easily visualized by switching the order of the summation over $m'$.

$$H^{(1)}_Q = \frac{-\omega_0^2}{2\omega_0 \tau_C} \sum_{m'=-2}^{2} \sum_{m=-2}^{2} (-1)^{m'+m} R_{z,m'} R_{z,-m} \left[ e^{i(m'+m)\omega_0 t_1} \left[ T_{z,m'}, T_{z,m} \right] - e^{im\omega_0 t_1} \right].$$

(34)

The time dependence of Equation 34 is eliminated for the first term by setting $m'=m$, and for the second term by setting $m'=0$. The integration of these time-independent terms gives:

$$H^{(1)}_Q = \frac{-\omega_0^2}{2\omega_0 \tau_C} \sum_{m'=-2}^{2} \sum_{m=-2}^{2} (-1)^{m'+m} A_{z,m'} A_{z,-m} \left[ T_{z,m'}, T_{z,m} \right] - \frac{-\omega_0^2}{2\omega_0} \left[ T_{z,0}, T_{z,0} \right].$$

(35)

We rearrange this slightly to the form in which it is commonly reported

$$H^{(1)}_Q = \frac{\omega_0^2}{2\omega_0} \sum_{m'=-2}^{2} \sum_{m=-2}^{2} (-1)^{m'+m} A_{z,m'} A_{z,-m} \left[ T_{z,m'}, T_{z,-m} \right] + (-1)^{m+1} A_{z,m} A_{z,-m} \left[ T_{z,0}, T_{z,0} \right].$$

(36)

There are two terms in this first-order average Hamiltonian. A quick inspection of the commutation rules for spherical tensors[15, 16] reveals that the first term commutes with the Zeeman interaction while the second does not. In other words, the first term of Equation 36 is diagonal in the Zeeman basis, while the second term is not, and this distinction defines secular and non-secular Hamiltonians, respectively. Thus, the secular terms of Equation 36 are identical to the result from perturbation theory. The total secular average Hamiltonian, up to first order, is written such that the quadrupole coupling constant is included in the space tensor, (replacing $R_{z,m}$'s with $A_{z,m}$'s to denote this slight difference):
\[ H_Q^{(0)} = A_{20} T_{20} + \frac{1}{2\omega_Q} \sum_{m=\pm 1, \pm 2} A_{2,m} A_{2,-m} [T_{2,m}, T_{2,-m}], \]  
\tag{37}

The non-secular second term of Equation 36 is neglected on the basis that it would become time dependent if we transformed into an interaction frame determined by \( H_Q^{(0)} \) and would be truncated in this frame since \( |H_Q^{(0)}| \gg |H_Q^{(1)}| \). This form is convenient for numerical simulations since all constants are built into the space tensor, simplifying complications that arise when attempting to delineate between externally represented constants (such as \( \omega_Q \)) and the proper form for the principal components of the space tensors (\( A_{lm} \)'s).

### 2.2.4. Sample Rotation Introduces High-Order Legendre Polynomials!

The efforts of the previous two sections can now be used to gain more insight into the solid-state spectra of quadrupolar nuclei. A significant feature of Equation 37 is the existence of tensor products. A tensor product results in a new set of spherical tensors in a basis of expanded rank, governed by the triangle rule which is well-known for the combination of angular momenta. For example, if two rank 2 tensors are multiplied, the result will be a linear combination of tensors of rank 1-4, with various fore factors which depend on Clebsch-Gordon coefficients. It is also useful at this time to introduce a fictitious spin-1/2 operator basis so that it is easy to treat subspaces of the Hamiltonian[18, 19]. Contracting the space tensors in Equation 37 and introducing the fictitious spin-1/2 operators gives

\[ H_Q^{(1)} = \sum_{l=0,2,4} \sum_{m=\pm \frac{1}{2}, \pm \frac{3}{2}, \ldots} C_{lm} R_{l0} I_{l}^{m,-m}, \]  
\tag{38}

where the dependence of the laboratory frame Hamiltonian on second and fourth rank geometric tensors is evident. The derivation for obtaining Equation 38 from 37 is provided in the appendix (Chapter 7). The summation over \( m \) simply accounts for all \( I_z \) subspaces and can be replaced by just one value of \( m \) to study the dynamics in that particular subspace. What is most interesting is how Equation 38 can be modified to account for spinning the sample about some given angle. The \( R_{l0} \) in Equation 38 are replaced with time dependent tensors given by

\[ R_{l0}(t) = \sum_m R_{lm} d_{m0}^l(\beta) e^{i\omega_m t}, \]  
\tag{39}
where $\beta$ is the angle of the spinning axis relative to the static field and $\omega_r$ is the rotation frequency. The time-dependent terms in Equation 39 (i.e. $m \neq 0$) will give rise to sidebands and will eventually be truncated in the fast spinning limit. So we look just at the time independent term ($m=0$), and identify the reduced Wigner elements $d_{m0}^l$ with Legendre polynomials in $\cos \beta$, giving

$$H_{Q,rot}^{(\ell)} = \left[ C_{0,\ell} R_{\ell 0} + C_{2,\ell} R_{2\ell} P_2(\cos \beta) + C_{4,\ell} R_{4\ell} P_4(\cos \beta) \right] I_{\ell}^{I - \ell},$$

where this is the Hamiltonian for the central $(\frac{1}{2}, \frac{1}{2})$ transition only. The second-order Legendre polynomial is commonly encountered in all other anisotropic interactions (CSA and dipole, e.g.), and the node for $P_2(\cos \beta)$ is $\beta = 54.74^\circ$, which is the well known magic angle. In the fast spinning limit, any second rank tensor interaction will be averaged to zero by rotating the sample about the magic angle. However the nodes for $P_4(\cos \beta)$ are $\theta_1 = 30.56^\circ$ and $\theta_2 = 70.12^\circ$. \textit{Equation 40 provides the crucial result for quadrupolar NMR in solids: there is no single axis about which a sample may be rotated, regardless of the rate, which will result in complete averaging of the quadrupolar coupling.}

For typical quadrupole couplings and static fields, the width of the central transition resonance line shape during MAS is on the order of 1-20 kHz. Examples of quadrupolar line shapes under MAS will be given in chapter 3.

### 2.2.5. Effective Quadrupolar Hamiltonian Under Radio-Frequency Irradiation

Motivated by the desire to treat polarization transfer dynamics (see chapter 4), a new description of quadrupolar nuclei under r.f. irradiation was recently given by Baldus et al.[20] by examining an effective r.f. Hamiltonian in an interaction frame determined by the quadrupole coupling. The derivation can proceed in the fictitious spin-$1/2$ operator formalism, or alternatively by expanding the spherical spin tensors into an eigenstate representation. Although the results are identical, the latter approach will be given here for completeness, while the former will be presented in section 4.2. Previously, the problem of modeling a quadrupolar nucleus under a spin-locking field was analyzed by describing a time evolution of the eigenstates [21, 22] (see section 3.5). One can compare both approaches with
the selection of a Schrödinger or Heisenberg picture [23] to describe the explicit time-
dependence of the system. The effective Hamiltonian is introduced here, while the
eigenstates treatment is given in section 3.5.

We begin by treating an isolated $S = 3/2$ spin during MAS. In the rotating frame, the
r.f. and quadrupolar terms in the Hamiltonian are

$$H = \omega_0 S_x + H_Q,$$  \hspace{1cm} (41)

where the isotropic chemical shift is neglected to simplify the analysis. The quadrupolar
contribution to the total spin Hamiltonian obtained in sections 2.2.2. and 2.2.3 is

$$H_Q = A_{20}(t) T_{20} + \frac{1}{\omega_0} \sum_{m=\pm 1, \pm 2} \frac{1}{m} A_{2m}(t) A_{2-m}(t) [T_{2m}, T_{2-m}],$$  \hspace{1cm} (42)

The time dependence of the spatial components can be described by a Fourier series
expansion in which the reduced Wigner elements $d_{nm}$ are evaluated at the magic angle $\theta_m$:

$$A_{2m}(t) = \sum_{n=\pm 1, \pm 2} A_{2n} d_{nm}(\theta_m) e^{i\omega_m t}.$$  \hspace{1cm} (43)

The tensor bases for the quadrupolar coupling have been given in Equations 10 and 11,
although for convenience the $\omega_Q$ has been factored into the spatial components so we use
$A_{lm}$'s to denote this minor distinction.

As in the spin 1/2 case, the calculation of an effective Hamiltonian proceeds by
transforming to an interaction representation. An analogous approach is used to describe the
influence of chemical shielding interactions in the context of homonuclear dipolar recoupling
[24, 25]. We simplify the problem using the lowest order contribution in Equation 42 (i.e.
$H^{(0)}_Q$) to define the unitary operator

$$U(t) = \exp \left[ -i \int_0^t [A_{20}(\tau) d\tau, T_{20}] \right].$$  \hspace{1cm} (44)

Following the notation of Vega [21, 22] the components of the r.f. interaction $S_x = 2C_x + R_x$
are given by

$$C_x = \frac{1}{2} \left( |\frac{3}{2}\rangle \langle \frac{1}{2}| + |\frac{1}{2}\rangle \langle \frac{3}{2}| \right), \quad \text{and}$$
$$R_x = \frac{\sqrt{3}}{2} \left( |\frac{3}{2}\rangle \langle \frac{1}{2}| + |\frac{1}{2}\rangle \langle \frac{3}{2}| + |\frac{3}{2}\rangle \langle \frac{1}{2}| \right).$$  \hspace{1cm} (45)
The transformation \( \tilde{H}_Q = e^{-i\zeta(t)T_{20}}S_x e^{i\zeta(t)T_{20}} \) is easily performed using the eigenbasis of the single transition operator \( S_x(\{\frac{3}{2}, \frac{1}{2}, \frac{1}{2}, \frac{1}{2}\}) \). In this representation, the spin tensor component of the quadrupolar coupling can be expressed as:

\[
T_{20} = \frac{1}{\sqrt{6}} \left[ \left( |\frac{1}{2}\rangle\langle\frac{3}{2}| + |\frac{3}{2}\rangle\langle\frac{1}{2}| \right) - \left( |\frac{1}{2}\rangle\langle\frac{1}{2}| + |\frac{1}{2}\rangle\langle\frac{1}{2}| \right) \right].
\]  

(46)

Thus, only the single-quantum coherence operator on the satellite transitions is altered by the transformation of Equation 44:

\[
R_x \rightarrow e^{2i\frac{3}{2}\zeta(t)} \left( |\frac{1}{2}\rangle\langle\frac{3}{2}| + |\frac{3}{2}\rangle\langle\frac{1}{2}| \right) + e^{-2i\frac{3}{2}\zeta(t)} \left( |\frac{1}{2}\rangle\langle\frac{1}{2}| + |\frac{1}{2}\rangle\langle\frac{1}{2}| \right).
\]  

(47)

Using

\[
S_zS_y + S_yS_z = i\frac{\sqrt{3}}{2} \left( \left( |\frac{1}{2}\rangle\langle\frac{3}{2}| + |\frac{3}{2}\rangle\langle\frac{1}{2}| \right) - \left( |\frac{1}{2}\rangle\langle\frac{1}{2}| + |\frac{1}{2}\rangle\langle\frac{1}{2}| \right) \right).
\]  

(48)

we obtain:

\[
\tilde{H} = \omega_x(2C_x + \cos(\zeta(t))R_x + \sin(\zeta(t))(S_zS_y)).
\]  

(49)

The time-dependent spatial components of the quadrupolar interaction,

\[
\zeta(t) = \sqrt{6} \sum_{m'=-2}^{2} \frac{d_{0m}(\Theta, \gamma)A_{2m}(\alpha, \beta, \gamma)}{im'\omega_r} \exp[im'\omega_r t] - 1,
\]  

(50)

might be further simplified using a Fourier Bessel function analysis [26]. Equation 49 can also be compared to the results for a spin \( S = 1 \) system [2, 27] in which the anticommutator of Equation 49 represents anti-phase single-quantum magnetization. In addition, identifying \( R_x \) with \( S_x \) and neglecting the central transition operator leads to the \( S = 1 \) result. In the spin 3/2 case, the anti-phase coherence operator (Equation 49) describes a rotation between the central and triple-quantum transition subspaces and leads to continuous coherence exchange at a rate of multiples of the MAS frequency. This RIACT phenomenon [28], which may be used for efficient excitation and reconversion of triple quantum coherence, will be developed in detail in sections 3.5 and 3.6. Equation 49 will be analyzed in more detail in Chapter 4.
2.3. Numerical Simulations

2.3.1. Solving the Liouville von Neumann Equation

The time dependent Schrödinger equation,
\[
\frac{d}{dt} |\psi(t)\rangle = -iH(t) |\psi(t)\rangle,
\]
(51)
can be rewritten in terms of the density operator, \( \rho = |\psi(t)\rangle \langle \psi(t)| \) for a pure state,
\[
\frac{d}{dt} \rho(t) = -i[H(t), \rho(t)].
\]
(52)
Equation 52 is the Liouville von Neumann equation, which can be solved to give
\[
\rho(t) = U(t) \rho(0) U^{-1}(t),
\]
(53)
\[
U(t) = Te^{-iH(t)dt},
\]
(54)
where \( T \) is the Dyson time order operator which has the effect of splitting up and ordering the propagator into sufficiently small time periods such that the Hamiltonian is self-commuting in each time period. If we choose time periods which are sufficiently small to permit the approximation that the Hamiltonian is time-independent over these small increments, then the propagator is simply:
\[
U(t) = \exp \left[ -iH(t_f)\Delta t - iH(t_f - \Delta t)\Delta t ... - iH(t_i)\Delta t \right].
\]
(55)
It is a computationally straightforward problem to evaluate incremental propagators and operate in time steps of \( \Delta t \) on a density operator (see Appendix 7). At any time during or after the propagation of the density operator, the expectation value of an operator \( O \) can be recorded by taking
\[
\langle O \rangle = Tr\{O\rho(t)\}.
\]
(56)
By following this general prescription, accurate simulations of NMR experiments can be conducted without having to solve the time dependent Schrödinger equation.

2.3.2. The GAMMA Simulation Environment

An open-source software project that was recently contributed to the NMR community is the General Approach to Magnetic resonance Mathematical Analysis package, GAMMA, developed to provide a completely general, object oriented C++ code base for carrying out
NMR simulations[29]. Classes such as tensors, vectors, spin operators, matrices, etc. are implemented with the operations needed for manipulating them, such as tensor products and Wigner rotations. GAMMA is a cross-platform package which provides a complete set of classes and operations, allowing for the design of nearly any arbitrary NMR simulation following the basic prescription laid out in section 2.3.1.

Information on downloading, installing and running programs in the GAMMA environment can be found with the URL http://gamma.magnet.fsu.edu A GAMMA NMR simulation is written by programming in standard C++, using the classes and operations supplied by libraries in GAMMA. The program is compiled as a C++ program, being sure to include the libraries at compile-time. It is instructive to consider a sample program in order to demonstrate the utility of programming in an object oriented environment designed for NMR simulations.

An example is given in Figure 2.4 in which a powder-averaged, static line shape for a spin-1/2 nucleus under a chemical shielding Hamiltonian is simulated. This shows, for example, how Euler rotations and powder averaging are implemented in GAMMA. It is beyond the scope of this report to analyze the algorithm line-by-line, but the most important features to recognize in the code in Figure 2.4 are the abstraction and the data-hiding, two of the fundamental principles of C++ object-oriented programming. Specifically, GAMMA allows for the design of C++ code which resembles the form of analytic theory, and hides the underlying data (such as the explicit tracking of individual matrix elements as would be required in plain C). For completeness an example of the output of this program is shown in Figure 2.5.
Figure 2.4. A calculation to simulate static CSA linewidths written in the GAMMA C++ programming environment. Note that this employs a rational powder-averaging algorithm which is more efficient than performing a summation over a completely random series of Euler angles[30].
Figure 2.5. A simulation of a static CSA line shape using the program demo_csa.cc. Parameters were ($\delta = 4$ kHz, $\eta = 0$, $R = 0$), using the definitions of Equation 5.

2.3.3. Quadrupolar Simulation Interface (QSI)

A graphical user interface has been implemented along with a suite of highly configurable GAMMA programs to create an integrated environment for the design, execution and visualization of simulations of quadrupolar effects in solid-state NMR spectroscopy. The parameter space in quadrupolar simulations can be daunting, as will be seen later in Chapter 3, and one of the main goals of QSI is to provide an environment in which it is intuitive and easy to configure and execute simulations. QSI allows the user to run simulations interactively or as batch jobs, preserving the ability to view the results of each calculation as soon as it is finished. QSI saves a master file which is a complete record of the simulations that may be re-opened at any later time to browse the data and resume the session. A more detailed description is given in Appendix 7.2.
2.4. References


3. High Resolution NMR of Quadrupolar Nuclei in Solids

3.1. Magic-Angle Spinning (MAS) Quadrupolar Line Shapes

3.1.1. Fast MAS Line Shapes of Quadrupolar Nuclei

The quadrupolar Hamiltonian under fast magic-angle spinning retains anisotropic, time-independent terms proportional to a fourth order Legendre polynomial. We write this by evaluating the Hamiltonian obtained in section 2.2.4. at the magic angle:

\[ H_{Q,\text{rot}}^{(1)} = \left[ C_{0,\frac{1}{2}} R_{\theta\theta} + C_{4,\frac{1}{2}} R_{\phi\phi} P_4(\cos \beta_{MA}) \right] I_z^{5/2}. \]  

Equation 1 can be evaluated to give the resonance frequency for the central transition, which may be integrated over all powder orientations to provide quadrupole line shapes in the fast-spinning limit, as given by Sun et al[1]. Alternatively, sideband-free line shapes can also be simulated by computing a propagator for one rotor period and using the density operator recipe in section 2.3 to stroboscopically compute an NMR signal. A Floquet theory approach may also be used for the simulation of quadrupole line shapes with accurate sideband shapes and intensities[2]. In Figure 3.1, the rotor-synchronized density operator algorithm was used to generate a series of magic-angle spinning quadrupole line shapes for typical S=3/2 parameters. The characteristic second-order quadrupolar line shapes in Figure 3.1 illustrate that it is not feasible to record isotropic spectra of quadrupolar nuclei in conventional MAS spectroscopy, even at high MAS rates and currently attainable static fields.
Figure 3.1. Calculated static and fast-MAS spectra of an isolated quadrupolar nucleus. Parameters are $e^2 qQ/h = 3.0$ MHz, $\eta=(0.0-1.0)$. The truncation of the quadrupole coupling by the Zeeman interaction is illustrated by comparing the MAS columns for 9.4 Tesla ($^{23}$Na=105 MHz) and 17.6 Tesla ($\gamma^{23}$Na=198 MHz), where the second-order quadrupole broadened line shapes narrow by a factor of 2 at higher field. MAS simulations employed rotor-synchronized evolution and fast MAS (200 kHz) to give pure central-transition line shapes. All calculations used 4000 crystallite orientations.
The richness of spectral features does, however, allow for the accurate determination of quadrupole coupling parameters in MAS spectra. As mentioned previously, this sensitivity of the quadrupole coupling to local structure has been used to significantly refine the X-ray crystal structure of a small inorganic phosphate[2].

3.1.2. Review - MAS Spectroscopy of Quadrupolar Nuclei

The development of spin-1/2 methodology in solids focused first on learning how to obtain high-resolution spectra. This has been achieved with the use of magic-angle spinning, which removes chemical shift and dipolar broadening, in combination with a variety of powerful decoupling schemes to remove residual hetero- and homo-nuclear dipolar couplings. Once it became possible to resolve all resonances in a sample, focus was shifted to restoring interactions, usually in indirect dimensions, to recover greater structural information while preserving resolution. Significant advances in this respect have resulted in several powerful solid-state NMR experiments for measuring structural parameters in biological solids [3-7].

In comparison, the development of quadrupolar NMR in solids has proceeded at a slower pace due to the difficulties of resolving inequivalent sites and obtaining isotropic spectra. It has long been recognized, in cases of quadrupole couplings such that \( e^2 qQ / h \approx \text{MHz} \), that only the central transition is observable in MAS spectroscopy of half-integer quadrupolar nuclei. In combination with moderate to high static fields, the characteristic second-order line shapes of the central transition can be measured [8-10] and quadrupole parameters extracted (recall Figure 3.1). This approach of recording the central transition signal was sometimes misleadingly termed ‘high resolution’ quadrupolar NMR, where such language is usually used to indicate truly isotropic spectroscopy. It has also been known for some time that the nutation rate of the central transition depends on the quadrupole coupling[11, 12]. When the quadrupole coupling is vanishingly small, the nutation rate of the central transition is equal to the strength of the applied field, \( \omega_1 \), as is the case for spin-1/2 nuclei. If the quadrupole coupling is instead in a regime \( \omega_Q >> \omega_{RF} \), then the nutation rate becomes \( (S+\frac{1}{2})\omega_1 \). For this reason care should be taken when setting pulse lengths in central transition spectroscopy to choose a 45° pulse width for \( S=3/2 \), a 33° pulse for \( S=5/2 \), and so on.
A variation on the method of measuring central transition line shapes under MAS is to choose an angle other than the magic-angle which best narrows both the second and fourth order Legendre polynomials of Equation 2.40 [13]. A significant complication is that such variable-angle spinning (VAS) does not fully average the chemical shift or dipolar coupling interactions. In addition, it is challenging to accurately calibrate the rotation angle since there is no convenient observable with which to accurately measure an arbitrary spinner angle. VAS linewidths, although somewhat narrowed in comparison to MAS, are still on the order of the second-order quadrupole coupling (i.e. kHz). Thus VAS is not a good choice for general quadrupolar spectroscopy.

However the form of the Hamiltonian suggests that there may be ways to record isotropic spectra by making the spinner axis time dependent. This has been accomplished using discrete hopping of the spinner axis (Dynamic-Angle Spinning -DAS), and continuous modulation of the spinner axis (Double-Rotation - DOR). These relatively recent techniques provided the first isotropic spectra of quadrupolar nuclei and are reviewed in section 3.2 in order to understand their advantages and limitations. Also, DAS serves as a conceptually identical model for a newer experiment that measures isotropic spectra with a *fixed spinner axis*: multiple-quantum magic-angle spinning (MQMAS)[14]. Instead of mechanically modifying the space tensor, MQMAS takes advantage of spin-operator degrees of freedom to refocus the fourth-order Legendre polynomial terms in the high-order quadrupolar Hamiltonian. The theory of MQMAS is presented in section 3.3 along with several applications and improvements in sections 3.4-3.6 which have made it possible to obtain quantitative, isotropic spectra with good sensitivity. We conclude that, while still in its infancy, MQMAS already shows the requisite performance characteristics for studying challenging biological solids.

---

4 The magic-angle of the sample spinning assembly is accurately set by maximizing the sideband manifold in $^{79}$Br MAS spectra of KBr, where the bromine chemical shift anisotropy is extremely large and small mis-settings of the magic angle will be evident in the K$^{79}$Br sideband intensities.
3.2 Double Rotation (DOR) and Dynamic-Angle Spinning (DAS)

3.2.1. Review – Zeros of the Legendre polynomials:

In chapter 2, we demonstrated why magic-angle spinning cannot reduce the quadrupolar Hamiltonian to isotropic terms only. We give the Hamiltonian for the central transition here again:

\[ H_{Q,\text{rot}}^{(1)} = \left[ C_{0,1} \mathcal{R}_{00} + C_{2,1} \mathcal{R}_{20} P_2(\cos \beta) + C_{4,1} \mathcal{R}_{40} P_4(\cos \beta) \right] I_0^{\frac{\hbar}{2} - \frac{\hbar}{2}}, \]  

where \( \beta \) is the angle between the spinner axis and the static field. The Legendre polynomials have the form of single-electron orbitals, as shown in Figure 3.2, giving the nodes \( \beta = 54.74^\circ \) for \( P_2(\cos \beta) \), and \( \theta_1 = 30.56^\circ \) and \( \theta_2 = 70.12^\circ \) for \( P_4(\cos \beta) \).

Specifically, the Legendre polynomials correspond to \( d_{z^2} \) and \( g_{2z^2} \) orbitals, respectively. The DAS and DOR methods are designed to exploit the nodal properties of Equation 2 by the introduction of an additional time dependence.
3.2.2. Double Rotation Spectroscopy (DOR)

Logically, the most straightforward way to approach Equation 2 is to impose a second rotation on the sample, which will then be spinning simultaneously about two angles. This has been reported as the double-rotation (DOR) method in which the spinner assembly consists of an inner and an outer rotor[1, 15, 16]. The outer rotor is oriented at 54.74° to average the second order terms, while the inner rotor is oriented 30.56° relative to the outer one to average the fourth order terms. This arrangement is illustrated in Figure 3.3, where the inner rotor can be visualized as precessing about the axis of the outer rotor.

The double rotation method is well-suited to low magnetic fields, where the quadrupole-induced isotropic shift can improve the resolution of lines, although diminishing signal-to-noise will always be a limiting factor at lower fields. Typical spinning rates are $\omega/2\pi \sim 1$ kHz for the outer rotor and $\omega/2\pi \sim 5$ kHz for the inner rotor. Considering that quadrupole linewidths can span 10-20 kHz under fast MAS, it is clear that the slow DOR spinning rates are a limiting factor in sensitivity. Sensitivity is further compromised by the poor filling factor of the sample in the r.f. coil, which is about 10%. In typical MAS coil/spinner
assemblies, filling factors are approximately 50-60\%\). Finally, the DOR double-rotor system is technically demanding and costly.

### 3.2.3. Dynamic Angle Spinning (DAS)

An alternative to the continuous time dependence imposed by double rotation is to impose a discrete sequence of rotor axes to remove all interactions which transform under rotation as second and fourth order Legendre polynomials\([17, 18]\). In fact, it turns out that evolution of central-transition coherence over only two successive rotor axes will refocus \(\textit{both}\) the second and fourth order Legendre polynomials. Starting with Equation 2, we can write a general effective Hamiltonian for this process of two successive evolution times, which we denote \(t_1\) and \(kt_1\), where \(k\) is an arbitrary constant which will be determined later.

\[
H_{\text{DAS}}(t_1 + t_2) = \sum_{l=2,4} C_{l,\frac{1}{2}} R_{l0} \left[ P_l(\cos \beta_1) + k P_l(\cos \beta_2) \right].
\] (3)

Ignoring the \(l=0\) terms since these are already isotropic, we seek solutions such that

\[
H_{\text{DAS}}(t_1 + t_2) = \sum_{l=2,4} C_{l,\frac{1}{2}} R_{l0} \left[ P_l(\cos \beta_1) + k P_l(\cos \beta_2) \right] = 0.
\] (4)

It turns out that, since \(k\) is just an experimental variable, there are a continuum of solutions \((k, \beta_1, \beta_2)\) satisfying Equation 4. There is also one natural DAS angle pair which requires no scaling \((k=1)\), and this occurs for \((\beta_1 = 37.38^\circ, \beta_2 = 79.19^\circ)\). For an appropriate angle pair and scaling factor, Equation 4 predicts that an echo will form at the end of the second evolution and the value of the signal at the echo maximum will be determined by isotropic terms in Equation 3 only. The pulse sequence and rotor-axis timing for a DAS experiment are shown in Figure 3.4. A complete DAS experiment must therefore be repeated for a large number of \(t_1\) increments, since it is only the value of the signal at the echo maximum (i.e. \(t_1 + t_2\)) which is free of anisotropic terms, and so an ‘isotropic spectrum’ is generated by sampling the echo maxima for a series of \(t_1\) values.
The time required for switching the spinning axis is typically about 100ms. This introduces the possibility for spin diffusion, which could occur by chemical exchange, dipole-dipole cross-relaxation, or through a secondary spin bath as in proton-driven spin diffusion[19].

The DAS experiment is an inherently two-dimensional experiment since it involves two separate evolution times \((t_1 + k t_1)\) and \(t_2\). Therefore it is straightforward to process DAS data sets to provide an isotropic spectrum in the \(f_1\) dimension and pure quadrupole line shapes along \(f_2\)[20, 21] corresponding to \((t_1 + k t_1)\) and \(t_2\), respectively.

As with DOR, DAS involves hardware that is complex and costly and there are no intrinsic means for accurately calibrating the DAS angles, short of performing several DAS experiments while varying \(k\). In addition to concerns over spin-diffusion, the relatively long angle-flipping time also requires sample with long \(T_1\)'s so that the magnetization does not decay during the angle switching. But DAS retains two important advantages over DOR. The first is the inherent nature of DAS which leads to 2D spectra which correlate the isotropic dimension with the anisotropic dimension. Second, DAS can be used to take advantage of the fact that the cross-polarization (CP) dynamics for a quadrupolar nucleus undergoing sample
rotation about an axis parallel to the static magnetic field are identical to the case of a static sample[22]. Jarvie et al. recently demonstrated the combination of a conventional DAS pulse sequence with a mixing period to perform CP at 0° orientation. This scheme results in isotropic, 2D heteronuclear correlation spectra between 23Na and 31P[23].

DAS and DOR represented significant advances in studying quadrupolar nuclei and both methods are in current use in many academic and industrial laboratories. However, from our brief discussion of the limitations of these techniques, it is clear that a more general methodology is needed. One such effort was reported by Lucio Frydman and John Harwood at the 1995 Experimental Nuclear Magnetic Resonance Conference (ENC) in Boston, MA, and renders the spin components of Equation 2 time dependent. This is described next in section 3.3.

3.3. Multiple-Quantum Magic-Angle Spinning

3.3.1. Simultaneous Averaging in Space and Spin Coordinates

In 1995, Frydman and Harwood developed an experiment for recording isotropic spectra of quadrupolar nuclei by correlating the evolution of a symmetric, multiple-quantum coherence, (m,-m), with the evolution of central transition coherence[14], (1/2,-1/2), during spinning about a fixed axis: the magic angle. We will develop an effective Hamiltonian for this process in a manner analogous to the treatment of DAS earlier, however we now include the chemical shielding to give a more general treatment. Let a spin system consist of half-integer spin nuclei (I>1) that are subjected to quadrupolar and chemical shielding interactions. In the rotating frame, the average Hamiltonian (to first order) can be written as[1]

\[ H = H_{cs} + H_{q}^{0} + H_{q}^{1}, \]

where \( H_{cs} \) is the chemical shielding Hamiltonian, and \( H_{q}^{0} \) and \( H_{q}^{1} \) are the zero'th- and first-order average Hamiltonians arising from the quadrupolar interaction. We are only concerned with the symmetric (m,-m) coherences, where \( m \) is the magnetic quantum number. In the following we only need to treat a single, isolated nucleus, and at any time the results can be generalized to a polycrystalline powder by integrating over all possible Euler angles which relate the PAS to the LAB frame.
Since the evolution of a symmetric coherence is unaffected by the first-order quadrupolar interaction, \( H_Q^0 \) can be neglected. For an \( S=3/2 \) nucleus, both \( H_{CS} \) and \( H_Q^1 \) can be expressed according to the fictitious spin-1/2 operators[11, 12] as

\[
H_{CS} = \sum_{l=0,2} \sum_{m} 2m R_{0l}^{CS} I_{Z}^{m,-m}, \tag{6}
\]

\[
H_Q^1 = \sum_{l=0,2,4} \sum_{m} C_{lm} R_{l0}^{Q} I_{Z}^{m,-m}, \tag{7}
\]

where

\[
C_{lm}(I) = \frac{\omega_Q^2}{\omega_0} \left[ a_{m,-m}^{(1)} C(2,2,l,1,-1) + a_{m,-m}^{(2)} C(2,2,l,2,-2) \right] \tag{8}
\]

and

\[
a_{m,-m}^{(1)} = m[4I(I+1) - 8m^2 - 1], \tag{9}
\]

\[
a_{m,-m}^{(2)} = m[2I(I+1) - 2m^2 - 1]. \tag{10}
\]

In Equation 8, \( C(2,2,l,1,-1) \) and \( C(2,2,l,2,-2) \) are the Clebsch-Gordon coefficients. \( R_{0l}^{CS} \) and \( R_{l0}^{Q} \) describe the orientation dependence of the chemical shielding and quadrupolar interactions, respectively. Before proceeding further, it is useful to illustrate the process we want to model by using a coherence transfer diagram, Figure 3.5. The diagram in Figure 3.5 should not be confused with a pulse sequence, but instead depicts the transformations we wish the spin system to undergo, regardless of how such transitions are effected.

\[\text{(S=3/2)}\]

\[\text{3}\]

\[\text{1}\]

\[\text{I}_Z\]

\[\text{1}\]

\[\text{3}\]

\[\text{0} \quad \text{Time} \]

\[\text{t}_1\]

\[\text{kt}_1\]

\[\text{"3Q"}\]

\[\text{"1Q"}\]

**Figure 3.5.** Coherence transfer diagram for the high-resolution MQMAS experiment for a \( S=3/2 \) nucleus in which a symmetric multiple-quantum coherence (3Q) evolves for a time \( t_1 \) and is then converted into central-transitions coherence for a second evolution time, \( kt_1 \). For higher spin numbers, e.g. \( S=5/2 \), the 3Q or 5Q may be correlated with the central transition to provide a high resolution MQMAS spectrum.
We wish to write a total propagator for the process in Figure 3.5, from which we can then deduce an effective Hamiltonian. If the \((m,-m)\) coherence \((m\neq 1/2)\) is allowed to evolve for a period of time \(t_1\), a propagator for this interval, \(U(t_1)\), can be written as

\[
U(t_1,0) = \exp[-iH_{m,-m}t_1].
\]  

(11)

If the \((m,-m)\) coherence is transferred into the central \((1/2,-1/2)\) transition and then allowed to evolve for another period of time \(t_2=kt_1\), the total propagator becomes

\[
U(t_2,0) = U(t_2,t_1)\Re U(t_1,0) = \exp[-iH_{1/2,-1/2}t_2] \Re \exp[-iH_{m,-m}],
\]  

(12)

where \(\Re\) represents the effect of a single RF pulse or a series of RF pulses that transfers the \((m,-m)\) coherence into the \((1/2,-1/2)\) central transition. Therefore, the effective Hamiltonian for the total evolution period, \(t_1+t_2\), is given by

\[
H_{\text{eff}} = \frac{1}{1+k} \left( \sum_{l=0} (2m+k)R_{10}^{CS} + \sum_{l=0,2,4} [C_m(l) + kC_{1/2,l}(l)]R_{10}^{Q} \right) \Omega_{\text{SFC}}^{1/2,-1/2}.
\]  

(13)

Equation 13 represents a result similar to Equation 4 for DAS, except that in this case the spinner axis is fixed during the entire experiment. Under the sample rotation condition, \(R_{10}^{CS}\) and \(R_{10}^{Q}\) become time-dependent and can be written using the Wigner rotation matrices as

\[
R_{10}^{\lambda}(t) = \sum_{m'=-l}^{l} D_{m,m'}^{(l)}(\Omega_{\text{SFC}}^{SFC}(t))R_{m'}^{\lambda},
\]  

(14)

where \(\lambda=CS,Q\) and \(\Omega_{\text{SFC}}^{SFC}(t)\) symbolizes the three Euler angles of the transformation to the laboratory frame from a sample-fixed-coordinate (SFC) frame. If the SFC frame is taken to be the principal axes system, then the \(R_{lm}^{\lambda}\) in Equation 14 can be substituted with the principal component \(\rho_{lm}^{Q}\). In general, \(R_{10}^{\lambda}(t)\) contains second- and fourth-rank Legendre polynomials for \(l=2\) and \(l=4\) respectively.

As indicated in Equations 6 and 7, the chemical shielding term contains only the second-rank Legendre polynomial, whereas the quadrupolar term contains both the second-
and fourth-rank Legendre polynomials. The effective Hamiltonian given in Equation 13 will not contain $R_{40}^0$ terms if the following condition can be satisfied:

$$C_{4m}(I) + kC_{4,1/2}(I) = 0 \quad (m = \frac{3}{2}, \frac{5}{2}, \ldots).$$

Under this condition, the effective Hamiltonian is analogous to that for the chemical shielding interaction. Therefore, MAS is sufficient to average the anisotropies which remain in this effective Hamiltonian, resulting in isotropic NMR spectra. It should be noted from Equation 13 that the effective Hamiltonian contains a time-independent part, which is the sum of isotropic chemical and quadrupolar shift terms ($l = 0$), and a time-dependent part that contains the chemical shift anisotropy and second-order quadrupolar interaction ($l = 2$). The latter time-dependent term contributes to rotational sidebands observed in multiple quantum NMR spectra. Since this strategy correlates multiple and single quantum coherences during magic-angle spinning, this method has been labeled the Multiple-Quantum Magic-Angle Spinning (MQMAS) experiment[14, 24]. Additionally, for $S=5/2$ nuclei, the MQMAS experiment can be carried out by correlating either the triple-quantum or the quintuple-quantum coherence with the central transition. These are distinguished as 3Q- and 5Q-MQMAS, respectively.

The effective Hamiltonian of Equation 13 can be experimentally realized in a manner conceptually identical to DAS. Where DAS correlates the evolution of central transition coherence at two different spinning axes, MQMAS correlates the evolution of a symmetric, multiple-quantum coherence, $(m, -m)$, with the evolution of central transition coherence, $(1/2, -1/2)$, for a sample rotating about the magic angle. And where DAS relies on spatial reorientation to average the high order quadrupolar Hamiltonian, MQMAS exploits the spin degrees of freedom to refocus the high order ($P_4(\cos\theta)$) quadrupolar term. The first MQMAS experiment used a three-pulse approach shown in Figure 3.6a[14], where a pair of pulses was used to generate triple-quantum coherence in $^{23}$Na($S=3/2$) or $^{55}$Mn($S=5/2$) nuclei, and a single third pulse reconverted the triple quantum coherence into observable central transition coherence.
Figure 3.6. RF pulse sequences for the 3Q-MQMAS experiment. (a) the three-pulse experiment used in the original report of MQMAS[14]. (b) a two-pulse 'nutation' sequence in which 3Q excitation and reconversion are each performed with a strong RF pulse. The phases of $\phi_1$ are stepped in 30-degree increments, while the acquisition mode is stepped in 90-degree increments in order to select for the correct 1Q-to-3Q-to-1Q coherence transfer pathway.

Just as in DAS, the pulse-sequence must be repeated for a series of $t_1$ increments in order to construct a high-resolution signal. The echo-maximum will be observed at time $t_2=kt_1$ where $k$ is given by Equation 15.

A significant strength of the MQMAS method is that it allows one to record an isotropic spectrum using conventional MAS probe technology. Limitations arising from filling factors and spinning rates, as in DOR, or from relaxation and spin diffusion effects, as in DAS, are not encountered with MQMAS. One of the initial limitations of MQMAS was in efficiently exciting and reconverting the multiple quantum coherences. For example, Frydman et al. only observed one of three crystallographically distinct sodium sites in their three-pulse MQMAS spectrum of Na$_2$HPO$_4$. For the MQMAS method to be useful in studying biological solids, the sensitivity issue must be addressed.
3.3.2. High Power RF Pulses Improve MQ Coherence Transfers

The sensitivity of MQMAS depends critically on efficient excitation of the multiple-quantum coherence. In contrast to situations in solution NMR studies where two radio-frequency (RF) pulses are necessary for generating multiple-quantum coherences, a single finite RF pulse is capable of exciting multiple-quantum coherence for half-integer quadrupolar nuclei in the solid state[11, 12, 25-28]. In this section, we demonstrate the utility of a two-pulse sequence (Figure 3.6b) in performing triple-quantum (3Q) MAS experiments[29, 30]. As an example, we will present $^{17}$O(S=5/2) NMR results for $^{17}$O-enriched hydroxyapatite (Ca$_5$(P$^{17}$O$_4$)$_3$OH) and $^{23}$Na(S=3/2) results for anhydrous $^{23}$Na$_2$SO$_4$ and $^{23}$Na$_2$HPO$_4$. The two-pulse sequence described here is shown to provide a higher and more uniform excitation of multiple-quantum coherence than the three-pulse sequence used previously.

In quadrupolar spin systems with I=3/2, the triple-quantum (3/2, -3/2) coherence can be excited by applying a weak RF pulse, i.e. $\omega_q < \omega_{RF}$[11, 12, 25]. To better understand the excitation efficiency of the triple quantum coherence by a finite RF pulse in rotating solids, we carried out several numerical calculations. Figure 3.7a shows the excitation profile of the triple-quantum (3/2,-3/2) coherence as a function of the pulse length, $P_1$, with different RF power levels. It is clear that, in the practical range of the RF power, increased RF field strength results in a higher efficiency for the excitation of the triple-quantum coherence. For example, the excitation efficiency of the triple-quantum coherence is increased by a factor of ~3 when the RF field is raised from 60 to 120 kHz. The triple-quantum excitation efficiency also depends on the strength of the quadrupolar interaction.

As seen in Figure 3.7b, the efficiency for the excitation of the triple-quantum (3/2,-3/2) coherence is decreased as the quadrupole coupling constant increases. However, it should be noted that the triple-quantum excitation efficiency decreases to zero at two extremes: (1) $\omega_Q = 0$ and (2) $\omega_Q >> \omega_{RF}$. 

Figure 3.7. Calculated coherence transfer efficiencies for RF pulses utilizing the GAMMA and NMRLAB simulation packages. The differences between the two columns are due to the use of non-secular terms in NMRLAB that are neglected in the GAMMA simulation. (a) Calculated excitation efficiency of 3Q coherence for $S=3/2$ nuclei as a function of the pulse length, for different RF field strengths. The quadrupole parameters for (a) are $e^2 q Q / h = 2.6$ MHz, $\eta=0.6$. (b) 3Q excitation efficiency as a function of the pulse length, and for different quadrupole coupling constants. Other parameters for (b) are $\omega_{RF} / 2\pi = 80$ kHz., $\eta=0.0$. (c) Calculated transfer efficiency from the 3Q coherence to the central-transition as a function of the pulse length, and for different RF field strengths. The same quadrupole coupling parameters were used for (c) and (a). MAS = 8 kHz for all calculations.

In triple-quantum experiments, it is necessary to transfer the triple-quantum coherence into the central ($1/2,-1/2$) transition. Therefore, the final sensitivity of the experiment depends not only on the triple-quantum excitation but also on the transfer efficiency. Figure 3.7c shows the calculated transfer profile in the two-pulse experiment as a function of the pulse length, $P_2$, with different RF field strengths. Again, the transfer efficiency increases as the RF field is increased. Interestingly, it seems that a ‘solid’ $180^\circ$ pulse of $P_2$ ($180^\circ$ pulse for the central transition)$[11, 12]$ always gives maximum transfer efficiency. As seen in Figure 3.7c,
the overall signal intensity of the two-pulse sequence described here can be as high as 40% of that arising from the central transition excited by a ‘solid’ 90° pulse. Our simulations also indicate that the triple-quantum excitation efficiency of the two-pulse sequence is an order of magnitude larger than that of the three-pulse sequence. These findings also agree with the results of Amoureux et al. who report MQ excitation and reconversion efficiencies for a single, finite pulse[29]. Also, for completeness, the calculated dependence of 3Q-to-1Q transfer, as a function of the pulse length and for different quadrupole couplings, is plotted in Figure 3.8. Similar to the 3Q excitation characteristics in Figure 3.7b, the 3Q reconversion efficiency is also reduced for increasing quadrupole couplings. Thus the use of hard ‘nutation’ pulses shows dependences on both the RF field strength and the quadrupole coupling. However, the two-pulse method shows promise for providing improved sensitivity in MQMAS experiments compared to the three-pulse approach, and particularly for recovering some MQ signal from sites with large quadrupole couplings.

![Figure 3.8](image)

**Figure 3.8.** Calculated transfer efficiency from the 3Q coherence to the central-transition coherence as a function of the pulse length, and for different quadrupole coupling constants (GAMMA/QSI). Other parameters are the same as for Figure 3.7b.

Next we demonstrate several two-pulse MQMAS spectra on S=3/2 and S=5/2 systems. An instructive illustration of MQMAS is to compare the time-domain signal recorded with single-pulse excitation to the isotropic FID generated in a complete MQMAS experiment. **Figure 3.9a** shows the free-induction-decay (FID) signal obtained in the conventional one-pulse MAS experiment for anhydrous Na₂SO₄, which decays to zero within 500 μs. **Figure**
3.9b displays the echo signal detected after the second pulse in the 3Q-MQMAS experiment with \( t_1 = 1.285 \) ms and \( t_2 = 1.000 \) ms. The echo resulting from the refocusing of the fourth-order Legendre polynomial is clearly seen at \( t_2 = k t_1 \). Figure 3.9c shows the echo maxima as a function of the total evolution time, \( t_1 + t_2 \). In contrast to the case in Figure 3.9a, the evolution of the echo exceeds 8 ms, indicating high resolution in the frequency domain. Indeed, the 1D 3Q-MAS spectrum, which is the Fourier transform of the echo evolution shown in Figure 3.9c, exhibits an isotropic peak with a line width of 109 Hz. By contrast the width of the MAS line shape exceeds 3 kHz.

Figure 3.9. (a) FID observed after one pulse under the MAS condition for anhydrous Na\(_2\)SO\(_4\). (b) One slice out of an MQMAS experiment: FID observed after the second pulse in the 3Q-MQMAS experiment with a delay between the first and second pulses of \( t_1 = 1.285 \) ms. (c) Composite FID formed from a series of echo maxima, each similar to (b), as a function of the total evolution time \( t_1 + t_2 \). The echo maximum in (b) appears at \( t_2 = k t_1 \), where the evolution corresponding to this echo is \( t_1 (1 + k) \), which is reflected in (c).
Similarly, two-pulse 3Q-MQMAS experiments can also be applied to other half-integer quadrupolar nuclei such as $^{17}$O (I=5/2). The 1D $^{17}$O 3Q-MQMAS spectrum of solid $^{17}$O-enriched hydroxyapatite (Ca$_5$(P$_{13}$O$_{34}$)$_3$OH) is shown in Figure 3.10, together with the static and MAS spectrum of the sample. The line width observed in the 3Q-MAS spectrum is approximately 330 Hz, which is an order of magnitude smaller than the 5.5 kHz width observed in the single-pulse MAS spectrum. Analysis of the $^{17}$O MAS spectrum yielded the following parameters: $\delta_{iso}=117$ ppm relative to H$_2^{17}$O, $\omega_0/2\pi=240$ kHz ($e^2qQ/h=4.8$ MHz), and $\eta=0.2$.

![Figure 3.10](image-url)  
$^{17}$O NMR spectra of hydroxyapatite. (a) Static, (b) MAS, and (c) 3Q-MQMAS. The sample spinning frequency used for obtaining (b) and (c) was 11.3 kHz. In (c) the $t_i$ increment was 12.6 $\mu$s. A total of 125 $t_i$ increments were collected. For each $t_i$ increment, a total of 624 transients were recorded. The recycle time was 1 s. The RF pulse lengths were 8.0 and 2.5 $\mu$s for the 3Q excitation and reconversion pulses, respectively.
The isotropic $^{17}$O chemical shift found for hydroxyapatite is in agreement with those reported for simple inorganic phosphates[31]. The quadrupole coupling constant determined for hydroxyapatite is also consistent with the NQR result reported for solid KH$_2$PO$_4$[32]. Interestingly, rotational side-bands are observed in the $^{17}$O 3Q-MAS spectrum of hydroxyapatite shown in Figure 3.10c. In 3Q-MAS experiments, where the isotropic echoes appear at $t_2=kt_1$, the apparent spinning frequency is scaled by a factor of 1+k, where k is 1.58 for $I=5/2$ nuclei. It is noted that a smaller value of k, 0.78, is required for spin-3/2 nuclei[14]. Similar scaled rotational sidebands have also been observed in DAS spectra[1]. As indicated by Equation 13, the isotropic line position observed in a 3Q-MAS spectrum is different from that found in the corresponding MAS spectrum. As seen in Figure 3.10, the offset of the isotropic peak in the $^{17}$O MAS line shape is approximately $-3.4$ kHz; however, the offset of the isotropic peak in the 3Q-MAS spectrum is $-0.1$ kHz.

3.3.3. MQMAS is a Two-Dimensional Experiment

Another sample that was chosen for testing the two-pulse sequence is anhydrous Na$_2$HPO$_4$, since this sample is known to contain three crystallographically distinct Na sites with different chemical shifts and quadrupole coupling constants[2]. As discussed earlier, the resonance positions in 1D 3Q-MAS spectra are determined by a linear combination of the isotropic chemical shift and isotropic second-order quadrupolar shift, and this dispersion is useful in distinguishing between crystallographically non-equivalent sites. Analogous to DAS experiments[17], 3Q-MQMAS intrinsically possesses two separate evolution times and can thus be presented in a two-dimensional (2D) fashion[24, 33, 34]. If the $t_2$ acquisition begins at the top of the echo, the resultant 2D FT spectrum will display isotropic peaks along the $\omega_1$ dimension and their corresponding anisotropic MAS line shapes along the $\omega_2$ dimension.

The 2D $^{23}$Na 3Q-MAS spectrum of anhydrous Na$_2$HPO$_4$ is shown in Figure 3.11. Along the $\omega_1$ dimension, the three crystallographically non-equivalent Na sites, Na(1), Na(2), and Na(3), are clearly observed. The line width of the three peaks is approximately $280$ Hz, which is significantly narrower than the width of the corresponding MAS line shape, $8.3$ kHz. The quadrupole coupling constant ($e^2qQ/h$) and asymmetry parameter ($\eta$) for the three Na sites in anhydrous Na$_2$HPO$_4$ were previously reported[2]: Na(1), $355$ kHz, 0.69; Na(2), $229$
kHz, 0.21; Na(3), 617 kHz, 0.27. The fact that there are three crystallographically non-equivalent Na sites in anhydrous Na$_2$HPO$_4$ makes the analysis of MAS spectra difficult. In the 2D 3Q-MQMAS spectrum, however, all three Na sites are clearly resolved. Furthermore, slice spectra can be displayed along the $\omega_2$ dimension from each of the isotropic positions. These slice spectra correspond to individual sub-spectra in the total MAS line shape and can be analyzed separately, yielding quadrupole parameters for each of the individual sites.

Figure 3.11. 2D $^{23}$Na 3Q-MQMAS spectrum of anhydrous Na$_2$HPO$_4$. The sample spinning frequency was 11.7 kHz. The $t_1$ increment was 25.7 ms. A total of 140 $t_1$ increments were collected. For each $t_1$ increment, a total of 112 transients were recorded. The recycle time was 2 s. The RF pulse lengths were 8.0 and 2.5 $\mu$s for the first and second pulses respectively. The $^{23}$Na MAS spectrum (top) and 1D 3Q-MQMAS spectrum (left) are also shown.

The 2D-MQMAS experiment is a widely employed method (for example, see Section 5.2 and references [35-42]). The proliferation of 2D-MQMAS applications has benefited
from extensive work by many groups to develop methods of acquiring pure-phase 2D-MQMAS spectra that are free of line shape distortions[43-47]. For example, it is often necessary turn the receiver on immediately after the reconversion pulse instead of at the echo maximum. In this variation, however, the quadrupole line shapes are skewed in the 2D plot. A ‘shearing’ phase transformation must be applied to the raw data to remove this determinate phase shift and tilt the signals in the $F_2$ dimension to be normal to the $F_1$ axis, as shown in Figure 3.11 [43]. However a full discussion of these methods is outside the scope of this report; please see the references for more information.

Since the positions of individual peaks in 3Q-MAS spectra are different from those in MAS, DAS or DOR spectra, caution must be exercised in interpreting 1D 3Q-MAS spectra. For example, the Na(3) site appears at the lowest frequency in the MAS and DOR spectra of anhydrous Na$_2$HPO$_4$, but it is at the highest frequency in the 1D 3Q-MAS spectrum (see Figure 3.11). For $I=3/2$ nuclei, the position of the isotropic peak in a 3Q-MAS spectrum is given by

$$\omega_{i1}^{3Q} = \frac{3+k}{1+k} \omega_{i1}^{3Q} + \frac{9}{10} \left( \frac{3-k}{1+k} \right) \frac{\omega^2}{\omega_0} \left( 1 + \frac{1}{3} \eta^2 \right).$$

Interestingly, the spacing between the three isotropic peaks in the $^{23}$Na 3Q-MAS spectrum of Na$_2$HPO$_4$ is greater than the difference between the centers of the MAS line shapes for individual sites. This indicates that 3Q-MAS spectra for spin-3/2 nuclei exhibit a higher resolution than do DAS and DOR spectra. For spin-5/2 nuclei, the shift difference between isotropic peaks in triple-quantum MQMAS spectra is smaller than that between the centers of individual line shapes, resulting in a poor resolution compared to DAS and DOR spectra. However, quintuple-quantum (5Q) MAS spectra for spin-5/2 nuclei still exhibit a higher resolution than DAS and DOR spectra have.

It is noted again that only one site, Na(2), was previously observed in the $^{23}$Na 3Q-MAS spectrum of anhydrous Na$_2$HPO$_4$ obtained with the three-pulse sequence[14]. This suggests that the two-pulse sequence described here provides a higher and more uniform triple-quantum excitation, which is important for detecting crystallographically non-equivalent sites. Of course the current 3Q-MAS experiment cannot be used as a quantitative
analysis. It is necessary to develop other techniques for uniform multiple-quantum excitation and this is addressed in section 3.6.

In summary, the MQMAS experiment described by Frydman and Harwood[14] is an important step towards the general goal of obtaining isotropic NMR spectra from quadrupolar nuclei. Here we demonstrate the utility of the two-pulse sequence in improving 3Q-MQMAS experiments in S=3/2 and S=5/2 nuclei. Its advantage over the three-pulse experiment is twofold. First, the two-pulse sequence has a higher efficiency in generating required multiple-quantum coherences. Second, the multiple-quantum coherence excitation in the two-pulse sequence is less sensitive to the magnitude of quadrupolar interactions, making it more likely to detect all crystallographically non-equivalent sites that may have different quadrupole coupling constants. The 3Q-MQMAS experiment can be interpreted in either a one- or two-dimensional fashion. While 1D 3Q-MAS experiments yield high-resolution isotropic spectra, the 2D presentation provides useful correlations between isotropic and anisotropic interactions.

3.4. High-Resolution Oxygen-17 NMR Spectroscopy of Solids

3.4.1. $^{17}$O NMR Parameters and Higher Sensitivity in MQMAS

Among quadrupolar nuclei, $^{17}$O ($S = 5/2$) has special importance because oxygen is the key constituent of many chemically and biologically important functional groups. For example, oxygen is often directly involved in hydrogen bonding, which is fundamental to physical and biological processes. In addition, $^{17}$O NMR parameters are sensitive to molecular structure and chemical environment, suggesting that direct observation of $^{17}$O spectra has the potential to yield valuable and previously inaccessible information[48]. Oxygen-17 chemical shifts span a range of approximately 1500 ppm and, along with the quadrupole coupling parameters, provide detailed information on hydrogen bonding effects, crystallographic symmetry, and molecular structure. In the cases where $^{17}$O MQMAS spectra are reported [30, 35-37], only compounds with moderate $^{17}$O quadrupole coupling constants,
≤ 5 MHz, were investigated. This fact reflects one of the major problems in $^{17}$O MQMAS studies, namely, the low sensitivity of the technique in systems with large quadrupole coupling constants. While $^{27}$Al (S=5/2) sites with quadrupole couplings of 8-9 MHz have been observed in MQMAS spectra [34, 39], the large gyromagnetic ratio of $^{27}$Al (e.g. $\gamma(^{27}$Al)/$\gamma(^{17}$O) = 1.92) provides a factor of 5 in signal-to-noise improvement in the directly detected dimension of $^{27}$Al MQMAS as compared to $^{17}$O. In this section we demonstrate that the problems encountered in $^{17}$O MQMAS experiments can be circumvented for an important class of $^{17}$O sites, i.e., H$_2^{17}$O and $-{^{17}$OH groups, with a combination of high frequency spinning (~20 kHz) and large $B_1$ fields (>120 kHz).

As shown in Figure 3.7 and Figure 3.8 (nutation vs. $e^2qQ/h$), and in agreement with other studies [25, 29, 49, 50], the strongest attainable $B_1$ field strengths are necessary for interconverting MQ coherences in systems with large quadrupole coupling constants. This requirement can be a significant burden on conventional MAS probes and high-power amplifiers, and specialized commercial probes (Bruker Instruments) have been developed to achieve power levels of 300 kHz for short pulse durations. Additionally, the well-known technique of rotor-synchronized acquisition [51] can also be used to improve the sensitivity of MQMAS experiments [34, 44]. Rotor synchronization of the total evolution period is accomplished by choosing the $t_i$ increment such that $(1 + k)t_i = \tau_e$, where $\tau_e$ is the time for one rotor period. Here we combine these features in $^{17}$O MQMAS experiments and report results for PO$_4$, H$_2$O and -OH containing compounds.

**Experimental Details**

All solid state $^{17}$O NMR spectra were obtained on a custom-designed NMR spectrometer operating at 53.93 MHz for $^{17}$O nuclei (9.4 T). Static $^{17}$O NMR spectra were obtained using the echo sequence described by Kunwar et al. [52]. The custom designed MAS probe was equipped with a 3.2 mm spinning assembly (Chemagnetics, Inc., Fort Collins, Colorado) which allowed sample spinning at 20-25 kHz. The $B_1$ field strength at the $^{17}$O frequency was 100-135 kHz. All $^{17}$O triple-quantum (3Q) MAS experiments were performed with the two-pulse sequence with a 24-step phase cycling scheme [14, 24]. Typical pulse widths for 3Q excitation and 3Q-to-1Q conversion were 5.5 and 1.8 $\mu$s,
respectively. For $^1$H containing compounds, proton decoupling was employed during both evolution and acquisition periods. All NMR interaction parameters presented in Table 3.1 were obtained from comparison of MAS line shapes and MQMAS line positions [30, 43]. All $^{17}$O chemical shifts were referenced to H$_2^{17}$O with an external sample. The samples of $^{17}$O-labeled phosphates and Ca($^{17}$OH)$_2$ were prepared by the literature methods [53-55] with $^{17}$O-enriched H$_2$O (containing 34% $^{17}$O). Ba(ClO$_3$)$_2$.H$_2$$^{17}$O was prepared by recrystallizing the compound from $^{17}$O-enriched water (containing 50% $^{17}$O). Enriched water was subsequently recovered on a vacuum line. Oxygen-17 enriched water was obtained from ISOTEC Inc. (Miamisburg, Ohio).

3.4.2. $^{17}$O MQMAS in PO$_4$, H$_2$O and –OH Moieties

Oxygen-17 MAS NMR spectra of four $^{17}$O-enriched phosphate samples are shown in Figure 3.12. Unique NMR line shapes arising from the second-order quadrupolar interaction are observed, with line widths ranging from approximately 5 to 7 kHz. Although it is possible to estimate $^{17}$O quadrupole parameters from the MAS spectra, no information concerning the crystallographic equivalence of the oxygen atoms can be obtained. In contrast, as shown in Figure 3.13, the $^{17}$O MQMAS spectra of the samples consist of isotropic peaks significantly narrower than the second-order quadrupolar line shapes observed in the MAS spectra. For Ca$_5$(P$^{17}$O$_4$)$_3$(OH), the $^{17}$O MQMAS spectrum exhibits two isotropic peaks. This doublet structure was not observed in our previous study [30] due to factors which constrained resolution in the $t_1$ dimension. Here we acquired for longer times in the $t_1$ dimension; additionally the higher MAS rate may have removed some residual dipolar couplings. It should be noted that size of the dipole coupling is scaled by the order ‘m’ during the MQ evolution so that fast MAS and high power decoupling can be of critical importance, particularly for 5Q-MQMAS.
Figure 3.12: $^{17}$O MAS spectra of (a) Ca$_5$(P$^{31}$O$_4$)$_3$(OH), (b) CaHP$^{31}$O$_4$·2H$_2$O, (c) KH$_2$P$^{31}$O$_4$, and (d) NH$_4$H$_2$P$^{31}$O$_4$. The MAS frequency was 19.8 kHz.

Figure 3.13: $^{17}$O 3Q-MQMAS spectra of (a) Ca$_5$(P$^{31}$O$_4$)$_3$(OH), (b) CaHP$^{31}$O$_4$·2H$_2$O, (c) KH$_2$P$^{31}$O$_4$, and (d) NH$_4$H$_2$P$^{31}$O$_4$. The MAS frequency was 19.8 kHz. The $t_f$ increment was synchronized with the sample spinning period, 50.5 $\mu$s. For each $t_f$ increment, 552 transients were accumulated. A total of 40 $t_f$ increments were collected. Each MQMAS experiments took approx. 6 hrs.

The significant improvement of resolution and sensitivity in this work results from the combination of fast sample spinning (ca. 20 kHz) with rotor-synchronized $t_f$ acquisition [34, 44]. The advantage of the rotor-synchronized $t_f$ acquisition is that signal intensities in the sidebands are folded into the center band. Although at this spinning frequency, rotational sidebands due to the CSA interaction are almost fully averaged, the magnitude of the quadrupolar coupling will lead to rotational sidebands. The total spectral width in the
isotropic $^{17}$O MQMAS spectra is determined by $\nu_R / (1 + k)$ where $\nu_R$ is the spinning frequency and $k$ equals 19/12 for $S = 5/2$ nuclei[14]. Therefore, rapid sample spinning (~20 kHz) is required to obtain a sufficient spectral window in the isotropic dimension. The crystal structure of $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ indicates that the $\text{PO}_4$ tetrahedron consists of three crystallographically distinct oxygen atoms, $O_1$, $O_2$, and $O_3$ with a population ratio of 1:1:2 in the unit cell [56]. Examination of the P-O bond distances reveals that $O_1$ and $O_2$ are similar and distinctively different from $O_3$ ($\text{P-O}_1 = 1.533$, $\text{P-O}_2 = 1.544$, and $\text{P-O}_3 = 1.514 \text{ Å}$). The quadrupole coupling parameters for the three environments are nearly identical and so the relative intensities in the MQMAS spectrum can be taken to be quantitative. Thus our observation of a 1:1 doublet in the $^{17}$O MQMAS spectrum is consistent with the crystal structure.

The crystal structure of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ belongs to the space group, $Ia$, which yields four non-equivalent oxygen atoms in the $\text{PO}_4$ tetrahedron with the P-O distances of 1.69, 1.58, 1.69 and 1.34 Å [57]. In the $^{17}$O MQMAS spectrum of $\text{CaH}^{17}\text{O}_4 \cdot 2\text{H}_2\text{O}$, two isotropic peaks are observed with an approximate 1:3 intensity ratio. Our observation supports the conclusion of the non-centrosymmetric space group $Ia$. Both $\text{KH}_2\text{PO}_4$ and $\text{NH}_4\text{H}_2\text{PO}_4$ crystallize in the tetragonal space group $I\bar{4}2d$ at room temperature [58]. In each of the two compounds, all four oxygen atoms of the $\text{PO}_4$ tetrahedron are crystallographically equivalent. Indeed, single resonances are observed in the $^{17}$O MQMAS spectra of these two compounds.

Since a potentially important application of high-resolution solid state $^{17}$O NMR is the study of hydration of biological macromolecules, we also investigated the efficiency of MQMAS in obtaining spectra of crystalline hydrates and hydroxides, where $^{17}$O quadrupole couplings are larger (~7 MHz) than in the phosphates. Figure 3.14 shows $^{17}$O NMR spectra of $\text{Ba(ClO}_3)_2 \cdot \text{H}_2^{17}\text{O}$ and $\text{Ca(}^{17}\text{OH})_2$ obtained under static, MAS and MQMAS conditions.
As expected, the static and MAS $^{17}$O NMR spectra of these crystalline hydrates are significantly broader than those of the phosphates. It is seen in Figure 3.14b that rapid sample spinning at 20 kHz significantly reduces the rotational sideband intensities, thus enhancing the sensitivity. Analysis of the static and MAS $^{17}$O NMR spectra of Figure 3.14 yields the quadrupole coupling parameters which are given in Table 3.1. For Ba(ClO$_3$)$_2$•H$_2^{17}$O our analysis yields $e^2qQ/h = 6.8$ MHz, which is somewhat smaller than the 7.61 MHz determined at 77 K by NQR [59]. As seen in Figure 3.14a, the $^{17}$O MQMAS spectrum of Ba(ClO$_3$)$_2$•H$_2^{17}$O exhibits an isotropic peak indicating only one water molecule in the asymmetric unit cell, consistent with the neutron diffraction structure [60]. The 100 Hz line width of the $^{17}$O MQMAS spectrum is about 150-fold narrower than that of the $^{17}$O MAS spectrum. Similarly, the $^{17}$O MQMAS spectrum of Ca($^{17}$OH)$_2$ exhibits an isotropic line narrower than that of the corresponding MAS spectrum by a factor of 100, and is consistent with one crystallographically distinct hydroxyl group [61].
Table 3.1. Oxygen-17 NMR parameters obtained from solid state $^{17}$O NMR spectra. Chemical shifts are referenced to external H$_2^{17}$O liquid.

All $^{17}$O NMR parameters obtained for compounds studied in this work are listed in Table 3.1. It should be noted that a significant portion of the line width observed in the $^{17}$O MQMAS spectra of the phosphates arises from one-bond, indirect coupling $J(^{17}$O, $^{31}$P), which is approximately 90 Hz for the [PO$_4$]$^{3-}$ group [31]. Indeed, $J$-coupling has been observed in the $^{11}$B ($S = 3/2$) MQMAS spectra of a borane phosphite adduct [62]. In the $^{17}$O ($S = 5/2$) triple-quantum-MAS spectra, however, the spectral splitting from $J(^{17}$O,$^{31}$P) would be reduced to 0.55 $J(^{17}$O,$^{31}$P) [62]. Therefore, the true line width in the $^{17}$O MQMAS spectra of the phosphates is estimated to be on the order of 100 Hz (approx. 1.8 ppm at 9.4 T), a value comparable to the line widths observed for Ba(ClO$_3$)$_2$•H$_2$$^{17}$O and Ca($^{17}$OH)$_2$.

In summary, the practicality of obtaining high-resolution solid state $^{17}$O MQMAS NMR spectra in samples exhibiting large quadrupole couplings has been demonstrated. The resolution in the $^{17}$O MQMAS spectra is approximately 30- to 150-fold higher than that found...
in the $^{17}$O MAS spectra, permitting the detection of crystallographically distinct oxygen sites. Such spectral details are not observable in the $^{17}$O MAS spectra because of the second-order quadrupolar broadening. Combining high magnetic fields (e.g., 750 MHz), fast sample spinning (e.g., > 25 kHz), rotor-synchronized acquisition, and NMR probes that can deliver high RF power, $^{17}$O MQMAS NMR can clearly be applied to a variety of solid materials including biologically important macromolecules.

3.5. Quantitative Multiple-Quantum Magic-Angle Spinning (MQMAS) NMR

In this section, we describe a new approach for observing multiple-quantum (MQ) NMR spectra of $S=3/2$ nuclei with magic-angle spinning (MAS) that employs the Rotation-Induced Adiabatic Coherence Transfer (RIACT) that occurs between triple-quantum (3Q) and central-transition (1Q) coherences in $S=3/2$ systems. In contrast to currently available coherence-transfer techniques, RIACT is relatively insensitive to the magnitude of the quadrupole interaction for $(e^2 qQ/h \leq 5$ MHz) for both 3Q excitation and 3Q-to-1Q conversion. Thus, RIACT provides a means of extracting quantitative information about site populations from isotropic MQMAS NMR spectra. This will be illustrated with $^{23}$Na ($S=3/2$) MQMAS spectra of a series of sodium salts exhibiting crystallographically distinct sites. The spectra provide quantitative measurements of quadrupolar parameters, chemical shifts, and relative site populations for each of the crystallographically distinct sodium sites.

Experimental Details:

Solid-State NMR. All sodium salts studied in this work were obtained from Mallinckrodt, Inc. and the solid-state NMR experiments were performed on a custom-designed NMR spectrometer operating at 105.22 MHz for $^{23}$Na nuclei. The MAS probe was equipped with a 5mm spinner assembly (Doty Scientific, Inc., Columbia, SC), and typical
spinning speeds were 5-10 kHz. In the $^{23}$Na NMR experiments, the radio-frequency field strengths were 80 kHz, corresponding to $^{23}$Na 90 pulse lengths of ~3.1 us. A sample of solid sodium bromide was used to align the magic angle (using 79Br NMR signals) and to calibrate the radio-frequency field strength at the $^{23}$Na NMR frequency. More detailed experimental parameters are given in the figure captions.

### 3.5.1. Rotation-Induced Adiabatic Coherence Transfer (RIACT) in Half-Integer Quadrupolar Nuclei

A major problem with two-pulse MQMAS experiments presented so far[29, 30] is that both the excitation of MQ coherence and its conversion into observable single-quantum (1Q) coherence depend strongly upon the magnitude of the quadrupolar interaction. This has led to NMR spectra where crystallographically distinct sites give rise to isotropic lines whose intensities are significantly different from those expected on the basis of the site population. A case which illustrates this point concerns the $^{23}$Na NMR spectra of anhydrous Na$_2$HPO$_4$ which has three crystallographically non-equivalent Na sites with relative populations of 1:1:2 with $e^2qQ/h$ of Na(1) 1.37, Na(2) 2.13, and Na(3) 3.70 MHz, respectively[2] (see Figure 3.11). In the original three-pulse MQ experiment[14] the NMR line corresponding to the Na(3) site was absent, and in our spectra with the two-pulse sequence [30] its intensity was approximately 1/3 that of the Na(1) site, whereas it should have been twice as strong. Thus, with the present methodology the signal intensities in the spectra do not reflect the relative populations of the individual sites. Since it is axiomatic that quantification of signal intensities in NMR spectra is crucial in applications to the analysis of inorganic, organic, or biological materials, it is desirable to correct this deficiency.

The concept of 3Q<->1Q coherence transfer in quadrupolar systems was first described in the seminal paper by Vega[63] who focused principally on the central-transition (1Q) signal during spin-locking. Here we show direct experimental evidence for 3Q excitation and 3Q-to-1Q conversion via RIACT. Furthermore, we demonstrate that signal intensities in RIACT spectra are relatively insensitive to the magnitude of the quadrupolar interaction, thus permitting quantitative measurements of site populations. We illustrate the
new method with an analysis of $^{23}\text{Na}$ isotropic NMR spectra for a series of sodium salts exhibiting crystallographically distinct sites.

In the rotating frame, the spin Hamiltonian for an $S=3/2$ nucleus in a strong radio frequency field can be written as [63]

$$H = \omega_i S_x + \frac{Q}{2} \left[ S_z^2 - \frac{1}{3} S(S+1) \right]. \quad (13)$$

where $Q$ is the first-order quadrupole splitting and $\omega_i$ is the radio frequency field strength in angular frequency units. For a static solid, $Q$ has the following form:

$$Q = \frac{\omega_0}{2} \left( 3 \cos^2 \theta - 1 - \eta \sin^2 \theta \cos \phi \right) \quad (14)$$

where

$$\omega_0 = \frac{3e^2 qQ}{2S(S+1)\hbar} \quad (15)$$

In Equation 14, $\eta$ is the asymmetry parameter, and $\theta$ and $\phi$ are the polar and azimuthal angles orienting the direction of the applied magnetic field in the principal axes system of the electric-field-gradient (EFG) tensor. The eigenstates and eigenvalues of the spin Hamiltonian (Equation 1) have been previously derived[11, 64] and a typical energy level diagram is shown in Figure 3.15. If $|c_z\rangle = \frac{1}{\sqrt{2}}(|\frac{1}{2}\rangle \pm |\frac{-1}{2}\rangle)$ and $|t_z\rangle = \frac{1}{\sqrt{2}}(|\frac{3}{2}\rangle \pm |\frac{-3}{2}\rangle)$ are chosen as bases, the eigenstates of the Hamiltonian can be expressed as:[64]

$$\psi_1 = \sin(\theta_+ + \frac{\pi}{3})|t_+\rangle - \cos(\theta_+ + \frac{\pi}{3})|c_+\rangle \quad (16a)$$
$$\psi_2 = \cos(\theta_+ + \frac{\pi}{3})|t_+\rangle + \sin(\theta_+ + \frac{\pi}{3})|c_+\rangle \quad (16b)$$
$$\psi_3 = \cos(\theta_- - \frac{\pi}{3})|t_-\rangle + \sin(\theta_- - \frac{\pi}{3})|c_-\rangle \quad (16c)$$
$$\psi_4 = \cos(\theta_- - \frac{\pi}{3})|t_-\rangle + \sin(\theta_- - \frac{\pi}{3})|c_-\rangle \quad (16d)$$
where

\[
\tan 2\theta_+ = \frac{-\sqrt{3}Q}{4\omega_1 + Q} \tag{17}
\]

\[
\tan 2\theta_- = \frac{-\sqrt{3}Q}{4\omega_1 - Q} \tag{18}
\]

Clearly, when \(|Q| \gg \omega_1\), the eigenstates of the spin Hamiltonian are \(|c_z\rangle\) and \(|t_z\rangle\) [63], and populations in these states give rise to central-transition (\(\frac{1}{2}, \frac{-1}{2}\)) and triple-quantum (\(\frac{3}{2}, \frac{-1}{2}\)) coherences, respectively. When \(Q\) is comparable to \(\omega_1\), neither \(|c_z\rangle\) nor \(|t_z\rangle\) are eigenstates of the spin Hamiltonian.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3_15.png}
\caption{Energy level diagram for the spin Hamiltonian given in Equation 13. When \(\left|\frac{Q}{\omega_1}\right| \gg 1\), the eigenstates of the spin Hamiltonian are \(|c_z\rangle\) and \(|t_z\rangle\).}
\end{figure}
Suppose that the density matrix of the original spin state is $\rho(0) = I_x^{(23)}$ and that $|Q| >> \omega_r$. As Vega showed[63], since $I_x^{(23)}$ is characterized by populations of the wavefunctions $|c_\downarrow\rangle$, which are eigenstates of the spin Hamiltonian given in Equation 13, the spin system should remain in its original state, i.e., being “spin-locked”. During MAS, the first-order quadrupole splitting, $Q$, becomes time dependent, so that the eigenstates of the spin Hamiltonian also change with time. Under such circumstances, the initial eigenstates of the spin Hamiltonian, $|c_\downarrow\rangle$, will not remain as eigenstates; therefore the initial coherence, $I_x^{(23)}$, will be lost in the course of the spin-locking period. Assuming an axially symmetric EFG tensor ($\eta = 0$) for simplicity, the time-dependent first-order quadrupole splitting, $Q(t)$, can be expressed in the following form,[63]

$$Q(t) = \frac{\omega_r}{2} = \left[ -\sqrt{2} \sin 2 \beta \cos(\omega_r t + \gamma) + \sin^2 \beta \cos(2\omega_r t + 2\gamma) \right],$$  \hspace{1cm} (19)$$

where $\beta$ is the angle between the unique axis of the EFG tensor for a particular crystallite and the sample rotation axis, $\gamma$ is a rotation of the EFG tensor about the spinning axis, and $\omega_r$ is the sample spinning frequency. Note that $Q(t)$ has components which oscillate at $\omega_r$ and $2\omega_r$, respectively. If the changes of $Q(t)$ are adiabatic – slow enough so that the spin system remains in its eigenstates—then as $Q(t)$ reverses sign due to sample rotation, the eigenstates of the spin Hamiltonian are changed from $|c_\downarrow\rangle$ and $|c_\uparrow\rangle$ to $|t_\downarrow\rangle$ and $|t_\uparrow\rangle$, respectively[63]. Thus the initial populations in $|c_\downarrow\rangle$ become populations in $|t_\downarrow\rangle$ accordingly. For crystallite orientations where the component oscillating at $\omega_r$ is predominant, the sign reversal of $Q(t)$ occurs twice per rotor period, $\tau_r$. Thus, the initial coherence, $I_x^{(23)}$, is converted into the triple-quantum coherence, $I_x^{(14)}$, at $\tau_r/2$. It then returns to its original state at the end of a rotor cycle. So the following cycles occur during the spin-locking period:

- populations transfer cycle: $|c_\downarrow\rangle \rightarrow |t_\downarrow\rangle \rightarrow |c_\uparrow\rangle \rightarrow \ldots$
- coherence transfer cycle: $I_x^{(23)} \rightarrow I_x^{(14)} \rightarrow I_x^{(23)} \rightarrow \ldots$
Similarly, if the initial coherence is $I^{(14)}_x$, it is converted to $I^{(23)}_x$ at $\tau_r/2$, and then returns to $I^{(14)}_x$ at $\tau_r$. For crystallite orientations where the component oscillating at $2\omega_r$ is dominant, the aforementioned cycles occur twice per rotor period and therefore maximum $I^{(23)}_x \leftrightarrow I^{(14)}_x$ transfer occurs at $\tau_r/4$ and $3\tau_r/4$. The RIACT phenomenon for $S=3/2$ nuclei is conceptually identical to the well-known population inversion for two-level systems induced by adiabatic passage in continuous wave NMR (see Abragam, p.44[65]). Based upon this concept, several NMR techniques have been recently developed in order to measure internuclear distances between spin-1/2 and quadrupolar nuclei [66-71].

Vega introduced an adiabaticity parameter, $\alpha$, and defined the adiabatic condition

$$\alpha = \frac{\omega^2}{\omega_c^2 \omega_r} \gg 1$$

(20)

Since the first-order quadrupole splitting is orientation dependent, Equation 20 should not be treated as a strict constraint for the adiabatic condition. Even when $\alpha \leq 1$, there is a significant portion of crystallites satisfying the adiabatic condition. Other definitions of the adiabaticity parameter are discussed by Hayashi[72] and by Baltisberger et al[22].

The pulse sequences used for observing the RIACT phenomenon for $S=3/2$ nuclei are depicted in Figure 3.16. In Figure 3.16a is shown the standard spin-locking sequence where the initial central-transition coherence, $I^{(23)}_x$, is created by a phase-alternated 45 degree pulse (an effective 90 degree pulse for the central transition of $S=3/2$ nuclei) and the central-transition coherence is observed after spin-lock. To observe the evolution of the 3Q coherence as a function of the spin-locking time, $\tau_{SL}$, an additional radio frequency pulse must be added at the end of $\tau_{SL}$ (see Figure 3.16b), so that the 3Q coherence can be converted to detectable central-transition coherence.
Figure 3.16. Multiple-Quantum pulse sequences used in this section. (a) Sequence for spin-locking (SL) the central transition. (b) Sequence for measuring the 3Q coherence created by RIACT during the spin-locking time. (c) The standard two-pulse (nutation) sequence for MQMAS experiments. (d) The RIACT (I) sequence for MQMAS. (e) The RIACT(II) sequence for MQMAS. In parts d and e, the spin-lock time was always set to $\tau, 4$. 

$$\phi = \{0, \pi/6, 2\pi/6, \ldots\}$$

$$\psi = \{(0,90,180,279),(...),(...)\}$$
It is also important to use phase cycling that selects the coherence transfer pathway of \(0 \rightarrow (-3) \rightarrow (-1)\) [14]. Experimental results for solid NaNO$_2$ (1) are shown in Figure 3.17, where oscillatory behavior is observed for both the 1Q and 3Q coherences. It is important to emphasize that 3Q excitation did not appear at \(\tau_r / 2\), but rather at \(\tau_r / 4\) and \(3\tau_r / 4\). This observation indicates that for a significant number of crystallites, \(Q(t)\) changes sign four times per rotor cycle. It can be readily shown from Equation 19 that for one-third of the crystallites, \(Q(t)\) reverses sign four times per rotor cycle. As we will show later, setting \(\tau_{SL}\) to \(\tau_r / 4\) instead of to \(\tau_r / 2\), always produces superior results.

![Figure 3.17](image)

**Figure 3.17.** Experimental results for the oscillatory behavior of the $^{23}$Na 3Q and 1Q coherences via RIACT in NaNO$_2$ (1). The sample spinning frequency was 5 kHz (giving a rotor period of 0.2 ms). The quadrupole coupling parameters of 1 are \(e^2 qQ / h = 1.1\) MHz, \(\eta = 0.11\).

Clearly, the RIACT process provides an effective mechanism for 3Q$\leftrightarrow$1Q transfer, and can be used either for generating 3Q coherence from the central-transition coherence or for converting 3Q to the central-transition coherence. More importantly, such 3Q$\leftrightarrow$1Q transfers are expected to be independent of the magnitude of the quadrupole interaction, provided that the adiabatic condition is satisfied. This property of RIACT will be verified by numerical calculations presented in the following section.
3.5.2. Sensitivity of MQMAS Experiments

The pulse sequences used for MQMAS experiments are also depicted in Figure 3.16c-e. Figure 3.16c is the standard two-pulse sequence for MQ experiments [24, 30, 33, 42] where two nutation pulses are employed for 3Q excitation and 3Q-to-1Q conversion, respectively. The 3Q coherence created by the first nutation pulse is allowed to evolve for t₁, and then is converted to 1Q coherence for detection by the second nutation pulse. At the time t₂=kt₁, where k=7/9 for S=3/2 nuclei, an echo will form. Sampling the top of the echo as a function of the total evolution time t₁+t₂ will yield a free-induction decay (FID) that consists of information about isotropic chemical shifts and isotropic second-order quadrupolar shifts, but is free of second-order quadrupolar broadening. [14]

The RIACT(I) sequence shown in Figure 3.16d utilizes a nutation pulse for 3Q excitation and a spin-locking pulse for 3Q-to-1Q conversion. As already discussed in the previous section, when τ_{SL} is chosen to be τₘ/₈, the 3Q coherence at the beginning of the spin-locking period will be converted into central-transition coherence by RIACT. In the RIACT(II) sequence (Figure 3.16e), both 3Q excitation and 3Q-to-1Q conversion are achieved by RIACT. The first 45-degree pulse is used to create I₂₃, which is then converted to 3Q coherence via RIACT during the first spin-locking pulse. In the discussion that follows, we investigate how these three different pulse sequences depend upon the magnitude of the quadrupole interaction.

MQMAS Experiments by the Two-Pulse Sequence. It has long been known that MQ excitation efficiency from a nutation pulse depends strongly upon the magnitude of the quadrupole coupling constant [27, 63, 64]. When ω_q >> ω₁, the 3Q excitation for S=3/2 systems goes as ω₂ [25, 64]. Numerical calculations on the 3Q excitation from a nutation pulse as a function of both radio frequency power and quadrupole coupling constant have been previously described (Section 3.3) [30]. Using a simplified model, Medek et al. [24] also evaluated the 3Q excitation and 3Q-to-1Q conversion by nutation pulses. All these previous studies indicated that both 3Q excitation and 3Q-to-1Q conversion efficiencies decrease substantially as the magnitude of the quadrupole coupling constant is increased. Here we present more complete results from numerical calculations about the sensitivity of the two-
pulse nutation sequence as a function of the quadrupole coupling constant. As illustrated in Figure 3.18, both 3Q excitation and 3Q-to-1Q conversion decrease monotonically with the increase of the quadrupole coupling constant, except for very small quadrupole coupling constants, i.e., \( \omega_Q < \omega_1 \). Therefore, the peak intensities in MQ spectra obtained with the nutation method do not reflect directly the site populations. For a given \( e^2 q Q / h \) value, both the 3Q excitation and 3Q-to-1Q conversion increase as the radio frequency field strength is increased; however, the 3Q excitation appears to be more sensitive to the field strength.

Figure 3.18. Calculated 3Q excitation (top) and 3Q-to-1Q conversion (bottom) by a nutation pulse as a function of \( e^2 q Q / h \) at different radio frequency power levels. The RF carrier is adjusted to be on-resonance by accounting for the second-order isotropic shift. The MAS frequency was 10 kHz and the r.f. powers are given in the legend. 1000 crystals were used for each point. Intensities are normalized to the intensity of the central transition following a theoretically 'perfect' 45° pulse.
It is also clear from Figure 3.18 that the 3Q-to-1Q conversion is less efficient than the 3Q excitation. In fact, the low 3Q-to-1Q conversion efficiency is always a severe limiting factor in MQ experiments. Another observation from Figure 3.18 is that, for small quadrupole couplings and high r.f. field strengths, the 3Q excitation efficiency can exceed unity, which is defined as the central-transition coherence following an ideal 45° pulse. Qualitatively, this may be understood as a consequence of inducing spin-population in the outer levels to transfer into symmetric 3Q coherence. Enhancement of the central-transition polarization via the satellite populations has already been demonstrated in the context of static[73-75] and spinning solids[76, 77], but no investigation of these processes in the context of MQ coherence transfer has been reported.

**MQ Experiments by RIACT.** As shown in the preceding section, the excitation efficiency and line intensities by the nutation method are strongly dependent on $e^2qQ/h$, and therefore it is difficult to extract quantitative information about relative site populations from MQ spectra obtained with this approach. It is anticipated, however, that RIACT should be less sensitive to the quadrupole couplings provided that the adiabatic condition is approximately satisfied. Calculated results for the dependence of RIACT upon the magnitude of $e^2qQ/h$ are presented in Figure 3.19, where the carrier frequency is adjusted to the isotropic quadrupole-induced shift for every quadrupole coupling. Both the 3Q excitation and 3Q-to-1Q conversion processes are much less sensitive to variations of $e^2qQ/h$ than those from the nutation method. For example, on varying $e^2qQ/h$ from 1.0 to 4.0 MHz (typically found for 23Na), the 3Q excitation by RIACT ($\tau_{sl} = \tau_r / 4$ and $\omega_r / 2\pi = 100$ kHz) is attenuated by less than 10%. In contrast, over the same range of $e^2qQ/h$, the 3Q excitation by the nutation method is attenuated by approximately a factor of 4 (see Figure 3.18).

Four additional points should be mentioned about Figure 3.19. First, superior results are always obtained for $\tau_{sl} = \tau_r / 4$. In contrast, for $\tau_{sl} = \tau_r / 2$, the numerical calculations (not shown) indicate that both 3Q excitation and 3Q-to-1Q conversion efficiencies decrease significantly in the range of $e^2qQ/h = 2.5 - 4.0$ MHz.
Figure 3.19. Calculated results for 3Q excitation (top) and 3Q-to-1Q conversion (bottom) by RIACT ($T_{SL} = \tau, /4$) as a function of $e^2qQ/h$ at different radio frequency power levels. The sample spinning frequency is 10 kHz.

Second, compared with the results from the nutation method (Figure 3.18), RIACT exhibits substantially higher 3Q-to-1Q conversion efficiency, but lower 3Q excitation efficiency. Therefore the RIACT(II) sequence is capable of providing reliable information about site populations, and the RIACT(I) sequence should have the best sensitivity in terms of the signal-to-noise ratio. Third, the calculated curves for 3Q excitation and 3Q-to-1Q conversion via RIACT are approximately identical. This is a consequence of the symmetry of RIACT. Finally, for larger $e^2qQ/h$ values, a smaller fraction of crystallites satisfy the adiabatic condition, and therefore the overall RIACT efficiency declines.
As Vega noted previously,[63] the RIACT process between 3Q and 1Q coherences is also sensitive to resonance off-sets. In particular, an off-resonance radio frequency field would convert $I_{x}^{(23)}$ to $I_{z}^{(14)}$ rather than to the 3Q coherence, $I_{x}^{(14)}$, because, under the off-resonance condition, $|t_{+}\rangle$ and $|t_{-}\rangle$ are no longer the eigenstates of the spin system. Potentially, this could be a severe limitation of the RIACT method. However, it should be noted that the first-order degeneracy of the two eigenstates, $|t_{+}\rangle$ and $|t_{-}\rangle$, is actually lifted by the presence of the spin-locking field[64]; therefore $|t_{+}\rangle$ and $|t_{-}\rangle$ will remain as eigenstates of the system provided that the resonance offset, $\Delta \omega$, is smaller than the energy difference between $|t_{+}\rangle$ and $|t_{-}\rangle$, i.e.

$$\Delta \omega < \frac{1}{2} \left( \frac{\omega_{1}}{\omega_{0}} \right)^{2} \omega_{1}$$

(21)

Figure 3.20. Calculated offset dependence of 3Q-to-1Q conversion by RIACT ($\tau_{SL}=\tau_{s}/4$) as a function of the quadrupole coupling $e^{2}qQ/h$ (top) and the r.f. field strength $\omega_{r}/2\pi$ (bottom). Top: $\omega_{r}/2\pi=80$ kHz. Bottom: $e^{2}qQ/h=1.8$ MHz.
To better understand the limitations of the RIACT method arising from the resonance offset effect, we carried out several numerical calculations, the results of which are presented in Figure 3.20. As expected, the 3Q-to-1Q conversion efficiency is decreased as the resonance offset increases. Again, compared to $\tau_{sl} = \tau_r/2$, $\tau_{sl} = \tau_r/4$ is found to be a better choice since it is less sensitive to the resonance offset. It is also noted in Figure 3.20 that for larger $e^2qQ/h$ values, the maxima are shifted to lower frequencies as a result of the second-order quadrupole shift. Therefore, if there are several sites present in a sample, it is recommended that the spin-locking field be set approximately to the center of the powder line shape arising from the site with the largest quadrupole coupling constant. A striking feature of the results shown in Figure 3.20 is that with high spin-locking fields, the 3Q-to-1Q efficiency becomes much less sensitive to the resonance offset, a result in agreement with the prediction of Equation 21. Based upon the numerical calculations, it can be concluded that, for $e^2qQ/h<3.0$ MHz, the reduction in 3Q-to-1Q conversion efficiency arising from a resonance offset of 4 kHz (corresponding to 38 ppm for $^{23}$Na nuclei at 9.4 T) is less than 10%. With a spin-locking field of 160 kHz, however, the RIACT method can be extended to handle an offset range of more than 12 kHz.

$^{23}$Na NMR spectra of solid $\text{Na}_4\text{P}_2\text{O}_7\cdot10\text{H}_2\text{O}$ (2) are shown in Figure 3.21. The static $^{23}$Na NMR spectrum (Figure 3.21a) and the X-Ray crystal structure[78] of 2 suggest the presence of two crystallographically distinct Na sites with different quadrupole coupling constants. It is clear from the MAS spectrum (Figure 3.21b) of 2 that the Na(1) site has a much smaller quadrupole coupling constant than Na(2). The crystal structure of 2 indicates that one Na site is in an octahedral environment with six oxygen atoms from pyrophosphate groups, while for the other Na octahedron, two of the six oxygen atoms are from water molecules with rather long Na---O distances.[78] Therefore, based upon the magnitude of the quadrupole coupling constants, Na(1) is assigned to the Na site surrounded by six oxygen atoms from pyrophosphate groups.
In each of the $^{23}$Na MQMAS spectra of 2 (Figure 3.21c-e), two well-resolved isotropic lines with widths of 180 Hz are observed. As Medek et al.[24] and Wu et al.[30] demonstrated, accurate information concerning chemical shifts and quadrupole parameters can be extracted directly from two-dimensional (2D) MQ spectra. From the 2D $^{23}$Na MQ spectra of 2, the following parameters were determined: Na(1), $\gamma_{iso}=0.9\pm1$ ppm, $e^2qQ/h \leq$ MHz; Na(2), $\gamma_{iso}=4.8\pm1$ ppm, $e^2qQ/h=2.00\pm0.05$ MHz, $\eta=0.2 \pm 1$. It is noted from Figure 3.21 that different line positions are observed in the static and MQ spectra. More specifically, whereas the signal from the Na(1) site is found to appear at higher frequency than that from Na(2) in the static spectrum, it is at a lower frequency position in the MQ spectrum. Such changes of line position in MQ spectra have been previously discussed.[30]

According to the crystal structure of 2[78], the two Na sites are equally populated in the unit cell. However, in the $^{23}$Na MQMAS spectrum obtained with the nutation method (Figure 3.21c), the line arising from the Na(2) site is of significantly less intensity than that
from Na(1). The ratio of integrated area under the two lines is Na(1):Na(2)=1:0.12. As noted above, this is due to the lower excitation efficiency of Na(2) which has a larger quadrupole coupling constant. With the RIACT(II) sequence, however, the two lines have approximately equal intensities, Na(1):Na(2) =1:0.87 (Figure 3.21e). It is also worth noting that, as the isotropic peak intensity of Na(2) is enhanced by the RIACT sequence, the 2\'nd low-frequency rotational sideband associated with the Na(2) site (ca. – 8kHz) becomes observable. Finally, as expected, the spectrum obtained with the RIACT(I) sequence (Figure 3.21d) demonstrated the best overall sensitivity but is not quantitative.

To further test the applicability of the RIACT technique in obtaining quantitative MQMAS spectra, we investigated a series of sodium salts, each of which consists of crystallographically distinct Na sites with a wide range of quadrupole coupling constants. Detailed results from quantitative analysis of the \(^{23}\text{Na}\) MQ spectra of trisodium citrate dihydrate, \(\text{Na}_3\text{C}_6\text{H}_5\text{O}_7\cdot2\text{H}_2\text{O}\) (3), anhydrous \(\text{Na}_2\text{HPO}_4\) (4), and \(\text{Na}_2\text{B}_4\text{O}_7\cdot10\text{H}_2\text{O}\) (5) are listed in Table 3.2. and the spectra are graphed in Figure 3.22.

<table>
<thead>
<tr>
<th>Site</th>
<th>Site</th>
<th>(\varepsilon^2qQ/\hbar^a)</th>
<th>(\eta^b)</th>
<th>(\delta_{iso}(\text{ppm}))</th>
<th>Theory(^c)</th>
<th>Nutation</th>
<th>RIACT-I</th>
<th>RIACT-II</th>
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<td>0.2</td>
<td>4.8</td>
<td>0.12</td>
<td>0.45</td>
<td>0.87</td>
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<td>1.36</td>
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</tr>
</tbody>
</table>

\(^a\) Errors are estimated to be 0.10 MHz  
\(^b\) Errors are estimated to be 0.1  
\(^c\) From crystallographic symmetry of the unit cell

Table 3.2. Quantitative results for \(^{23}\text{Na}\) MQMAS spectra of sodium salts.
Anhydrous Na₂HPO₄ (4) is an excellent test sample, which is known to consist of three crystallographically distinct Na sites with different quadrupole coupling constants[2]. The $^{23}\text{Na}$ MQ spectra of 4 have also been reported recently with both the three-pulse[14] and the two-pulse sequences[24, 30]. However, in all previously obtained $^{23}\text{Na}$ MQ spectra of 4, the signal arising from the Na(3) site was either absent[14] or weak[24, 30], since the Na(3) site exhibits a relatively large quadrupole coupling constant, $e^2 qQ/h = 3.7$ MHz[2]. This can be seen in Figure 3.22 where the $^{23}\text{Na}$ MQ spectrum of 4 obtained with the nutation sequence consists of three isotropic peaks with the integrated peak ratio Na(1):Na(2):Na(3) = 1.00:0.53:0.31. With the RIACT(I) sequence, both the Na(3) and Na(2) signals were enhanced. In addition the signal-to-noise ratio of the MQ spectrum was also improved, as expected from the earlier discussion. With the RIACT(II) sequence, the integral ratio observed in the MQ spectrum becomes 1.00:0.80:1.93, approaching the ratio expected from the crystal structure of 4[2], Na(1):Na(2):Na(3) = 1:1:2. Similar to the case of 2, significant intensity was observed at the second low-frequency rotational sideband associated with the Na(3) site. The $^{23}\text{Na}$ quadrupole parameters and chemical shifts in 4 were determined from the 2D MQ Spectra: Na(1), $\delta_{iso} = 4.5 \pm 1$ ppm, $e^2 qQ/h = 1.31 \pm 10$ MHz, $\eta = 0.2 \pm 0.1$; Na(2), $\delta_{iso} = 3.4 \pm 1.0$ ppm, $e^2 qQ/h = 2.04 \pm 10$ MHz, $\eta = 0.7 \pm 0.1$; Na(3), $\delta_{iso} = 8.8 \pm 1.0$ ppm, $e^2 qQ/h = 3.84 \pm 1$ MHz, $\eta = 0.3 \pm 0.1$. These results are in agreement with those previously determined from DOR and MAS spectra of 4.[2]
Figure 3.22. MAS and MQMAS spectra of a series of sodium salts. Results are summarized in Table 3.2. Typical nutation pulses were 6.0 and 2.5 μs for the first and second pulses, respectively. MAS rates were 10-12 kHz, and the RIACT times were always set to \( TSL = \tau/4 \). For the MQMAS spectra the \( \tau_t \) increments were 25.7 μs, and typically 96-150 \( \tau_t \) steps were collected.

As seen from Table 3.2. and Figure 3.22, the RIACT(II) sequence is capable of yielding reasonably reliable information about relative populations among crystallographically distinct sites. Two possible sources of error from the quantitative analysis of MQ spectra are worth mentioning. First, in order to prepare an initial spin state, \( \tau_s^{(23)} \), that contains accurate site population, a small excitation pulse[79, 80], instead of a 45-degree pulse, should be employed in the RIACT(II) sequence. However, this may attenuate the sensitivity of the MQ experiments. Second, if crystallographically distinct sites have different relaxation times in the rotating frame \( (T_{1p}) \), errors will be introduced into the peak intensities in MQ spectra, especially for long rotor cycles (i.e. slow spinning speeds). In the
present study, the spinning speeds are on the order of 10 kHz ($\tau, / 4 = 25\mu$s), where the ($T_{1p}$) effect is negligible for sodium salts.

In summary, we have described a new method of obtaining MQ spectra for $S=3/2$ nuclei, from which quantitative information on relative site populations can be extracted. The new method is straightforward to implement and is relatively insensitive to the magnitude of the quadrupole interaction for $e^2 qQ / h < 4$ MHz, which is the practical range of quadrupole coupling constants for $^7$Li, $^{11}$B, $^{23}$Na, and $^{87}$Rb nuclei. In addition, the RIACT method also enhances the sensitivity of MQ experiments over a large range of quadrupole coupling constants. We found that it is necessary to set the spin-locking time to $\tau, / 4$ rather than to $\tau, / 2$ in order to obtain the best experimental results.

**Figure 3.23.** Eigenstate diagram obtained from diagonalization of Equation 13 for a $S=5/2$ nucleus. The RIACT effect recouples the $5Q$ coherence with the central transition.

Finally, we note that the RIACT concept is general to coherence transfer between the central transition and the outermost symmetric multiple-quantum coherence of any half-integer quadrupolar nucleus during magic-angle spinning. This is illustrated by the eigenstate diagrams for $S=5/2$ in **Figure 3.23.**, which indicates that the $5Q$- and central-transition (1Q)
coherences interchange in the limits $Q/\omega_c >> 1$ and $Q/\omega_c << -1$. The RIACT effect for a $S=5/2$ nucleus is seen in simulations monitoring the $5Q$- and $1Q$-coherence during spin-locking, as shown in Figure 3.24. Recently, $5Q$-MQMAS obtained via RIACT have shown considerable promise for $^{27}$Al in zeolite systems\(^6\). It must be emphasized that RIACT only recouples the outer MQ coherence with the central-transition. The use of transverse pulses to recouple coherences other than these in $S\geq5/2$ nuclei will result in some residual transfer (e.g. $3Q$ coherence in $S=5/2$), but the method is inefficient and cannot be explained as a RIACT transfer but rather as a complex superposition of nutation effects with rotation-induced population transfers.

\[ S=5/2 \]

![Simulation of RIACT transfer under 10 kHz MAS between the central-transition and the five-quantum coherence for a $S=5/2$ nucleus with a Larmor frequency of 54 MHz with the quadrupole parameters $e^2qQ/h = 1.5$ MHz, $\eta = 0.0$. This situation corresponds to oxygen-17 at 9.4 T, where a smaller quadrupolar coupling is chosen to illustrate the RIACT transfer. The carrier is adjusted to be on-resonance with the total isotropic quadrupolar shift, and $\omega_c$=100 kHz. (1000 crystallites)](image)

\(^6\) Private correspondences with Prof. Dominique Massiot and Prof. Jean-Paul Amoureaux, 1998.
3.6. Performance Characteristics of Adiabatic Coherence Transfer

3.6.1. Revisiting the Adiabaticity Parameter

The phenomenon of rotation-induced adiabatic coherence transfers has so far been used for interconverting coherences in half-integer quadrupolar nuclei. However a detail has not been discussed so far is that Vega classified spin-locking phenomena into three general types of behavior [63], parameterized by the unit-less adiabaticity number

$$\alpha = \frac{\omega_i^2}{\omega_q \omega_R},$$

In the ‘sudden –passage’ regime, $\alpha<<1$, the MAS modulation of the Hamiltonian is fast relative to the magnitude of the RF field in the rotating frame. The motion of the Hamiltonian changes so fast (i.e. ‘suddenly’) that the nuclear spin states do not follow the eigenstates. This leads to effective spin-locking of the coherence along the r.f. field. This case will be examined again in the context of the cross-polarization experiment in Chapter 4. At the other extreme is the ‘adiabatic’ regime, in which the Hamiltonian changes slowly relative to the r.f. field strength, $\omega_i$, and the nuclear spin states continuously track the eigenstates. This behavior results when $\alpha>>1$ and is useful in the context of MQMAS, which is the subject of this Chapter. Finally, when $\alpha\sim1$, an intermediate condition should result in which the spin states are neither locked nor undergoing coherent adiabatic passages but are in some poorly defined intermediate state that is not well modeled in this simple picture. These three cases are experimentally illustrated for $^{23}$Na in NaNO$_2$ in Figure 3.25, where three different r.f. powers were used to create the three different spin-locking conditions. Also, a 1.5 kHz offset is introduced (dashed lines) to illustrate the perturbation to the eigenstates due to a resonance offset. These experiments reproduce well the three regimes predicted by the adiabaticity parameter, $\alpha$. 
Figure 3.25. Spin-locking profiles measured for $^{23}$Na in NaNO$_2$. Using the pulse sequence of Figure 3.15a. Three regimes of alpha are (a) sudden-passage, (b) intermediate, and (c) adiabatic, and were observed by adjusting the magnitude of the applied transverse RF field. MAS rate is approx. 10 kHz, giving a rotor period of 100 μs.

In Figure 3.25a the coherence is almost completely spin-locked, while in Figure 3.25c there is a steady beating observed between the single- and triple-quantum subspaces. And Figure 3.25b shows the intermediate state in which neither of the extreme behaviors governs the spin dynamics. These observations therefore confirm Vega’s approximations in applying the adiabaticity theorem to the spin-locking problem.

As shown in section 3.5, a number of crystallites will behave in the adiabatic passage regime even when $\alpha \sim 1$, which is seen by a residual beating in Figure 3.25b. This can be understood by recalling that the quadrupole frequency ($Q$ in Equation 13-14) is dependent
upon crystallite orientation. A distribution in $Q$ (i.e. $\omega_Q$) corresponds to a distribution in $\alpha$. Another interesting result from section 3.5 is that the influence of the resonance offset on the eigenstates was not a serious limitation when using moderate to high r.f. field strengths to interconvert MQ and central transition coherence. In spite of the excellent predictive power of $\alpha$, we have shown two examples of how the experimental performance of RIACT deviates from qualitative theoretical predictions. We conducted a more detailed investigation of RIACT characteristics to try to gain improved insight into quadrupolar spin dynamics. In the following, two additional characteristics of RIACT are presented which again contradict predictions that are based solely on $\alpha$. We are led to adopt a picture in which RIACT and nutation effects are competing, and use this to develop an amplitude-modulated, or shaped, RIACT pulse which delivers greater sensitivity and more accurate site quantitation in multiple-quantum experiments.

### 3.6.2. Fast MAS Suppresses Nutation and Enhances RIACT

The adiabaticity parameter, $\alpha$, is inversely proportional to the MAS frequency, $\omega_r$. We would expect that, as the MAS frequency increases and $\alpha$ becomes smaller, the efficiency of adiabatic passages should decrease. Such a situation is of concern since it is critical to reach high MAS rates in quadrupolar NMR so that the spinning sidebands do not overlap with the centerband line shape, which is generally on the order of 5-20 kHz. These spinning frequencies are routinely available with commercial spinning assemblies that employ 3.2 mm and 2.5 mm (outer diameter) sample rotors which are able to achieve rates of 25-32 kHz. There are two additional benefits of the reduced dimensions that are contributing to an increasingly widespread adoption of these ultra-fast MAS systems. First, sample size requirements are much smaller, allowing for the study of just a few milligrams of a typical solid. Second, the decreased coil cross-section means it is easier to achieve higher flux densities inside the coil for the applied r.f. power. For these reasons, such miniature sample spiners are very desirable for nearly all modern SSNMR experiments. It is therefore critical to develop MQ coherence transfer methods which are robust under very fast MAS.

Several tests were conducted to investigate the effect of the MAS rate on RIACT efficiency. First, the RIACT offset performance for 3Q coherence excitation was numerically
evaluated for MAS frequencies of 5 kHz, 10 kHz, and 20 kHz, shown in Figure 3.26. In Figure 3.26, the calculations indicate a significant improvement in the offset performance of 3Q excitation with respect to the MAS frequency. Further, there is little change in the on-resonance efficiency.

![Diagram](image)

**Figure 3.26.** Numerical Simulation of 3Q excitation for $S=3/2$ via RIACT for 5, 10 and 20 kHz spinning frequencies, where the 3Q coherence is detected at $\tau_{sl} = \tau_r / 4$ over a range of offset values as indicated in the figure. Extensive testing for convergence was used to minimize the number of crystal orientations to 200; $\omega_c/2\pi = 100$ kHz, $\eta = 0.0$, $e^2qQ/\hbar = 3.0$ MHz. A Larmor frequency of 105 MHz was used, corresponding to $^{23}$Na at 9.4 Tesla.

The simulations suggest that faster MAS may result in higher overall 3Q excitation by recovering MQ coherence from a larger percentage of the crystallites which contribute to the central-transition line shape. This can be tested looking at the effect that varying the MAS rate has for RIACT efficiencies in nuclei with different quadrupole couplings. Calculations of 3Q excitation as a function of MAS rate for an $S=3/2$ nucleus with $e^2qQ/\hbar = 2.0$ MHz and with $e^2qQ/\hbar = 4.0$ MHz are shown in Figure 3.27. It is seen that changing the MAS rate has a more pronounced effect for the curve representing the 4 MHz quadrupole coupling constant.
Figure 3.27. Calculated 3Q excitation efficiencies for nutation and RIACT methods as a function of MAS spinning frequency. Curves are graphed for $\hat{e}^2Q/h=4.0$ MHz (open circles) and for $\hat{e}^2Q/h=2.0$ MHz. The left axis is arbitrarily scaled to the maximum 3Q efficiency for $\hat{e}^2Q/h=2.0$ MHz; $\omega_f/2\pi=100$ kHz, $\eta=0.0$. An excess of crystallite orientations, 2000, was used to eliminate any doubt of insufficient powder averaging. The r.f. was applied at 0 kHz, thus introducing resonance offsets into both sets of curves (2.0 MHz and 4.0 MHz).

We already know from Figure 3.20 that the tolerance to resonance offsets decreases with increasing quadrupole coupling. In other words, any improvement in the offset performance will benefit sites with larger quadrupole couplings more than sites with smaller couplings. Thus, for the moderate 2.0 MHz coupling in Figure 3.27, the RIACT offset profile is already sufficient to span the central transition line shape and increasing the offset performance should not change the total sensitivity. For a 4 MHz quadrupole coupling, the offset profile spans 2-3 kHz ($\omega_f/2\pi=10$ kHz, data not shown) while the central transition line shape has a breadth of about 10 kHz; thus broadening the RIACT offset curve with fast MAS should improve the overall efficiency. This qualitative prediction is well reproduced in Figure 3.27.

The presence of non-zero 3Q coherence via RIACT in the static limit is attributed to nutation effects. From Figure 3.27 we conclude that RIACT efficiencies for nuclei with large quadrupole couplings in fact increase for MAS rates up to 20 kHz before starting to decline.
Since *both* $\omega_1$ and $\omega_2$ appear in the denominator of $\alpha$, this effect of improved adiabatic passages occurs for decreasing $\alpha$! The nutation method is also calculated in Figure 3.27 where the efficiency of 3Q excitation by nutation decreases almost linearly from the static case. A significant observation from Figure 3.27 is that 3Q excitation via RIACT at $\omega_1 > 27$ kHz is higher than that for nutation. We wish to attempt to experimentally verify the trends predicted in Figure 3.27, however it is not possible to design an experiment which exactly reproduces the conditions in Figure 3.27.

Figure 3.28. Measurement of the total recovery of MQ coherence via different transfer methods at 9.4 Tesla for (a) $I_x$-3Q-1Q transfer in $^{23}$Na$_2$SO$_4$ ($S=5/2$, $\omega_Q = 433$ kHz, $\eta = 0.6$) and (b) $I_x$-5Q-1Q transfer in $^{17}$O in Hydroxyapatite ($S=5/2$, $\omega_Q = 240$ kHz, $\eta = 0.0$) versus MAS frequency, and comparing nutation and RIACT schemes. RF field strengths were about 150 kHz ($^{23}$Na) and 100 kHz ($^{17}$O). RIACT spin-lock times were set at $\tau/4$; nutation pulses were optimized at each spinning frequency.
However, we can still measure the influence of MAS rate on coherence transfers by observing the total signal recovered for the first slice of an MQMAS experiment. This monitors the amount of coherence that survives 3Q excitation and reconversion, beginning from initial $I_s$ populations. The results for two model systems are given in Figure 3.28. Even though the approach in Figure 3.28 is an approximation to Figure 3.27, the validity of this approximation is evident in the good agreement between the RIACT curves in Figure 3.27 and 3.28, which increase in overall sensitivity up to MAS rates of 20 kHz, and for $S=3/2$ and $S=5/2$ nuclei. Poor signal-to-noise for 5Q excitation and reconversion by nutation in Figure 3.28b obscures our ability to see trends in the MAS dependence in this case. Based on these simulations and results we conclude that faster MAS broadens the offset performance of RIACT, improving signal intensities for samples with large quadrupole coupling constants.

There are two effects resulting from magic-angle spinning. The first is to cause partial averaging of the term that recouples the single and triple quantum subspaces, while the second is the continuous modulation of eigenstates. Both effects will decrease nutation efficiencies. The use of high-power r.f. nutation pulses to excite multiple quantum coherences has been reported by several authors [11, 12, 25] and can be predicted analytically through a fictitious spin-1/2 operator analysis of the Hamiltonian that includes the lowest-order term only ($H_Q^{(0)}$). Nielsen and coworkers have examined the nutation of the central transition under MAS and observed it to be strongly dependent on the MAS frequency[26, 27]. This is to be expected since MAS causes $H_Q^{(0)}$, the term that induces nutation, to oscillate about 0. And, as we have already studied extensively, MAS also leads to adiabatic passages (RIACT) that are useful for interconverting coherences in MQMAS experiments.

A qualitative physical picture for understanding the improved RIACT efficiencies at higher MAS rates is that, as the MAS rate increases, some crystallites that would nutate under static or slow-MAS conditions into other coherences, such as double-quantum coherence for example, instead undergo adiabatic passages.
An additional comparison of the performance of RIACT at fast MAS with nutation is shown in Figure 3.29 with $^{23}$Na MQMAS spectra of the salt Na$_2$HPO$_4$, acquired with the RIACT-II and two pulse nutation MQMAS sequences. In Figure 3.29a, the nutation method at $\omega/2\pi=19.46$ kHz gives incorrect relative site populations which are known from crystallographic studies to be Na(3):Na(2):Na(1)=2:1:1. The RIACT-II sequence at $\omega/2\pi=19.46$ kHz (Figure 3.29b), also utilizing rotor-synchronized acquisition, dramatically improves the intensity for Na(3). Just from inspection it can be appreciated that the correct intensities, Na(3):Na(2):Na(1)=2:1:1, are recovered. The two-pulse nutation scheme provides higher sensitivity only for the site with the smallest quadrupole coupling, Na(1). Although the MAS rates in Figure 3.29 are below the 27 kHz threshold predicted earlier, the RIACT-II method at 19.46 kHz has been demonstrated to be a quantitative technique at fast
MAS as well as competitive with the sensitivity achieved via nutation.

To summarize this section, we conclude that RIACT is a sensitive method for interconverting coherences under very fast MAS, in contrast with the decreasing sensitivity of the nutation approach. This aspect of RIACT is not predicted by the adiabaticity parameter, $\alpha$. However, since the nutation approach is a property of the low-order Hamiltonian ($H_Q^{(0)}$) in static half-integer quadrupolar nuclei, we have confirmed and extended results observed by others that MAS interferes with the nutation process. It is anticipated that the use of very high spinning frequencies will increase as samples exhibiting increasingly large quadrupole coupling come under scrutiny.

### 3.6.3. Amplitude-Modulated Pulses Improve RIACT

From section 3.6.2, a picture of coherence transfer for half-integer quadrupolar nuclei in rotating solids as a combination of competing nutation and RIACT effects was formed. We now introduce a second unusual aspect of RIACT and will see that this is again a result of competing nutation and RIACT effects.

![Figure 3.30](image)

**Figure 3.30.** Comparison of RIACT 3Q excitation efficiency versus quadrupole coupling at 100 kHz and 150 kHz field strengths shows that higher power results in poorer efficiency for small $e^2qQ/h$. $\omega_0$=10 kHz.
As we have shown in Figure 3.19, increasing the power of the spin-locking pulse improves the transfer efficiency for RIACT for large quadrupole couplings. But an aspect of that figure which was not discussed was the efficiency of RIACT for nuclei with smaller quadrupole coupling constants. Figure 3.30 focuses on this issue for RIACT spin-locking with $\omega_r/2\pi = 100$ kHz and 150 kHz. An unexpected result is encountered where, for small quadrupole couplings, RIACT transfer is more efficient for the lower RF field strength (100 kHz). Increasing the r.f. field increases $\alpha$, implying that a greater number of crystallites should undergo adiabatic passages. But this does not appear to be the case. To consider how nutation effects might explain the behavior in Figure 3.30, recall that the 3Q transfer by nutation (Figure 3.18) is strongest for nuclei with small quadrupole couplings, and also exhibits a steep dependence on $\omega_r$. These nutation effects can be seen in simulations of RIACT transfers, shown in Figure 3.31, where the appearance of high frequency wiggles become more significant with higher r.f. power.

![Figure 3.31](image_url)

**Figure 3.31.** Calculations of 3Q coherence during r.f. spin-locking for an $S=3/2$ nucleus with $e^2qQ/h=1.0$ MHz, $\eta=0.0$, $\omega_r/2\pi=10$ kHz, for one rotor period ($\tau_r = 100 \mu s$). $\omega_r/2\pi = (a) 50$ kHz, (b) 100 kHz, and (c) 150 kHz. In (a)-(c) the increase in nutation with r.f. field is seen with the increase in the beating pattern superimposed on the RIACT transfer. r.f. power is ramped in (d) from 0-150 kHz.
It should be noted from Figure 3.31 that the frequency of the beating increases with the applied r.f. field, as expected for nutation. We can use this to explain why the RIACT efficiency in Figure 3.30 actually decreases in the region of $e^2 q Q / h = 0.2 - 2 \text{ MHz}$ when the r.f. power (and thus $\alpha$) is increased. We see that increasing the r.f. power causes more spins with small quadrupole couplings to undergo nutation, detracting from the RIACT efficiency. This sacrifices overall sensitivity and also compromises the quantitative performance of RIACT. In Figure 3.31b we apply a ramped RIACT profile and observe that the nutation effects have been almost completely removed, even though the maximum power at the finish of the r.f. ramp is 150 kHz. By comparing Figure 3.31a and (d), we see that the ramped RIACT pulse has even less interference from nutation effects than the very low power $\omega_r/2\pi = 50$ kHz flat r.f. RIACT pulse. The maximum power levels for all four RIACT curves can be compared with the result that the ramped pulse achieves the highest maximum transfer efficiency. So we conclude that suppressing nutation effects in RIACT can improve sensitivity for crystallites which experience interference from nutation.

**Figure 3.32.** Comparison of the quadrupole coupling profile calculated for a shaped RIACT pulse, set to $\tau_{SL} = \tau / 3$, to the profiles of Figure 3.30. Quantitation is restored to the region of small $e^2 q Q / h$. 

\[
\text{Shape} = \sin(\pi t), \quad x = 0 - 0.8\pi
\]
Using this information, a ramped, amplitude-modulated RIACT pulse was empirically and experimentally optimized. Numerical simulations of the optimal shape with respect to the quadrupole-coupling profile are shown in Figure 3.32. Note that the timing for the transfer has been extended to $1/3\tau_r$, which agrees with the delayed maximum which resulted for the ramped spin-locking pulse in Figure 3.31. Figure 3.32 shows an improvement in sensitivity in the range $e^2qQ/h = 0.2-3$ MHz. The efficiency profile of the shape is very level for the region $e^2qQ/h < 1$ MHz; thus the enhancement from the shaped RIACT depends upon the quadrupole coupling in such a way as to restore the quantitative nature of RIACT in this region. We demonstrate this by using shaped pulses for both spin-locking times of the RIACT-II pulse sequence for $^{23}$Na in Borax (see Figure 3.33c). The MQMAS spectra acquired using constant-field pulses for both spin locking times in RIACT-II ($\tau_{sl}=\tau_r/4$) or using one shaped pulse for reconversion, do not reflect the correct 1:1 site intensities. Using shaped RIACT transfers for both steps ($S^2$-RIACT) results in an MQMAS spectrum with 1:1 peak heights as well as higher overall sensitivity.

![Figure 3.33](image)  
Figure 3.33. Use of constant-field and shaped RIACT for $^{23}$Na 3Q-MQMAS spectra of Borax ($Na_2B_4O_7\cdot10H_2O$): (a) two-pulse nutation MQMAS, (b) RIACT-I using one shaped pulse for reconversion, and (c) full $S^2$-RIACT which yields the correct 1:1 intensity. $\omega_p=100$ kHz, MAS=10 kHz. Quadrupole parameters obtained previously[81] are Na(2): $e^2qQ/h=0.92$ MHz, $\eta=0.2$; Na(1) $e^2qQ/h=0.58$ MHz, $\eta=0.0$. 
Figure 3.33 confirms one of the critical predictions of the calculations in Figures 3.31-32, that the enhancement is dependent on the quadrupole coupling in a way that leads to quantitative MQMAS spectra. As emphasized in Section 3.5, RIACT is a symmetric process with respect to the two steps of MQ excitation and reconversion. It was found that the best results were obtained when the pulse shapes were symmetrized; the symmetrized $S^2$-RIACT is shown later in Figure 3.34b.

3.6.4. A Comparison of Methods.

There is a critical need to optimize the efficiency of MQ coherence transfers to enable the study of quadrupolar nuclei in challenging samples such as biopolymers. Of equal importance is the need to examine how the quadrupolar coupling influences the efficiency and outcome of these MQ experiments. In the last several sections we have devoted much attention to ways of improving the signal-to-noise of MQ experiments while eliminating dependence on the quadrupolar coupling. We conclude this section and the chapter with a comparative study of the efficiencies of the MQ coherence transfer techniques currently in use in modern quadrupolar spectroscopy. The scope of the experiments shown in the section is limited to the $S=3/2$ case and results are shown for $^{23}$Na NMR in a NaNO$_2$ powder sample.

Experimental Details

All experiments were conducted on a 4.7 Tesla custom-designed spectrometer and a transmission-line probe utilizing a 5mm Chemagnetics (Varian Inc.) spinning assembly. The MAS rate was 6.6 kHz for all experiments, with high- and low-power pulses corresponding to nutation rates of 110 and 25 kHz respectively, as measured in NaBr. The low-power setting was used for pulses which selectively excite central transition coherence, while the high power setting was used for all coherence transfer steps for best efficiency.

The schemes for interconverting coherences that were tested in this section are illustrated in Figure 3.34. Four distinct processes were considered and are illustrated by the four cases (a-d). In many cases, these were mixed to determine optimal coherence transfer conditions. The earliest approach, used by Frydman et al. in their initial study, is a three-
pulse method for exciting multiple quantum coherence which is borrowed from solution-state methodology. This approach suffered from a poor overall efficiency and was unable to excite appreciable multiple quantum coherence for nuclei exhibiting medium to large quadrupole coupling constants. This method is no longer in use in the literature and will not be reported on here.

Figure 3.34. Four general methods for interconverting multiple quantum coherences in half-integer quadrupolar nuclei are illustrated in the context of an MQMAS experiment, where $t_i$ represents the incrementable delay during which MQ evolution takes place. Single, ‘nutation’ pulses may be used as shown in (a). Spin-locking RIACT pulses are shown in (b). Sine-shaped ($S^2$) RIACT, where $\tau_{SL}=.33\tau_r$ is shown in (c) and the fast-amplitude modulated (FAM) pulse scheme is shown in (d).
We briefly review these four approaches and then compare the efficiencies of the methods with experimental data. All of the experiments in Figure 3.34 may be modified to include a z-filter before acquisition which, in combination with the States-Ruben-Haberkorn approach of acquiring separate real and imaginary FIDs, will result in pure-phase 2D spectra. Figure 3.34a shows a two-pulse ‘nutation’ scheme, where a strong r.f. pulse recouples the initial Zeeman population to MQ coherence. The nutation method is particularly effective for exciting MQ coherence for moderate quadrupole couplings, and efficiencies are usually 40-80% of the central transition Zeeman polarization. The reconversion of MQ coherence to central-transition coherence by nutation is very inefficient, and typical recovery is 10-20%. Both steps are very sensitive to the magnitude of the quadrupole coupling and their efficiencies decrease rapidly with increasing quadrupole coupling.

This problem was addressed by Wu et al. in 1996 with the introduction of rotation-induced adiabatic coherence transfer (RIACT), first proposed by A. Vega. As RIACT is an adiabatic process, this method is very insensitive to the quadrupole coupling. The MQ coherence excitation and reconversion steps are symmetric and efficiencies are typically 30-40% each. RIACT suffers principally from mediocre offset performance which can attenuate the efficiency when the large quadrupole coupling results in a very broad MAS line shape. However strong RF pulses (100-150 kHz) overcome this limitation. Being insensitive to the quadrupole coupling, MQMAS spectra obtained using RIACT pulses in both steps, as shown in Figure 3.34b, can be considered quantitative within 5-10% error.

The RIACT approach contrasts the nutation approach. RIACT performs best for moderate to large quadrupole couplings which are needed for the transfer to take place. For nuclei with smaller quadrupole couplings, nutation is very favorable and competes directly with the RIACT process. Thus, for small quadrupole couplings \( (e^3qQ/h < 1.0 \text{ MHz}) \), the RIACT process ceases to be quantitative and suffers from poor transfer efficiencies. A shaped pulse scheme was developed to improve the performance of RIACT for sites with small quadrupole coupling constants. A sine shape which ramps the r.f. power causes crystallites which would normally be nutated to participate instead in the RIACT process. This pulse scheme is illustrated in Figure 3.34c. This sine-shaped \((S^2)\)-RIACT utilizes an average power that is less than the constant-amplitude RIACT pulse and has the effect of restoring MQ transfer efficiency for sites with small quadrupole couplings by suppressing
competing nutation effects. In samples that consist only of sites with small $e^2qQ>h$, r.f. powers of 100 kHz are suitable for realizing the improvement. However the lower average power means that the offset dependence is exacerbated for sites with large quadrupole couplings, and so larger r.f. fields (120-150 kHz) are needed in such cases. The beneficial effect of $S^2$-RIACT is illustrated for NaNO$_2$ in Figure 3.35. In this case, an 11 % enhancement versus constant amplitude RIACT is realized. In fact the improvement can be seen to be even higher as the sideband intensities are clearly increased in the $S^2$ spectrum.

![Figure 3.35](image)

Figure 3.35. ($^{23}$Na) 3Q-MQMAS spectra of NaNO$_2$ at 4.7 Tesla comparing constant amplitude(CW) and shaped(SHP) implementations of the RIACT(II) pulse scheme for exciting and reconverting multiple quantum coherence using the spin-locking time of one-third of a rotor period. $\omega/2\pi 6.6$ kHz.

In cases where the quadrupole coupling is small, spin-locking times of $0.33\tau_r$ result in a slight improvement (data not shown) for constant-field RIACT. Thus the 11% enhancement is only a lower bound when compared to RIACT timed to $1/4\tau_r$.

A new approach for interconverting coherences was introduced by Vega et al. at the 1999 Experimental Nuclear Magnetic Resonance Conference (ENC) in Orlando, Florida, and was demonstrated for the reconversion of 3Q to central transition coherence for $^{23}$Na in model
compounds. The scheme is illustrated in Figure 3.34d where a nutation pulse is used for creating MQ coherence and a fast-amplitude modulated pulse follows the MQ evolution. The frequency profile of this pulse is such that the average power at the r.f. carrier frequency is zero but non-zero at frequencies of $\pm 1/\tau_c$ relative to the carrier, where $\tau_c=4\delta$ is the period of one FAM unit. This can be thought of as irradiating the side transitions[76, 77], but a theoretical description is outside the scope of this report; please see the mentioned references for more details. However Vega et al. examine the eigenstates in a tilted frame and show that this process is adiabatic. As such, it should be insensitive to the quadrupole coupling and be advantageous for replacing the low-efficiency nutation method for MQ coherence reconversion. In particular Vega et al. have reported a three-fold improvement over nutation and claim that accurate site quantitation is achieved with this FAM method.

![Figure 3.36](image)

*Figure 3.36.* $({}^{23}\text{Na})$ 3Q-MQMAS spectra of NaNO$_2$ at 4.7 Tesla (200 MHz for 1H) at 6.6 kHz MAS. A comparison of MQ coherence transfer schemes is given, where 'NUT' indicates a short nutation pulse, RIACT is a spin-locking pulse for one quarter or one third of a rotor period as indicated, and FAMx5 is a 5-cycle Fast-Amplitude-Modulated pulse.
In the following we now examine experiments which employ the four concepts discussed previously and evaluate the relative performance of these experiments. In Figure 3.36, a number of experiments are compared which were designed to optimize the overall efficiency for $^{23}$Na 3Q-MQMAS in NaNO$_2$ when 110 kHz r.f. fields are available. The double nutation approach, while a factor of 2-3 better than the three-pulse method discussed previously, is clearly seen to be poor for modern MQMAS spectroscopy, even in the favorable case of the small quadrupole coupling exhibited by this sample. Since 3Q excitation by the nutation method is still more effective than RIACT for small quadrupole couplings, three experiments are shown which combine nutation and RIACT for excitation and reconversion steps respectively. When the quadrupole coupling is low, RIACT is better for $1/3 \tau_r$ rather than $1/4 \tau_r$. Thus even a shaped pulse at $.25 \tau_r$ is slightly less sensitive than a CW pulse at $.33 \tau_r$, while the most sensitive NUT-RIACT approach is achieved with a shaped RIACT for $.33 \tau_r$. Finally, the nutation-FAM approach is found to give the highest signal-to-noise. However, Vega at al. are unable to recover correct site intensities for all three sites in Na$_2$HPO$_4$ where this is routinely achieved with a RIACT-II scheme for such r.f. powers. The most likely explanation is that, since Vega et al. reported FAM for 3Q reconversion only, the nutation pulse used for 3Q excitation compromised the relative 3Q excitation efficiencies. However an examination of the offset performance of the FAM pulse train has not been reported and this represents an area in which potential errors have not been ruled out.

Two other studies of the FAM approach were undertaken to investigate the experimental parameters of the FAM pulse train, since this method shows much promise in this preliminary study. Two aspects of the FAM cycle parameters were investigated and are shown in Figure 3.37. The dependence of the overall MQMAS signal intensity as a function of the subunit $\delta$ is shown in Figure 3.37a. It is found that the efficiency is largely insensitive to mis-settings of the FAM cycle period as there is a decrease in intensity of only 5% over the range $\delta = [1.2,1.6]$ $\mu$s. In Figure 3.37b, the sensitivity to the number of cycles is examined with a similar result that the FAM reconversion is very tolerant to any mis-setting. The overall MQMAS intensity varies by approximately 6-8% in the range $n=[2,6]$, where $n$ is the number as cycles as shown in Figure 3.37b. Thus, Figure 3.37 shows that the FAM
approach is a viable and robust method for reconverting MQ coherence into central transition coherence.

(a)

(b)

Figure 3.37. (a) $^{23}$Na 3Q-MQMAS spectra of NaNO$_2$ at 4.7 Tesla utilizing the Fast-Amplitude Modulated pulse scheme (FAM) consisting of 5 cycles and varying the time $\delta$ as indicated in the pulse sequence of Figure 1. The vertical scaling is arbitrary, however the intensity decreases by only 5% for $\delta = [1.2,1.6]$. (b) With $\delta$ fixed at 1.68 ms, this shows the relative MQMAS intensity for different numbers of the FAM cycles ($n$ in Figure 1).

Two outstanding issues remain to be considered at this point. The FAM method should also be applicable for exciting multiple MQ coherence when used after a selective $\pi/2$ pulse, as shown for RIACT in Figure 3.34b. This application of FAM, while straightforward, has not yet been demonstrated in the literature. On the other hand, more investigation into the quantitative nature of the FAM transfer efficiency is required since the data presented by Vega et al. do not appear to recover accurate relative site intensities in all cases.
In summary we see that the nutation method is clearly a poor choice for MQ reconversion, being 2-3 times less sensitive compared to RIACT and FAM methods. For best site quantitation, $S^2$-RIACT at moderate r.f. strengths is the most accurate reporter on relative intensities of inequivalent sites in the crystallographic unit cell. If relative quantitation is not required, then the highest S/N ratios in MQMAS experiments will be obtained using Nutation-FAM combination for moderate to small quadrupole couplings and Nutation-RIACT for moderate to large quadrupole couplings.
3.7. References


4. Multi-spin Correlation Methods Involving Quadrupolar Nuclei

Hetero- and homo-nuclear polarization transfer methods [1, 2] among spin 1/2 nuclei are routinely employed to circumvent long relaxation times (T1), enhance polarization, and determine structural parameters[3-5] and through-space connectivities [6, 7] in solids. In solid-state NMR experiments, the efficiency of the underlying dipolar transfer is, generally, explicitly dependent on the size of the dipolar coupling, and these experiments can also be useful for simplifying spectra of complex spin systems[8, 9].

But unlike the spin 1/2 situation, where the applied radio frequency (r.f.) field is the most important contribution to the spin system Hamiltonian, the quadrupolar coupling usually dominates the spin dynamics under static [10, 11] or MAS conditions [12, 13]. The quadrupolar interaction is generally not truncated by the Zeeman interaction even under the highest available spinning frequencies and magnetic fields [14-17]. The relative ease with which interactions may be turned ‘on’ and ‘off’ in the evolution periods of spin-1/2 experiments is due to the fact that the effects of RF irradiation can be described very simply for spin-1/2 nuclei. However, the application of π pulses and spin-locking fields in quadrupolar spin systems leads to non-trivial behavior which can only be treated exactly with
numerical calculations. Thus the development of correlation experiments in quadrupolar NMR has not matched the successes of spin-1/2 methods development.

Methods have been proposed to yield structural information either by dephasing and subsequent detection of spin-1/2 polarization in the presence of a ‘recoupled’ dipolar interaction to a nearby quadrupole [18, 19], or by exploiting the difference in longitudinal relaxation times [20] of a spin 1/2 nucleus and its quadrupolar partner. These techniques are most easily employed when spectral resolution of the spin 1/2 resonances permits the observation of each resonance individually. One particular drawback is that it is challenging to measure the quadrupolar interaction in these experiments.

As was motivated in Chapter 1, quadrupolar nuclei hold tremendous promise for illuminating the most significant regions of biological macromolecules, and this stresses the need for developing correlation spectroscopy and filtering experiments involving quadrupolar nuclei.

In this chapter, two new experiments are described for obtaining hetero- and homonuclear correlations in sections 4.1. and 4.2., respectively.

4.1. Multiple-quantum MAS Cross Polarization in Quadrupolar Spin Systems

4.1.1. Limitations of cross-polarization in quadrupolar NMR

One of the few correlation experiments currently available for quadrupolar spin systems is the cross-polarization (CP) method during magic-angle spinning (CP-MAS), in which polarization can be transferred to or from the central transition of a half-integer quadrupolar nucleus. Before and up to the development of MQMAS, many authors exploited CP to study connectivities in a variety of solids[21-26]. More recently, CP has been incorporated into MQMAS schemes to yield isotropic heteronuclear correlation (HETCOR) spectra for half-integer quadrupolar nuclei coupled to spin-1/2 species [27, 28]. But this
central-transition CP, termed here single-quantum CP, suffers from poor transfer efficiencies due to the challenge of spin-locking a quadrupolar nucleus during magic-angle spinning.

Specifically, the ‘sudden-passage’ condition,

\[ \alpha = \frac{\omega^2}{\omega \omega_0} \ll 1, \]

must be satisfied to spin-lock the central transition and this requires very low r.f. powers. In the sudden-passage limit, if the time dependence of the first-order quadrupole term in the Hamiltonian is fast compared to the rate of precession of the spin about the spin-locking field, then the eigenstates cannot follow the Hamiltonian and are considered ‘locked’. Because the well-known Hartmann-Hahn matching condition on the sidebands must be satisfied (\(\omega_{ij} \approx 2\omega_{1S} \pm n\omega_r\)) [12], very low r.f. powers must be employed on the paired nucleus as well. Both nuclei are therefore poorly spin-locked with r.f. fields that can easily be smaller than the linewidths of the resonances being studied [26], and the CP transfer does not achieve any enhancement of the central-transition polarization when compared to the signal following a simple selective 90° pulse. The CP-MAS signal has been observed to be on the order of 20-40 % of the signal obtained from a one-pulse experiment [12]. Further, as already stressed in Chapter 3, the two coherence transfer steps in MQMAS experiments will further attenuate the available signal when CP is combined with MQMAS. A method is desired which allows for the use of stronger r.f. powers on the spin-1/2 partner, and which improves the S/N ratio in a MQMAS-HETCOR experiment.

The method of multiple quantum cross polarization between an I=3/2 and an I=1/2 spin during magic angle spinning is presented to address these limitations. Experimental and theoretical results for \(^{23}\text{Na}-^1\text{H}\) pairs will be described that elucidate the transfer mechanism and the beneficial effect of amplitude modulations of the CP spin-locking pulse. Two applications of this approach, dipolar filtering and correlation, are described in the context of high resolution MQMAS spectra. We also demonstrate the improved transfer characteristics of an amplitude-modulated TQCP transfer.
4.1.2. Theory: Two Spins under RF Irradiation

In spin 1/2 applications, a theoretical analysis of the CP dynamics is often performed by considering an isolated \( I = 1/2, S = 1/2 \) spin pair [29]. In this case, strong radio frequency (r.f.) fields dominate all other interactions in the system Hamiltonian and the spin lock field can be described in a tilted rotating frame in which the eigenvectors are related to the high-field eigenstates by a simple rotation given by \( \exp[i(I_y + S_y)\frac{t}{2}] \). In this frame, the initial conditions \( I_x, S_x \) are diagonal which justifies the concept of polarization transfer under strong r.f. fields. The dynamics and matching conditions in an isolated two-spin system can easily be derived using average Hamiltonian theory [30] (AHT) or Floquet theory [31-33].

In contrast to the spin 1/2 case, Vega has shown [12, 13] that the quadrupolar contribution usually dominates the r.f. Hamiltonian for an \( S = 3/2 \) system, even during rapid MAS modulation. We first examine the effective Hamiltonian derived in section 2.2.5. to confirm the spin-lock behavior of an isolated quadrupolar nucleus as demonstrated by Vega[13] and then extend the treatment to two dipolar coupled spins under simultaneous r.f. irradiation. The effective Hamiltonian derived in section 2.2.5. for a single spin under r.f. irradiation is:

\[
\hat{H} = \omega_s (2C_x + \cos(\zeta(t)) R_z + \sin(\zeta(t))(S_x S_x + S_y S_y)).
\]  

As discussed in section 3.5, the RIACT phenomenon [34] is due strictly to the time-dependence of the lowest order quadrupolar Hamiltonian under magic-angle spinning. Since Equation 1 is obtained neglecting the high-order quadrupolar term, an appropriate test is to verify that Equation 1 leads to coherence transfer between central and triple-quantum coherence during magic-angle spinning. In Figure 4.1a the spin-lock behavior predicted by using the effective Hamiltonian of Equation 1 is compared to the result of an exact numerical simulation (Figure 4.1b) using the first and second-order secular quadrupolar Hamiltonians (\( e^2 qQ/\hbar = 1.2 \text{ MHz}, \text{MAS} = 6 \text{ kHz} \)). In the usual fashion, polarization transfer is measured by the expectation value determined from the equation of motion

\[
\langle A \rangle(t) = Tr\{A\sigma(t)\},
\]
with $A = T_x$ and $\sigma(t) = U(t)C_xU^{-1}(t)$. Here, the triple quantum coherence is defined by:

$$T_x = \frac{1}{2}\left(\langle \frac{3}{2}\rangle\langle \frac{1}{2}\rangle + \langle \frac{1}{2}\rangle\langle \frac{3}{2}\rangle\right).$$

\[ (3) \]

Figure 4.1. Comparison of a) evolution of triple-quantum coherence using the effective Hamiltonian given in Equation. (1) and b) the exact numerical prediction using the Hamiltonian of Equation. 2.37. MAS spinning frequency and r.f. field strength were set to 6 and 50 kHz, respectively. The offset was chosen to compensate the isotropic quadrupolar shift. A quadrupolar coupling of 1.2 MHz was assumed. Simulations were performed in the GAMMMA environment [35]. Comparison of triple-quantum coherence transfer using the effective Hamiltonian of Equation. (4) (c) and (d) using the exact Hamiltonian of Equation. 2.37 including the heteronuclear dipolar contribution as given in Equation. 4. A dipolar coupling of 1.5 kHz was assumed. An r.f. field strength of 94 kHz was employed on the I spin.
The MAS modulated quadrupolar coupling acts as a recoupling element that connects central and triple-quantum subspaces in a periodic manner. Also note from Equations 1 (and Equation 2.50) that at multiples of a rotor period \( t = T_r \), a non-vanishing amount of triple quantum coherence remains that is equivalent to the transfer under static conditions. In addition, the dependence of the transfer rate \( \zeta(t) \) upon the Euler angles \((\alpha, \beta, \gamma)\) determines the efficiency of the MQ exchange. This behavior is in close analogy to the case of hetero- or homonuclear dipolar recoupling of spin 1/2 nuclei under MAS.

Due to the explicit definition of the spherical tensor operators, the total Hamiltonian of a dipolar coupled (I=1/2, S=3/2) spin pair can easily be included in Equation 1. Since the heteronuclear dipolar contribution commutes with the zero'th order quadrupolar interaction, it is invariant to the interaction transformation and can be directly included in Equation 1 to give:

\[
\hat{H} = \omega_I I_x + D(t) I_z S_z + \omega_S (2C_x + \cos(\zeta(t))R_x + \sin(\zeta(t))(S_y S_x + S_z S_y)),
\]

where \( D(t) \) represents the MAS modulated dipolar coupling (e.g. Equation 2.43) with:

\[
D_{\pm 1} = \frac{D'}{2\sqrt{2}} \sin(2\beta_d)e^{\pm i\gamma_d},
\]

\[
D_{\pm 2} = \frac{D'}{4} \sin^2(\beta_d)e^{\pm 2i\gamma_d},
\]

and

\[
D' = -\frac{\gamma_k I_\gamma J \hbar}{4\pi r^3},
\]

where \( \beta_d \) and \( \gamma_d \) are the polar angles that describe the direction of the internuclear dipolar vector \( \mathbf{r} \) in a rotor-fixed coordinate system with its z axis along the sample rotation axis. \( \gamma_k \) represents the gyromagnetic ratio for spin I and S, respectively. Obviously, the spin operators are now given by the product base \(|m,n\rangle\) with \( m = \pm \frac{1}{2} \) and \( n = \pm \frac{1}{2}, \pm \frac{3}{2} \).

Based on the results of Equations 1-5 we will attempt to predict the CP dynamics for the cases of weak and strong r.f. fields applied to the S spins under simultaneous I spin irradiation (of strength \( \omega_I \)).
Weak r.f. fields. If we consider very small r.f. fields, we can neglect the r.f. perturbation contribution in Equation 4 and elucidate the coherence transfer dynamics by transforming into a tilted frame

$$\bar{H}^{T} = \exp[-i(\Theta_{s}I_{y} + \Theta_{s}C_{y})]\bar{H}\exp[i(\Theta_{s}I_{y} \Theta_{s}C_{y})],$$

(6)

with $\Theta_{s} = \Theta_{s} = \frac{\pi}{4}$. In this frame, the r.f. contributions are diagonal and time-independent as in the usual spin 1/2 situation. The Hamiltonian commutes with the initial condition $C_{x}$ and spin-locking is possible. Application of Floquet[36] or average Hamiltonian theory[37] then gives the zero and double quantum matching conditions,

$$\omega_{s} = 2\omega_{s} = n\omega_{r},$$

(7)

which result in the observable signal (central transition coherence)

$$\langle C_{x}(t) \rangle = \pm \frac{1}{2}[1 - \cos(d_{n}(\Theta_{r})t)]\sin(\Theta_{r})d\Theta_{r},$$

(8)

assuming $\sigma(0) = I_{x}$. Note that Equation 7 accounts for the effective nutation rate of the central-transition of the S spin. This behavior is analogous to transient oscillations observed both in the liquid [38] and the solid state of spin 1/2 systems [39]. Experimental results (data not shown) on a number of different $^{23}$Na compounds confirm the validity of this concept for quadrupolar species. Also note that in this approximation, no triple quantum coherence is generated. For these reasons, we will refer in the following to coherence transfer in the presence of small r.f. fields as single-quantum cross-polarization (SQCP) transfer. For increasing r.f. fields the zero and double quantum conditions of Equation 7 will be less defined, and we expect a superposition of positive and negative transfer amplitudes to attenuate the transfer efficiency.

Strong r.f. fields. We now turn to the case in which the time-dependent RIACT effect in Equations 1 and 5 cannot be neglected. As shown in the single spin $S = 3/2$ case, this regime corresponds to the case of strong r.f. fields. Additional insight might be gained by introducing a new tilted coordinate system (defined by $\frac{\pi}{2}I_{y} + \Theta_{s}(t)C_{y}$, where $C_{y}$ represents a transformation in which the r.f. term is diagonal). In this frame, the effective dipolar coupling will be further modulated by the time-dependent r.f. terms $\zeta(t)$, leading to additional TQCP matching conditions (i.e. $I_{x} \rightarrow T_{x}$ transfer) that may deviate from the result of Equation 17.
Straightforward diagonalization of the Hamiltonian of Equation 17 e.g. shows that the eigenvalues are now explicitly dependent on $\zeta(t)$. These non-zero contributions will also lead to an explicit dependence of the matching conditions upon the single crystallite orientation defined by the Euler angles given in Equations 5 and 2.10. As in the extreme case of NQR [40], TQCP transfer will also occur for non-integer multiples of the spinning frequency around the $n = 0, \pm 1, \pm 2$ matching conditions. Likewise, the buildup of triple quantum polarization will be governed by the combined modulation of RIACT quadrupolar transfer and the generally slower dipolar transfer $I \rightarrow S$. This behavior is illustrated in Figures 4.1c and 4.1d, again comparing the exact numerical results using Equation 2.37 (and including the $I$ spin terms of Equation 15) to the theoretical predictions of Equation 4. In this case, an $I,S$ dipolar coupling of 1.5 kHz was assumed and r.f. fields of $\omega_r = 94$ kHz and $\omega_s = 50$ kHz were employed. For a MAS spinning frequency of 6 kHz this condition corresponds to the $n = -1$ condition of Equation 7. Again, both Hamiltonians lead to similar transfer characteristics with a monotonic signal growth modulated by the RIACT transfer. In analogy to the low r.f. field case outlined previously, we will in the following use the term triple quantum cross polarization (TQCP) whenever the CP experiment is optimized for transfer steps involving triple-quantum coherence. This behavior could be further described by a ‘second averaging’ [41] approach in which a slow coherence transfer $I_x \rightarrow C_x$ (given by the dipolar coupling constant) at the SQ conditions of Equation 7 is followed by a fast (MAS dictated) RIACT-transfer from single to triple quantum coherence to generate the TQCP signal. Alternatively, Floquet theory could be used after a Fourier analysis of the MAS modulated r.f. field to elucidate the TQCP matching profile.

Additional simulations for a dipolar coupled $^1H-^{17}O$ spin system indicate that the same mechanism outlined above also leads to the creation of 5-quantum coherence in a $I=1/2$, $S=5/2$ spin system. Using the same simulation environment, it is also possible to observe the excitation of triple quantum coherence in a spin $I=1/2$, $S=5/2$ system such as recently reported by Ashbrook et al. [42]. However, our numerical results do not agree with the experimental findings of Ashbrook et al. [42] which might be explained by a combined influence of nutation and spin locking effects in a spin $5/2$ system.
4.1.3. Triple-Quantum Cross-Polarization Results

To test the theoretical predictions of the previous section, we have employed the pulse sequence depicted in Figure 4.2a, where 3Q coherence resulting from CP is immediately converted into observable central transition coherence by a RIACT pulse. We have generally observed a superior reconversion efficiency of the RIACT pulse in comparison to nutation approaches [1, 10, 11, 34, 43]. The phase of the spin-locking pulse on the S channel is cycled in 30° steps synchronously with phase cycling of the receiver by 90° steps to detect only 3Q coherence. Additionally, the sequence employs spin-temperature alternation [1] of the CP signal to suppress directly excited signals.

![Figure 4.2](image)

**Figure 4.2.** a) Pulse sequence to observe triple quantum cross polarization (TQCP). After an initial 90 pulse on the I spins (in our case \(^1\)H), polarization transfer occurs during a subsequent mixing time (typically 1 -10 ms). Triple quantum coherence is subsequently reconverted by P\(_2\), which may be a nutation or RIACT [34] pulse. b) Optimized TQCP experiment in the context of a high resolution MQMAS [44, 45] experiment. Adiabatic modulation of TQCP is introduced on the spin 1/2 (I spin) channel and a MQ evolution time (3Q, S=3/2) after TQCP mixing is added.

Experimental results on Na-Acetate are shown in Figure 4.3 for which we observe substantial triple quantum polarization transfer in the range \(\omega_{RF,1H} \in [80,120]\) kHz. After a mixing time of 2 ms, the resulting triple quantum signal was recorded as a function of proton
r.f. field strength. For comparison, numerical simulations using quadrupolar, dipolar and r.f. parameters as given in the figure caption are shown in Figure 4.3. The quadrupolar parameters ($v_Q = 1.6$ MHz, $\eta = 0.7$) were obtained using a Floquet analysis [14-17, 33, 46] in the GAMMA simulation environment [35]. The general trend is well reproduced although with a smaller total matching range. This discrepancy can be at least partially attributed to r.f. inhomogeneity effects that are known to significantly broaden the matching profile in spin 1/2 applications [39]. Moreover, multi-spin flips among nearby protons can further influence the experimental results [37].

Figure 4.3. Comparison between experimental and numerically predicted 3Q matching as observed in Na-acetate for a contact time of 2 ms and variable $^1$H r.f. field strength. For the numerical simulation an isolated two spin (I=1/2, S=3/2) system was assumed using r.f. fields of 94 and 50 kHz on I and S spins, respectively. As in Fig.1, a dipolar coupling of 1.5 kHz was used.

In spin 1/2 HHCP experiments, considerable progress has recently been made to enhance the polarization transfer with adiabatic [47] transfer methods - e.g. by varying r.f. field amplitudes [7, 39] or the MAS spinning frequencies [48]. In the present case, adiabatic variation of the dipolar coupling Hamiltonian should not be confused with the 'adiabatic
passage' spin locking regime defined in chapter 3 [12, 13]. Adiabatic CP transfer is most efficiently achieved if the rate of amplitude change is smaller than the dipolar coupling and much smaller than the quadrupolar coupling that drives RIACT transfer. We may therefore expect a gain in TQCP transfer efficiency if the amplitude variation is slow in comparison to these interactions and does not interfere with the RIACT transfer. This is a good assumption in light of section 3.6 which demonstrates RIACT transfer for amplitude-modulated spin-locking pulses. This is most easily achieved performing an amplitude variation on the I spins (Figure 4.2b). It should also be noted that the matching profile is characterized by a broad distribution of matching conditions and we may expect an increased overall transfer efficiency by sweeping the spin-lock field. To discriminate between both effects, additional experiments that reverse the adiabatic transfer [7, 39] could be employed. In the present context, it is sufficient to study the resulting signal enhancement that is experimentally illustrated in Figure 4.4. In agreement with numerical simulations (not shown) we observed an enhancement of more than 50%. For sodium acetate, an optimum amplitude sweep of 8% about the average power at maximum transfer (corresponding to $\Delta \omega_1 \pm 3$ kHz) was observed.

![Figure 4.4](image-url)

**Figure 4.4.** Comparison of experimentally observed signal enhancements resulting from a tangential modulation of the I spin r.f. field strength. Radio frequency modulations (as indicated by $\Delta$) are centered on the maximum TQCP matching condition determined experimentally in Figure 4.3.
Of particular interest is the relative sensitivity of the TQCP method with respect to SQCP during MQMAS experiments. In general, the TQCP method eliminates the excitation (or reconversion) step connecting central- and triple-quantum coherence. A comparison can be attempted by accurately optimizing the SQCP and TQCP matching conditions and comparing the following transfer steps

\[ I_x \xrightarrow{SQCP} C_x \xrightarrow{nutation} T_x \xrightarrow{RIACT} C_x \], and
\[ I_x \xrightarrow{TQCP} T_x \xrightarrow{RIACT} C_x \].

One should keep in mind that experimental parameters can seriously influence this comparison. For the SQCP method, the polarization transfer efficiency is influenced by offset terms and the superposition of zero- and double quantum terms (see Equation 7). In general, careful adjustment and stability of the r.f. power level are required to ensure efficient transfer. In addition, a reduced r.f. bandwidth for SQCP, which could be serious in the presence of large chemical shift terms, will result in a reduced efficiency for the transfer. Both effects are less problematic for the TQCP approach for which high r.f. fields can be employed over a wide range of r.f. power levels. Experimentally, we have observed TQCP transfer efficiencies for Na-acetate that were at least a factor 2 higher than for the SQCP approach. On the other hand, numerical predictions indicate that the SQCP is superior in the presence of small dipolar couplings. These predictions were experimentally observed on anhydrous Na\textsubscript{2}HPO\textsubscript{4} in which the dipolar \textsuperscript{1}H-\textsuperscript{23}Na couplings are known to be smaller [49]. While in the context of dipolar filtering this behavior can further simplify the spectral analysis, we are currently also investigating experimental modifications that enhance the TQCP efficiency for larger distances.
To illustrate the filtering that can be achieved with TQCP we performed $^{23}$Na experiments on a test sample consisting of Na-acetate and aprotic sodium sulfate. The left column of Figure 4.5 shows a standard one pulse decay spectrum under MAS and a TQCP-filtered MAS spectrum. A superposition of two line shapes is expected in the one-pulse MAS spectrum corresponding to the residual second-order quadrupolar broadening under MAS. In the TQCP filtered spectrum, no signal due to sodium sulfate is observed. In the right-hand column of Figure 4.5 we show two MQMAS spectra (using the $t_1$ evolution in Figure 4.2b) which demonstrate the utility of the TQCP filter. Only the isotropic signal from sodium acetate is retained in the TQCP-MAS experiment.

In spin 1/2 cases, the polarization transfer characteristics of an isolated spin 1/2 pair are symmetric, i.e. the efficiency of the transfer is not influenced by the initial condition (non-zero polarization on I or S spin). Our theoretical and numerical results indicate that this
behavior also applies to the multiple-quantum transfer methods discussed in this contribution. It is thus also possible to directly transfer triple quantum coherence from a quadrupolar nucleus to the spin 1/2 nucleus with equal efficiency. Starting with a conventional MQMAS experiment, in which multiple quantum coherence is evolved for time $t_1$ on the quadrupolar nucleus, a subsequent TQCP transfer to the spin 1/2 coupling partner followed by $t_2$ detection would provide a high resolution, 2D heteronuclear correlation spectrum. This modification of the experimental techniques outlined in Figure 4.2 would also be advantageous whenever the spin 1/2 spin lattice relaxation time is significantly longer than the quadrupolar $T_1$ relaxation time.

4.1.4. Summary

Heteronuclear polarization transfer represents one of the key elements for structure elucidation in spin 1/2 applications. In these cases, the cross polarization dynamics in an isolated two-spin pair are well understood.

In this section we have investigated extensions of this approach to heteronuclear spin systems involving quadrupolar nuclei. In the quadrupolar case, a more detailed analysis of the influence of r.f. and dipolar interactions under MAS is needed. We have shown that an effective Hamiltonian can be derived to describe the central transition spin locking behavior of a quadrupolar nucleus. In the same formalism, the RIACT concept to excite and reconvert multiple-quantum coherence can be understood as a MAS induced rotation between central and triple quantum transition subspaces of a spin $S=3/2$ spin system.

The effective Hamiltonian derived for an isolated quadrupolar spin under MAS also provides analytical insight into the dynamics of an I,S spin system subjected to r.f. fields and MAS. In particular, we have shown that by employing medium size (i.e. in the order of 50 kHz) r.f. fields on the quadrupolar nucleus it is possible to directly cross polarize into the triple-quantum coherence. Thus, TQCP transfer can act as a dipolar filter for the subsequent high-resolution (MQMAS) part of the experiment. This scheme can also be adapted for a 2D HETCOR experiment (displaying high resolution in 2 dimensions), by introducing an additional evolution time after the preparatory $\pi/2$ pulse on the spin 1/2 nuclei. Comparative studies between a conventional single quantum (SQ) CP experiment and the TQCP method
indicate that the latter approach leads to a 2-5 times higher overall transfer efficiency for large or medium-size dipolar couplings. For small dipolar couplings (below 500 Hz) this gain cannot be realized and further investigations are required to optimize the transfer for longer distances (i.e. smaller dipole couplings). Preliminary results indicate that adiabatic TQCP techniques can substantially improve the transfer efficiency, where adiabaticity refers to a slow variation of the r.f. field amplitude on the spin 1/2 nucleus during TQCP mixing.

The outlined concept of direct creation of multi-quantum coherence during the CP process might also be employed in the context of generating double quantum coherence among strongly dipolar coupled spin 1/2 nuclei such as $^{13}$C-$^1$H or $^1$H-$^3$H systems.

4.2. Radio-Frequency Mediated Dipolar Recoupling Among Quadrupolar Nuclei

In section 4.1 a method for cross-polarization was presented which provides improved sensitivity in CP-MQMAS spectra and allows for the measurement of heteronuclear correlations. In this section, it is demonstrated that through-space recoupling among quadrupolar spins can be achieved by applying an appropriate spin-locking radio-frequency field to a system of dipolar coupled quadrupolar nuclei[50]. Results are presented for $^{23}$Na-$^{23}$Na spin pairs in Na$_2$HPO$_4$. The rotary resonance condition in quadrupolar systems will be compared to that for spin-1/2 systems to illustrate the influence of the quadrupole coupling on the spin dynamics. Adiabatic modulation of the r.f. amplitude is employed to improve the recoupling effect.

4.2.1. Homonuclear Recoupling and Rotary Resonance

Biomolecular structure determination by NMR depends upon the ability to measure internuclear dipolar couplings for constraining the conformation of the molecule under study and for unambiguously assigning all lines in the NMR spectra[1, 51-53]. An example of the
importance of correlation spectroscopy in determining assignments in quadrupolar spin systems will be given later in Section 5.2.

As of this writing, we are aware of only one technique for performing homonuclear correlations in quadrupolar spin systems under magic-angle spinning[54], which is accomplished by sweeping through the well-known R² condition [48, 55-57] for quadrupolar-broadened MAS line shapes by ramping the MAS frequency during the mixing period. On the other hand, dynamic-angle spinning probes can be switched to zero-degrees to enable rf-driven spin-diffusion[58], but this fails to average all anisotropic interactions during the mixing time (e.g. CSA, quadrupole, dipole). Both approaches require a modulation of spatial degrees of freedom and more approaches are required to provide homonuclear recoupling techniques in quadrupoles using a fixed spinner axis and frequency.

However, recoupling methods for spin-1/2 systems that rely on the use of r.f. fields cannot be readily applied in quadrupolar spin systems due to the complications of nutation and RIACT processes resulting from the influence of the quadrupolar coupling during r.f. irradiation (for recent reviews of spin-1/2 methods see [59, 60]). To extend spin-1/2 methods to quadrupolar nuclei, two aspects of the quadrupolar coupling must be considered.

Additional shift terms. In the spin-1/2 case it may be assumed that, under conditions of very fast MAS, only the isotropic chemical shifts remain. However for S>1, the second-order quadrupolar broadening remains, even under fast MAS, and represents an orientation dependent shift term that can affect dipolar transfer.

Zero’th Order Quadrupolar Hamiltonian. In spin-1/2 systems it can generally be assumed that the applied r.f. field is the largest term in the Hamiltonian. In quadrupolar spin systems, the zero’th order quadrupole coupling (A₂₀T₂₀) is on the order of MHz and leads to effects such as scaled nutation frequencies and adiabatic passages which are absent in spin-1/2 systems.

In this section we extend the rotary-resonance phenomenon, observed in dipolar[61, 62] and CSA[63] interactions, to quadrupolar spin systems. In spin-1/2 systems, these effects have been applied for determining local spin geometries[64, 65], for adiabatic 2D correlation spectroscopy[66], and for the detection of spin pairs by dipolar filtering[67]. The rotary resonance condition is satisfied for a spin pair when the sum of the r.f. spin-locking field strengths for the two nuclei equals the MAS frequency,
\[ n\omega_r = \omega_{1,\text{eff}} + \omega_{2,\text{eff}}, \quad n = 1, 2, \ldots \]  

Then the dipolar coupling is reintroduced into the Hamiltonian for hetero-[61] or homonuclear[62] spin pairs. The resonance condition of Equation 9 has been derived for spin-1/2[61, 62] and quadrupolar[50] systems, where the difference is in the forms of the effective r.f. fields. We will not give the derivations here, but instead contrast the effective fields required under spin-1/2 and quadrupolar cases to illustrate how quadrupolar effects are included in Equation 9. The two cases are described in Table 4.1.

<table>
<thead>
<tr>
<th>Rotary Resonance Conditions</th>
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<tbody>
<tr>
<td>Spin 1/2</td>
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<tr>
<td>[ \omega_{1,\text{eff}} = \sqrt{\omega_i^2 + \Omega_{1,\text{CS}}^2} ]</td>
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Table 4.1. Effective r.f. fields which create rotary resonance recoupling in spin 1/2 and spin 3/2 systems according to Equation 9. \[ \Omega_{1,\text{CS}} \] and \[ \Omega_{1,\text{CS+Q}} \] represent isotropic shift terms due to the chemical shift only and the combined chemical and quadrupolar shifts, respectively.

In the spin 1/2 case, the effective field is simply \( \omega_i \) modified for a resonance offset. In the spin 3/2 case, two more modifications to the effective field can be seen. The first is that the effective nutation rate of the central transition, \( (S+1/2)\omega_i \), replaces the applied r.f. field, \( \omega_i \). Second, the quadrupolar broadening of the central transition line shape is treated as an additional chemical shift.

The parameter

\[ \kappa = \frac{\omega_{\text{RF}}}{\omega_r} \]

will be helpful in identifying the conditions needed to satisfy Equation 9. For instance, the need to apply a spin-locking pulse to the quadrupolar spin systems requires that the sudden-passage regime described by Vega[13] be fulfilled by using fast MAS and small r.f. fields (see also sections 3.5-3.6). Effective spin-locking of the central transition for \(^{23}\text{Na}\) in \( \text{NaNO}_2 \) was experimentally demonstrated previously in Figure 3.25. In order to determine the range of \( \kappa \) values over which acceptable spin-locking is achieved, we have carried out a number of calculations of the spin-locking efficiency of the central transition and these are shown in Figure 4.6.
Figure 4.6. Time evolution of central transition coherence as a function of applied r.f. field strength for an S=3/2 nucleus. The Quadrupolar coupling and MAS spinning frequency were kept constant at 3 MHz and 10 kHz, respectively. All numerical simulations were carried out with the effective Hamiltonian derived in section 2.2.5. Ideal spin-locking is marked by the shaded line. As expected, the spin-lock behavior generally decreases for increasing r.f. fields with minima at K=n/2 (n=1,2,...). For values between k=[0.1,0.3] the time evolution can be approximately described by a spin-locked central-transition coherence that is only weakly modulated by time dependent terms. These results are in agreement with additional simulations using different quadrupole coupling and MAS frequencies.

In Figure 4.6, with e²qQ/h=3.0 MHz and ω₀=10 kHz, effective spin-locking is obtained in the range K=[0.1,0.3], corresponding to r.f. fields of 1-3 kHz, in agreement with the spin-locking results of Figure 3.25. Interference effects are also observed at K=n/2 (n=1,2,...), in agreement with the study of Sun et al.

Using such r.f. fields, the rotary resonance condition in Equation 9 can now be tested numerically for an isolated, S=3/2 spin pair, as shown in Figure 4.7 for several different values of K. Quadrupole couplings of 1.2 MHz and 2.4 MHz were used for the spin pair however, to simplify the analysis, chemical shift offsets were chosen so that isotropic shifts
could be neglected in Equation 9. That is, the effective field is just \((S+1/2)\omega_i\). A homonuclear dipole coupling of 2 kHz was assumed. The simulation procedure started the spin system with central-transition coherence on one spin, I, and used the detection operator \(S_x^{23}\) to monitor the build-up of central-transition coherence on the dipolar coupled S spin.

![Figure 4.7](image_url)

**Figure 4.7.** Numerical simulation of dipolar recoupling in a dipolar coupled quadrupolar two-spin system. At time \(t=0\), the system is prepared with central-transition coherence on the first (i.e.-I) spin and the polarization transfer on spin S is monitored for different r.f. fields. The MAS frequency is 10 kHz for all curves. Polarization transfer is observed at the \(n=1\) and \(n=2\) rotary resonance conditions (\(K=.25, K=.50\)).

Transfer at \(\kappa=0.25\) occurs at a higher efficiency than for \(\kappa=0.50\). This is due to the magnitude of the recoupling element, which is actually larger for the \(n=1\) condition\[50\], and is also the result of a competing single-quantum resonance\[50\]. Also, it should be observed that the coherence transfer is negative, as expected for a double-quantum process\[68\].

Finally, the second order quadrupolar shift does not allow the rotary resonance condition to be satisfied for all spins, since \(\Omega_{i,cS+Q}\) is orientation dependent. An amplitude modulated pulse should make it possible to satisfy the recoupling condition for a larger number of crystallites, as show in spin 1/2 systems\[67\]. Tangential shapes of the r.f.
amplitude[69] are chosen for the experiments in this section but also obeying the restriction \( \kappa(t) = \frac{1}{4} \pm \frac{i}{4} \).

### 4.2.2. 2D \textsuperscript{23}Na–\textsuperscript{23}Na Homonuclear Correlation via Rotary Resonance

The test sample chosen for this study was anhydrous disodium hydrogen phosphate, Na\textsubscript{2}HPO\textsubscript{4}, which has been extensively studied and contains three distinct sodium sites in the crystallographic unit cell[49]. The quadrupolar and chemical shift parameters are well known and MAS frequencies of 8 kHz or higher at 11.75 Tesla (500 MHz for \textsuperscript{1}H) are sufficient to remove all rotational sidebands.

**Figure 4.8.** Pulse sequences used in this study. (a) Experiment to observe the central transition coherence as a function of the spin-locking time or the r.f. field strength. (b) a two-dimensional dipolar correlation experiment to perform recoupling among quadrupolar nuclei. All \( \pi/2 \) pulses are actually \( \pi/4 \) pulses so as to be selective for the central transition. After the evolution time, \( t_1 \), dipolar recoupling occurs during the mixing time, \( t_{\text{mix}} \), in which the r.f. field is modulated tangentially about the \( K=0.25 \) condition. A z-filter facilitates phase cycling by destroying unwanted coherences.
Although the $^{23}\text{Na}$ r.f. fields were measured externally with an NaBr sample, calibrating the powers for very low fields by this method is not sensitive enough for setting the rotary resonance conditions. To determine these fields more accurately and identify the recoupling conditions, a spin-locking experiment, as diagramed in Figure 4.8a, was carried out and the results are shown in Figure 4.9. The central-transition coherence was measured after a 1 ms spin-locking pulse over a series of closely spaced r.f. field strengths.

![Figure 4.9](image)

**Figure 4.9.** Central transition coherence for $^{23}\text{Na}$ in Na2HPO4 as a function of the r.f. field strength for 1 ms spin-locking time and $\omega_{0}=8.3$ kHz. The figure shows the signal for a 1D slice taken through the region of the line shape which corresponds to Na(1) (see Figure 3.11).

It can be seen from Figure 4.9 that characteristic minima occur at $\kappa=n/2$ ($n=1,2,\ldots$), where the MAS frequency is 8.3 kHz, and a small minimum occurs also at $\kappa=0.25$, which indicates the rotary resonance. Having identified the recoupling condition in Figure 4.9, a 2D correlation experiment may be carried out in the manner shown in Figure 4.8b. The method of States, Ruben and Haberkorn was used for phase-sensitive detection[70]; low r.f. fields of $\omega_{0}=5-10$ kHz were used to ensure efficient excitation of the central transition coherence without competing nutation effects.
Figure 4.10. Two dimensional correlation patterns for anhydrous Na2HPO4 for mixing times of (a) 0.5 ms, and (b) 6 ms using the pulse sequence of Figure 4.8b. All diagonal contours are positive, while crosspeak contours represent negative intensity.
Figure 4.10 shows the results of the 2D experiment for two mixing times in order to verify the effect of the recoupling condition. Optimization of the carrier frequency was required in order to satisfy Equation 9, since the shift terms cannot be disregarded as we did for Figure 4.7. A 0.5 ms mixing time was used in Figure 4.10a, where the absence of crosspeaks indicates that no recoupling effects are taking place, as expected. In Figure 4.10b, the same experiment is shown for a 6ms mixing time and substantial double-quantum transfer is seen to have occurred. The crosspeak intensities are negative, although this is not directly shown in the figure. One consequence of this is that some cancellation of signal results when crosspeaks are close to the diagonal signal. Contour levels have been chosen to emphasize the most intense portions of the crosspeaks.

Correlations are clearly observed in Figure 4.10b among different regions of the total MAS line shape in Na$_2$HPO$_4$. It is reasonable in fact to separate the one broad spectrum into three regions and notice that appreciable crosspeak intensity is observed for the (Na$_1$,Na$_3$) and (Na$_2$,Na$_3$) spin pairs. This supports the distances determined by the recent X-ray & NMR study.

However care should be taken to interpret any exact distance information from this spectrum. Although the use of the shaped pulse provides a more broadband dipolar transfer over all three regions, it is difficult to know the extent of the dipolar transfer and also to predict dipolar transfer in a three-spin quadrupolar system. One possibility for extracting more quantitative information from such spectra is to perform numerical simulations of the entire recoupling pattern (i.e. Figure 4.10b) in the spirit of techniques used for static tensor analysis [71-74]. Nonetheless, the rotary resonance method has confirmed the known spin topology in this sample.
Figure 4.11. (a) One dimensional slice along the position pointed to by the arrow in Figure 4.10b. The negative crosspeak intensity is clearly seen. The numbering system is also indicated here to show the three roughly distinct spectral regions corresponding to the three Na sites. (b) a drawing of the local structure in Na2HPO4 which highlights the three Na positions, and shows the larger distance between Na1 and Na2.

Additional experiments (not shown) suggest that varying the carrier frequency by several kHz may be useful in the context of suppressing dipolar transfers in certain regions of the spectrum. This is attributed to the influence of the chemical shift terms, \( \Omega_{i,cs+Q} \), in Equation 9. However, off-resonance irradiation compromises the spin-locking efficiency (see Figure 3.25) and in general care should be taken to set the carrier for on-resonance spin-locking. This technique is most effective for situations when differences in chemical shifts
between the observed species are small. Also, since spin-locking is more challenging for nuclei exhibiting large quadrupole couplings, it is advantageous to choose the r.f carrier position near to the isotropic shifts of these sites.

4.2.3. Summary: Isotropic Methods and Biological Solids

A means for homonuclear correlation in quadrupolar nuclei has been described for spin 3/2 systems. Requiring low r.f. fields and moderate MAS rates, and using shaped spin-locking pulses for improved transfer, this method is accessible to most hardware configurations. From consideration of the rotary resonance conditions in Equation 9 and Table 4.1, this effect should also be observable for higher spin quantum numbers where the principal change to consider will be in the effective nutation rate of the central transition. To summarize, this represents a more general approach to observe through-space correlations in quadrupolar multi-spin systems.

To incorporate a high-resolution, isotropic dimension, it is noted that since this technique occurs in the 2-3 subspace, it can be inserted at the point of echo formation (kt_r) in the MQMAS experiment. In such a scheme, the initial selective π/2 pulse in Figure 4.8b is not required, but an additional evolution time is introduced which significantly extends the total experimental time required. Conversely, approaches are currently under consideration which modify the original MQMAS experiment in order to provide a high-resolution dimension in the 2D correlation without the additional evolution period.

In the context of biological solids, it should be noted that in addition to providing correlation information which may assist in assignments, this method can be used to distinguish between exchange processes and through-space proximity, an approach which is frequently used in solution NMR[51].
4.3. References


73. Robyr, P., 1994, ETH, Zurich.

5. Applications in Biological Solids

5.1. Introduction

The principle conclusion of the efforts described in Chapters 2-4 is that over the last 4-5 years quadrupolar NMR has been substantially improved, through theoretical treatments and new experimental methods, well beyond the 1-pulse, central transition spectroscopy under magic-angle spinning. At this stage we are prepared to utilize quadrupolar NMR to address questions in biological solids.

Two studies are presented to demonstrate the value of performing quadrupolar NMR experiments in biological solids. The first, described in section 5.1, involves the direct observation of boron (\(^{11}\)B, \(S=3/2\)) in the binding site of an enzyme-inhibitor complex. This study is a straightforward example of the use of the quadrupole coupling to gain insight into the structure of the catalytic triad in \(\alpha\)-lytic proteases. This will show that both the effective site-specific labeling of the boron and the sensitivity of the quadrupole coupling allow for studying the binding site geometry. In addition, this will confirm that signal-to-noise,
resolution and other potential spectroscopic limitations do not inhibit the acquisition of quadrupolar NMR spectra of biomolecules. Section 5.2 describes an extensive solid-state NMR study of sodium ($^{23}$Na, S=3/2) in guanine rich DNA oligonucleotides that demonstrates the combination of a suite of techniques to perform complete resonance assignments. The ability to assign sodium lines using complimentary experiments at high field illustrates the relative maturity of quadrupolar NMR over just a few years ago.

5.2. $^{11}$B NMR of an Enzyme-Inhibitor Complex

5.2.1. Peptide Boronic Acids Have Two Binding Modes

Peptide boronic acids act as inhibitors of many important proteases with a high degree of specificity and affinity[1]. In 1988, Bachovchin et al. [2] demonstrated the lack of a common structural mechanism of inhibition of α-Lytic Protease (α-LP) by peptide boronic acids. In the well-known active site of α-LP, the catalytic triad (Asp-102, His-57, Ser-195), the boron could bind to either Ser-195 or His-57 depending on the substituents of the boron inhibitor. This picture was both confirmed and clarified by Bone et al.[3], who demonstrated with x-ray crystallography the existence of two binding motifs in a series of α-LP-inhibitor complexes, where the inhibitor series was MeOSuc-Ala-Ala-Pro-Boro-X (X=Val, Ala, Ile, Phe, Nle). The binding with X=Val, Ala, Ile and Nle, is characterized by a covalent bond between the O' of Ser-195 and the boron; but in the complex formed with X=Phe, the boron covalently bridges Ser-195 and His-57. The two binding schemes are illustrated in Figure 5.1. Tsilikounas et al. [4] collected solution $^{11}$B NMR spectra and presented compelling evidence that the boron environments in R-Boro-X are similar, on the basis of their chemical shifts, for all X = Phe, Val, Ala, Ile, and Nle.
Although the distinction between serine-bound and serine-histidine bridging binding motifs is established, the symmetry of covalent bonding about the boron nucleus is not clearly understood[3, 4]. It is agreed that, for the Ser-195 adduct in Figure 5.1, the $O'$ in the serine sidechain binds to the boron above the R-Boro-Val plane and induces tetrahedral covalent symmetry at the boron. However, Bone et al. interpret the structure of the bridging arrangement (Figure 5.1(top)) as preserving the trigonal-planar symmetry about the boron, while the $^{11}$B NMR study by Tsilikounas et al. suggests that the boron is tetrahedral. To
determine if it is possible for solid-state NMR to contribute to this question, we have examined a controlled inhibitor/enzyme complex.

There are several justifications for applying solid-state NMR to this class of enzyme/inhibitor complexes. First, solution NMR does not allow general access to quadrupole coupling parameters, which may resolve the bond symmetry issue of boron in α-LP-inhibitor complexes. The use of SSNMR of quadrupolar nuclei to resolve structural details in greater detail than x-ray crystallography has already been demonstrated in the study of Baldus et al. involving $^{23}$Na[6]. Second, the $^{11}$B-solution NMR study indicated that the enzyme/inhibitor complexes were not in the extreme narrowing regime and displayed solids-like spectral features including a second-order-quadrupolar isotropic shift[4].

![Diagram of phenyl boronic acid](image)

**Figure 5.2.** $^{11}$B NMR spectra of phenyl boronic acid for (a) static, (b) MAS–12 kHz, and (c) MQMAS experiments at 7.4 Tesla (101 MHz for $^{11}$B, 317 MHz for $^1$H). Note two distinct sites in the MQMAS spectra.
We first examined a model boronic acid, phenyl boronic acid. Figure 5.2 shows static, MAS and MQMAS $^{11}$B NMR spectra of phenyl boronic acid. Numerical fits to the line shape give the quadrupole coupling parameters $e^2 q Q / h = 3.15$ MHz, and $\eta = 0.55$. The value for $e^2 q Q / h$ is somewhat larger compared to trigonal BO$_3$ in inorganic solids [7]. However, as Turner et al. demonstrated, the large quadrupole coupling is diagnostic for the trigonal geometry. In addition it should be noted that the MQMAS reveals two crystallographically inequivalent boron sites which are nearly degenerate in the MAS spectrum.

5.2.2. $^{11}$B NMR confirms binding mode

The inhibitor used for this example is H-Ala-Boro-pinacol (see Figure 5.3), a simple substrate-analogue inhibitor of serine proteases which is, in fact, the protected intermediate in the synthetic route to R-Boro-X inhibitors[1].

The $^{11}$B MAS spectra of the free inhibitor and the $\alpha$-lytic protease complex are shown in Figure 5.4. The spectrum of H-Ala-Boro-pinacol exhibits a quadrupole line shape from which we obtain: $e^2 q Q / h = 2.86$ MHz, and $\eta = 0.48$. These compare well with the values for phenyl-boronic acid and indicate trigonal bonding at the boron. In contrast, the $\alpha$-LP-inhibitor complex gives a narrow line partially obscured by amorphous, free inhibitor which is present in large (7x) excess in this sample.
The reduced quadrupole coupling for the boron in the complex must be due to a higher symmetry, which can result from octahedral or tetrahedral coordination. Octahedral coordination is clearly not physically realistic and thus we conclude that the boron coordination has been distorted from a planar arrangement towards a tetrahedral one [7]. The $^{11}$B quadrupole coupling for the complex is estimated from Figure 5.4 to be $\epsilon^2 qQ/h \approx 0.6$ MHz.

Thus, by inspection of the quadrupole coupling and knowledge of quadrupole parameters in model systems we can conclude that the mode of binding of H-Ala-Boro-Pinacol is similar to that illustrated in the bottom panel of Figure 5.1. This is expected, however, since the cyclic diester (pinacol) prevents the loss of an $-$OH group to form a bridging Ser-His adduct, and so the complex can only form a tetrahedral serine adduct.

Figure 5.4. $^{11}$B MAS spectra (10 kHz) at 7.4 T (101 MHz for $^{11}$B) of (a) the free H-Ala-Boro-Pinacol inhibitor and (b) the complex of a-lytic protease with the inhibitor.
In summary, we draw three significant conclusions from our study of an enzyme-inhibitor complex. First, direct observation of the catalytic triad was demonstrated without isotopic enrichment, exemplifying the potential for quadrupolar NMR to access the active sites of proteins and nucleic acids. Second, we found that the free and bound inhibitor samples yielded well-defined, second-order line shapes, demonstrating that crystallization procedures could be avoided for these samples. Finally, the quadrupole coupling confirmed the predicted boron binding geometry and was demonstrated to be a sensitive reporter to local bonding and symmetry.
5.3. $^{23}\text{Na}$ NMR in a DNA Quadruplex at High Field

Guanine rich DNA oligonucleotides can form structures characterized by several planes of four guanines each of which are stabilized by hydrogen bonding contacts and by monovalent cations which reside in the cavities of such planes [8-11]. Such G-quartet structures are proposed to form at the ends of telomeres, imparting resistance to chemical modification to the chromosome, and are currently under intense study as potential targets for inhibiting telomerase activity in cancerous cells[12-14]. Recent investigations into understanding the stabilization of guanine quartets include the substitution of different cations into the quartets [15, 16] and the development of ligands which bind to tetraplex-forming DNA[13, 14]. In particular, stabilization of G-quartet structures may inhibit telomerase, the action of which is believed to extend lifetimes of cancer cells (see Sun et al. [17] and references therein). We report the direct NMR observation of $^{23}\text{Na}$ in a d[TG$_4$T]$_4$ sample where three classes of sodium are resolved and assigned.

5.3.1. Guanine Quartets are Stabilized by $^{23}\text{Na}$

Guanine-rich DNA oligonucleotides may form single or multistranded structures which consist of planes defined by four guanine nucleotides each and a monovalent cation. Four strands may join together axially to form the four ‘corners’ of a set of stacked G-quartet planes, as observed in [d(TG$_4$T)]$_4$ (G=Guanine, T=Thymine). Another involves just two strands, where each strand folds back once, as observed for [d(G$_4$T$_4$G$_4$)]$_2$. These structures are given in Figure 5.5, where each structure exhibits characteristic guanine planes. The thymine nucleotides are not displayed in Figure 5.5 in order to facilitate the identification of the quartets and, in the case of d(TG4T), to observe the positions of the bound Na$^+$ nuclei.
The 'top' views are oriented with the channel formed by the stacked quartets normal to the paper. The side views are obtained by performing a 90° end-over-end rotation. Note that the G-quartets are extremely planar in \([d(TG_4T)_4]\) while the quartets in \([d(G_4T_4G_4)_2]\) are more distorted. The structure of \([d(TG_4T)_4]\) turns out to be more complex than it initially appears. Four strands of TG₄T associate to form a four-rung, cylindrical ladder structure. Two of these units stack end-on-end to create a ladder of 8 planes. This dimerization provides the binding site for the central Na⁺. Finally, in the high-resolution structure of d(TG₄T) a belt of Ca²⁺ ions was found circling the interface between the two units\[20\]. By attracting a hydration shell, the Ca²⁺ ions facilitate hydrogen-bonding contacts for joining the quadruplex units.
Figure 5.6 illustrates the numbering scheme that will be used to identify the seven channel ions in [d(TG₄T)]₄. Figure 5.6 also shows the exterior guanine quartet of d(TG₄T) where the sodium ion resides within the G-quartet plane. While Na(4,4') reside in-plane, the remaining interior Na⁺ ions are complexed in between the guanine planes. Na(1) occupies a perfectly octahedral site between the two [d(TG₄T)]₄ units. This heterogeneous distribution of the cation binding geometry has not been observed in other G-rich systems which form quartets[15, 16]. For example, NMR studies have indicated that both [d(G₄T₄G₄)]₂ and [d(G₃T₄G₃)]₂ bind cations in octahedral, interplanar sites.

![Figure 5.6](image)

Figure 5.6. The structure of the outermost quartet of d(TG₄T) illustrating the arrangement of guanine bases to form the G-quartet plane. It is significant that for this particular quartet, the stabilizing cation is in the guanine plane.
5.3.2. Sensitivity of Quadrupolar NMR at High Field – $^{23}$Na NMR in [d(TG, T)]

The importance of high fields in quadrupolar NMR can be readily understood from examination of the Hamiltonian which describes the coupling of the nuclear quadrupole moment, inherent to any nucleus possessing spin $S>1/2$, to the surrounding electric field gradient. We have seen that, for half-integer quadrupolar nuclei, the quadrupolar coupling must be evaluated to first and second order in perturbation or average-Hamiltonian theory. Applying these corrections we find that the central ($1/2,-1/2$) transition is not affected by the first order term which is quadratic in the spin quantum number $I$. The high order term does apply but can leave residual broadening in the MAS spectrum of several kHz (see Figure 3.1). There is no one angle about which the sample may be spun to eliminate all anisotropies. The width of the residual MAS line shape may be estimated as

$$\Delta \propto \left( \frac{e^2 q Q / h}{\gamma B_0} \right)^2,$$

Equations 1 and 2 show that, for a quadrupolar nucleus satisfying $\omega_z > e^2 q Q / h >> \omega_I$, doubling the strength of the static field halves the MAS line width to provide a 2:1 increase in signal-to-noise. Thus, for half-integer quadrupolar nuclei, the sensitivity difference becomes

$$S = \left( \frac{B_0'}{B_0} \right)^3,$$

The line narrowing at high field improves resolution and enhances sensitivity. In the case of 17.6 Tesla versus 9.4 Tesla, an overall sensitivity enhancement of 4.8 is realized. This is a
non-trivial gain and, when studying large biomolecules, the highest possible fields are needed to detect and resolve resonances arising from quadrupolar nuclei.

This is illustrated in Figure 5.7 which shows one-pulse $^{23}$Na MAS-NMR spectra of d[TG₄T]₄ at 9.4 Tesla (105.22 MHz for $^{23}$Na) and 17.6 Tesla (198 MHz for $^{23}$Na) where the high field truncation reveals three well-resolved sodium environments with good signal to noise. A comparison of sensitivity is complicated by extensive hardware differences between the two spectrometer and probe systems, but it should be noted that the spectrum acquired at 9.4 Tesla employed ten times the number of transients as that acquired at 17.6 Tesla. Three lines are observed at 6.8, 0, and −19 ppm. These are labeled Na(1), Na(2) and Na(3) respectively.

![Figure 5.7](image-url)

**Figure 5.7.** Magic-angle spinning $^{23}$Na spectra of [d(TG₄T)₄] at (a) 9.4 Tesla from 1012 transients and 7 kHz MAS and (b) 17.6 Tesla from 96 transients and 4.5 kHz MAS. Shifts are externally referenced to 0.1 M NaCl.
5.3.3. Low Temperature Studies and Sample Preparation

The resolution of the high field MAS spectrum is very striking in Figure 5.7 as the residual linewidths are very small, ≤ 1 kHz, at half-maximum. A possible additional source of line narrowing that should be considered is motional averaging of the quadrupole coupling tensor. To investigate this we ran a series of low-temperature $^{23}$Na spectra in a dewared, variable-temperature probe capable of magic-angle spinning at temperatures ranging down to -130°C at 7.4 Tesla. Although the lower static field limits resolution, there are a number of ways in which intrinsic motions of the sample can be detected. A standard approach is to monitor the MAS line shape as a function of decreasing temperature. If the quadrupole tensor becomes motionally constrained at low temperature, this will be indicated by additional spectral broadening. This temperature series is shown in column (a) of Figure 5.8. An extension of this method is to observe the effect of decoupling the proton spin bath as a function of temperature since the dipolar coupling will also be reintroduced as sample motions become constrained. The proton decoupled spectra are given in column (b) of Figure 5.8. Finally we introduce a new approach which has not been previously reported. In column (c) of Figure 5.8 we measure 3Q excitation via a single nutation pulse. It is well-known (e.g. Figures 3.6-7) that the 3Q nutation efficiency is a sensitive function of the quadrupole coupling constant [22-24]. The lowest order quadrupole coupling ($\omega_q R_{20} T_{20}$) provides the coupling element needed to excite MQ coherence; in the approximation $\omega_q << \omega_0$, the magnitude of this coupling element is proportional to $\omega_0^3/\omega_q$. However, in the limit of vanishing $\omega_0$, no 3Q excitation is possible. This is the regime in which we are interested, where motional averaging of the quadrupole coupling will prevent 3Q excitation.
Figure 5.8. Low-temperature $^{23}$Na MAS spectra of [d(TG₄T)]₄ at 7.4 Tesla (83 MHz for $^{23}$Na) using (a) Hahn-Echo MAS spectra without decoupling and (b) with decoupling, and (c) triple-quantum filtered spectra obtained from the pulse sequence Figure 3.15b. MAS = 4.6 kHz. 1824 transients were acquired for each spectrum with a recycle time of 1 second.

We first note the increased signal-to-noise that results from higher Boltzmann populations and reduced thermal noise at lower temperatures. However, we also make three observations of motional effects from the low temperature series. First the Na(1,2) region is slightly broadened on progressing to low temperature. Second, proton decoupling has no effect at
15°C but results in significant improvement in signal-to-noise at -130°C. And lastly, there is almost no 3Q signal at 15°C but substantial excitation of 3Q coherence at -130°C, unambiguously demonstrating a progression from a vanishing to a measurable quadrupole coupling without any large change in the linewidths. The 3Q filter also provides the clearest evidence that the motion does not completely freeze until below -40°C. In summary, there is substantial motion in the sample yet, upon freezing, the line widths do not change significantly. This is explained if we assume that the majority of the room temperature line width is due to heterogeneous broadening resulting from a non-crystalline sample. Then the quadrupolar coupling in the frozen, static limit must be too small to be observed with central transition spectroscopy. This is not surprising since, at 17.6 Tesla where the three linewidths are approximately 0.8-1 kHz, a moderate quadrupole coupling of \( e^2 qQ / h = 0.5 \) MHz results in a MAS line that is only 100 Hz at half-height. Moreover, typical quadrupole coupling for \(^{23}\text{Na}\) in ionic salts are \( e^2 qQ / h \leq 1\) MHz (e.g. sodium citrate, borax, sodium acetate, etc.). This also means that the resolution between the Na(1,2) and Na(3) regions in Figure 5.8 indicates the existence of at least two sites which differ on the basis of their chemical shift and not due to quadrupole induced shifts. The 3Q filtering approach introduced here for gauging sample motions is in the same spirit as the well-known single-quantum deuterium experiments. In \(^2\text{H} (S=1)\) solid-state NMR, quadrupole couplings are measured for a series of temperatures and used to test motional models. Where deuterium studies fit the single-quantum line shapes to extract quadrupole parameters, the 3Q filter indicates the quadrupole coupling through the efficiency of 3Q excitation.

Further, there is no indication that the sodium ions might be undergoing exchange since the Hahn-echo spectra are able to clearly resolve the Na(1,2) and Na(3) regions at all temperatures. Therefore the motions are attributable to the stacked quartets themselves. Isotropic reorientation or end-over-end rotation both incur obvious steric and energetic violations. In a solid or semi-solid environment, axial rotation, similar to axial diffusion in phospholipids, is suggested to explain the motions in this sample. In principle we could measure the magnitude of the quadrupole coupling as a function of 3Q excitation and extract an estimate of the rate of axial diffusion. However such a procedure should first be extensively applied in model systems and this is beyond the scope of the present study.
Experimental

Samples of \([d(TG_4T)_4]\) are generously supplied by Dr. Nicholas Hud and Professor Juli Feigon of the University of California, Los Angeles. In order to reduce heterogeneous broadening and to ensure proper hydration, the group at UCLA proposed a method for packing the sample into the NMR sample rotor termed ‘slow-drying’. The slow-drying approach consists of drying 20-30 µL quantities of concentrated \([d(TG_4T)_4]\) inside the NMR rotor under anhydrous atmosphere (P=1atm) over a period of several hours. In combination with slow centrifugation (<1g) of the entire rotor during the drying, a pellet forms at the bottom. After the pellet is dry, the process is repeated (~3-4x) until the rotor is filled. A transmission-line probe designed by Chad Rienstra and utilizing a Chemagnetics (Varian Associates, Inc.) 3.2 mm spinning assembly was used for all experiments at 17.6 Tesla.

In Figure 5.9 $^{23}$Na spectra of a lyophilized sample at 13.8 Tesla and of a slow-dried sample at 17.6 Tesla are compared.

![Figure 5.9](image)

**Figure 5.9.** $^{23}$Na MAS spectra of \([d(TG_4T)_4]\) dried by (a) lyophilization and (b) the slow-drying method. The lyophilized sample was run at 13.8 Tesla while the slow-dried sample was run at 17.6 Tesla.

Although the comparison is complicated by the differing static fields, an important difference is the absence of a broad signal in Figure 5.9b, which is centered beneath the Na(2) signal in Figure 5.9a and is approximately 15-20 ppm in breadth. In general freeze-drying can introduce severe amorphous broadening and can remove structurally significant water
molecules. As discussed earlier, hydrogen bonding contacts from water molecules attracted to the Ca\textsuperscript{2+} belt appear to be critical for allowing the stacking of two [d(TG\textsubscript{4}T)\textsubscript{4}] units. We propose that the lyophilized sample, in addition to increased amorphous character, may not exhibit this stacking. Hence, the center sodium ion, Na(1), would not exist in the lyophilized sample. The total chemical shift changes substantially for Na(2), is unchanged for Na(1), and differs only slightly for Na(3). The significance of these shifts will be discussed in the next section after their assignments are demonstrated.

### 5.3.4. Complete Assignments from 2D NMR at High Field

In spite of the extremely low 3Q excitation and transfer efficiencies at room temperature, we were able acquire a 2D-MQMAS spectrum at 9.4 Tesla, utilizing the Nutation-RIACT approach to provide optimal sensitivity. The acquisition for each slice was turned on at the echo position (t\textsubscript{2}=kt\textsubscript{1}) in order to give a ‘sheared’ 2D plot in which the signals along F\textsubscript{2} are normal to F\textsubscript{1}. It should be noted that, given the small quadrupole couplings, a shaped RIACT pulse would have provided an improvement in sensitivity, however the shaped method had not been fully developed.
Figure 5.10. Sheared $^{23}$Na 2D-MQMAS spectrum at 9.4 Tesla is shown along with an MAS spectrum above. 64 $t_1$ slices were acquired with 3600 transients per slice and a 1 second recycle delay. $\omega_z=170$ kHz.

The signal at $-19$ ppm shows a skewed line shape which is not normal to the $F_1$ (high-resolution) axis. This indicates a heterogeneous distribution of sites with similar quadrupole couplings but differing chemical shifts[25, 26]. The approximate shift position at $-19$ ppm differs somewhat from the $-13$ ppm chemical shift observed for surface sodium ions in a lyophilized sample of calf-thymus DNA [27], which also displayed an amorphous distribution of the sodium sites on and near the DNA surface. This chemical shift difference is reasonable in light of the non-Watson&Crick base pairing of G-quartet structures[8, 9] that leads to four-stranded helices instead of double helices and therefore presents a different surface for bound
ions. We therefore assign the Na(3) line in Figure 5.7 to sodium ions on the surface of the DNA. More evidence in support of this assignment will come in the experiments at high field that are required to provide information on Na(1) and Na(2), which are severely overlapped in Figure 5.10.

The canonical approach for improving resolution and elucidating assignments in spin 1/2 systems is to perform correlation spectroscopy in order to establish sequential connectivities between the nuclei in the sample. We have carried out a $^{23}$Na-$^{23}$Na correlation experiment by applying a standard three pulse sequence which increments chemical shift evolution after the first $\pi/2$ pulse, stores magnetization along the longitudinal axis for mixing using a second $\pi/2$ pulse, and then observes chemical shift evolution again after a final $\pi/2$ pulse[28]. This is typically referred to as an exchange or solid-NOESY experiment, and crosspeaks in the indirect dimension may arise due to true chemical exchange, direct dipolar cross-relaxation, or proton driven spin diffusion during the mixing period. The pulse sequence is displayed in Figure 5.11.

![Figure 5.11. Pulse sequence for the ‘solid-NOESY’ or exchange experiment. For quadrupolar nuclei, the three $\pi/2$ pulses must be adjusted for the effective nutation rate of the central transition $\omega_{eff}=(S+1/2)\omega$.](image)

A low MAS rate (920 Hz) and a relatively long mixing time (8 ms) were used to observe a correlation between Na(1) and Na(3), shown in Figure 5.12. This correlation could not be observed at higher MAS frequencies (2 – 6 kHz) and is attributed to proton-driven spin diffusion. The crosspeaks are not perfectly symmetric. However this is not unusual in solid-state 2D NMR, where asymmetric crosspeak intensities may result when the initial populations of the two exchanging species are not equal. The difficulty of applying true $\pi$ and $\pi/2$ pulses to quadrupolar nuclei is well known from the nutation behavior of the central transition in half-integer quadrupolar nuclei[22, 24], and inequivalent initial populations may be due nutation effects in these spectra.
Figure 5.12. Homonuclear correlation experiment following the pulse sequence of Figure 5.11 with $\tau=8$ ms and $\omega_0=920$ Hz. 72 $t_1$ increments with 96 transients each were collected. Recycle time was 3.5 seconds, and the temperature was -4°C±1°C. The Na(1) peak exhibits a shorter relaxation time and almost no signal survives the 9 ms mixing time.

The existence of this correlation demonstrates a connectivity between surface sodium, Na(3), and some other well-defined site on the DNA. This is evidence that Na(1) is due to channel sodium ions. It is also be noted that the correlation in Figure 5.12 corresponds to the edge of Na(1), 4 ppm, and not to the center of Na(1).

The nutation behavior may be exploited to gain further insight into the three resonances observed in Figure 5.7. As shown by Vega et al. and Ernst et al.,[22, 24] there are two regimes for the nutation rate of the central transition of a half-integer quadrupolar nucleus:

$$\omega_{\text{eff,}1}^\text{CT} = (S+\frac{1}{2})\omega_{\perp}, \quad \omega_Q >> \omega_{\perp},$$

$$\omega_{\text{eff,}1}^\text{CT} = \omega_{\perp}, \quad \omega_Q < \omega_{\perp}. \quad (4)$$
Thus the nutation rate reports on the magnitude of the quadrupole coupling. Although the general magnitude of the quadrupole coupling is usually obvious from MAS spectra, the amorphous broadening demonstrated with the low temperature studies inhibits the ability to evaluate the quadrupole coupling based on the 1D MAS linewidths. A 2D-MAS nutation experiment is obtained by applying an incrementable pulse to the spin system followed by direct observation of the signal. Standard two-dimensional processing in magnitude mode should reveal the nutation frequencies of the lines in the spectrum. The pulse sequence is shown in Figure 5.13a, along with the singly processed spectrum in Figure 5.13b, where the Fourier-transform has been applied to each slice along $t_2$. The indirect dimension in Figure 5.13b is the time-domain nutation spectrum. Even before the second Fourier-transform, it is clear that Na(1) and Na(3) are nutating in the regime $\omega_Q >> \omega_1$, while Na(2) behaves in the limit of a small quadrupole coupling.
The nutation frequencies can be read from the complete frequency domain 2D spectrum (Figure 5.14), where Na(1) and Na(3) are nutating at $2\omega_1$, and the nutation rate for Na(2) is $\omega_1$. Noting also that Na(2) is observed at 0 ppm, it is straightforward to assign this line to free sodium on the bases of the vanishing quadrupole coupling and the chemical shift that is identical to the external reference.

Having accounted for free and surface sodium sites, we now ask if it is reasonable to assign Na(1) to sodium ions in the channel of [d(TG,T)]$_4$ (Figure 5.6). In addition to the unusual chemical shift, a strong indicator of such an assignment is the observation in Figure 5.12 of the coupling between Na(3) and Na(1) which indicates that the surface sodium sites are proximate to a non-amorphous, chemically distinct class of sodium which must reside on the interior. We note also that Na(1) nutates at twice the applied r.f. field strength, thus indicating a rigid, non-symmetric environment that would result in a measurable quadrupole coupling. Based on the chemical shift, the correlation spectroscopy, and the quadrupole coupling we conclude that Na(1) must correspond to channel sodium ions.
We turn to investigate the possibility of improving the resolution in the Na(1) region with the goal of detecting distinct channel sites. Another homonuclear correlation experiment was carried out to try to detect correlations within Na(1) and is shown in Figure 5.15. There is evidence of a weak crosspeak in Figure 5.15 between the edge and the center of the Na(1) line. This would compliment the observation in Figure 5.12, where the Na(3) is correlated to the same edge of Na(1), suggesting that surface sodium, Na(3), is correlated with the outermost channel ions which are logically the closest to the surface.

![Figure 5.15](image)

**Figure 5.15.** Homonuclear correlation as in figure 5.11, with τ=3ms and ω₀=2.9 kHz. 64 t₁ increments with 64 transients each were collected. Recycle time was 3 seconds, and the temperature was -1.9°C±0.5°C.

The distinction between at least two types of channel Na ions is expected on the basis of the binding symmetry, since Na(4,4’) are bound in the guanine plane, while the remaining five
are bound in between guanine planes with octahedral coordination. Due to the differing symmetries, the quadrupolar couplings for \(\text{Na}(4,4')\) are expected to differ from those for \(\text{Na} (3,3',2,2',1)\). The differences in the isotropic quadrupole shifts may explain the ~3ppm shift. However, there may also be a contribution from the isotropic chemical shift since \(\text{Na}(4,4')\) are exposed to the surface. Unfortunately, the crosspeak in Figure 5.15 is weak and highly asymmetric and it is difficult to state this conclusion with certainty. The asymmetry might be addressed by applying very short pulses in order to obtain quantitative excitation of the central transition[29-31], while improved transfer should be seen with slower MAS and longer mixing times.

Finally, we return to Figure 5.9 in which the lyophilized and slow-dried spectra are compared and note that there is no shift in the position of the Na(1), suggesting that this feature is a constant of sample preparation. This is consistent with our assignment of Na(1) to channel sodium ions which reside in the interior of the DNA quadruplex. The Na(2) line narrows and shifts substantially to 0 ppm, indicating that this resonance is very sensitive to sample preparation. In fact, the Na(2) line in the slow-dried sample likely corresponds to NaCl microcrystals, given the chemical shift and the nature of the slow-drying method. In contrast, the lyophilized sample would appear to result in an amorphous distribution of free Na\(^{+}\) ions. Finally, the surface sodium line (Na(3)) does not shift significantly, indicating that both samples exhibit four stranded quadruplex structures, although the lyophilized sample is postulated to be highly amorphous and have few if any stacked quadruplexes. The existence of quadruplexes in the lyophilized sample is also confirmed by the presence of the Na(1) resonance.

In summary, we have assigned three lines in the \(^{23}\text{Na}\) NMR spectrum of \([\text{d(TG}_4\text{T)}]_4\) through the combination of a suite of techniques including 2D homonuclear correlation and 2D MAS nutation spectroscopy at high field. In addition, we introduced the 3Q filter as a means of detecting motional effects in low temperature studies and find that the DNA does exhibit substantial motions which could be explained by axial rotations. Finally, preliminary results suggest that the resonance corresponding to channel ions may be separated into planar Na ions at the edges (4ppm) and the remaining octahedral Na ions in between the planes (6.8 ppm). We illustrate these results with a diagram of \([\text{d(TG}_4\text{T)}]_4\) in Figure 5.16.
Figure 5.16. Representation of assignments determined by solid-state NMR for 23Na in [d(TG4T)4] in which three general classes of sodium are assigned to free, surface, and channel ions.
5.4. Direct Observation of Quadrupolar Nuclei in Biological Solids

Two studies have been presented in which quadrupolar nuclei were directly observed in biological solids, one a protein system and the other DNA. In both cases, it was demonstrated that the signal due to the active site could be distinguished from any background signals. A suite of complimentary techniques was introduced to permit assignments in the presence of multiple sites. The ability to assign multiple lines represents the first critical step in extracting further structural information.

Two novel strategies for exploiting aspects of the quadrupolar coupling were introduced: (1) the use of multiple-quantum filtering, sensitive to motional averaging of the quadrupole coupling tensor and (2) two-dimensional MAS nutation spectroscopy, which probes quadrupole couplings obscured by amorphous broadening.

These results are very encouraging of the prospect that quadrupolar NMR can be applied to address relevant questions regarding biological solids. In the first study, we demonstrated that the quadrupole coupling clarifies the binding symmetry in the catalytic triad of an α-Lytic Protease/inhibitor complex. The ability to extract geometrical information from the active site without any isotopic enrichment and in just a few hours of spectrometer time is a compelling illustration of the potential of quadrupolar NMR. In the second study, the assignments of three lines were obtained despite sample motions, amorphous broadening, and varying quadrupolar couplings. The molecular mass of the four stranded \([d(TG_4T)]_4\) is approximately 7.6 kDa. Our study of a multispin system in a large biomolecule illustrates that quadrupolar NMR at high field is not limited to simplistic or model systems.
5.5. References


6. Summary and Future Directions

6.1. Methods Development in Quadrupolar NMR

The isotropic MQMAS experiment has been extended to yield quantitative site populations with increased signal to noise through RIACT methodology. Current attention is now focused on exploiting the polarization in the side (satellite) transitions to gain further overall enhancements. One of the most promising directions of quadrupolar research is the development of correlation experiments. A multiple-quantum cross-polarization experiment has been described here which is ideally suited for improving the sensitivity of heteronuclear correlations in isotropic MQMAS spectra. A homonuclear correlation experiment has also been described which is an extension of the r.f. driven rotary resonance effect that was first observed in spin-1/2 methods. In general, continued development of quadrupolar methodology should continue to take lessons from the impressive accomplishments in the spin-1/2 community. A compounding challenge, though, is the need to combine correlation spectroscopy with MQMAS spectroscopy to provide structural information in true high-resolution dimensions. Such combined experiments require extensive phase cycling, and the implementation of pulsed field gradients to simplify phase lists as well as remove artifacts due
to errors in phase cycles should be developed for such applications. So far, there have been a paucity of investigations into pulsed field gradients for MQMAS [1, 2].

Also, it should be noted that the dipolar interaction is scaled by the MQ order during the multi-quantum evolution period, $t_1$, in MQMAS experiments. Studies of broadening mechanisms which contribute to residual MQMAS linewidths are sparse, although one study has shown the beneficial effect of TPPM decoupling [3] for reducing heteronuclear proton couplings during MQ evolution [4]. A related issue is the source of rotational sidebands in the isotropic dimension of MQMAS experiments. This has been recently addressed, where the influence of r.f. pulses and the zeroth-order quadrupole coupling have been shown to contribute to the sideband manifold [5, 6]. However more work is still required to extend and clarify these results.

The use of a 3Q filter to detect motions in amorphously broadened samples shows considerable promise for gaining information from samples which do not crystallize well. As biophysical studies develop an increasingly dynamic view of biochemical processes, sensitive methods for characterizing motions should become important. Also, the 2D MAS Nutation experiment was particularly helpful in solidifying the assignments in multispin systems. Experiments which combine this with MQMAS methodology may reveal more information and are deserving of more investigation.

In the spirit of brevity, it should be clear that methodology for quadrupolar solid-state NMR is an underdeveloped area in which new insights are revealed at a remarkable pace. Discovering new ways to exploit the quadrupole coupling in half-integer quadrupolar nuclei to yield structural and dynamical information is a true frontier in solid-state NMR.

6.2. Biological Solids

It has been demonstrated in two very different systems that quadrupolar NMR has the sensitivity and relative maturity to access a range of challenging problems in biochemical research. In combination with high static fields, advances in r.f. technology and sample spinning designs, the types of systems which are accessible will only increase. Nuclei which have been previously 'invisible' to other methods were shown to be accessible with current
SSNMR techniques, and in combination with new techniques presented here. Oxygen-17 remains among the most significant and challenging quadrupolar nuclei in biological solids and continued investigations at higher field represent an ideal future direction for this work. Fundamental studies into the sensitivity of the dependence of the $^{17}$O quadrupole coupling on parameters such as hydrogen bonding, for example, have not been rigorously reported in the literature.

### 6.3. References

7. Appendices

7.1 Supplemental Calculations

7.1.1. Formulating the tensor product in the high order quadrupolar Hamiltonian

A convenient manner in which to sum the high order term in the quadrupolar Hamiltonian is

\[
H_Q^{(1)} = \frac{\omega_Q^2}{2\omega_0} \sum_{m=-1,2}(\text{m})R_{2,m}R_{2,-m}[T_{2m},T_{2,-m}]
\]

\[
= \frac{\omega_Q^2}{\omega_0} \sum_{m=1,2}(\text{m})R_{2,m}R_{2,-m}[T_{2m},T_{2,-m}]
\]

\[
= \frac{\omega_Q^2}{\omega_0} \left(R_{2,1}R_{2,-1}[T_{21},T_{2,-1}] + (\frac{1}{2})R_{2,2}R_{2,-2}[T_{22},T_{2,-2}] \right). \tag{1}
\]

We must now transform the \( R_{2,m} \)’s into the laboratory frame to give

\[
H_Q^{(1)} = \frac{\omega_Q^2}{\omega_0} \left(\sum_{j,k=-2}^{2} \left( D_{j,1}^2 D_{k,-1}^2 \rho_{j,2} \rho_{2k}[T_{21},T_{2,-1}] \right) + \sum_{j',k'=-2}^{2} \left( D_{j',2}^2 D_{k',-2}^2 \rho_{j',2} \rho_{2k'}(\frac{1}{2})[T_{22},T_{2,-2}] \right) \right). \tag{2}
\]

The tensor product is now expressed in a larger basis by applying two identities in series which are provided by Rose[1]. First we contract the rotation matrices using the Clebsch-Gordon coefficients \( C(l_1 l_2 l; \mu_1 \mu_2) \):

\[
D_{j_1,m_1}^{l_1} D_{j_2,m_2}^{l_2} = \sum_l C(l_1 l_2 l; \mu_1 \mu_2) C(l_1 l_2 l; m_1 m_2) D_{j_1,m_1}^{l_1} \tag{3}
\]

to give

\[
H_Q^{(1)} = \frac{\omega_Q^2}{\omega_0} \left( \sum_{j,k=-2}^{2} \left( \sum_{l=0}^{4} C(2l,1,1) C(2l,jk) D_{j,k}^{l} \rho_{j,2} \rho_{2k}[T_{21},T_{2,-1}] \right) + \sum_{j',k'=-2}^{2} \left( \sum_{l=0}^{4} C(2l,1,-1) C(2l,j'k') D_{j',k'}^{l} \rho_{j',2} \rho_{2k'}(\frac{1}{2})[T_{22},T_{2,-2}] \right) \right). \tag{4}
\]
Next, we substitute Equation 3 into
\[ \sum_{\mu_1 \mu_2} D_{\mu_1 \mu_2}^j D_{\mu_1 \mu_2}^j \rho_{\mu_1 \mu_2} = \sum_l \sum_{\mu} C(l_1 l_2 l_1 m_1 m_2) D_{\mu_1 \mu_2}^j \rho_{l_1 \mu} \rho_{l_2 \mu_2} \]

(5)
to derive the following identity
\[ \sum_{\mu_1 \mu_2} \sum_{l=0}^{\infty} C(l_1 l_2 l_1 m_1 m_2) D_{\mu_1 \mu_2}^j \rho_{l_1 \mu} \rho_{l_2 \mu_2} \]
\[ = \sum_{\mu} \sum_{l_1} \sum_{m_1} C(l_1 l_2 l_1 m_1 m_2) D_{\mu \mu_1 \mu_2}^j \rho_{l_1 \mu} \rho_{l_2 \mu_2} \]
\[(6)\]

Equation 6 can be applied to simplify each term in Equation 4,
\[ H_Q^{(1)} = \frac{\omega_0^2}{\omega_0} \sum_{l=0}^{\infty} \sum_m \left( C(22l;1,-1) D_{m,0}^l \left[ T_{21}, T_{2,-1} \right] + C(22l;2,-2) D_{m,0}^l \left( \frac{5}{12} \left[ T_{22}, T_{2,-2} \right] \right) \right) \]
\[(7)\]

However, this can be further simplified by recognizing that each of the two terms corresponds to the transformation of a rank 4 tensor out of the PAS frame, in analogy to Equation 2.6.
Thus Equation 7 gives
\[ H_Q^{(1)} = \frac{\omega_0^2}{\omega_0} \sum_{l=0}^{\infty} \sum_m R_{l,m} \left( C(22l;1,-1) \left[ T_{21}, T_{2,-1} \right] + C(22l;2,-2) \left( \frac{5}{12} \left[ T_{22}, T_{2,-2} \right] \right) \right) \]
\[(8)\]

From Bowden et al. the tensor commutators for S=3/2 are [2, 3]
\[ \left[ T_{21}, T_{2,-1} \right] = -\frac{5}{3} T_{10} - 4\sqrt{\frac{5}{3}} T_{30} \]
\[ \left[ T_{22}, T_{2,-2} \right] = \frac{12 \sqrt{5}}{5} T_{10} - 2\sqrt{\frac{5}{3}} T_{30} \]
\[ T_{10} = I_Z, \quad T_{30} = \sqrt{\frac{7}{30}} \left( 5I_Z^2 - 3I(I+1) + 1 \right) I_Z \]
\[(9)\]

Before bringing everything together, note that we can expand the operator \( I_Z \) into a sum of fictitious spin-1/2 operators for arbitrary \( I \) as
\[ I_Z = \sum_{m=\frac{1}{2}, \ldots, S} 2m l_{m}^{\pm} \]
\[(10)\]

By inserting equations 9 and 10 into Equation 8, we have
\[ H_Q^{(1)} = \sum_{l=0}^{\infty} \sum_{m=\frac{1}{2}, \ldots, S} C_{l,m} R_{l,0} \left[ I_Z \right] \]
\[ C_{l,m} = \left( a_{m}^{(1)} C(22l;1-1) + a_{m}^{(2)} C(22l;2-2) \right) \]
\[ a_{m}^{(1)} = 4m \left[ \frac{5}{3} l(I+1) - 2m^2 - 1 \right] \]
\[ a_{m}^{(2)} = 2m \left[ \frac{5}{3} l(I+1) - m^2 + 1 \right] \]
\[(11)\]
If it were not for the spin tensor commutators, Equation 11 would completely general for arbitrary spin. The recipe for calculating tensor commutation is also given by Bowden[2, 3]. If I is restricted to spin-3/2, the result of Wu et al. is obtained [4].

7.1.2. Effective Hamiltonian of a Quadrupolar Nucleus During R.F. Irradiation.

This is a supplement for the derivation of an effective Hamiltonian which is useful for the treatment of the triple-quantum cross-polarization (TQCP) effect and for the quadrupolar HORROR -analogue experiment. Although the calculation is straightforward, this supplement aims to make the procedure as accessible as possible and is divided into three sections:

(1). The initial rotating frame Hamiltonian
(2). Some properties of diagonal matrices
(3). The quadrupolar interaction frame.

(1) The starting point is the ‘rotating frame’ Hamiltonian for a quadrupolar nucleus under an applied r.f. field:

\[ H = \omega_S S_x + H_Q, \]  \hspace{1cm} (1)

where \( H_Q \) is given as

\[ H_Q = A_{20}(t)T_{20} + \sum_{m=\pm 1/2} \frac{1}{\omega_0} A_{2m}(t)A_{2-m}(t)[T_{2m},T_{2-m}]. \]  \hspace{1cm} (2)

which is understood as either a combination of the first and second order perturbation results, or as a combination of the 0'th order average Hamiltonian with the secular terms of the 1'st order average Hamiltonian[5], both cases are treated in chapter 2.

Likewise, the first term is the standard result for transforming an r.f. term into the rotating frame. This is illustrated here. Let a laboratory frame Hamiltonian be given as

\[ H_{lab} = H_{Zeeman} + H_{r.f.} \]

\[ = -\gamma S H_Z S_Z - 2\gamma S H_{1S} \cos(\omega_{df} t) S_x \]  \hspace{1cm} (3)
where the nucleus is denoted by the spin quantum number \( S \), the gyromagnetic ratio is \( \gamma_s \), the angular frequency of the radiation is \( \omega_{\text{rf},s} \), and the magnetic component of the r.f. field is \( H_{15} \). As in chapter 2, the convention of writing the Hamiltonian in units of angular frequency (i.e., \( \frac{1}{\hbar} \)) is taken here. Additionally, it is often conventional to introduce the factor of 2 in the r.f. term.

To transform into the doubly rotating frame, we write Equation 3 as

\[
H_{\text{lab}} = \omega_{\text{rf},s} S_z - (\gamma_s H_0 - \omega_{\text{rf},s}) S_z - 2\gamma_s H_{15} \cos(\omega_{\text{rf},s} t) S_x
\]  

(4)

where we recognize this to have the form

\[
H_{\text{lab}} = H_0 + H_1
\]

\[
H_0 = \omega_{\text{rf},s} S_z
\]

\[
H_1 = -(\gamma_s H_0 - \omega_{\text{rf},s}) S_z - 2\gamma_s H_{15} \cos(\omega_{\text{rf},s} t) S_x
\]

and the rotating frame Hamiltonian will be given by

\[
H^* = e^{iH_0 t} H e^{-iH_0 t}
\]  

(6)

When evaluating equation 6, it is important to note that when \( H_0 \) and \( H_1 \) commute then \( H_1 \) is left unchanged. Otherwise, it is straightforward to perform the rotations that results from inserting Equation 5 into Equation 6 and these have already been shown in chapter 2. We have now

\[
H^* = -(\gamma_s H_0 - \omega_{\text{rf},s}) S_z - 2\gamma_s H_{15} \cos(\omega_{\text{rf},s} t)(S_x \cos(\omega_{\text{rf},s} t) - S_y \sin(\omega_{\text{rf},s} t)),
\]  

(7)

which simplifies to

\[
H^* = -2\gamma_s H_{15} \cos(\omega_{\text{rf},s} t)(S_x \cos(\omega_{\text{rf},s} t) - S_y \sin(\omega_{\text{rf},s} t))
\]

(8)

if the applied field is assumed to be on-resonance with the Zeeman frequency. However, it is often desired to treat off-resonance effects and so, for completeness, this off-resonance term is kept for this section. The final step then is to take an average over one period or cycle of the Zeeman frequency \( \tau_{\text{cycle}} = 2\pi / \omega_{\text{rf},s} \)

\[
\langle H^* \rangle = \frac{1}{\tau_c} \int_0^{\tau_c} H^*(t) dt.
\]  

(9)

Over periodic limits, the integral of any odd function vanishes, so the term involving \( \cos(\omega_{\text{rf},s} t) \sin(\omega_{\text{rf},s} t) S_y \) can be ignored. For reference we give the integral
\[
\int \cos^2(at)\,dt = \frac{1}{2}t + \frac{1}{4a}\sin(2at),
\]
and the final result becomes
\[
\overline{H}^* = \omega_{15}S_x + (-\gamma_5H_0 - \omega_{\tau,5})S_z
\]
where \(\omega_{15} = -\gamma_5H_{15}\) is the amplitude and, again, \(\omega_{\tau,5}\) the frequency, of the applied field. If the frequency of the applied r.f. field is identical to the Zeeman frequency, the second term is 0, which leaves the sole term \(\overline{H}^* = \omega_{15}S_x\) as it appears in Equation 1.

(2) Some properties of diagonal matrices.

Before carrying out the explicit calculation, some aspects on the properties of diagonal matrices are reviewed. In particular, note that \(T_{20}\) is a diagonal operator. Now, let \(\hat{P}\) be a square, diagonal matrix (\(n\times n\)):

\[
\hat{P} = \begin{bmatrix}
p_{11} & 0 \\ \vdots & \ddots \\ 0 & \cdots & p_{nn}
\end{bmatrix}
\]

Then
\[
(\hat{P})^k = \begin{bmatrix}
p_{11}^k & 0 \\ \vdots & \ddots \\ 0 & \cdots & p_{nn}^k
\end{bmatrix}
\]

and
\[
e^{\hat{P}} = \begin{bmatrix}
e^{p_{11}} & 0 \\ \vdots & \ddots \\ 0 & \cdots & e^{p_{nn}}
\end{bmatrix}
\]
since
\[
e^{\hat{P}} = \sum_{k=0}^{\infty} \frac{(\hat{P})^k}{k!} = 1 + \hat{P} + \frac{(\hat{P})^2}{2} + \frac{(\hat{P})^3}{6} + \ldots
\]

Now we evaluate an example to see how Equations 12-15 apply. Consider
The quadrupolar interaction frame.

The Hamiltonian in Equation 1 is now re-expressed in an interaction frame, recognizing, similar to the preceding treatment to obtain the rotating frame result, that

\[ H = H_0 + H_1 \]

\[ H_0 = \omega S_x \]  \hspace{1cm} \text{(17)}

\[ H_1 = H_0 \]

The new interaction frame will be given by

\[ H' = U(t)S_xU^{-1}(t) \]

\[ = e^{i\Delta \tau_0}S_xe^{-i\Delta \tau_0}. \]  \hspace{1cm} \text{(18)}

We proceed to explicitly show this calculation using an eigenbasis approach, beginning with the detailed expressions for \( S_x, H', \), and \( T_{20} \):

\[ S_x = \left( \frac{1}{2}\right| \frac{1}{2} \rangle + \frac{1}{2}\left| \frac{3}{2} \right. \rangle + \frac{\sqrt{3}}{2}\left( \frac{1}{2}\left| \frac{1}{2} \rangle \right. + \frac{1}{2}\left| \frac{1}{2} \rangle + \frac{1}{2}\left| \frac{3}{2} \rangle \right. \right) \]

\[ H' = e^{i\Delta \tau_0}S_xe^{-i\Delta \tau_0} \]

\[ T_{20} = \frac{1}{36} \left( \left( \frac{3}{2}\right| \frac{3}{2} \rangle + \frac{1}{2}\left| \frac{1}{2} \rangle \right. \right) - \left( \frac{1}{2}\left| \frac{1}{2} \rangle + \frac{1}{2}\left| \frac{3}{2} \rangle \right. \right) \]

\[ e^{\frac{i^2}{4}\left( \left( \frac{1}{2}\right| \frac{1}{2} \rangle + \frac{1}{2}\left| \frac{1}{2} \rangle \right. \right)^2 + \frac{i}{6}\left( \left( \frac{1}{2}\right| \frac{1}{2} \rangle + \frac{1}{2}\left| \frac{1}{2} \rangle \right. \right)^3} \]

\[ = \left( 1 + \frac{1}{2}\left( \frac{1}{2}\right| \frac{1}{2} \rangle + \frac{1}{2}\left( \frac{1}{2}\right| \frac{1}{2} \rangle \right) + \frac{i}{6}\left( \left( \frac{1}{2}\right| \frac{1}{2} \rangle + \frac{1}{2}\left( \frac{1}{2}\right| \frac{1}{2} \rangle \right)^3 + \ldots \right) \left( \frac{1}{2}\right| \frac{1}{2} \rangle \right) \]

\[ = 1 + \frac{1}{2} + \frac{i}{6} + \ldots \left( \frac{1}{2}\right| \frac{1}{2} \rangle \right) \]

\[ = e^{i\Delta \tau_0} \]

\[ \text{(3). The quadrupolar interaction frame.} \]
\[ H^- = \]
\[ e^{-i \frac{\alpha_{30}^2}{\sqrt{6}} \left( \frac{1}{2} \right) \left( \frac{1}{2} \right) e^{i \frac{\alpha_{30}^2}{\sqrt{6}}} \] 
\[ + e^{-i \frac{\alpha_{30}^2}{\sqrt{6}} \left( \frac{1}{2} \right) \left( \frac{1}{2} \right) e^{i \frac{\alpha_{30}^2}{\sqrt{6}}} \] 
\[ + e^{i \frac{\alpha_{30}^2}{\sqrt{6}} \left( \frac{1}{2} \right) \left( \frac{1}{2} \right) e^{i \frac{\alpha_{30}^2}{\sqrt{6}}} \] 
\[ + e^{-i \frac{\alpha_{30}^2}{\sqrt{6}} \left( \frac{1}{2} \right) \left( \frac{1}{2} \right) e^{i \frac{\alpha_{30}^2}{\sqrt{6}}} \] 
\[ + e^{i \frac{\alpha_{30}^2}{\sqrt{6}} \left( \frac{1}{2} \right) \left( \frac{1}{2} \right) e^{i \frac{\alpha_{30}^2}{\sqrt{6}}} \].

(20)

The exponentials are then carried through:

\[ H^- = \frac{1}{2} \left( \left| \frac{1}{2} \right> \left< \frac{1}{2} \right| + \left| \frac{1}{2} \right> \left< \frac{1}{2} \right| + e^{i \frac{\alpha_{30}^2}{\sqrt{6}}} \left( \frac{1}{2} \right) \left( \frac{1}{2} \right) e^{-i \frac{\alpha_{30}^2}{\sqrt{6}}} \right] \] 

(21)

**7.1.3. Computing an exponential operator.**

For the numerical simulation of NMR observables, a propagator of the form \( U = e^{-iHt} \) must be computed. This is accomplished by invoking the well-known theorem that, for a Hermitian matrix, a unitary matrix \( D \) can be formed from the eigenvectors of the Hermitian matrix such that

\[ DHD^+ = H^{(\text{diag})}, \]
\[ H = D^+ H^{(\text{diag})} D, \]  

(22)

where \( D^* \) is the transpose complex-conjugate of \( D \), and a matrix is unitary if \( D^* = D^{-1} \).

Then the propagator becomes

\[ e^{-i(D^* H^{(\text{diag})} D^t)} = D^+ e^{-i H^{(\text{diag})} t} D. \]

(23)

This is now easily evaluated by exponentiating each element of the diagonal matrix and operating with \( D^* \) and \( D \). We justify this result by expanding the exponential.
\[ e^{-iD^*H_{\text{diag}}D\tau} = \sum_{k=0}^{\infty} \frac{(-iD^*H_{\text{diag}}D\tau)^k}{k!} \]
\[ = \left(-iD^*H_{\text{diag}}D\tau\right)^0 + \left(-iD^*H_{\text{diag}}D\tau\right)^1 + \frac{1}{2} \left(-iD^*H_{\text{diag}}D\tau\right)^2 + \ldots \]
\[ = D^* \left[ 1 + \left(-iH_{\text{diag}}\tau\right) + \frac{1}{2} \left(-i\tau\right)^2 \left(H_{\text{diag}}^2\right) + \ldots \right] D \]
\[ = D^* e^{-iH_{\text{diag}}\tau} D \]

### 7.1.4. Rotations of Spin Operators

The NMR Hamiltonian can be written as the product of two second-rank tensors, as described in Chapter 2 and equation 2.2: a tensor describing the spatial dependence of a given coupling and a tensor for the spin operator components. Equations 2.4-2.6 described how the spatial tensors can be expressed in different coordinate systems in order to treat effects such as sample rotation and powder averaging. We show here how to perform rotations of the spin tensors. Such rotations are imposed by applied r.f. fields, not by mechanical reorientation of the sample, since the spin angular momenta are quantized relative to the external, static magnetic field, \( B_0 \). This class of transformations is used for the rotating frame transformation (that is, a frame rotating at the Zeeman frequency as in section 7.1.1.) , and for transformations into other interaction frames (see sections 2.2.3. and 2.2.5.). The operations take the general form

\[ H_{(\text{new})} = U^{-1}H_{(\text{old})}U, \]
\[ U = e^{-i\alpha S_\alpha}, \alpha = x, y, z \] (25)

Often, as in the rotating frame transformation, the rotation angle is time dependent:

\[ U(t) = e^{-i\omega S_\alpha} \] (26)

where \( \omega \) might be a Zeeman or other interaction frequency. It is given here how to treat transformations such as those in Equations 25 and 26. The derivations of these are straightforward and are given, for example, by Slichter (see pages 26-28)[6]. All such rotations of spin angular momentum can be summarized by the examples given in Figure 7.1.
Figure 7.1. Rotation of spin angular momentum operators through an arbitrary angle \( \theta \).

Although Figure 7.1. does not illustrate every possible rotation (see Haeberlen [7], Appendix A), a comparison of the figure and the solutions shows that the solutions are very intuitive and all others may be quickly deduced from the ones given here.

### 7.1.5. Computer Program for Calculating Clebsch-Gordon Coefficients

The computation of Clebsch-Gordon coefficients is important to the determination of the refocusing constant \( k \) in MQMAS experiments. The constant \( k \) also determines the spectral width in the isotropic dimension and the effective MAS rate as well. The computer program is designed after the formulas given by Rose (page 39).[1]
The following program, written in standard C, is presented in this thesis by the author under the terms of the General Purpose Software License, either version 2 or higher, a copy of which can be obtained from.

The Free Software Foundation, Inc.
59 Temple Place
Suite 330
Boston, MA 0211-1307

/**************************************************************************
/** Program to Calculate Clebsch-Gordon Coefficients and present
/** the answer in decimal form; therefore a very slight error exists
/** in roughly the 10'th or 12'th decimal place and is likely to be
/** negligible for all practical considerations.
/**
/** Usage: I. This code is self contained; it does not need to be
/** linked against any special libraries during compilation.
/**
/** II. The notation is as follows. The C-G Coefficients
/** describe how to express a state, which involves
/** coupled angular momenta, as a sum of vectors in the uncoupled basis. This is for combining two
/** angular momenta: j1 and j2 are the individual angular momenta; j3
/** is the total angular momentum such that j3 = (j1+j2), (j1+j2-1),...|j1-j2|)
/** Then m1 and m2 are the individual projections onto the z-axis of j1 & j2 and m3=m1+m2.
/**
/** CREDIT: This follows from Rose (pg. 39)
/**
/** D. Rovnyak, Oct. 5, 1995
/****************************************************************************/

#include <stdio.h>
#include <stdlib.h>
#include <math.h>

/**Function Declarations***********************************************/

double forefactor(float jl, float j2, float j3, float ml, float m2, float m3);
double sumfactor(float jl, float j2, float j3, float ml, float m2, float m3, int v);
void checkint(float ma, float mb, float mc);
double fact(float i);

/**Function Definitions***********************************************/
double forefactor(float jl, float j2, float j3, float ml, float m2, float m3){
    double numerator, denominator, answer;
    numerator=fact(j3+jl-j2)*fact(j3-jl+j2)*fact(jl+j2-j3)*fact(j3+m3)*fact(j3-m3)*pow(2,j3+1);  
    denominator=fact(jl+j2+j3+1)*fact(jl-ml)*fact(jl+ml)*fact(j2-m2)*fact(j2+m2);
    answer=sqrt(numerator/denominator);
    return(answer);
}
double sumfactor(float jl, float j2, float j3, float ml, float m2, float m3, int v) { 
    double numerator, denominator, answer;
    numerator=fact(j2+j3+ml-v)*fact(j1+ml)*pow(-1,v+j2+m2);
denominator=fact(v)*fact(j3-j1+j2-v)*fact(j3+m3-v)*fact(v+j1-j2-m3);
answer=numerator/denominator;
return(answer);
}

void checkint(float ml, float m2, float m3) {
if ( (ml+m2) != m3) {
    printf("ml + m2 != m3 !!!!!!\n"); exit(-1); }
}

double fact(float i) {
    long int count=1, dummy=i;
double sum;
if ((i-dummy)!=-0.0) {
    printf("Fraction argument to factorial!\n"); exit(-1); }
if (i<0) {
    printf("Negative argument to factorial!\n"); exit(-1); }
if (i==0.0) return(1);
sum=i;
for ;(i-count)>0;count++) {
    sum*=(i-count);
}
return(sum);
}
/**Main Program**************************************************/
main() {
float j1, j2, j3, ml, m2, m3;
int loop=1, v=0;
double factor, sum=0.0, answer;

printf("Enter j1, j2, j3, ml, m2, m3: ");
scanf("%f %f %f %f %f %f", &j1, &j2, &j3, &ml, &m2, &m3);

/**Check that ml+m2=m3***/
checkint(ml, m2, m3);

/**Calculate forefactor***/
factor=forefactor(j1, j2, j3, ml, m2, m3);

/**Perform sum over legal values of v***/
for(;loop==1;v++){
    if ( ((j3-j1+j2-v)>=0) && ((j2+j3+ml-v)>=0) && ((j1-ml+v)>=0)
        && ((j3+m3-v)>=0) && ((v+j1-j2-m3)>=0) )
        sum+=sumfactor(j1, j2, j3, ml, m2, m3, v);
else loop=0;
}

V=-1;

for(;loop==1;v--){
    if ( ((j3-j1+j2-v)>=0) && ((j2+j3+ml-v)>=0) && ((j1-ml+v)>=0)
        && ((j3+m3-v)>=0) && ((v+j1-j2-m3)>=0) )
        sum+=sumfactor(j1, j2, j3, ml, m2, m3, v);
else loop=0;
}

answer=factor*sum;
printf("\nC(%.lf,%.lf,%.lf,%.lf,%.lf,%.lf)=%.16lf\n", j1,j2,j3,ml,m2,m3,answer);
7.2. A Graphical Simulation Environment for Quadrupolar NMR

Research in solid-state NMR relies heavily upon numerical simulations to test and verify new ideas. The success of numerical calculations in NMR spectroscopy can be attributed to the relatively simple forms of the Hamiltonians, the ability to perform piece-wise-constant density matrix propagation, and the availability of algorithms which run efficiently on modern computer hardware. Developing new methods for performing NMR simulations has become a research discipline in its own right[8-11].

Numerical simulations have been a fundamental component of the work shown in this thesis, particularly when investigating issues of sensitivity of MQMAS experiments. Here it was found that the efficiencies of multiple quantum coherence transfers were dependent on a great number of variables including the quadrupole coupling, the r.f. field strength, the resonance offset, the MAS frequency, the approximations to the quadrupolar Hamiltonian, etc. In order to observe meaningful trends in these simulations it is necessary to manage and keep track of all of these variables and more, carefully changing only one at a time. During the course of this thesis, a software project evolved to meet such needs, and will be referred to as the Quadrupolar Simulation Interface, or QSI.

The source code for QSI is currently available at http://tachyon.mit.edu, however future releases may occur on the Griffin group web page. The source code will not be reported here for a number of reasons, the first being that is available in the public domain on the world-wide-web. Moreover, much of the code is devoted to creating the user interface, and this is a matter which is not of significant academic interest here. But most important, QSI is an evolving project and any record of the source code here would be a snapshot that would serve little purpose since updates may appear every 3-4 months.

7.2.1. The Simulation Code Base

All simulations are based upon the GAMMA C++ platform for general magnetic resonance simulations[11, 12]. This is a peer-reviewed, extensively tested suite of C++ class
libraries (e.g. tensors, operators, rotation matrices, etc.). GAMMA is principally designed for NMR simulations and is starting to find growing popularity in the NMR community. GAMMA takes full advantage of the architecture of the C++ language, which is an object-oriented language that offers data hiding and the well-known class structure.

The graphical interface is written with Tcl/Tk, however the use of short command-line scripts requires that the platform for QSI be unix, even though Tcl/Tk is a cross-platform scripting language. Eliminating the dependence of QSI's operations on unix shell commands is, of course, an area for future work in order for QSI to be useful on a wider number of platforms. Both Tcl/Tk, and GAMMA are routinely available under Microsoft Windows 98 or Windows NT. QSI was developed on a pentium workstation running the Linux operating system. Such workstations offer impressive performance in the sub-$1k price range and their numbers are growing in academic research laboratories; hence the restriction of QSI to unix-type machines is not necessarily a limitation for the immediate future.

The plotting environment is externally supplied by either Ace/Gr (often known as XMGR) or GnuPlot, where QSI can spawn either of these programs.

### 7.2.2. The Root Window of QSI

In Figure 7.1. a grayscale screenshot of the root window of the QSI environment is displayed. This offers all of the functionality which is built into QSI and illustrates the manner in which QSI is intended to be used. As described briefly in section 2.3.3., a user conducts simulations in a 'session'. A master file can be saved which records and organizes every data set generated during that session and every parameter list also. The 'session' may be closed and reopened at any time to review the data, and add or remove simulations as needed. We discuss the general features of the QSI environment next by examining the elements of the main screen with which the user interacts.
Figure 7.1. Screen capture of the root window of QSI version 0.30, converted to greyscale. This shot is taken during a session in which a number of simulations have already been carried out.

We note three general areas: a toolbar at the top of the widow from which the majority of operations are initiated, a console window to the right of the QSI logo and located roughly in the middle of the entire frame, and finally a large double-columned window which is called the session log.

**Toolbar.** The toolbar is where the user generally begins a QSI session. After starting the QSI program, the user may specify under the ‘session’ button the name of the master file to be saved, the user’s name, and a working directory in which to store the data that is generated. At any time during the session, the user may periodically save their work under the ‘session’ menu. The actual simulations are configured by clicking on either the ‘1_spin’ or ‘2_spin’ buttons to view a list of available modules. It is important to stress here that QSI is not an environment for general solid-state NMR simulations. Instead, through highly configurable modules, QSI presents the user with a variety of pre-programmed experiments for which the user must supply all variables or accept defaults. Later in this section, the RIACT module will be considered in detail. To view the results of a simulation, the user may highlight any
number of sets which are displayed in the session log and then choose among a variety of display options under the ‘view’ menu. As discussed earlier, the ‘view’ option currently supports XMGR and GnuPlot. Finally, the toolbar offers a help page which implements pop-up messages to provide easy access to explanations on a number of topics. Pop-up messages have been incorporated in all regions of QSI, including the simulation modules and a user may, at any time, turn off the balloon help with a switch that is located under the ‘session’ menu.

Console Log. Any messages generated by the simulation are displayed to the console. In addition, a progress bar is located just above the console window for monitoring the time of running calculations. Messages generated internally by QSI are also displayed to the console window. Figure 7.1 shows some examples of the types of messages which are frequently displayed, including notifying the user of the isotropic quadrupolar shift involved in a given calculation, or informing the user on the progress of a batch job.

Session Log. Every time a simulation is carried out, an entry is recorded in the session log which identifies the file name of the data set that was created and the full parameter list used in that simulation. The session log keeps track of every simulation performed and greatly simplifies the task of reviewing work that has already been done. Entries in the session log may be renamed or deleted. Highlighting any number of sets in the session log selects those sets to be viewed externally. Also, a basic set of operations is for multiplying, adding or subtracting any two sets. There is no known limit to the number of sets which QSI can keep track of. This is likely dictated by the particular implementation of Tcl and may be platform dependent. For all practical purposes, a user can create an arbitrary number of data sets in one QSI sessions.

QSI does not represent the data from simulations in any kind of internal format. Rather, each simulation creates a separate data file which represents all information in ascii formatted columns. Thus one QSI session may generate a large number of data files. This has been an intentional decision since it eliminates the need to export data for use in other
applications, such as for preparing figures or for performing fitting routines. A feature for organizing data sets is the QSI working directory, which is simply a sub-directory specified by the user that QSI uses for storing all data files. It should also be noted that QSI records a time stamp for when the session (i.e. master file) was initiated and the most recent time that it was modified.

### 7.2.3. A QSI Module – Multiple Quantum Coherence Transfer

The most straightforward way to see how QSI enables a user to easily configure and run advanced quadrupolar simulations is to show one of the simulation modules. The most sophisticated module offered by QSI is that for simulating a rich variety of coherence transfer phenomena in half-integer quadrupolar nuclei in solids, and a picture of this module is shown in Figure 7.2. We describe the features of this in detail to illustrate the wide range of options available in calculating coherence transfer effects in isolated half-integer quadrupolar nuclei.

![Figure 7.2. The QSI module for configuring and running simulations which investigate coherence transfer effects in half-integer quadrupolar nuclei.](image-url)
This module is used most often for simulating RIACT phenomena and so we will refer to it often as the ‘riact’ module for ease of discussion. The top portion gives a brief overview of the purpose of the module, and one of the first things we note from the description is that the module is general for all half-integer quadrupolar nuclei.

The general purpose of the ‘riact’ module is to monitor coherences in half-integer quadrupolar nuclei during r.f. irradiation and magic-angle spinning. The first line is a list of radio-button choices for choosing the type of experiment which is desired. In Figure 7.2 the time evolution of a coherence during r.f. irradiation is selected. Other options allow a user to record the intensity of a given coherence over a range of resonance offsets, or quadrupole coupling, or MAS frequencies. The ‘Timing’ variable is measured in units of the rotor period and tells QSI how long to evolve the density matrix for. For the three profiles (Offset, Quadrupole Coupling, MAS frequency), this is the time at which a single point is collected, after which the evolution is repeated again for a different Offset, or Quadrupole coupling or MAS frequency.

The next frame is a set of tools for designing pulse shapes so that the r.f. amplitude may be modulated during the evolution of the coherence. A ‘view shape’ button is implemented to allow the user to see the r.f. amplitude profile that will be used by QSI before running any simulation. The button marked ‘other’ is given for a section in the source code of the riact module where a user may program in any other specialized shapes which they require. Also, the phase of the pulse relative to the observed coherence is specified to allow for the observation of either RIACT or nutation effects.

An extensive parameter list follows, all elements of which are intuitively presented with an identifying tag on the left and the required units on the right. It should be noted that there are a number of choices for the initial and observed coherences and that the ‘riact’ module deals with how to determine the forms of the operators and density matrices for arbitrary half-integer S. Also, QSI implements the formulas of Suzukawa et al.[10] for intelligent powder averaging for reducing computational time. These are two examples of important details in simulations which are handled transparently in QSI. Near the bottom are two radio-buttons for choosing the Hamiltonian that will be operative for evolving the spin system. The user may select one or both approximations (given by average-Hamiltonian theory or perturbation theories in Chapter 2) in the Hamiltonian. Finally, the user specifies
whether the simulation that has been configured will be run interactively or placed into a batch queue that will be run at a separate time.

The batching procedure places an entry into the session log with an additional tag ‘-batch-’. After any number of batch entries are created, the batch engine is started with a switch located under the ‘session’ toolbar.

7.2.4. **Current QSI Modules**

Other modules in QSI include those for simulating static or MAS line shapes of half-integer quadrupolar nuclei. For MAS line shapes, algorithms for the infinite-spinning rate assumption and for the floquet theory treatment are available for simulating pure centerband spectra or complete center and sideband spectra, respectively. These modules are useful for fitting the characteristic second-order line shapes in order to extract quadrupole coupling parameters. A module is also available for simulating the line shape of a quadrupolar nucleus under spin-locking, an area of some recent investigation by other authors that has not been considered here [13, 14]. In the category of two spins, a module for the simulation of the triple-quantum CP effects reported in Section 4.1 is available. Also, a module for simulating the spectra of spin-1/2 nuclei which are dipolar coupled to quadrupolar nuclei is given as well.

Clearly this is not a complete set of simulations for describing arbitrary quadrupolar effects. This does provide a robust, easily configured set of tools for running those simulations which are most relevent to recent developments in quadrupolar NMR. It also provides a number of relevent and complex examples of GAMMA code to researchers wishing to customize their own simulations.

There are a number of current directions for QSI, such as the implementation of more controls in the Hamiltonian. One other area of interest is that we are not aware of an easy-to-use tool for solid-state NMR simulations that would be useful in classroom instruction. Further developments of QSI should take potential teaching applications into mind.
7.3. Characterization of a CN Network Solid with NMR and High Field EPR [15]

Considerable attention has been focused on developing a synthetic route to a carbon-nitrogen material with mechanical and thermal properties comparable or superior to those of diamond. To date, no substance with the desired C₃N₄ stoichiometry in a silicon-nitride crystal lattice has been reported. One of the principal difficulties in the pursuit of ultra-hard carbon-nitrogen (CN) solids is the characterization of amorphous CN samples. We describe a solid-state NMR study of a paracyanogen-like solid utilizing ¹³C-¹⁵N Adiabatic-Passage Hartmann Hahn Cross-Polarization (APHH-CP) to perform dipolar filtering, and show that this method is well-suited for recoupling ¹³C-¹⁵N in network solids. In addition, high-frequency electron paramagnetic resonance (EPR) indicates a density of electron spins of approximately 1x10¹⁷ e-/cm³. We conclude by discussing how NMR and EPR data may be useful for optimizing CN-polymer samples as potential precursors for ultra-hard carbon nitrogen solids.

Introduction

Modern, high-performance materials are required for applications in computing (heat conduction and insulation), industrial processes (hardness and machinability) and medicine (bio-implants and surgical tools). Examples of synthetic materials which have emerged to address these markets include phosphazenes, synthetic diamonds, boron nitride, and many plastics. A recent approach for the development of new materials is the use of theoretical models to predict, from first-principles only, properties of hypothetical solids. It has been proposed that a crystalline solid with C₃N₄ stoichiometry in a β-Si₃N₄ crystal lattice would have a bulk modulus comparable or greater to that of diamond, have a high thermal conductivity, and be optically transparent[16-18]. Synthetic routes aimed at such carbon-nitride samples commonly produce amorphous powders or films with few or no crystalline regions [19-25]. The analysis of amorphous powders is difficult since many techniques such as Rutherford back-scattering (RBS), X-Ray photoelectron spectroscopy (XPS), and electron energy loss spectroscopy (EELS), probe primarily sample surfaces. While quantitating atom ratios and revealing hybridization, these methods are unable to describe complete local structural motifs of the bulk material.
Solid-state nuclear magnetic resonance (SSNMR) is used in a diverse range of disciplines to characterize local structure in crystalline and amorphous solids [26, 27]. Here, we utilize Bloch-decay and cross-polarization experiments, together with the adiabatic passage Hartmann-Hahn cross-polarization (APHH-CP) [28, 29] method to transfer polarization between $^{13}$C and $^{15}$N nuclei a paracyanogen-like material. The dipolar filtering of APHH-CP is able to improve resolution, facilitate assignments, and provide connectivity information.

It is of additional interest that network solids can possess significant concentrations of paramagnetic impurities [30, 31]. Paramagnetic defects may be useful for improving spectral density, for electron paramagnetic resonance (EPR) studies, and as paramagnetic centers to enhance NMR spectra through dynamic nuclear polarization [30, 32]. And EPR studies at high frequencies (>100GHz) are attractive since sample sizes are very small and isotopic labeling is not required. We have therefore also acquired 139.5 GHz electron paramagnetic resonance (EPR) spectra for additional insight into paracyanogen-like materials.

### 7.3.1. NMR in CN Solids

We briefly discuss the NMR spectroscopy of materials which exhibit covalent, high-dimensional bonding. These systems may be aprotic and amorphous, and these two characteristics significantly affect the acquisition of NMR spectra.

There are few motions of the lattice in the NMR frequency range for superhard materials such as diamond and silicon nitride, and the recovery time of the spins (i.e. $T_1$) following a single excitation pulse can be hours, days, or longer. For an isotopically labeled, crystalline material such as $\beta$-Si$_3$N$_4$, it is still possible to obtain one-pulse spectra [33-35], although recycle times on the order of 1 hour are required. Both $^{29}$Si and $^{15}$N spectra of $\beta$-Si$_3$N$_4$ reveal narrow, well-resolved lines with good signal to noise ratios.

There may not be a synthetic protocol to introduce spin labels into a given network solid. Isotopic labeling of specific sites presents additional challenges but would not even be meaningful if there is not a well-defined unit cell. In the case of thin-film preparations, where film thickness is measured on micrometer or sub-micrometer scales [22, 36, 37],
samples on the milligram scale may not even be attainable. A typical solid-state NMR rotor accommodates 40-60mg. With the introduction of MAS spinning systems using 2.5mm and 3.2mm sample spinning rotors, reasonable filling factors of the NMR coil can be obtained with as little as 1-5mg of material.

Although one of the principal advantages of structural studies via solid-state NMR is the ability to study a powdered sample, another effect to consider is that CN solids may be so amorphous that the NMR spectra can be composed of featureless, inhomogeneously broadened line shapes. In comparing the $^{29}$Si NMR spectra of the crystalline and amorphous forms of $\beta$-Si$_3$N$_4$, the linewidths increase by factors of 10-100 over those observed for the polycrystalline sample[33, 35] [33, 35]. As the chemical shift anisotropy scales with the applied magnetic field, inhomogeneously broadened NMR linewidths will increase at higher applied fields, in contrast to the sensitivity or resolution enhancement that would be realized for a polycrystalline powder.

The structural information that may be available from applying NMR to network CN solids must be evaluated. For the case of polycrystalline materials, Harris and coworkers demonstrated that the $^{15}$N chemical shift distinguished clearly between the alpha- and beta- crystalline forms of silicon nitride[33]. However in an unknown sample it may not be straightforward to determine if multiple lines indicate a phase-mixture or a single unit cell with multiple sites, a distinction which could be facilitated with spin-spin correlation information. With well-resolved lines, the extraction of chemical shift anisotropy tensors by MAS sideband analysis[38, 39] or single-crystal studies[26] is straightforward. Two-dimensional NMR methods to measure dipolar or scalar couplings would require prohibitively long acquisition times due to poor relaxation rates, although pulsed field gradients could be used to limit the number of transients needed per $t_1$ evolution step. For the case of amorphous solids we consider $^{13}$C NMR spectra of carbon-hydrogen powders [31, 40, 41] which exhibit broad features in the sp$^2$ and sp$^3$ regions of the spectrum. These regions may be quantitated to obtain the relative concentrations of hybridized carbon, but site resolution is not possible in these samples and it is not meaningful to describe a repeated structural unit in a completely disordered solid. While short relaxation times would permit the acquisition of two-dimensional correlation spectra, the lack of spectral resolution could severely compromise the interpretation of any
observed correlations. For broad-line spectra, echo-detection is important as the short free-induction decay can be obscured by receiver saturation following probe ring-down.

And finally, as already mentioned, network solids can possess significant concentrations of paramagnetic impurities in the form of dangling bonds[30, 31]. The issues of observing nuclei which are dipolar coupled to unpaired electrons will be discussed later in the text.

### 7.3.2. Recoupling in Amorphous Carbon-Nitrogen Solids

As is well-known, the dipolar coupling between nuclei scales as \(1/r^3\), where \(r\) is the internuclear distance. Due to this dependence, the dipolar coupling is significant for short internuclear distances \((r < 5\text{Å})\) and many experiments have been developed which reintroduce the coupling during magic-angle spinning (for reviews see Bennett et al. [42] and Griffiths et al. [43]). In this section we describe the criterion for choosing a recoupling experiment applicable to carbon-nitrogen network solids. Since site-specific labeling in network solids is not likely to be possible and recoupling of rare nuclei such as \(^{13}\text{C}\) and \(^{15}\text{N}\) is not feasible in natural abundance spin systems, we restrict our focus to the case of uniformly labeled, network solids.

Homonuclear correlations will give information on one nuclear species, and will be poorly resolved if amorphous broadening is severe. A heteronuclear correlation method would isolate the portions of the carbon spectrum which are dipolar coupled to nitrogen and vice versa. For the general case of an amorphous CN solid, a heteronuclear correlation approach would provide connectivity information and improved resolution. Such an experiment must be sufficiently broad-banded to recouple evenly across an entire line shape, and a one dimensional acquisition scheme must be possible in anticipation of potentially long recycle times.

Among heteronuclear recoupling schemes, methods have been developed to recouple isolated spin pairs (REDOR [44], TEDOR [45]), selectively recouple CN pairs in multiply labeled samples (FDR [46]), and perform broadband recoupling in multi-spin systems (RFDR-CP [47], APHH-CP [28, 29]). The adiabatic-passage Hartman-Hahn cross polarization (APHH-CP) experiment, first described for \(^1\text{H}\) to \(^{13}\text{C}\) polarization...
transfer [48, 49], has recently been utilized for recoupling two low-gamma species such as $^{13}\text{C}$ and $^{15}\text{N}$ [28, 29]. Unlike other techniques, the theoretical efficiency of adiabatic cross-polarization is 100%.

![Diagram of the Adiabatic Polarization Hartmann Hahn Cross Polarization pulse sequence, shown here for polarization transfer first from $^1\text{H}$ to $^{13}\text{C}$ via conventional cross-polarization followed by APHH-CP from $^{13}\text{C}$ to $^{15}\text{N}$. An even offset profile of at least 10 kHz is obtained when $\Delta = 2$-3 kHz.](image)

**Figure 1.** Diagram of the Adiabatic Polarization Hartmann Hahn Cross Polarization pulse sequence, shown here for polarization transfer first from $^1\text{H}$ to $^{13}\text{C}$ via conventional cross-polarization followed by APHH-CP from $^{13}\text{C}$ to $^{15}\text{N}$. An even offset profile of at least 10 kHz is obtained when $\Delta = 2$-3 kHz.

The APHH-CP experiment is illustrated for $^{13}\text{C}$ to $^{15}\text{N}$ polarization transfer in Figure 1. The order of transfer may be reversed to observe a dipolar-filtered $^{13}\text{C}$ spectrum. The experiment can begin with a conventional cross-polarization from the proton to carbon spins, as shown in Figure 1, or with a direct excitation of carbon transverse magnetization with a single pulse timed such that $\omega_1 t = \pi/2$ (where $\omega_1 = 2\pi v_{\text{RF}}$ is the angular nutation rate). For optimal signal-to-noise, of course, $^1\text{H}$-$^{13}\text{C}$ CP should be performed adiabatically as well. Next, a spin-locking pulse of constant power is applied to the $^{13}\text{C}$ channel with a tangential sweep of the RF power on the $^{15}\text{N}$ channel. The value of $\Delta$ indicated in Figure 1 for the RF sweep range is chosen to be in the 2-4 kHz range.
While a larger value of $\Delta$ may be chosen to broaden the offset performance, it is necessary to scale the contact time to longer values such that the transfer remains adiabatic. A value of $\Delta=2$ kHz provides a flat transfer region of about 10 kHz centered around the carrier frequency[28, 29], which represents a range of at least 330ppm for $^{15}$N and 200ppm for $^{13}$C at 4.7 Tesla. The experiment shown in Figure 1 is implemented in a one-dimensional fashion and provides spectral filtering according to $^{13}$C-$^{15}$N connectivities. An incrementable evolution before the $^{13}$C-$^{15}$N cross-polarization could be introduced to provide a two-dimensional heteronuclear correlation spectrum; however, because of the long $T_1$'s involved, this may not be the optimal approach for studying samples with long relaxation times.

It is well-known that cross-polarization in an isolated two-spin system is possible only when a matching condition is satisfied which depends on the spinning frequency such that

$$\omega_1(^{13}\text{C}) = \omega_1(^{15}\text{N}) \pm n\omega_r \quad , \quad n = 1,2.$$  

(1)

Here $\omega_1(^{13}\text{C})$ and $\omega_1(^{15}\text{N})$ denote the RF power amplitudes applied to the carbon and nitrogen channels respectively, and $\omega_r$ is the spinning frequency of the sample about an axis tilted at an angle of 54.74 degrees relative to the external static field. For APHH-CP, the rate of polarization transfer is determined by the magnitude of the particular dipolar coupling element. For spin-locking periods of 1-2 ms, the $n=1$ condition generally provides the best transfer efficiency. When $n=0$, polarization transfer via the dipolar coupling is forbidden; however, transfer through the scalar coupling ($J$) can occur at long mixing times and for large values of $J$. Given its high transfer efficiency, ease of implementation, broad offset profile, and versatility in distinguishing dipole and $J$ recoupled spectra, the APHH-CP method was selected for this study.

Experimental

Sample Preparation. Samples of K$^{13}$C,$^{15}$N and Na$^{13}$C,$^{15}$N were obtained from Cambridge Isotope Laboratories (Andover, MA 01810) and used without further
purification. *Preparation of Hg(CN)₂*: HCN was generated by addition of NaCN or KCN in aqueous solution to sulfuric acid; the HCN was transferred through a condenser tube into a reaction vessel containing an excess of mercuric oxide, HgO, suspended in water. Hg(CN)₂ is very water soluble and hence the excess HgO is removed by filtration; subsequent evaporation with heat yields a white powder. Hg(CN)₂ is both heat and light sensitive. *Preparation of paracyanogen-like solid*: The Hg(CN)₂ was annealed under vacuum and at high temperature; for details see Stevens et al. [50]. Samples were baked at 100 deg. Celsius for approx. 12 hours before they were packed into rotors.

**Caution:** Consult your departmental and institutional safety officers before working with HCN.

**Nuclear Magnetic Resonance:** NMR experiments were performed at room temperature on custom-designed spectrometers operating at 4.8 T (200 MHz for ¹H) and 7.4 T (317 MHz for ¹H). Three-channel, custom-designed probes incorporated 5mm and 4mm Chemagnetics spinner assemblies and implemented transmission line principles to deliver radio-frequency power to the coil. Typical RF fields for cross-polarization and APHH-CP ranged from 30-50kHz depending upon the matching conditions. A ¹⁵N-Acetyl-Valine (NAV) sample was used to set the appropriate RF matching condition for the APHH-CP. Proton decoupling fields of 100kHz or greater were employed. Magic-angle spinning rates were controlled to within a few Hertz and typical rates used in this study were in the 6-8kHz range. All ¹³C chemical shifts are referenced externally to glycine such that the carbonyl line is defined to be 177ppm. All ¹⁵N chemical shifts are referenced externally to NAV such that the nitrogen shift in NH₄¹⁵NO₃ is defined to be 0 ppm. **Electron Paramagnetic Resonance:** EPR data were recorded at room and low temperature (10 K) with a custom-designed pulsed EPR spectrometer operating at 139.5 GHz (5 Tesla) [51].

### 7.3.3. Results

For our study of NMR in network-bonded CN solids, we selected a material which is prepared by thermal annealing of mercuric(II) cyanide Hg(CN)₂ [52]. This synthesis was first performed by Guy-Lussac, and the resulting solid is believed to be an amorphous
carbon-nitrogen polymer, often denoted paracyanogen (pCN). Paracyanogen has been explored as a precursor for metallic CN polymers [53] and ultra-hard carbon-nitride [50, 54]. A convenient isotopic enrichment method exists for paracyanogen through the synthesis and subsequent annealing of $^{13}$C,$^{15}$N-mercuric cyanide (see experimental section for details). Moreover, the structure of paracyanogen has not been demonstrated in the literature, although recent studies have ruled out several proposed models [53, 54], shown in Figure 2a, and explored the high-temperature and high-pressure phase diagram [50] of pCN. Structures such as those in Figure 2a are highly ordered and their extended conjugation would lead to metallic character [53]. More recently, the structure of Figure 2b which is composed of chains of sp$^2$ carbon and nitrogen with pendant nitriles, was suggested to be more representative of the physical properties of pCN [50, 54]. Two samples were made for this study, denoted pCN($^{13}$C) and pCN($^{13}$C,$^{15}$N). pCN($^{13}$C) is a fine, black powder and was generated by high-temperature, vacuum annealing of 25%-$^{13}$C, Hg(CN)$_2$. The second sample, pCN($^{13}$C,$^{15}$N), was generated by annealing of 100%-$^{15}$N, 25%-$^{13}$C, Hg(CN)$_2$.

![Figure 2](image)

**Figure 2.** a) Structures proposed for paracyanogen which possess a high degree of order and extended conjugation; b) amorphous chains of sp$^2$ carbon and nitrogen with pendant nitriles proposed [54, 55] to explain the low order and electrically insulating nature of paracyanogen.

**Nuclear Magnetic Resonance.** In Figure 3 we show $^{13}$C echo-detected CP-MAS spectra of pCN($^{13}$C) obtained at 4.8T and 7.4T. Figure 3 exhibits lines at 163ppm and 157ppm
as well as a broad feature at 120ppm. There are no lines in the sp$^3$ region (0-70ppm). The lines at 163 and 157ppm correspond to sp$^2$ carbon, while the broad 120ppm resonance is assigned to nitrile groups. The $^{13}$C chemical shift in nitriles is very sensitive to local structure and hence this feature experiences considerable amorphous broadening.

![Figure 3](image)

**Figure 3.** Echo-detected $^{13}$C cross-polarization spectra of pCN($^{13}$C) with proton decoupling at 4.8 T ($\omega_0/2\pi[^{13}$C$]$=50MHz) and 7.4 T ($\omega_0/2\pi[^{13}$C$]$=80MHz). Asterisks denote spinning side bands. Recycle times were 4s and 8s (4.8T, 7.4T). Spectra are referenced to an external glycine sample.

Increasingly longer recycle delays were required at 7.4T and 9.4T (not shown), suggesting a sharply declining spectral density. We note that it was not possible to obtain direct-excitation spectra at 14.1T. The spectral resolution is only slightly improved at 7.4T, indicating at least partial amorphous character of this solid. Asymmetric doublets for spin-1/2 nuclei coupled to quadrupolar nuclei are most readily observed for $^{13}$C resonances in nitrile groups [56], however the nitrile region is too broad to observe this effect in our spectra. The $^{13}$C linewidths of pCN($^{13}$C) and pCN($^{13}$C,$^{15}$N) are also not
measurably different. This is reasonable considering that asymmetric splitting was also not observed in $^{29}\text{Si}$ spectra of $\beta$-Si$_3$N$_4$.[33]

It is remarkable that the $^1\text{H}$ cross-polarization experiment can be applied as this sample is expected to be aprotic. To better understand this behavior we compare the $^{13}\text{C}$ echo detected spectra of pCN($^{13}\text{C}$) obtained via cross-polarization and one-pulse excitation, as shown in Figure 4. The spectra are normalized arbitrarily to the broad 120 ppm feature to facilitate their comparison. The differing excitation profiles suggest that the line at 163ppm is more strongly coupled to the proton bath than the 157ppm line due to its enhanced relative intensity in the CP spectra. We also performed a CP build-up experiment in which the intensity of the $^{13}\text{C}$ CP-MAS signal is monitored as a function of the contact time. The build-up of the CP signal intensity corresponded to a time constant of $T_c=0.17\text{ms}$, indicating that the observed resonances do not correspond to $^{13}\text{C}$'s with directly bound $^1\text{H}$'s [57, 58]. The proton decoupling strength was approximately 100kHz, which is sufficient to completely remove any dipolar couplings not averaged by MAS in the $^{13}\text{C}$ spectra.

![Figure 4](image)

Figure 4. Echo-detected $^{13}\text{C}$ MAS NMR spectra of pCN($^{13}\text{C}$) via direct-excitation (dashed trace) and cross-polarization (solid trace). Spectra have been normalized to the broad feature at 120ppm to emphasize the relative excitation efficiencies.
A typical $^{15}$N CP spectrum is shown in Figure 5. Resonances are observed at -279 ppm, -252 ppm, and -189 ppm, along with a broad shoulder at -120 ppm. The $^{15}$N CP-MAS spectrum is clearly richer than the $^{13}$C spectra obtained either by cross-polarization or direct-excitation. From $^{15}$N NMR spectroscopy in silicon nitrides and other nitrogen ceramics [33, 34], this sensitivity of the $^{15}$N shifts is expected. From an $^{15}$N CP rise-time experiment, it was found that the upfield portion of the $^{15}$N spectrum (-252 ppm, -279 ppm) reaches its maximum intensity at about 150 µs, but the downfield portion (-128 ppm, -189 ppm) continues to increase in signal after 5 ms. Therefore the upfield portion of the spectrum corresponds to nitrogen nuclei with directly bonded protons, whereas the downfield portion is coupled very weakly to the proton bath. This is illustrated by the shaded regions in Figure 5. From Figure 5 it is possible to identify regions corresponding to imine (-189 ppm), secondary amine (-252 ppm, -279 ppm), and nitrile (-120 ppm) groups.

![Figure 5](image_url). Echo-detected $^{15}$N cross-polarization spectra with proton decoupling of pCN($^{13}$C,$^{15}$N) at 4.8 Tesla.
Dipolar recoupled spectra employing the APHH-CP sequence are shown for $^{15}\text{N}$ to $^{13}\text{C}$ transfer and $^{13}\text{C}$ to $^{15}\text{N}$ transfer in Figures 6 and 7 respectively. The pulse sequence of Figure 1 was employed where, in each case, initial magnetization was prepared with cross-polarization from protons. The APHH-CP experiments are shown for $n=1$ and $n=0$ matching conditions for the middle and top spectra respectively. The signal-to-noise in the spectra ranges from $10$ to $10^2$, each spectrum requiring approximately 12 hours of acquisition time. The coherence transfer observed in the $n=0$ case probably occurs via proton-proton spin flips and not via the J-coupling as a signal is observed in Figure 6 ($n=0$) for the secondary amines, for which one bond $^{13}\text{C}$$^{15}\text{N}$ J couplings are of the order of 10Hz. Scalar transfer would be expected in the nitrile and imino regions (-128ppm,-189ppm) of the $^{15}\text{N}$-detected APHH-CP spectrum as these groups will have the largest $^{13}\text{C}$$^{15}\text{N}$ one-bond J couplings. Mixing times up to 7ms were employed in an attempt to observe scalar transfer but this was unsuccessful. Probably even longer contact times are needed as well as a higher enrichment of $^{13}\text{C}$ (25% in this study). However the $n=1$ spectra provide crucial information for resolving sites and improving assignments. The downfield shoulder of the $^{15}\text{N}$ CP spectrum is resolved at -128ppm in the APHH-CP($n=1$), consistent with typical nitrile shifts and with an increased intensity relative to the -189 ppm resonance that is to be expected from the larger $^{13}\text{C}$$^{15}\text{N}$ dipolar coupling of the nitrile compared to the imine. The relative transfer efficiencies in the upfield region (-252ppm, -272ppm) suggest a stronger CN interaction at -252ppm than -272ppm. Assignments will be treated in more detail in the discussion.
Figure 6. $^{13}$C-detected APHH-CP at 4.8 T, as per the pulse sequence of Figure 1 modified for initial CP from $^1$H to $^{13}$C followed by APHH-CP from $^{13}$C to $^{15}$N.

Figure 7. $^{15}$N-detected APHH-CP at 4.8 T using the pulse sequence of Figure 1.
Electron Paramagnetic Resonance. Direct-excitation $^{15}$N NMR spectra could not be obtained due to the presence of very intense, short-$T_2$ signals which introduced large distortions in the spectra. This may be attributed to paramagnetic Nitrogen atoms, which are expected in lattice defects when a nitrogen atom occupies a carbon position, or when a polymer segment terminates with a divalent nitrogen. We have confirmed the existence of paramagnetic centers with EPR experiments obtained at 139.5 GHz, shown in Figure 8. A single absorption is observed at room and low temperature (10 K). The low temperature spectrum, obtained by integrating the spin-echo intensity over the magnetic field sweep, gives an absorptive line shape as shown in Figure 8. The signal in this sample only permitted continuous wave EPR (CW-EPR) at room temperature which results in a derivative spectrum as shown in the inset of Figure 8. A field sweep range of 0.5 Tesla (about the central field of 5 T) allows detection of paramagnetic centers with $g$-values of 2.15 - 1.85.

![Echo-detected EPR T=10K](image)

**Figure 8.** EPR spectrum (main) obtained by integration of the spin-echo intensity over the field sweep at 10 Kelvin. A sweep of 0.5 Tesla at room temperature is shown in the inset. The $g$-anisotropy is negligible, and $g_{iso}=2.0032$. 
The line shape is only slightly asymmetric, indicating a small g-anisotropy with an estimated isotropic g-value of 2.0032. Magnetic field scans over half a Tesla detected no other paramagnetic signals, showing that metallic impurities are not present. The EPR signal intensity increases at lower T in a Boltzmann-like fashion as expected for an isolated paramagnetic center in an electrically insulating material. Without knowing the molecular unit of this material, it is difficult to specify the spin density; however, the spin echo intensity was compared to standard solutions of different organic radicals, indicating an approximate spin count of $1 \times 10^{17}$ e-/cm$^3$. EPR spectra for pCN($^{13}$C) and pCN($^{13}$C,$^{15}$N) are identical. Additionally, the observation of spin echoes at low temperature indicates that electron-nuclear double-resonance (ENDOR) experiments, which detect electron-nuclear dipolar couplings, are possible.

### 7.3.4. Discussion

Materials which exhibit covalent, high-dimensional bonding may be aprotic and amorphous, and these characteristics can significantly affect the acquisition of NMR spectra. Recycle times up to an hour or longer may be required [33-35] due to the near absence of local motions in the NMR time scale, or amorphous broadening [31, 40, 41] could limit site resolution to the point that some materials cannot even be described by repeated structural units.

Thus, the observation of resolved resonances in the $^{13}$C and $^{15}$N NMR spectra (Figures 3 and 5) indicates that this pCN-like material is composed of a repeated structural motif. However the degree of order is insufficient to provide NMR linewidths normally observed for crystalline phase samples, or to motionally constrain the solid since NMR recycle times of 2-3 seconds provide optimal signal-to-noise at 4.7 Tesla. This disparity is reconciled first by our knowledge of NH moieties whose motions may provide frequencies on the NMR time scale. Second, we note that field-dependent studies show a rapidly declining spectral density at higher static fields, indicating some degree of extended, three-dimensional bonding in this solid. An explanation consistent with these
findings is that a repeated, two-dimensional structural unit exists along with significant covalent cross-linking which constrains motions and introduces structural heterogeneity.

The current data is insufficient to fully constrain the local structure of this solid, however we are able to make assignments of the spectra to chemical moieties and we discuss their significance here. From the $^{13}$C spectra we rule out the presence of sp$^3$ carbon and identify two sp$^2$ carbon lines and a broad nitrile resonance. This observation agrees with previous hypotheses for the structure of paracyanogen-like solids [54] which describe chains of sp$^2$ carbon and nitrogen with cyano groups hanging off the chains. The absence of sp$^3$ carbon indicates that water molecules do not add across double bonds, an effect seen in paracyanogen-like solids prepared by reactive sputtering [54] and also in protonated silicon nitride powders [35]. Other studies have observed that reactive sputtering leads to dangling bonds on carbon nuclei, which are reactive centers for water addition reactions [31]. In contrast our sample was prepared by a thermal annealing process. Although samples were stored in air before measuring, controlled exposures to moisture were not undertaken. The addition of water in this fashion would result in protons dipolar coupled to sp$^3$ carbon nuclei, which would thus be easily detected in CPMAS spectra. The sp$^2$ chemical shifts agree with imine resonances observed in nitrogenated amorphous C:H films [59]. It is also significant that the difference in chemical shift between the two sp$^2$ carbon species is well resolved (5 ppm). A distribution of carbon bound to one, two, or three nitrogen atoms, or one or more cyano groups, would be expected to result in a completely amorphous spectrum. This reinforces our assertion above that resolved lines must indicate the existence of a regular chemical unit. Finally, a broad signal is present at about 120 ppm, consistent with literature values for nitrile groups [60].

Turning to the nitrogen spectra, a nitrile region at -128 ppm is resolved by correlating the $^{15}$N-CPMAS spectrum with the APHH-CP (n=1) spectrum, as shown in Figure 7. Without the $^{13}$C-$^{15}$N correlation to resolve the cyano region, this assignment would be difficult as the signal at -128 ppm is weak. In fact, the weak polarization of the cyano region in the $^{15}$N CPMAS spectrum probably explains the absence of intensity for the nitrile region in the $^{13}$C-detected APHH-CP (Figure 5, n=1). We assign the -189 ppm
$^{15}$N region to imine groups (C=N-R) based on the large intensity of the peak in the APHH-CP (n=1), which suggests strong CN dipolar coupling, and the chemical shift which is well outside the nitrile and amino regions. The imino region may be broadened by heterogeneity in R, which may be C or N, but is still well resolved from the secondary amine groups at -252 ppm and -279 ppm, which are typical for R*NHR shifts [60]. Here we propose to assign these to C*NHC and C*NHN moieties respectively due to the fact that their intensities are similar in CPMAS spectra but differ by a factor of two in the APHH-CP. This is reasonable considering that the adiabatic-transfer efficiency should be identical for both sites due to the even offset profile of APHH-CP [29] and the small difference in isotropic shifts. A significant observation is that the breadth of the imine region and the presence of two distinct amine resonances both suggest N-N bonding, a feature that is absent from Figure 2b. The presence of N-N bonding has also been supported by thermal decomposition studies [50].

The APHH-CP spectra provide dipolar filtering information which improve resolution and facilitate assignments, as in the case of the nitrile region which is resolved at -128 ppm in Figure 7 (n=1). However, in the n=0 case, the $^{15}$N-detected APHH-CP experiment suggests scalar carbon-to-nitrogen transfer for the secondary amines which is clearly not physical based on typical $J_{CN}$ values for a sigma bond. Mixing times in excess of 7ms and an increased $^{13}$C enrichment level (>25%) are proposed to overcome the difficulty of observing the scalar transfer, but the presence of proton-assisted polarization transfer is a complicating factor in this sample. In the absence of protons this mechanism is not available and, for aprotic network solids, APHH-CP at n=0 has considerable potential.

The problem of observing an NMR signal for a nucleus dipolar coupled to one or more unpaired electrons has been discussed by Nayeem et al. [61], who showed that the interaction is inhomogeneous even in the presence of dipolar coupled, unpaired electrons, but only if the g-anisotropy is large. Such an interaction was observed for proton-electron pairs, but also in $^{13}$C spectra by Pan et al. who observed spinning sidebands over a 2000ppm range in amorphous carbon [31]. Extended spinning sideband manifolds were not observed in the $^{13}$C or $^{15}$N spectra of our compounds, in agreement with our direct observation of minimal g-anisotropy. The observation of a single, almost featureless EPR
line ($\Delta B \approx 20$ Gauss) leaves open the possibility of free electrons localized on carbon instead of nitrogen, for which a substantial hyperfine coupling, resulting in typical three line($^{14}$N) or two line($^{15}$N) splittings up to 30 Gauss, is usually expected ([62, 63] and references therein) [30]. The narrow observed EPR line width can be due either to a small hyperfine coupling or to electron exchange ([63] pg. 199). Although the $^{15}$N NMR spectra suggest free electrons localized on nitrogen, the EPR spectra do not unambiguously support this assignment. $^{15}$N ENDOR experiments are required to resolve this disparity.

Finally, we evaluate the potential for magnetic resonance data to provide a set of structural criteria in the bulk material which may determine the suitability of such samples as possible precursors for high-dimensional, sp$^3$-hybridized, carbon-nitrogen network solids. The temperature-pressure composition diagram of a similarly prepared sample has been previously reported [50] in which it was found that kinetic decomposition to form N$_2$ was favored over thermodynamic conversion of sp$^2$- to sp$^3$-carbon, even at very high temperatures and pressures. Such a material is unsuitable as a precursor for network bonded, covalent CN solids so long as the decomposition to N$_2$ remains favored. Both NMR and EPR data suggest means by which a paracyanogen-like material can be tested for suitability for high-pressure and high-temperature composition analyses. The line at -279ppm in the $^{15}$N spectra indicates N-N bonds which can condense to N$_2$ during decomposition. Synthetic preparations can be attempted to minimize this feature. Similarly, elimination of cyano groups removes sp$^1$ carbon from the sample and may assist in favoring the thermodynamic process over the kinetic one. Monitoring the appropriate lines in the $^{13}$C and $^{15}$N spectra will report on the relative cyano concentrations in the bulk. Of course observation of $^{13}$C signals in the sp$^3$ region would be valuable. The presence of directly bound protons can be tested with simple cross-polarization build-up experiments, as in this study, or with dipolar dephasing or separated local field spectroscopies.

Additionally, preliminary $^{13}$C electron-nuclear double-resonance (ENDOR) experiments have been performed at low temperature which reveal a complex distribution of dipolar couplings. Detailed modeling of this distribution may lead to a measure of the
proximity of $^{13}$C to paramagnetic nitrogen. In particular, a decrease in N-N bonding may be detected in this way by observing an increase in the average dipolar coupling in the $^{13}$C ENDOR spectra.

Using NMR and EPR to provide feedback data versus variations in the synthetic parameters leading to CN-polymers, it may be possible to develop a paracyanogen-like material with minimal N-N bonding, reduced hydrogenation, and greater sp$^3$ character, and thus whose temperature/pressure composition diagram may favor thermodynamic rearrangement over kinetic decomposition.

In summary, we have performed a study of an isotopically enriched, paracyanogen-like solid, which possesses both short relaxation times and resolved spectral regions. These spectral characteristics permitted the use of adiabatic-passage Hartmann-Hahn CP between $^{13}$C and $^{15}$N to identify and assign three carbon sites and four nitrogen sites. We find that APHH-CP is a robust method for recoupling in CN solids and providing information not readily available in conventional direct-excitation spectra. We also showed the existence of two NH moieties by CP build-up experiments and note that preparations of pCN-like solids, whether by RF sputtering or thermal annealing, seem to be susceptible to hydrogenation mechanisms. Although we are not able to unambiguously determine the local structure of this pCN-like solid, we have demonstrated that water molecules do not add across C=N double bonds, confirmed the presence of nitrile groups, provided evidence for N-N bonding, and shown the existence of paramagnetic nitrogen nuclei. In particular, we note that the good resolution of many spectral regions is difficult to reconcile for an amorphous solid. Thus, in consideration of a rapidly declining spectral density, we propose that a repeated, two-dimensional structural motif exists with three-dimensional cross-linking. Finally, we have described methods which would permit the optimization of amorphous CN materials for high-temperature, high-pressure composition analyses.
7.4. References


Curriculum vitae

Name: David S. Rovnyak
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Education

Publications


Additional Experience
1993-1999: Teaching Assistant. Course 5.60 "Thermodynamics and Kinetics" for three semesters including in-class demonstrations.

Research Presentation


Rovnyak, D. Research lecture (20 minutes). 44th International Conference on Analytical Sciences and Spectroscopy, Queens University, Kingston, Ontario Canada. (August 9-14, 1998) "Solid-State NMR of Quadrupolar Nuclei in Biological Solids"

