

MECHANISMS AND OPTIMIZATION METHODS OF
 ^{13}C DYNAMIC NUCLEAR POLARIZATION

by

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*This dissertation
is dedicated to
Erin and Bernadette*

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 ^{13}C DYNAMIC NUCLEAR POLARIZATION

by

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Nuclear magnetic resonance (NMR) is a technique that probes the microscopic environment of molecules by investigating the interaction between nuclear magnetic moments and the magnetic field in which they reside. Nuclear magnetic moments, however, are very weak, causing the Boltzmann polarization of nuclei and hence the NMR signal to be very small. This may be resolved through the use of dynamic nuclear polarization (DNP), a process by which high electron polarization is transferred to nuclei through microwave irradiation near the electron resonance. In this work, primary focus is given to dissolution DNP in which nuclei are highly polarized at cryogenic temperature and then rapidly dissolved with a superheated solvent. This process results in a liquid sample whose NMR signal is enhanced many thousand-fold over thermal equilibrium. While applications of this technique are abundant, there are many unanswered questions surrounding the underlying physics and methods of optimization of the DNP process. In this work, some of these open questions are investigated through development of instrumentation and exploration of DNP free radicals. In particular, the construction of two high-magnetic field DNP polarizers are discussed, one of which is the first of a new generation of cryogen-free polarizers. Furthermore, the water-soluble DNP free radicals TEMPO and trityl are thoroughly investigated with a specific emphasis on the addition of paramagnetic ion complexes. Over the course of these experiments,

several paramagnetic agents were tested for the first time and proven to be effective to a similar degree as the field standard gadolinium. Additionally, the link between shortened electronic T_1 and improved DNP efficiency was confirmed through the study of transition metal complex dopants to DNP. A number of supporting experiments are also discussed, including Earth's field NMR and classification of free radicals by UV-Vis spectrophotometry and electron paramagnetic resonance. Finally, several basic molecular imaging applications of dissolution DNP are presented highlighting one of the many possible uses of hyperpolarized ^{13}C NMR spectroscopy. Ultimately, this dissertation presents and discusses a number of novel methods by which ^{13}C dynamic nuclear polarization may be optimized, paving the way for further study into the physics and applications of this technique.

TABLE OF CONTENTS

ACKNOWLEDGMENTS	v
ABSTRACT	viii
LIST OF FIGURES	xiii
LIST OF TABLES	xxv
CHAPTER 1 INTRODUCTION	1
1.1 Nuclear Magnetic Resonance and Magnetic Resonance Imaging	1
1.1.1 Introduction to NMR and MRI	1
1.1.2 Relaxation and Line Broadening	5
1.1.3 Chemical Shift and J-Coupling	8
1.2 Electron Paramagnetic Resonance	9
1.3 Dynamic Nuclear Polarization	11
1.4 Introduction to Free Radicals	15
1.5 Biomedical Applications of Dissolution Dynamic Nuclear Polarization	15
CHAPTER 2 DYNAMIC NUCLEAR POLARIZATION MECHANISMS	25
2.1 DNP at Low Magnetic Fields	25
2.1.1 The Overhauser Effect	25
2.2 DNP at High Magnetic Fields	29
2.2.1 Solid Effect	30
2.2.2 Cross Effect	31
2.2.3 Thermal Mixing	32
CHAPTER 3 INSTRUMENTATION	37
3.1 6.4 T DNP Hyperpolarizer	37
3.1.1 Design and Construction	38
3.1.2 System Testing	44
3.2 Cryogen-Free DNP System	47
3.2.1 Design and Construction	48
3.2.2 System Testing	52
3.3 Construction of a 0.35 T NMR system	56

3.4	Construction of an Earth's Magnetic Field NMR System	61
CHAPTER 4 OPTIMIZATION OF DISSOLUTION DNP		65
4.1	Free Radicals	65
4.1.1	Electron Paramagnetic Resonance	66
4.1.2	UV-Vis Spectrophotometry	66
4.1.3	Free Radical Concentration	76
4.2	Isotopic Labeling of the 4-Oxo-TEMPO Free Radical	77
4.3	DNP Using TEMPO Derivatives	80
4.4	The Effect of Isotopic Labeling on Solid-State Polarization Enhancement and Relaxation in Sodium Acetate	83
4.5	Additives to DNP using Trityl	87
4.5.1	Ln(III)-DOTA Complexes	88
4.5.2	Trimeric Gadolinium Complex	99
4.5.3	Transition Metals	104
4.6	Measuring ^{13}C Spin Lattice Relaxation Time at Cryogenic Temperatures Using a Sweepable Magnet	115
4.7	Summary of DNP Optimization Studies	121
CHAPTER 5 METABOLIC STUDIES USING DISSOLUTION DNP		122
5.1	Hyperpolarization of ^{13}C and ^{15}N -labeled Substrates	122
5.2	Yeast Metabolism	125
5.2.1	Conventional NMR of Living Yeast	125
5.2.2	Hyperpolarized ^{13}C Studies of Living Yeast	128
5.3	Prostate Cancer Metabolism	129
5.3.1	Conventional NMR using PCA Extracts	130
5.3.2	Hyperpolarized ^{13}C and ^{15}N Magnetic Resonance	131
CHAPTER 6 LOW MAGNETIC FIELD NMR		135
6.1	Earth's Field Nuclear Magnetic Resonance (EFNMR)	135
6.1.1	Terranova EFMRI	136

CONCLUSION	139
APPENDIX A NMR PULSE SEQUENCES	141
APPENDIX B THERMAL MIXING NUMERICAL SIMULATIONS	144
APPENDIX C OPERATION OF HOMEBUILT DNP POLARIZERS AT UTD . . .	146
APPENDIX D CELL CULTURE PROTOCOL	162
APPENDIX E PROTOCOL FOR <i>IN VITRO</i> HYPERPOLARIZED MRS EXPERIMENTS	176
REFERENCES	178
BIOGRAPHICAL SKETCH	197
CURRICULUM VITAE	

LIST OF FIGURES

1.1	Schematic showing the simplified acquisition of an MRI. (a) The slice select gradient is used to select an imaging plane in the z direction. (b) Then, a phase shift in the y direction is induced using the phase encoding gradient. (c) Finally, an NMR echo is monitored in the presence of the frequency encoding gradient, giving location in the x direction.	5
1.2	Spin-lattice and spin-spin relaxation times calculated as a function of correlation time using Equations 1.16 and 1.17 for protons in water at a magnetic field of 9.4 T.	7
1.3	Theoretical J-Coupling peaks for various numbers of identical spin $\frac{1}{2}$ nuclei. The theoretical relative intensities of the peaks are given by Pascal's Triangle (shown on the left).	9
1.4	The X-band EPR magnet and spectrometer at the University of Texas at Dallas NanoTech Institute	10
1.5	Schematic showing the splitting of energy levels when hyperfine coupling is present	12
1.6	Polarization of Electron, ^1H , and ^{13}C as a function of temperature in fields corresponding to the Oxford's commercial HyperSense polarizer (left), the homebuilt polarizers at the University of Texas at Dallas (middle), and at the strongest magnetic field available for high resolution NMR (right). The vertical line denotes a temperature readily achievable using liquid helium.	13
1.7	The structures of the free radicals studied in this work that are not in the nitroxide class.	17
1.8	The structures of the nitroxide free radicals studied in this work.	18
1.9	Tracking of the six carbons of glucose (and fructose) through glycolysis. After glycolysis, the products (pyruvate) could have a number of final destinations, but most common are introduction into the TCA cycle or lactic acid fermentation (mammalian cells) or anaerobic fermentation (yeast cells).	20
1.10	^{13}C NMR spectra acquired from prostate cancer (PC-3) cells <i>in vitro</i> after the application of hyperpolarized $[1-^{13}\text{C}]$ pyruvate by the author. The spectra displayed is the sum of 60 spectra taken every 4 s with a 10° RF pulse.	22
1.11	The TCA cycle, showing the substrates that are useful for hyperpolarized ^{13}C magnetic resonance. Circles denote carbon atoms and are color-coded to show the possible metabolic fate of ^{13}C atoms of a given location.	23
1.12	The first step of the Kennedy Pathway in which choline is converted to phosphocholine.	24
2.1	Possible states and transitions in a two-spin system	27

2.2	A visualization of the “well-resolved solid effect” showing the DNP enhancement as a function of irradiation frequency. The inset window is an illustration of the classic ”flip-flip” view of the solid effect.	31
2.3	Available states and transitions for a system containing a nucleus and two electrons. The general system is shown in (a) while (b) and (c) depict the system when the electron resonances are separated by the the nuclear Larmor frequency. The transitions shown in (b) [(c)] correspond to irradiation at the first [second] electron’s resonance, yielding negative [positive] enhancement	33
2.4	Schematic showing the heat transfer among the reservoirs in the Thermal Mixing process. By irradiating a sample with microwaves, the EDS is cooled through contact to the EZS, which allows for cooling of the NZS, and hence polarization enhancement.	34
3.1	Design of the homebuilt UTD hyperpolarizer. Figure adapted with permission from A. Kiswandhi, P. Niedbalski, C. Parish, S. Ferguson, D. Taylor, G. McDonald, and L. Lumata “Construction and ^{13}C Hyperpolarization Efficiency of a 180 GHz Dissolution Dynamic Nuclear Polarization System,” <i>Magn. Reson. Chem.</i> , vol. 55, pp. 828-836, Copyright © 2017 John Wiley & Sons, Ltd.	39
3.2	Top view of the connections of the KF-50 cross	40
3.3	NMR saddle coil used in the UTDallas hyperpolarizer.	41
3.4	The 180 GHz sweepable microwave source used for polarizing samples.	42
3.5	The UTD Hyperpolarizer system. In the background, the roots blower vacuum pump may be seen, while the magnet and polarizer components are seen in the foreground. It should be noted that this picture was taken during a time when the magnet was temporarily de-energized, so the metal table and ladder near the magnet do not pose a danger to life or equipment. These objects are kept outside the 5 G limit during normal magnet operation.	43
3.6	Schematic of the dissolution system used in the UTDallas Hyperpolarizer. . . .	44
3.7	Liquid helium consumption of the Janis LHe cryostat both when the rootsblower pump is on and when it is off. Figure adapted with permission from A. Kiswandhi, P. Niedbalski, C. Parish, S. Ferguson, D. Taylor, G. McDonald, and L. Lumata “Construction and ^{13}C Hyperpolarization Efficiency of a 180 GHz Dissolution Dynamic Nuclear Polarization System,” <i>Magn. Reson. Chem.</i> , vol. 55, pp. 828-836, Copyright © 2017 John Wiley & Sons, Ltd.	45

3.8	(a) Comparison of ^{13}C NMR signal of a sample of $[1-^{13}\text{C}]$ acetate doped with 15 mM trityl OX063 polarized at thermal equilibrium and after polarization for two hours. The center frequency is 68.768 MHz. (b) Polarization buildup curves for 3 M $[1-^{13}\text{C}]$ acetate doped with 15 mM trityl OX063 (blue circles) and 40 mM 4-oxo-TEMPO (red squares). Figure adapted with permission from A. Kiswandhi, P. Niedbalski, C. Parish, S. Ferguson, D. Taylor, G. McDonald, and L. Lumata "Construction and ^{13}C Hyperpolarization Efficiency of a 180 GHz Dissolution Dynamic Nuclear Polarization System," <i>Magn. Reson. Chem.</i> , vol. 55, pp. 828-836, Copyright © 2017 John Wiley & Sons, Ltd.	46
3.9	Hyperpolarized and thermal ^{13}C NMR signal taken using the SpinSolve NMR spectrometer following polarization by the home-built polarizer and dissolution. The initial sample was 3 M $[1-^{13}\text{C}]$ sodium acetate doped with 15 mM trityl OX063, which was reduced to about 37 mM $[1-^{13}\text{C}]$ sodium acetate post-dissolution. Figure adapted with permission from reference A. Kiswandhi, P. Niedbalski, C. Parish, S. Ferguson, D. Taylor, G. McDonald, and L. Lumata "Construction and ^{13}C Hyperpolarization Efficiency of a 180 GHz Dissolution Dynamic Nuclear Polarization System," <i>Magn. Reson. Chem.</i> , vol. 55, pp. 828-836, Copyright © 2017 John Wiley & Sons, Ltd.	47
3.10	The schematic for the cryogen free DNP system that was constructed. Figure adapted with permission from A. Kiswandhi*, P. Niedbalski*, C. Parish, Q. Wang, and L. Lumata "Assembly and Performance of a 6.4 T Cryogen-Free Dynamic Nuclear Polarization System," <i>Magn. Reson. Chem. Rapid Communication</i> , vol. 55, pp. 846-852, Copyright © 2017 John Wiley & Sons Ltd.	49
3.11	The technical drawing submitted to Cryomagnetics Incorporated for production	50
3.12	CAD drawings showing the various elements of the DNP probe that was built (a), (b), (c). Photographs of the completed probe (d) and the complete cryogen free DNP system (e). Figure adapted with permission from A. Kiswandhi*, P. Niedbalski*, C. Parish, Q. Wang, and L. Lumata "Assembly and Performance of a 6.4 T Cryogen-Free Dynamic Nuclear Polarization System," <i>Magn. Reson. Chem. Rapid Communication</i> , vol. 55, pp. 846-852, Copyright © 2017 John Wiley & Sons Ltd.	51
3.13	The sample temperature over time within the cryogen-free DNP sample space. This data was taken over 24 hours. Initially, magnet and microwave source are turned off. At 7,000 s, the ramp-up of the magnet to 6.423 T is begun, and halfway through the ramp-up at 8,000 s, the microwave is turned on. At 9,000 s the magnet reaches 6.423 T. At 11,000 s, the microwave is turned off and the magnet ramped further to 8.8 T, where it remains through the duration of displayed data	53

3.14	Microwave irradiation frequency sweeps for samples of 3 M [1- ¹³ C] sodium acetate doped with either 15 mM trityl OX063 or 40 mM 4-oxo-TEMPO. Figure adapted with permission from A. Kiswandhi*, P. Niedbalski*, C. Parish, Q. Wang, and L. Lumata “Assembly and Performance of a 6.4 T Cryogen-Free Dynamic Nuclear Polarization System,” <i>Magn. Reson. Chem. Rapid Communication</i> , vol. 55, pp. 846-852, Copyright © 2017 John Wiley & Sons Ltd.	54
3.15	(a) Representative hyperpolarized and thermal ¹³ C NMR spectra in the cryogen free DNP system operating at 6.423 T. (b) Polarization buildup curves for samples of 3 M [1- ¹³ C] sodium acetate doped with either 15 mM trityl OX063 or 40 mM 4-oxo-TEMPO. Figure adapted with permission from A. Kiswandhi*, P. Niedbalski*, C. Parish, Q. Wang, and L. Lumata “Assembly and Performance of a 6.4 T Cryogen-Free Dynamic Nuclear Polarization System,” <i>Magn. Reson. Chem. Rapid Communication</i> , vol. 55, pp. 846-852, Copyright © 2017 John Wiley & Sons Ltd.	55
3.16	Photographs of the top (left) and side (right) views of the permanent magnet NMR system constructed	56
3.17	The most basic NMR circuit made with two variable capacitors and a coil. One capacitor is used to tune the circuit resonance while the other is used for narrowing the range of frequencies over which the circuit is resonant, also known as matching.	58
3.18	Spin echo FID (left) and NMR spectrum (right) for a water sample in the 0.35 T permanent magnet NMR system using 1 (top), 10 (middle), and 1000 (bottom) scans	59
3.19	Diagram showing the proper pinning for operation of the YIG oscillator and associated amplifier	60
3.20	The calibration data for the YIG oscillator used in this work. Data was obtained by supplying a current to the oscillator and recording the frequency displayed on the spectrum analyzer.	61
3.21	Circuit schematics for the homebuilt EFNMR system. Top: Polarizing circuit, Middle: Transmitter circuit, Bottom: Receiver circuit. All relays are HE3351A0500 and all diodes are 1N4148 unless otherwise specified.	62
3.22	The EFNMR system built by the author with a example spectrum of a bulk water sample taken using the system	64
4.1	X-band EPR Spectra at room temperature for the nitroxide-based radicals studied in this work.	67
4.2	X-band EPR Spectra at room temperature for the radicals studied in this work that are not nitroxide based.	67
4.3	Evolution 220 Spectrophotometer with Peltier temperature control unit	69

4.4	UV-Vis spectra of 4-oxo-TEMPO at room temperature at various concentrations (left). Maximum absorbance of peak vs radical concentration (right).	70
4.5	UV-Vis spectra of 4-oxo-TEMPO, d ₁₆ at room temperature at various concentrations (left). Maximum absorbance of peak vs radical concentration (right). . . .	70
4.6	UV-Vis spectra of 4-oxo-TEMPO, ¹⁵ N at room temperature at various concentrations (left). Maximum absorbance of peak vs radical concentration (right). . . .	71
4.7	UV-Vis spectra of 4-oxo-TEMPO, ¹⁵ N, d ₁₆ at room temperature at various concentrations (left). Spectra at 31.25 μM was not obtained due to spectrometer malfunction. However, because the trend is clearly visible, this was not reproduced. Maximum absorbance of peak vs radical concentration (right).	71
4.8	UV-Vis Spectra of BDPA at room temperature at various concentrations (left). Maximum absorbance of low wavelength peak (middle) and high wavelength peak (right) vs. BDPA concentration.	72
4.9	Maximum absorbance of large peak (left) and small peak(right) of BDPA at 3 hour intervals. Decays are fit with a single exponential fitting.	73
4.10	UV-Vis Spectra of DPPH at room temperature at various concentrations (left). Maximum absorbance of low wavelength peak (middle) and high wavelength peak (right) vs. DPPH concentration.	73
4.11	Absorbance of DPPH at 522 nm over time in 30 minute intervals. Data are fit with a single exponential.	74
4.12	Absorbance of galvinoxyl at 307 nm over time in 30 second intervals. Data are fit with a single exponential.	75
4.13	Absorbance of blue radical between 580 and 790 nm (left). Absorbance at 715 nm as a function of time. Fitting was done using a single exponential.	75
4.14	(a) and (b) show DNP Buildups for [1- ¹³ C] samples doped with the 4-Oxo-TEMPO Variants in non-deuterated and deuterated solvents, respectively. (c) through (f) show a comparison of final ¹³ C NMR intensity (c and d) and build-up time constants (e and f) for non-deuterated and deuterated solvents. Figure adapted with permission from P. Niedbalski, C. Parish, A. Kiswandhi, and L. Lumata, “ ¹³ C Dynamic Nuclear Polarization Using Isotopically Enriched 4-Oxo-TEMPO Free Radicals,” <i>Magn. Reson. Chem.</i> vol. 54, pp. 962-967, Copyright © 2016 John Wiley & Sons Ltd.	79
4.15	(a) Polarization buildup curves for ¹³ C-labeled acetate doped with different derivatives of the TEMPO radical in a glassing matrix of 1:1 v/v ethanol:water. (b) Comparison of maximum polarization achieved by using each of the TEMPO derivatives. Figure adapted from “ ¹³ C Dynamic Nuclear Polarization Using Derivatives of TEMPO Free Radical,” <i>App. Magn. Reson.</i> vol. 48, 2017, pp. 933-942, P. Niedbalski, C. Parish, Q. Wang, A. Kiswandhi, and L. Lumata, Copyright © Springer-Verlag GmbH Austria 2017 With permission of Springer . . .	82

4.16	(a) Polarization buildup curves for ^{13}C -labeled acetate doped with different derivatives of the TEMPO radical in 1:1 v/v ethanol, $\text{d}_6\text{:D}_2\text{O}$. (b) Comparison of maximum polarization achieved by using each of the TEMPO derivatives in deuterated solvents. Polarization using TEMPO in non-deuterated solvents is shown for comparison. Figure adapted from “ ^{13}C Dynamic Nuclear Polarization Using Derivatives of TEMPO Free Radical,” <i>App. Magn. Reson.</i> vol. 48, 2017, pp. 933-942, P. Niedbalski, C. Parish, Q. Wang, A. Kiswandhi, and L. Lumata, Copyright © Springer-Verlag GmbH Austria 2017 With permission of Springer .	82
4.17	(a) Polarization buildup curves for ^{13}C -labeled acetate doped with different water soluble derivatives of the TEMPO radical in 1:1 v/v glycerol:water. (b) Comparison of maximum polarization achieved by using these select radicals. Polarization using TEMPO in non-deuterated 1:1 v/v ethanol:water is shown for comparison.	83
4.18	(a) ^{13}C polarization buildup curves for the differently isotopically labeled acetate molecules studied in this work. (b) Comparison of maximum polarization for these substrates. Figure adapted with permission from P. Niedbalski, C. Parish, A. Kiswandhi, Z. Kovacs, and L. Lumata, “Influence of ^{13}C Isotopic Labeling Location on Dynamic Nuclear Polarization of Acetate,” <i>J. Phys. Chem. A</i> vol. 121, pp. 3227-3233, 2017 Copyright 2017 American Chemical Society	85
4.19	(a) ^{13}C polarization decay monitored at 3.35 T and 1.4 K for different isotopically labeled versions of acetate. (b) Comparison of T_1 relaxation time for these substrates. Figure adapted with permission from P. Niedbalski, C. Parish, A. Kiswandhi, Z. Kovacs, and L. Lumata, “Influence of ^{13}C Isotopic Labeling Location on Dynamic Nuclear Polarization of Acetate,” <i>J. Phys. Chem. A</i> vol. 121, pp. 3227-3233, 2017 Copyright 2017 American Chemical Society	86
4.20	The DOTA ligand in which Dy^{3+} , Gd^{3+} , Ho^{3+} , and Tb^{3+} were chelated.	89
4.21	Field swept EPR spectra for trityl doped with each of the Ln(III) -DOTA complexes studied in this work. Spectra were measured at $T = 10$ K. Figure adapted from A. Kiswandhi, P. Niedbalski, C. Parish, P. Kaur, A. Martins, L. Fidelino, C. Khemtong, L. Song, A. D. Sherry, and L. Lumata, “Impact of Ho^{3+} -Doping on ^{13}C Dynamic Nuclear Polarization Using Trityl OX063 Free Radical,” <i>Phys. Chem. Chem. Phys.</i> , vol. 18, pp 21351 - 21359, 2016 with permission from the PCCP Owner Societies and from P. Niedbalski, C. Parish, A. Kiswandhi, L. Fidelino, C. Khemtong, Z. Hayati, L. Song, A. Martins, A. D. Sherry, and L. Lumata, “Influence of Dy^{3+} and Tb^{3+} Doping on ^{13}C Dynamic Nuclear Polarization,” <i>J. Chem. Phys.</i> , vol. 146, pp. 014303, 2017 with the permission of AIP Publishing.	90
4.22	Double exponential, stretched exponential, and single exponential fitting is shown for the collected inversion recovery data for the reference sample (15 mM trityl OX063) at temperatures of 100 K, 30 K, and 7.5 K. From this it is clear that the double exponential fitting results in the most consistent fit.	91

- 4.23 T_1^{-1} data obtained using double exponential fitting plotted versus temperature on a log-log scale. Power law fitting has been performed on segments of the plot and α is shown for each region and sample. Figure adapted from rA. Kiswandhi, P. Niedbalski, C. Parish, P. Kaur, A. Martins, L. Fidelino, C. Khemtong, L. Song, A. D. Sherry, and L. Lumata, "Impact of Ho^{3+} -Doping on ^{13}C Dynamic Nuclear Polarization Using Trityl OX063 Free Radical," *Phys. Chem. Chem. Phys.*, vol. 18, pp 21351 - 21359, 2016 with permission from the PCCP Owner Societies and from P. Niedbalski, C. Parish, A. Kiswandhi, L. Fidelino, C. Khemtong, Z. Hayati, L. Song, A. Martins, A. D. Sherry, and L. Lumata, "Influence of Dy^{3+} and Tb^{3+} Doping on ^{13}C Dynamic Nuclear Polarization," *J. Chem. Phys.*, vol. 146, pp. 014303, 2017 with the permission of AIP Publishing. 92
- 4.24 DNP microwave sweeps for samples doped with (a) gadolinium, (b) holmium, (c) dysprosium, and (d) terbium. The sweep of a control sample with no lanthanide is shown in each plot for reference. Figure adapted from A. Kiswandhi, P. Niedbalski, C. Parish, P. Kaur, A. Martins, L. Fidelino, C. Khemtong, L. Song, A. D. Sherry, and L. Lumata, "Impact of Ho^{3+} -Doping on ^{13}C Dynamic Nuclear Polarization Using Trityl OX063 Free Radical," *Phys. Chem. Chem. Phys.*, vol. 18, pp 21351 - 21359, 2016 with permission from the PCCP Owner Societies and from P. Niedbalski, C. Parish, A. Kiswandhi, L. Fidelino, C. Khemtong, Z. Hayati, L. Song, A. Martins, A. D. Sherry, and L. Lumata, "Influence of Dy^{3+} and Tb^{3+} Doping on ^{13}C Dynamic Nuclear Polarization," *J. Chem. Phys.*, vol. 146, pp. 014303, 2017 with the permission of AIP Publishing. 94
- 4.25 Buildup curves for ^{13}C sodium acetate doped with 15 mM trityl OX063 and further doped with the optimal concentration of an Ln(III)-DOTA complex. Figure adapted from reference [161] with permission from PCCP Owner Societies and from P. Niedbalski, C. Parish, A. Kiswandhi, L. Fidelino, C. Khemtong, Z. Hayati, L. Song, A. Martins, A. D. Sherry, and L. Lumata, "Influence of Dy^{3+} and Tb^{3+} Doping on ^{13}C Dynamic Nuclear Polarization," *J. Chem. Phys.*, vol. 146, pp. 014303, 2017 with the permission of AIP Publishing. 95
- 4.26 The ^{13}C DNP intensity achieved at 10,000 s for samples doped with varying concentrations of holmium, dysprosium, and terbium. For some samples, the build up curve was stopped prior to 10,000 s and the curve extrapolated to that time using a single exponential fit. Figure adapted from A. Kiswandhi, P. Niedbalski, C. Parish, P. Kaur, A. Martins, L. Fidelino, C. Khemtong, L. Song, A. D. Sherry, and L. Lumata, "Impact of Ho^{3+} -Doping on ^{13}C Dynamic Nuclear Polarization Using Trityl OX063 Free Radical," *Phys. Chem. Chem. Phys.*, vol. 18, pp 21351 - 21359, 2016 with permission from the PCCP Owner Societies and from P. Niedbalski, C. Parish, A. Kiswandhi, L. Fidelino, C. Khemtong, Z. Hayati, L. Song, A. Martins, A. D. Sherry, and L. Lumata, "Influence of Dy^{3+} and Tb^{3+} Doping on ^{13}C Dynamic Nuclear Polarization," *J. Chem. Phys.*, vol. 146, pp. 014303, 2017 with the permission of AIP Publishing. 96

4.27	(a) Decay of hyperpolarized ^{13}C signal after dissolution at 9.4 T and room temperature. Data are fit using Equation 4.6 and T_1 displayed for each sample. (b) Comparison of liquid-state enhancement when using each of the Ln(III) complexes.	99
4.28	The trimeric gadolinium complex studied as a DNP additive	100
4.29	(a) Microwave frequency sweeps for samples of 3 M $[1-^{13}\text{C}]$ sodium acetate in 1:1 v/v glycerol:water doped with 15 mM trityl OX063 and additionally doped with either no additive, 2 mM Gd^{3+} -DOTA, or 0.4 mM 3-Gd. (b) ^{13}C polarization buildup curves for the three samples described in (a). (c) 3-Gd Concentration dependence of the ^{13}C DNP intensity. ^{13}C DNP intensity achieved with 2 mM Gd^{3+} -DOTA-doping is shown for reference.	102
4.30	(a) Comparison of liquid state ^{13}C NMR enhancement for control, 3-Gd, and Gd^{3+} -DOTA samples. (b) Liquid state ^{13}C NMR signal relaxation for dissolutions of a 100 μL samples for the three aforementioned samples. Figure adapted with permission from P. Niedbalski, C. Parish, Q. Wang, A. Kiswandhi, Z. Hayati, L. Song, and L. Lumata, “ ^{13}C Dynamic Nuclear Polarization Using a Trimeric Gd^{3+} Complex as an Additive,” <i>J. Phys. Chem. A</i> , vol. 121, pp.5127-5135, 2017, Copyright 2017 American Chemical Society	102
4.31	(a) Field Swept EPR spectra for samples of 1:1 v/v glycerol:water containing 3 M $[1-^{13}\text{C}]$ sodium acetate, 15 mM trityl OX063, and either no additive, 2 mM Gd^{3+} -DOTA, or 0.4 mM 3-Gd (b) Temperature dependence of the trityl OX063 electronic relaxation rate for the aforementioned three samples. Figure adapted with permission from P. Niedbalski, C. Parish, Q. Wang, A. Kiswandhi, Z. Hayati, L. Song, and L. Lumata, “ ^{13}C Dynamic Nuclear Polarization Using a Trimeric Gd^{3+} Complex as an Additive,” <i>J. Phys. Chem. A</i> , vol. 121, pp.5127-5135, 2017 Copyright 2017 American Chemical Society	103
4.32	The NOTA ligand in which Co^{2+} , Cu^{2+} , and Mn^{2+} were chelated (left) and the ferumoxytol nanoparticle (right).	105
4.33	Normalized microwave irradiation frequency sweeps for samples of 3 M $[1-^{13}\text{C}]$ sodium acetate and 15 mM trityl OX063 doped with different concentration of the four different transition metals that were studied.	106
4.34	(a) ^{13}C polarization buildup curves for samples of 3 M $[1-^{13}\text{C}]$ sodium acetate and 15 mM trityl OX063 doped with different concentrations of the 3 metals chelated within NOTA. Data are normalized such that the control builds up to unity. (b) Dependence of ^{13}C DNP intensity on the concentration of transition metal ion complex.	107

4.35	(a) ^{13}C polarization buildup curves for samples of 3 M $[1-^{13}\text{C}]$ sodium acetate and 15 mM trityl OX063 doped with different concentrations elemental iron in the form of Feraheme. Data are normalized such that the control builds up to unity. (b) Dependence of ^{13}C DNP intensity on the iron concentration. Figure adapted with permission from P. Niedbalski, C. Parish, Q. Wang, Z. Hayati, L. Song, Zackary I. Cleveland and L. Lumata, "Enhanced Efficiency of ^{13}C Dynamic Nuclear Polarization by Superparamagnetic Iron Oxide Nanoparticle," <i>J. Phys. Chem. C</i> , vol. 121, pp. 19505-19511, 2017, Copyright 2017 American Chemical Society.	108
4.36	(a) Field swept EPR spectra of the trityl radical when doped with transition metals. (b) Temperature dependence of electron T_1 for the 5 samples studied. .	108
4.37	(a) Comparison of continuous wave EPR spectra of trityl OX063 and each of the transition metal ion complexes studied. (a) Co^{2+} -NOTA, (b) Mn^{2+} -NOTA, (c) Cu^{2+} -NOTA. In (d), the continuous wave EPR spectrum of trityl is shown with adequate resolution in the x axis to discern its features. All spectra are normalized.	111
4.38	(a) Relaxation of hyperpolarized ^{13}C NMR signal for transition metal ion complex-doped samples after dissolution. (b) Comparison of liquid-state ^{13}C NMR signal enhancement 8 s following dissolution for samples optimally doped with transition metal ion complexes	111
4.39	(a) Representative hyperpolarized and thermal signals for the dissolution of the 10 μL sample containing 3 M $[1-^{13}\text{C}]$ sodium acetate, 15 mM trityl, and 11 mM elemental iron post dissolution. (b) Decay of the hyperpolarized ^{13}C signal for control and different volumes of polarized sample containing the optimum concentration of iron. (c) Comparison of liquid state ^{13}C NMR signal enhancement for the different volume samples containing an optimum concentration of iron. Figure adapted with permission from P. Niedbalski, C. Parish, Q. Wang, Z. Hayati, L. Song, Zackary I. Cleveland and L. Lumata, "Enhanced Efficiency of ^{13}C Dynamic Nuclear Polarization by Superparamagnetic Iron Oxide Nanoparticle," <i>J. Phys. Chem. C</i> , vol. 121, pp. 19505-19511, 2017, Copyright 2017 American Chemical Society.	112
4.40	(a) Dependence of proton T_1 on iron concentration in water. Horizontal lines denote measured proton T_1 of a pure water control and a mock dissolution sample before and after filtration. (b) Dependence of absorbance at 302 nm on iron concentration in water. Horizontal lines denote absorbance of control and a mock dissolution sample before and after filtration.	113
4.41	(a) Inversion recovery data for a dissolution sample containing approximately 0.268 mM elemental iron (prior to filtration) that had been forced through a syringe filter. (b) Comparison of ^{13}C NMR signal decay after dissolution of a sample containing 11 mM (0.268 mM after dissolution) elemental iron before and after filtration. Post filtration data is simulated based on the T_1 found using inversion recovery shown in (a).	114

4.42	Schematic showing the experimental procedure used to measure ^{13}C spin lattice relaxation times at a large number of magnetic fields	116
4.43	The ^{13}C -labeled carboxyl molecules whose field dependence of ^{13}C relaxation was studied (a) Sodium Pyruvate, (b) Sodium Acetate, (c) Pyruvic Acid, and (d) Glycine	117
4.44	Sample spectra (left) and integrated intensity (right) for the decay of the hyperpolarized ^{13}C signal of sodium pyruvate at three different magnetic field strengths.	118
4.45	^{13}C T_1 shown vs. magnetic field in both (a) linear and (b) logarithmic scales. This is to emphasize both the vast difference between ^{13}C relaxation times and the power law field dependence observed ($T_1 = CB^\alpha$). Sodium pyruvate - $\alpha = 3.01 \pm 0.05$, Pyruvic acid - $\alpha = 3.1 \pm 0.1$, Glycine - $\alpha = 2.85 \pm 0.07$, Sodium acetate - $\alpha = 2.35 \pm 0.05$	119
5.1	A comparison of the ^{13}C NMR signal for hyperpolarized and thermal [$\text{U-}^{13}\text{C}_6,1,2,3,4,5,6,6\text{-d}_7$] glucose (left). Representative decay of the hyperpolarized ^{13}C NMR signal for the fastest relaxing peak (Peak (a)) and the slowest relaxing peak (Peak (h)) of the uniformly ^{13}C labeled glucose (right)	123
5.2	A comparison of the ^{13}C NMR signal for hyperpolarized and thermal [$2\text{-}^{13}\text{C}$] fructose (left). Representative decay of the hyperpolarized ^{13}C NMR signal for peak (a) (right). All three peaks had approximately the same relaxation time. .	124
5.3	A comparison of the ^{15}N NMR signal for hyperpolarized and thermal ^{15}N labeled choline (left). Decay of the hyperpolarized ^{15}N NMR signal and liquid state T_1 . .	125
5.4	Sample NMR spectra for metabolic studies in yeast using [$1\text{-}^{13}\text{C}$] glucose at 9.4 T (a) and 1 T (b). Plots showing the integrated intensity of the NMR spectra of metabolic reactants and products for different administered substrates at 9.4 T ((c)-(f)) and 1 T ((g) - (j)).	127
5.5	(a) Real time tracking of the metabolism of [$1\text{-}^{13}\text{C}$] pyruvate and its conversion to carbon dioxide and bicarbonate. The pyruvate data is scaled down so that it is more easily compared to the products. (b) Sum of the first 50 spectra measuring metabolism.	129
5.6	Sum of the first 50 spectra measuring yeast metabolism of [$2\text{-}^{13}\text{C}$] pyruvate. . . .	130
5.7	NMR spectrum of the extract of PC-3 cells that had been cultured in [$1\text{-}^{13}\text{C}$] choline for 24 hours. The single peak corresponds to phosphocholine, indicating that choline has been metabolized by the PC-3 cells.	131
5.8	(a) Decay of the ^{15}N hyperpolarized signal after administering hyperpolarized ^{15}N -labeled choline to a PC-3 cancer cell suspension. (b) Sum of the first 50 spectra measured after administering choline to the cells.	133

5.9	(a) Real time metabolism of PC-3 cells in suspension after administering hyperpolarized ^{13}C -labeled pyruvate. Scans were measured at 9.4 T (b) Sum of the first 50 spectra measured after administering pyruvate to the cells.	134
5.10	(a) Real time metabolism of PC-3 cells in suspension after administering hyperpolarized ^{13}C -labeled pyruvate. Scans were measured at 1 T	134
6.1	An MRI Image obtained of a custom 3-D printed phantom designed and printed by the author. The phantom was filled with water and measured using a gradient echo pulse sequence (See Appendix A) in the Terranova MRI.	136
6.2	Temperature dependence of proton relaxation times for water doped with varying concentrations of copper chloride measured at Earth's magnetic field.	138
A.1	The single pulse NMR experiment, which is the most basic NMR pulse sequence.	141
A.2	The Spin Echo Pulse Sequence	142
A.3	The inversion recovery pulse sequence used to measure T_1 of nuclei.	142
A.4	A simple MRI pulse sequence - Gradient Echo.	143
B.1	Numerical simulation of DNP spectra for ^{13}C polarization using a narrow line-width radical such as trityl. The electron relaxation rate was adjusted to simulate the addition of paramagnetic agent. Simulations are scaled such that $1/T_{1e} = 1$ has a maximum intensity of 1.	145
C.1	The toggle valve used in purging the system.	147
C.2	Standard parameters for acquiring microwave frequency sweep data (top row), killing the built-up polarization (middle row), and monitoring the DNP build-up curve (bottom row).	151
C.3	Left: The control panel for the MOKON chiller used to cool the helium compressor. Process pump and compressor controls are labeled. Right: The flow meter attached to the chiller. The flow indicator is labeled and shows an appropriate value for the running of the system.	154
C.4	The front panel of the helium compressor used in the cryogen-free magnet system. Important buttons and switches are indicated. Not pictured is the main breaker of the unit, which is a large switch under the main power. If the system trips, the breaker will need to be flipped.	155
C.5	The circulation pump used to bring the sample space temperature down to a base of 1.7 K.	156
C.6	The magnet power source used to energize the cryogen-free system. The series of images shows the process by which the limits are set. (a) Power source home page that is automatically displayed when the power is turned on. The SETUP menu is indicated (b) Power source SETUP menu with the LIMITS menu indicated. (c) Power source LIMITS menu with navigation arrows indicated. (d) LIMITS menu after a value has been changed. Select SAVE and EXIT to retain the input values and return to the main menu.	158

D.1	The laminar flow hood used for cell culture.	164
D.2	The incubator used for cell culture. The incubator should be kept at 37°C and 5% CO ₂ . Additionally, the water tray within the incubator needs to be kept filled with 1.5 L of sterilized distilled water.	165
D.3	The warmer that is used to thaw and warm media components to 37°C.	166
D.4	The image through an inverted microscope of cell dishes with (a) low confluency, (b) appropriate confluency for transferring cells, and (c) over-confluency at which point cells should be discarded.	168
D.5	A hemacytometer	170
D.6	View through a microscope of a hemacytometer. Count the cells in the squares indicated	170

LIST OF TABLES

1.1	Free electron and nuclear isotopes commonly used in NMR and their properties.	2
1.2	Free radicals to be studied. The “Nickname” of each free radical is how it will be referred to in the body of this work.	16
2.1	Maximum possible Overhauser enhancement for ^1H and ^{13}C	28
4.1	UV-Vis Spectrophotometry Results showing the primary UV-Vis peaks for each radical, as well as the dependence of absorbance (A) on concentration (C) and the decay time constant, determined by fitting a single exponential decay to time course data.	76
4.2	Optimal concentrations of free radicals for dissolution DNP	77
4.3	Concentrations of metals added to DNP samples. Optimum concentrations are shown in bold.	106
5.1	^{13}C location and anomer corresponding to NMR peak	124
5.2	The approximate maximum soluble concentration in 1:1 v/v glycerol:water of the substrates studied	132
C.1	Typical values for the sensor readings on the CP2800 Series Helium Compressor	155
D.1	Useful cell numbers and medium quantities for cell culture (Values are approximate)	169

CHAPTER 1

INTRODUCTION

1.1 Nuclear Magnetic Resonance and Magnetic Resonance Imaging

1.1.1 Introduction to NMR and MRI

Nuclear magnetic resonance (NMR) is a phenomena in which nuclear spins absorb and emit electromagnetic radiation at frequencies determined by the strength of a magnetic field in which they reside[1]. It was discovered independently by Purcell [2] and Bloch [3] in 1946 and since that time NMR spectroscopy has become an invaluable technique in physics, chemistry, biology, and many other scientific fields.

NMR is possible when a given nucleus has spin, which gives said nucleus a non-zero magnetic moment [4]. The nuclear spin is governed by the spin quantum number, the most basic unit of spin being that of the proton or neutron ($I = \frac{1}{2}$). Thus, different combinations of protons and neutrons within a nucleus will lead to spin in multiples of $\frac{1}{2}$ ($I = 0, \frac{1}{2}, 1, \frac{3}{2}$, etc). By setting an arbitrary z axis, the projection of the spin vector along z axis is then defined as

$$m = -I, -I + 1, \dots, I - 1, I. \quad (1.1)$$

This may loosely be considered as the direction of the spin angular momentum vector. In the absence of an external magnetic field, the direction of the nuclear spin angular momentum vector has no impact on the energy of a nucleus. Thus, for nuclei with non-zero spin, there is $(2I + 1)$ -fold degeneracy. However, the degeneracy is lifted by placing the nucleus in an external magnetic field, which results in Zeeman splitting. This splitting causes $(2I + 1)$ distinct energy levels which are described by the m quantum number.

Consider then a nucleus in an external magnetic field. If a nucleus is irradiated at a frequency of ν , then it may absorb a photon and become excited to a higher energy state.

The probability for this to occur is highest under the condition of resonance, when

$$E_i - E_j = h\nu \quad (1.2)$$

(where h is Planck's Constant and E_i and E_j are the energies of any two states) [1].

This energy difference between states depends on the magnitude of the external magnetic field as well as the strength of the magnetic moment of the nucleus in question and is governed by the equation

$$\Delta E_{ij} = \hbar\gamma B_0, \quad (1.3)$$

where $\hbar$ is the reduced Planck constant, B_0 is the magnitude of the external magnetic field, and γ is the gyromagnetic ratio of the nucleus. The gyromagnetic ratio is a proportionality constant between the spin and the magnetic moment (μ) of a nucleus [5], defined by:

$$\mu = \gamma\mathbf{I}. \quad (1.4)$$

The constant γ has the units rad/s/T. Often, the gyromagnetic ratio is expressed as $\gamma_n = \gamma/2\pi$, with units MHz/T. A list of nuclei commonly used in NMR, along with gyromagnetic ratios and natural abundances may be found in Table 1.1.

Table 1.1. Free electron and nuclear isotopes commonly used in NMR and their properties.

Species	Spin	$\gamma(10^7 \text{ rad/s/T})$	$\gamma_n \text{ (MHz/T)}$	Natural Abundance (%)
Free Electron	1/2	17608.60	28024.95	—
1H	1/2	26.7519	42.576	99.98
2H	1	4.1066	6.536	.015
3He	1/2	-20.38	-32.434	.00013
7Li	1/2	3.9371	16.546	92.58
^{13}C	1/2	6.7283	10.705	1.108
^{14}N	1	1.9338	3.077	99.635
^{15}N	1/2	-2.712	-4.316	.365
^{17}O	1/2	-3.6279	-5.772	.037
^{19}F	1/2	25.181	40.052	100
^{31}P	1/2	10.841	17.235	100
^{89}Y	1/2	-1.3155	-2.094	100

Equations 1.2 and 1.3 may be combined to show that the probability of a nucleus being excited to a higher energy state is greatest when

$$\nu = \gamma_n \times B_0 \quad (1.5)$$

This frequency is known as the Larmor frequency.

In any macroscopic sample, there will be an enormous number ($\sim 10^{23}$) of nuclear spins occupying the different available energy states [1]. All of these spins together may be described as a coherent ensemble, in which case the population of states at thermal equilibrium is determined using Boltzmann statistics [6]:

$$\frac{N_j}{N_i} = \exp\left(-\frac{\Delta E_{ij}}{kT}\right) \quad (1.6)$$

The NMR signal strength is dependent on the difference in populations between energy states, quantified by the polarization. The polarization is defined as

$$P = \frac{N(I) - N(-I)}{\sum_{m=-I}^I N(m)}. \quad (1.7)$$

Most commonly studied in NMR are spin $\frac{1}{2}$ particles, for which the polarization may be expressed as

$$P = \frac{N_+ - N_-}{N_+ + N_-} = \tanh\left(\frac{\Delta E}{2kT}\right). \quad (1.8)$$

Less common, but still somewhat heavily used are spin 1 particles, for which the polarization is

$$P = \frac{N_{+1} - N_{-1}}{N_{+1} + N_{-1} + N_0} = \frac{4 \tanh\left(\frac{\Delta E}{2kT}\right)}{2 + \tanh^2\left(\frac{\Delta E}{2kT}\right)}. \quad (1.9)$$

where ΔE is the energy difference between two adjacent states.

Non-zero polarization leads to a bulk magnetization in the sample, which may then be measured using NMR spectroscopy. Initially, the magnetization of the sample is parallel to the external magnetic field, usually referred to as the z direction. However, by applying a pulse of electromagnetic radiation at the Larmor frequency defined in equation 1.5, the

magnetization can be rotated into the xy plane. Once in the xy plane, the magnetization will precess about the external magnetic field at the Larmor frequency of the nuclei, which may be monitored to obtain the “NMR signal”. In practice, an NMR coil is used to apply a pulse to a sample of nuclear spins and the induced precession of magnetization causes a current in the same NMR coil. The signal retrieved is known as a free induction decay (FID) and may then be Fourier transformed into frequency space, giving the spectral signal of the sample.

An important development in NMR occurred in the 1970’s when Raymond Damadian [7], Peter Mansfield [8], and Paul Lauterbur [9] independently developed a method of imaging using NMR technology [10]. By applying known gradient magnetic fields across the sample, nuclear spins in different locations of the sample will precess at different Larmor frequencies. The strength and duration of the gradient fields are carefully regulated which allows the intensity of a given signal to be mapped to a location based on its frequency and phase, creating an image [11]. Specifically, there are three main gradients used in image formation: slice select, phase encoding, and frequency encoding. The slice select gradient is applied during NMR pulses to specify an imaging plane. Then, phase encoding and frequency encoding gradients are used to read out a specific location in that imaging plane (Figure 1.1). This method of imaging is widely known as magnetic resonance imaging, or MRI. MRI has become an indispensable tool in the medical field because, unlike other medical imaging technologies, it uses non-ionizing radiation and is non-invasive but still provides good spatial and contrast resolution [10]. It should be noted that although the principles of MRI are applicable to any nucleus with non-zero spin, generally only large- γ nuclei, specifically ^1H , have strong enough signal to be commonly imaged.

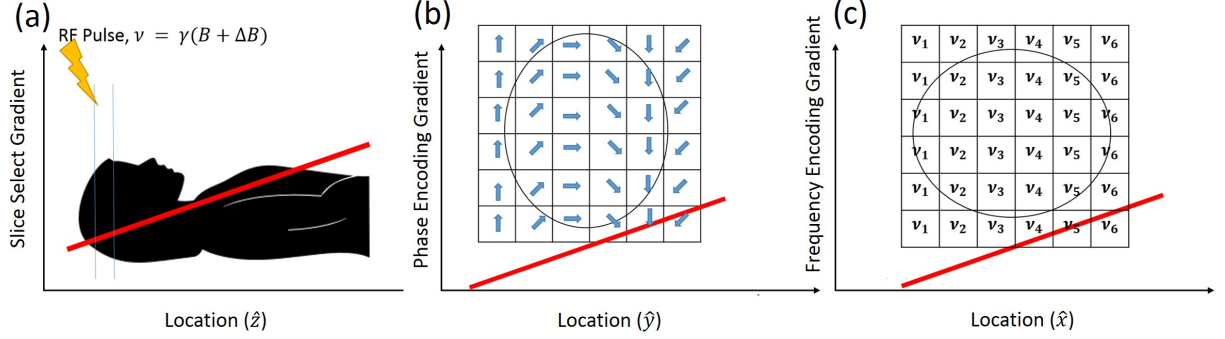


Figure 1.1. Schematic showing the simplified acquisition of an MRI. (a) The slice select gradient is used to select an imaging plane in the z direction. (b) Then, a phase shift in the y direction is induced using the phase encoding gradient. (c) Finally, an NMR echo is monitored in the presence of the frequency encoding gradient, giving location in the x direction.

1.1.2 Relaxation and Line Broadening

In an NMR experiment, after the polarization has been rotated by electromagnetic radiation, two powerful relaxation processes work to bring the sample back to thermal equilibrium. These processes are longitudinal and transverse relaxation.

Longitudinal relaxation refers to the realignment of the sample magnetization with the external field [5] and is governed by the equation

$$M_z(t) = M_0 \left[1 - \exp\left(-\frac{t}{T_1}\right) \right] \quad (1.10)$$

where M_z is the z -component of the magnetization, M_0 is the thermal equilibrium magnetization and T_1 is the time constant governing the relaxation [5].

Transverse (often called spin-spin or T_2) relaxation refers to the dephasing of spins in the xy plane that leads to a reduction in overall magnetization [5]. The equations governing this process are:

$$M_y(t) = M_0 \cos(\omega t) \exp\left(-\frac{t}{T_2}\right) \quad (1.11)$$

and

$$M_x(t) = M_0 \sin(\omega t) \exp\left(-\frac{t}{T_2}\right). \quad (1.12)$$

In this case, M_x and M_y are the components of the magnetization in the x and y directions, respectively, ω is the Larmor frequency of the nucleus, and T_2 is the time constant for the relaxation.

Typically, both types of relaxation are due to random field fluctuations. That is, spins can be considered to reside both in a constant magnetic field B_0 along the z axis and a time-dependent, small magnetic field in the xy plane, $B_\perp(t)$. This small fluctuating field is assumed to average to 0 ($\langle B_\perp(t) \rangle = 0$), so its magnitude is defined as $\langle B_\perp^2(t) \rangle \neq 0$. Furthermore, the fluctuation of the magnetic field is also characterized using the autocorrelation function $G(\tau) = \langle B_\perp(t)B_\perp(t+\tau) \rangle \neq 0$. Generally, a simplified exponential autocorrelation function is used.

$$G(\tau) = \langle B_\perp^2(t) \rangle e^{-\frac{\tau}{\tau_C}} \quad (1.13)$$

The value τ_C is known as the correlation time and refers to the time that it takes for a spin to rotate by 1 radian. Values of τ_C are typically in the range of picoseconds and can be increased by cooling a sample.

Rather than the autocorrelation function, its Fourier transform is often used for analysis. This is known as the spectral density function $J(\omega)$.

$$J(\omega) = 2 \int_0^\infty G(\tau) e^{-i\omega\tau} d\tau \quad (1.14)$$

More commonly, the normalized spectral density is used, which, for the exponential autocorrelation function (Equation 1.13) becomes

$$\mathcal{J}(\omega) = \frac{\tau_C}{1 + \omega^2 \tau_C^2} \quad (1.15)$$

By using a series of rate equations known as the Solomon equations [12], the relaxation times of nuclei may be written in terms of the spectral density function as

$$T_1^{-1} = \frac{3}{10} b^2 \{ \mathcal{J}(\omega_0) + 4 \mathcal{J}(2\omega_0) \} \quad (1.16)$$

and

$$T_2^{-1} = \frac{3}{20}b^2\{3\mathcal{J}(0) + 5\mathcal{J}(\omega_0) + 2\mathcal{J}(2\omega_0)\} \quad (1.17)$$

where ω_0 is the Larmor frequency and $b = -\frac{\mu_0}{4\pi} \frac{\hbar\gamma^2}{r^3}$ with γ the gyromagnetic ratio of the nucleus in question and r the internuclear distance. These equations are often used for calculating the relaxation times of simple systems such as pure water in a high magnetic field (Figure 1.2).

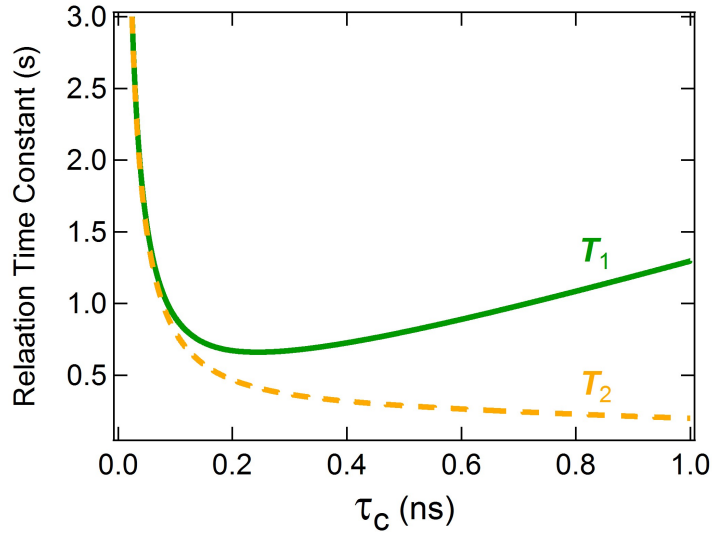


Figure 1.2. Spin-lattice and spin-spin relaxation times calculated as a function of correlation time using Equations 1.16 and 1.17 for protons in water at a magnetic field of 9.4 T.

While spin-lattice relaxation affects signal strength and the speed at which scans may be taken, spin-spin relaxation has a direct effect on the NMR spectrum. Specifically, the spectral peak is intrinsically broadened, sometimes called homogeneous broadening. Furthermore, when the magnetic field is not perfectly homogeneous, nuclei at different locations will experience different magnetic field strengths and thus precess at different frequencies. This will increase the rate of transverse relaxation, and the time constant that describes this adjusted relaxation rate is T_2^* [10]. The relaxation described by T_2^* also causes broadening of the NMR spectrum but in this case is known as inhomogeneous broadening.

In an arbitrary spectrum, the source of broadening may be determined by the shape of the spectral lines. Homogeneously broadened peaks have a Lorentzian lineshape, while inhomogeneously broadened peaks have a Gaussion lineshape [13]. For real situations in which both types of broadening are present, a convolution of Gaussian and Lorentzian lineshapes, known as a Voigt profile, may be used to determine the relative contribution of each type of broadening [14]. Homogeneous broadening is intrinsic to a given sample, but inhomogeneous broadening can be eliminated by using highly homogenous magnetic fields [15]. For example, a proton spectral resolution of 1 Hz at a field of 1 T requires homogeneity to 1 part in 10^8 . As a result, high resolution NMR is limited to magnetic fields that are highly homogeneous, with the requisite homogeneity becoming more difficult to achieve at higher fields.

1.1.3 Chemical Shift and J-Coupling

In a given sample for NMR, there are a huge number of spins in mutual interaction. Because of this, there are several features that can be used to understand the molecular environment of the nucleus in question. The presence of other magnetically active nuclei and electrons within the sample will cause a shift in the Larmor frequency of a given nucleus, called the chemical shift. This shift is unique to different molecular environments, and thus, the location of nuclear spin in a molecule may be identified by the location of the NMR peak with respect to a given reference. For ^1H , ^{13}C , and ^{29}Si , the reference used is tetramethylsilane (TMS) [5]. The chemical shift is measured in parts per million (ppm).

In addition to the chemical shift, the spins interact via dipolar and scalar coupling (sometimes referred to as J-coupling or indirect dipole-dipole coupling) [5, 6]. Dipolar coupling is a result of a nucleus directly interacting with the magnetic field emitted by its neighboring nuclei. This is the stronger of the two types of coupling, but in liquids tends to be effectively neutralized by rapid molecular tumbling. In solids, dipolar coupling is responsible for a widening of the NMR peak but can be reduced through the process of magic angle

spinning in which the sample is rapidly spun at the “magic angle” ($\theta = 53^\circ$) [16]. Scalar coupling arises from nuclei weakly magnetizing electrons within the molecule and the resulting magnetic field affecting each nucleus. This type of coupling is not averaged out by molecular tumbling and splits the signal into multiple peaks, the number depending on how many nuclei are involved in the coupling. In general, if a nucleus is scalar coupled to n equivalent spin- $\frac{1}{2}$ nuclei the NMR spectrum will show $(n + 1)$ peaks (Figure 1.3). However, if this splitting is unwanted, the nuclei may be decoupled by saturating the resonance of the coupled nuclei with RF irradiation while monitoring the target nuclei [17].

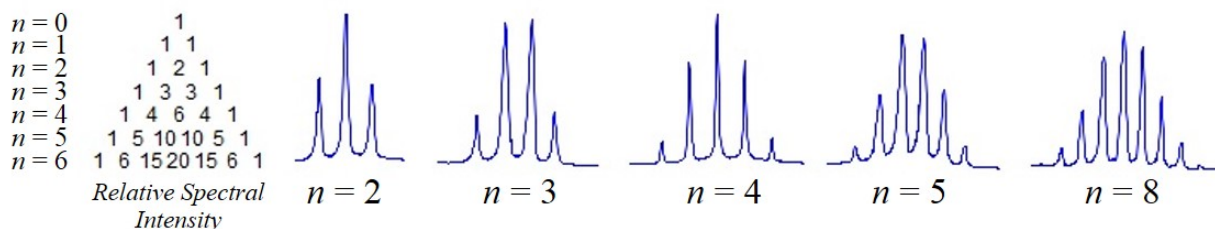


Figure 1.3. Theoretical J-Coupling peaks for various numbers of identical spin $\frac{1}{2}$ nuclei. The theoretical relative intensities of the peaks are given by Pascal's Triangle (shown on the left).

A final interaction that exists in NMR is the quadrupolar interaction. This interaction is much stronger than the chemical shift, scalar, and dipolar interactions but only occurs for nuclei with $I > \frac{1}{2}$. In such nuclei, the electric quadrupole moment of the nucleus interacts with electric field gradients caused by uneven charge distribution within the nucleus. This effect is apparent in NMR spectra by significant broadening of the peak and fast relaxation times [18].

1.2 Electron Paramagnetic Resonance

Electron paramagnetic resonance (EPR), sometimes referred to as electron spin resonance (ESR) or electron magnetic resonance (EMR), is a technique very similar to NMR. While

NMR studies the interaction of electromagnetic radiation with nuclei, EPR studies the interaction of electromagnetic radiation with with unpaired electron spins contained in atoms or molecules [19]. As can be seen in Table 1.1, the electron possesses a gyromagnetic ratio much greater than that of any nucleus. This leads to high electron thermal polarization (by Equation 1.6)) and thus, much greater signal strength when compared to nuclei at similar magnetic fields and temperatures.

EPR was pioneered in 1944 by Yevgeny K. Zavoisky when he found the spectrum of a hydrated copper chloride complex at a low field of 50 G [19, 20]. Very quickly, experiments at much higher fields near 4000 G were performed (Figure 1.4). This specific field was common because the microwave technology for the corresponding frequency range (9 GHz-X band) was well developed at this time due to its use in radar equipment during WWII. Eventually, as technology progressed, the use of stronger magnetic fields became common.

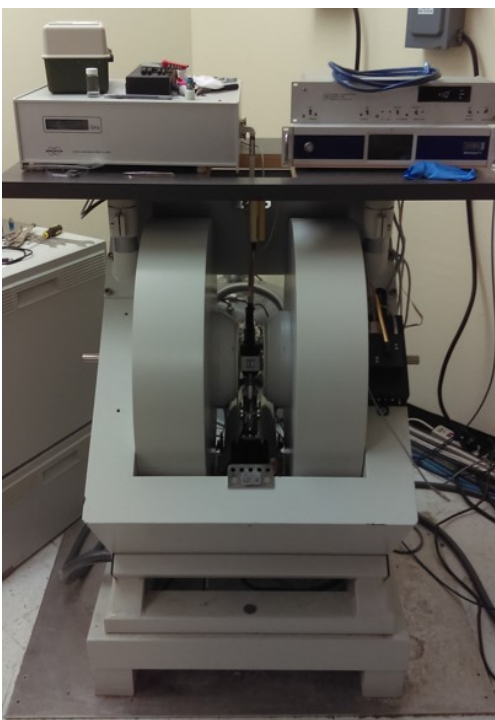


Figure 1.4. The X-band EPR magnet and spectrometer at the University of Texas at Dallas NanoTech Institute

The procedure for EPR is very similar to that of NMR though due to the strength of the magnetic moment, the frequency of the electromagnetic radiation used is typically in the GHz range rather than MHz as in NMR. While physically similar, the microscopic interactions that the electrons experience are described differently than the nuclear interactions in NMR.

There is an analog to the the NMR chemical shift known as the g factor [19, 20]. For a free electron, the g factor is 2.0023. Free radicals, such as those used in this work, tend to have a g value that is very close to that of a free electron. However, even a small difference from the free electron value can give information about the molecular environment in which the electron resides.

When the electron interacts with nearby nuclear dipole moments, it is known as the hyperfine interaction and leads to hyperfine splitting (Figure 1.5). When this occurs, the EPR spectrum is split into multiple peaks. Like scalar coupling in NMR, the number of lines depends on the number of nuclei to which an electron is coupled as well as the type of nuclei. If an electron is coupled to N identical spin I nuclei, there will be $(2NI + 1)$ lines in the EPR spectrum [20].

In liquid samples, the g factor and hyperfine interactions give the EPR spectrum its shape. In the solid state, dipolar coupling dominates the spectral shape, widening the peak(s) as in NMR [20]. Additionally, although every electron has spin $I = \frac{1}{2}$, certain coupled electron systems can simulate spins of greater than $\frac{1}{2}$ which introduces quadrupolar coupling effects, broadening the EPR spectrum.

1.3 Dynamic Nuclear Polarization

As alluded to above, NMR is highly specific and can provide a great wealth of information, but suffers from insensitivity making many experiments difficult, time consuming, or even impossible. One common way to overcome this is referred to as the “brute force” method of increasing polarization [21]. Because the signal strength at thermal equilibrium is dependent

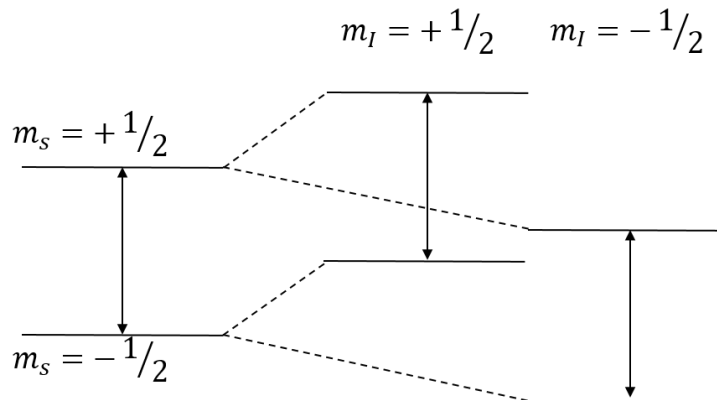


Figure 1.5. Schematic showing the splitting of energy levels when hyperfine coupling is present

on Boltzmann statistics, it may be increased either by increasing the external magnetic field or decreasing the temperature. Decreasing temperature, to a certain extent, is the more simple of these two. Even in the 1970's, temperatures in the millikelvin range were available for NMR [22]. However, the difficulty and expense of bringing a sample to millikelvin temperatures makes using extremely low temperatures relatively rare. In addition, at low temperatures, the sample will be in the solid state, requiring magic angle spinning for high resolution which becomes progressively more difficult as temperatures are lowered [16].

High magnetic fields for NMR are also difficult to achieve because as the field goes higher, the homogeneity of the field must be increased in order to maintain high resolution in the NMR spectra. The strongest magnetic field strength currently available for high resolution NMR is about 24.0 T [23], and even at this high field the proton polarization falls short of 30% at any easily achieved temperature. In Figure 1.6, the polarization of electrons, protons, and ^{13}C is shown as a function of temperature for three different magnetic field strengths, illustrating the stark difference between the thermal equilibrium polarization of electrons and nuclei.

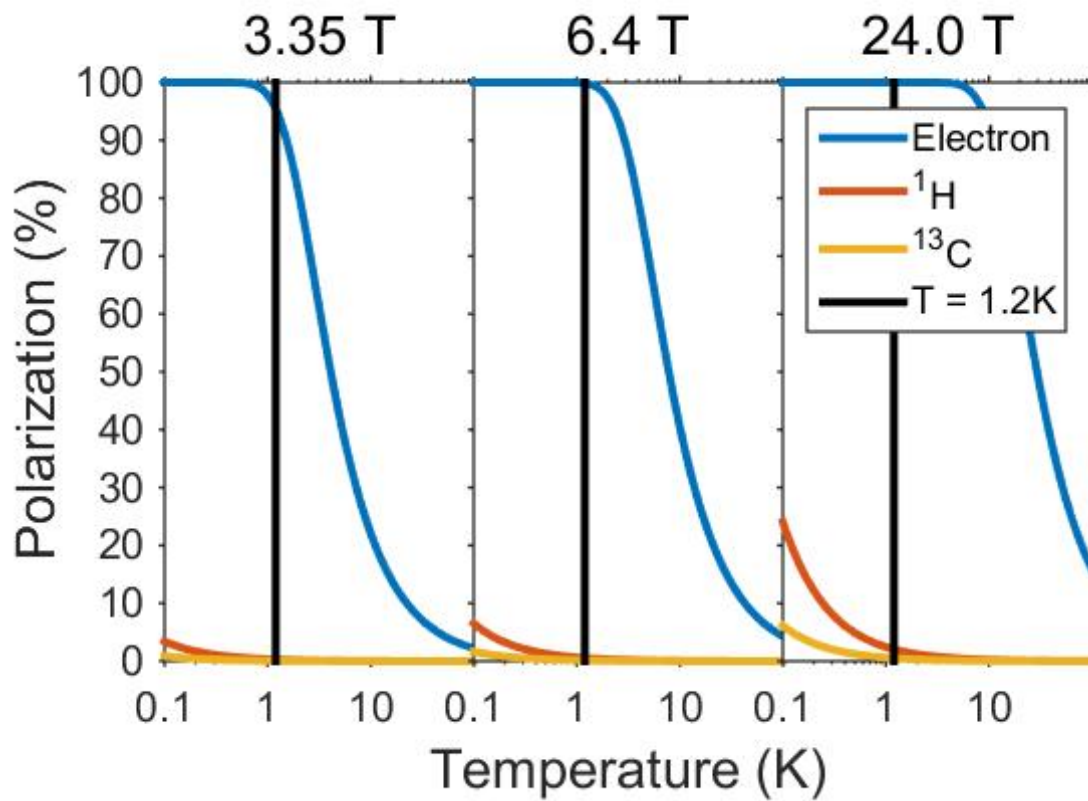


Figure 1.6. Polarization of Electron, ^1H , and ^{13}C as a function of temperature in fields corresponding to the Oxford's commercial HyperSense polarizer (left), the homebuilt polarizers at the University of Texas at Dallas (middle), and at the strongest magnetic field available for high resolution NMR (right). The vertical line denotes a temperature readily achievable using liquid helium.

A solution to the problem of low NMR sensitivity was first proposed by Albert Overhauser in 1953 [24]. He suggested that by irradiating a metal at the electron resonance frequency, the high electron polarization could be transferred to nuclei. Though originally met with great skepticism, Overhauser's work has become the basis for the large and growing field of dynamic nuclear polarization (DNP) [18]. Once verified [25], Overhauser's idea was rapidly expanded upon, allowing for polarization of not only metals as originally proposed, but also liquids, semiconductors, and non-conducting solids [26, 27]. It is important to note that DNP requires the presence of free electrons whose polarization may be transferred to nuclei.

Therefore, if a nonmetal is to be polarized, it must be doped with paramagnetic impurities (free radicals) [22, 28].

Research in DNP was originally driven by the desire to have solid polarized targets for use in nuclear and particle physics experiments [28]. However, the field has significantly evolved, and current research in DNP generally falls into one of two categories: Magic angle spinning (MAS) DNP [29] or dissolution (*ex situ*) DNP [30].

In MAS DNP, samples are both polarized and studied by NMR in a specially designed magic angle spinning probe. Typically, this type of DNP is performed at high magnetic fields using a high power gyrotron microwave source [31].

For dissolution DNP, samples of nuclear spins are typically polarized at intermediate magnetic fields ($\sim 3 - 5$ T) and liquid helium temperatures ($\lesssim 2$ K) [30, 32, 33]. Once significant polarization has been achieved, samples are rapidly dissolved by a superheated solvent and removed from the DNP magnet. This results in a “hyperpolarized” liquid that has an NMR signal enhanced many thousand-fold over thermal equilibrium. This hyperpolarized signal persists for a short time based on the T_1 of the nucleus being polarized. For ^{13}C nuclei (the most commonly studied), the signal lasts from as short as several seconds to as long as several minutes [34]. This work is primarily concerned with the field of dissolution DNP.

The efficiency of a given DNP experiment depends very strongly on the experimental conditions under which it takes place. The magnetic field, temperature, irradiation frequency, sample composition and state, nucleus to be polarized, and the concentration and EPR spectrum of the free radical being used all play a part in determining the overall polarization enhancement [27, 35, 36, 37, 38]. These experimental conditions determine which of four DNP mechanisms- Overhauser Effect, Solid Effect, Cross Effect, and Thermal Mixing- are dominant in a given experiment. Each mechanism will be discussed in detail in chapter 2.

1.4 Introduction to Free Radicals

The choice of free radical plays an essential role in DNP. A free radical is an atom, molecule or ion containing an unpaired electron [20]. In general, free radicals are very unstable and short-lived, but those used for DNP are relatively stable with long half-lives. The free radical provides the free electrons necessary for polarization and determines the DNP mechanism based on its EPR spectrum. The most common radicals in use for DNP belong to the family of the nitroxides (e.g. 4-Oxo-TEMPO) [36, 39, 40, 41] or the trityls (e.g. OX063) [33, 42, 43, 44]. Nitroxides have wide EPR linewidth, making them more efficient for polarizing large- γ nuclei such as ^1H [39, 45]. Trityls, on the other hand, have narrow EPR linewidths and are ideal for direct polarization of low- γ nuclei [32, 42]. Furthermore, polarization when using trityl can be further enhanced by the addition of gadolinium contrast agents [41, 43, 46]. Other radicals, such as DPPH [47], galvinoxyl [48], and BDPA [35] have been found to be successful polarizing agents as well. Additionally there are a number of novel radicals that have been used in dissolution DNP. These include BDPA radicals caged in polystyrene beads [49], TEMPO radicals attached to silica mesh [50], paramagnetic defects in carbon and silicon nanoparticles [51, 52], and non-persistent photo-induced radicals [53].

The primary focus of this work is to study and optimize the DNP efficiency when using both common and novel free radicals with an aim for dissolution DNP and biomedical applications. The radicals that will be studied are found in Table 1.2 and their structures in Figures 1.7 and 1.8.

1.5 Biomedical Applications of Dissolution Dynamic Nuclear Polarization

From the time of its invention, dissolution DNP has offered the possibility of performing biological studies that would otherwise be impossible. The primary example of this is real-time tracking of carbon metabolism. ^{12}C has no nuclear spin, so it is invisible to NMR or

Table 1.2. Free radicals to be studied. The “Nickname” of each free radical is how it will be referred to in the body of this work.

Free Radical	Synonym	“Nickname”
TEMPO	2,2,6,6-Tetramethyl-1-piperidinyloxy	TEMPO
4-Hydroxy-TEMPO	4-Hydroxy-2,2,6,6-tetramethylpiperidine 1-oxy	TEMPOL
Water Soluble-TEMPO	2,2,6,6-Tetramethyl-4-[1-oxo-6-(triethylammonio)hexylamino]-1-piperidinyloxy bromide	WS-TEMPO
4-Oxo-TEMPO	4-Oxo-2,2,6,6-tetramethyl-1-piperidinyloxy	TEMPONE
Deuterated 4-Oxo-TEMPO	4-Oxo-2,2,6,6-tetramethylpiperidine-d16-1-oxy	TEMPONE-d ₁₆
¹⁵ N 4-Oxo-TEMPO	4-Oxo-2,2,6,6-tetramethylpiperidine-1-15N-1-oxy	TEMPONE- ¹⁵ N
Deuterated ¹⁵ N 4-Oxo-TEMPO	4-Oxo-2,2,6,6-tetramethylpiperidine-d16,1-15N-1-oxy	TEMPONE- ¹⁵ N, d ₁₆
AZADO	2-Azaadamantane-N-oxy	AZADO
Polymer Bound TEMPO	(2,2,6,6-Tetramethyl-1-piperidinyloxy, free radical), polymer-bound	PS-TEMPO
Trityl OX063	tris{8-carboxyl-2,2,6,6-benzo(1,2-d:4,5-d)-bis(1,3)dithiole-4-yl}methyl sodium salt	Trityl
BDPA	1,3-bisdiphenylene-2-phenylallyl	BDPA
Water Soluble BDPA	1,3-bis(diphenylene)-2-phenylallyl	SA-BDPA
DPPH	2,2-diphenyl-1-picrylhydrazyl	DPPH
Octylphenyl DPPH	2,2-Di(4-tert-octylphenyl)-1-picrylhydrazyl, free radical	O-DPPH
Galvinoxyl	2,6-Di-tert-butyl- $\dot{I}$ ±-(3,5-di-tert-butyl-4-oxo-2,5-cyclohexadien-1-ylidene)-p-tolyloxy, free radical	Galvinoxyl
—	Tris(4-bromophenyl)ammoniumyl hexachloroantimonate	Blue
—	4-Phenacylidene-2,2,5,5-tetramethylimidazolidin-1-ylloxy	Phena

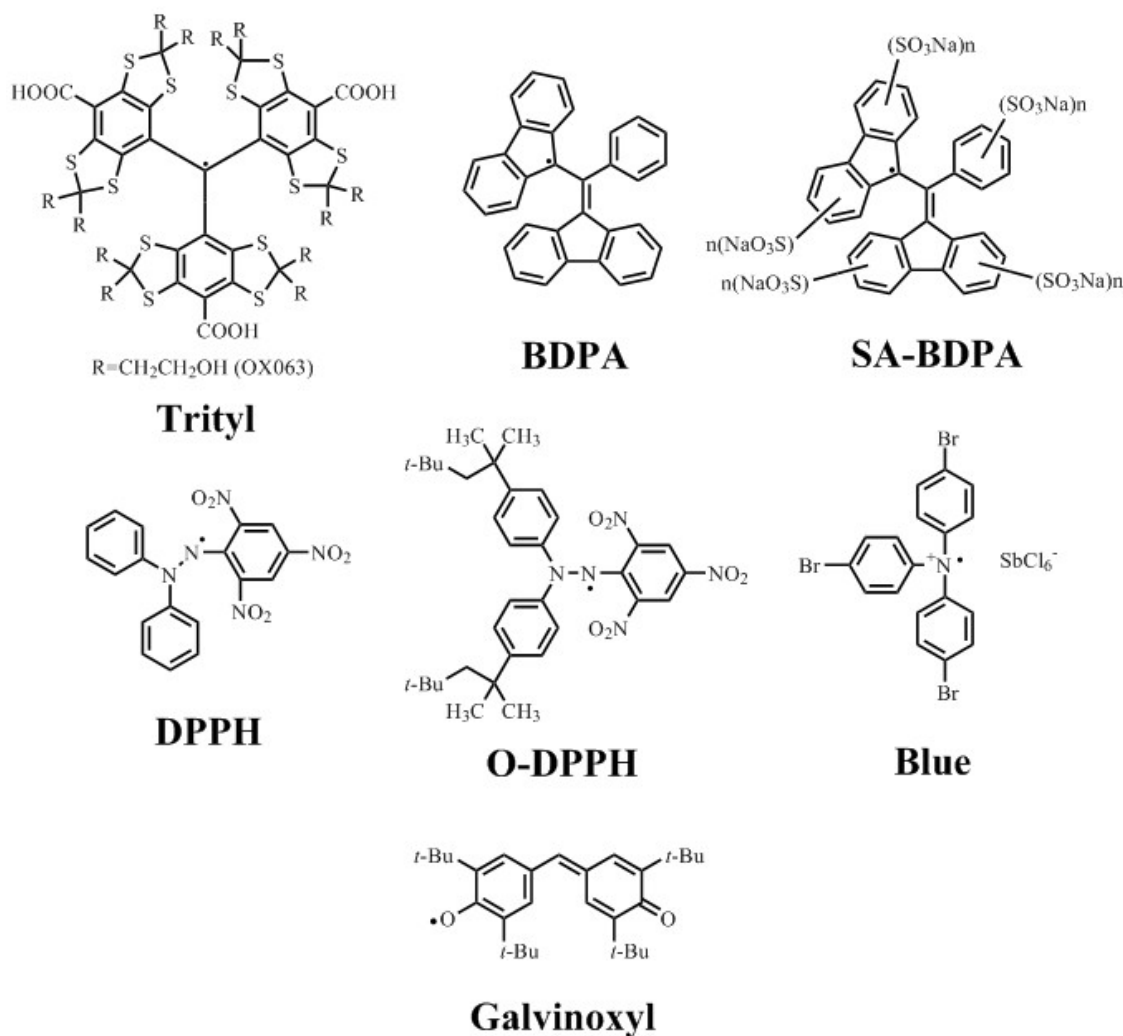


Figure 1.7. The structures of the free radicals studied in this work that are not in the nitroxide class.

MRI, while ^{13}C has very low natural abundance and a weak gyromagnetic ratio (see Table 1.1). The combination of these factors makes ^{13}C require long signal averaging times or bulk samples, both of which are impractical for real time *in-vitro* or *in vivo* studies.

With dissolution DNP, both the invisibility to NMR of ^{12}C and the low abundance and signal strength of ^{13}C become significant advantages. These ensure that there will be virtually no background when monitoring a hyperpolarized ^{13}C NMR or MRI signal. The situation is similar for nitrogen. ^{14}N is highly abundant, but has a smaller gyromagnetic ratio than

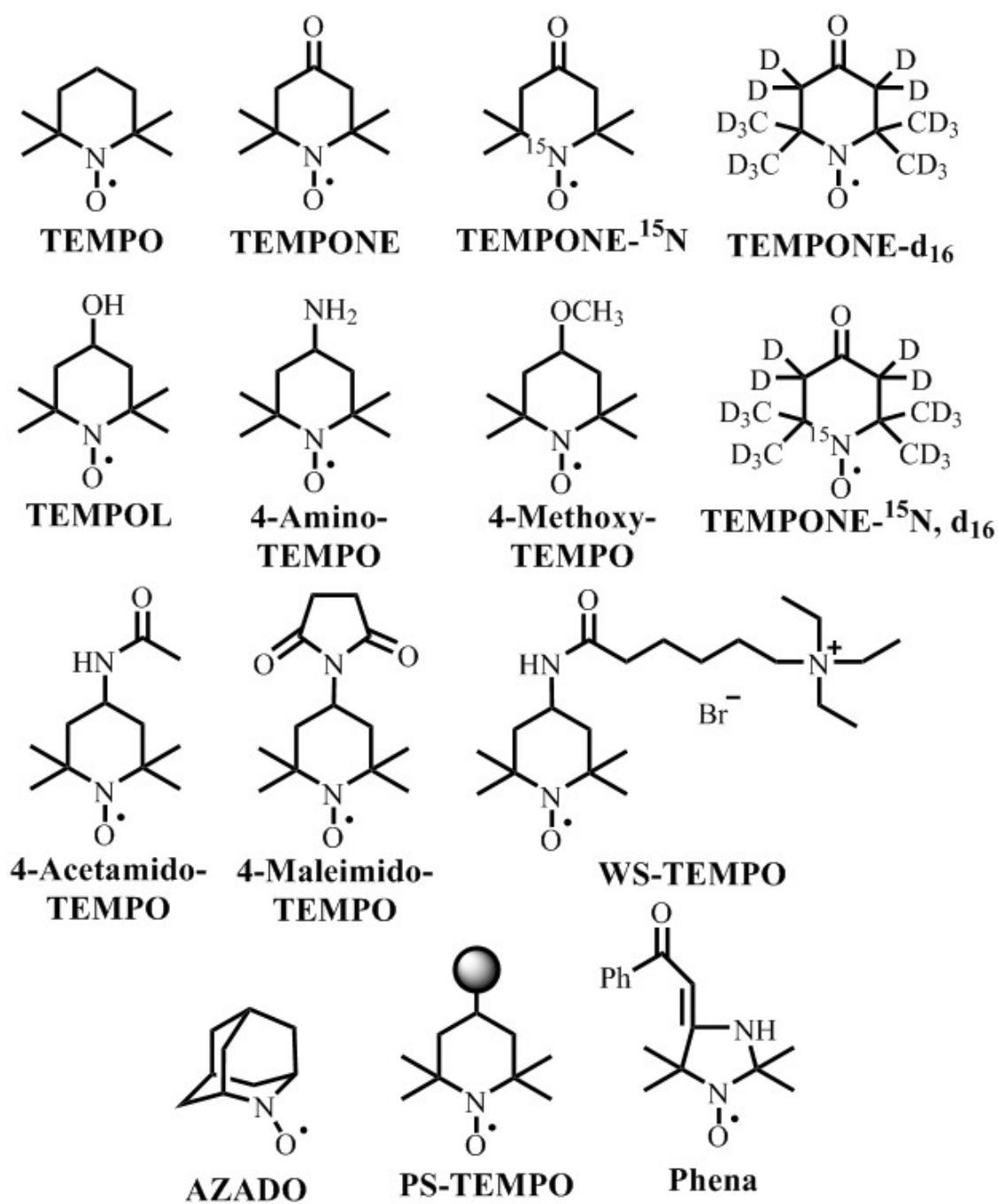


Figure 1.8. The structures of the nitroxide free radicals studied in this work.

does ^{13}C and with spin $I = 1$ has very fast relaxation time due to quadrupolar effects. ^{15}N , on the other hand, has very low natural abundance but is ideally suited for DNP NMR with spin $I = \frac{1}{2}$ and long T_1 .

These advantages of dissolution DNP have been utilized in a huge number of metabolic studies. The basic process is as follows: A ^{13}C -labeled biologically relevant substrate is hyperpolarized. The liquid collected after dissolution is then administered to suspended cells *in vitro*, perfused organs, or an organism[54, 55, 56]. The hyperpolarized ^{13}C NMR or MRI signal is then monitored until the substrate has relaxed back to thermal equilibrium. The ^{13}C chemical shift indicates what metabolites are being produced from the initial substrate.

A great deal of work has been put into finding substrates suitable for this type of metabolic imaging. Any substrate chosen must have a sufficiently long T_1 relaxation time, have an interesting metabolic pathway, have sufficient chemical shift separation from its metabolic products, and be metabolized within the imaging window afforded by the T_1 relaxation time.

For the most part, ^{13}C -labeled substrates have been used for metabolic imaging, though there has been research into ^{15}N [57, 58], ^6Li [59], and ^{89}Y [60, 61, 62]. For studies using ^{13}C , the substrate as well as the location of the nuclear spin must be chosen carefully to ensure adequate imaging time. Generally, substrates with a carboxyl location are ideal in order to isolate the ^{13}C from other nuclear spins, particularly ^1H . For carbons bonded to hydrogen atoms, ^2H can be substituted for ^1H to significantly improve the length of the ^{13}C liquid-state T_1 and thus imaging time window.

The study of metabolic pathways drives the choice of biomolecule. One of the characteristics of many diseases, particular cancers, is an overexpression of certain enzymes which alters the metabolic fate of biomolecules. Perhaps the most famous instance of this is the Warburg effect [63, 64]. In healthy cells, there is a comparatively low rate of glycolysis (Figure 1.9) producing pyruvate, most of which is then transferred to the mitochondria and the TCA cycle [65]. Most types of cancer cells, on the other hand, exhibit the Warburg

effect, which entails a very high rate of glycolysis and subsequent lactic acid fermentation with little pyruvate entering the TCA cycle.

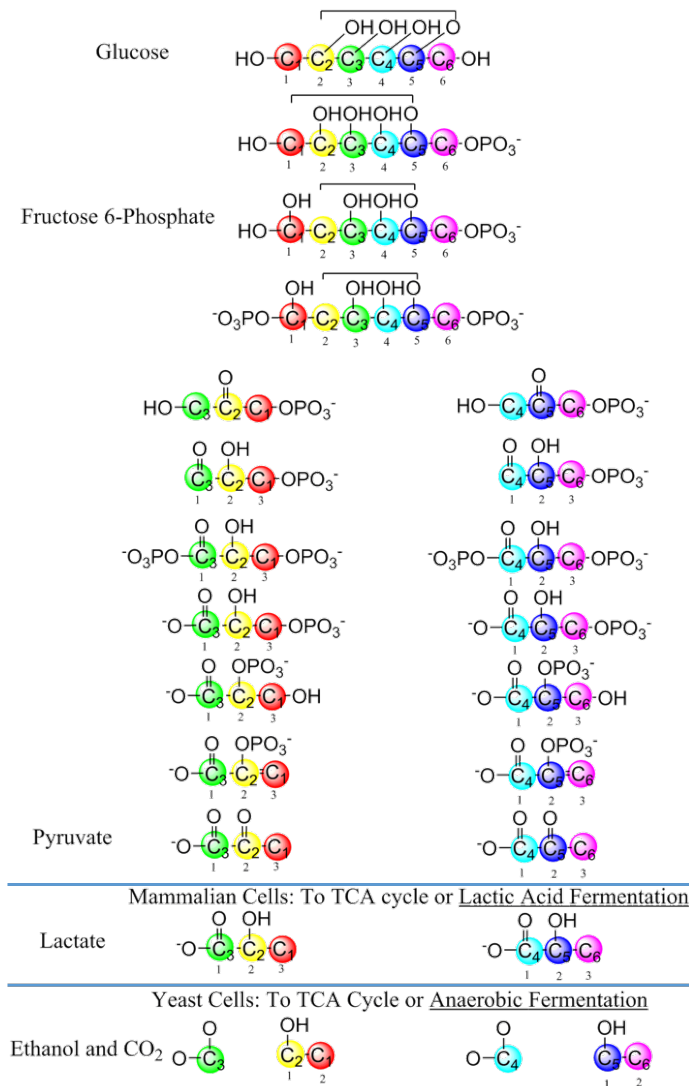


Figure 1.9. Tracking of the six carbons of glucose (and fructose) through glycolysis. After glycolysis, the products (pyruvate) could have a number of final destinations, but most common are introduction into the TCA cycle or lactic acid fermentation (mammalian cells) or anaerobic fermentation (yeast cells).

Being able to non-invasively monitor this particular effect in real time would be a huge boon both to cancer research and clinical diagnostics. Hyperpolarized ¹³C magnetic resonance, in principle, makes this possible. However, the ability, or lack thereof, of monitoring

the Warburg effect hinges on the choice of substrate. Given its prevalence as an energy-providing molecule and its location at the head of glycolysis, glucose is the obvious first candidate (See Figure 1.9). However, the glucose molecule has no carbon atoms that are isolated from hydrogen, which results in a short liquid-state ^{13}C T_1 , making metabolic studies difficult [66]. The most successful experiments using glucose have used $[1,2,3,4,5,6\text{-}^{13}\text{C}_6]$ glucose- d_7 (also known as $[\text{U-}^{13}\text{C}_6, \text{U-}^2\text{H}_7]$), that is, glucose with all of its carbon locations ^{13}C -labeled and all ^1H changed to ^2H . This particular isotopomer of glucose has a liquid state ^{13}C T_1 of up to 15 s, depending on the field and temperature conditions, which has allowed its use in studying bacteria [67], yeast [68], cancer cells *in vitro* [66, 69], and tumors *in vivo* [70].

Despite the moderate success of hyperpolarized glucose as a biomarker, its use is severely limited by its short relaxation time, and, as such, other substrates have also been tested for the study of glycolysis. Fructose too can enter glycolysis and has an isotopomer ($[2\text{-}^{13}\text{C}]$ fructose) with slightly longer T_1 than the labeled glucose described above [71]. However, by far the most successful biomolecule used in hypolarized ^{13}C metabolic studies is pyruvate [33, 54, 55, 56, 72, 73]. Both $[1\text{-}^{13}\text{C}]$ and $[2\text{-}^{13}\text{C}]$ pyruvate have long relaxation time (>30 s), are quickly metabolized, and are able to show metabolic paths of interest, most notably the Warburg effect and in some cases, the TCA cycle [34, 54, 74, 75, 76]. This has made pyruvate the standard by which all other DNP substrates are judged.

Hyperpolarized pyruvate has been used extensively in many different types of metabolic studies. By tracking the production of lactate, alanine, bicarbonate, and carbon dioxide, insights about cellular metabolism of pyruvate may be gathered (Figure 1.10). Initially, experiments were performed *in vitro*, such as *in vitro* cancer cell response to treatment [77] and studying metabolism of perfused rat organs [78, 79]. Now, *in vivo* studies are more popular and have been used to study animal metabolism in the kidneys [80, 81], heart [82, 83, 84], liver [85], brain [86, 87], prostate cancer tumors [88], carcinoma tumors [89,

90], and glioma tumors [91, 92]. Note that this list is by no means comprehensive. The crowning achievement of hyperpolarized ^{13}C pyruvate to date is a clinical study in which human patients with prostate cancer were studied, showing that hyperpolarized ^{13}C magnetic resonance can be used safely and effectively for human studies [73].

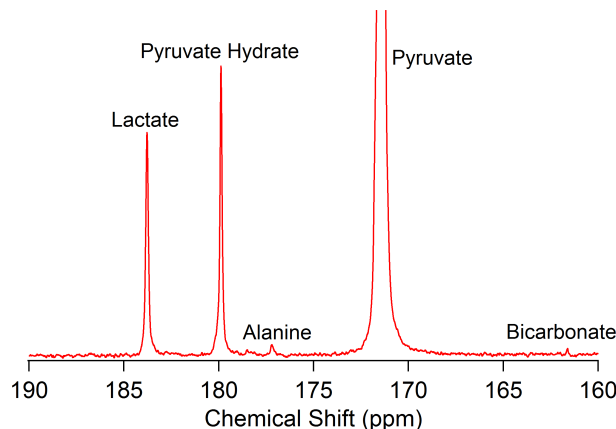


Figure 1.10. ^{13}C NMR spectra acquired from prostate cancer (PC-3) cells *in vitro* after the application of hyperpolarized $[1-^{13}\text{C}]$ pyruvate by the author. The spectra displayed is the sum of 60 spectra taken every 4 s with a 10° RF pulse.

The same metabolic processes as studied by pyruvate have also been imaged using hyperpolarized lactate [93, 94] and alanine [85, 95], though not with the success or ubiquity of pyruvate.

Of course, there are many other metabolic pathways of interest, and for these, different hyperpolarized ^{13}C substrates with long T_1 have needed to be found. In particular, substrates that have greater uptake into the TCA cycle (Figure 1.11) are highly desired because of the centrality of the TCA cycle to mammalian metabolism [65]. Unfortunately, many TCA intermediates cannot pass through the mitochondrial wall to where the cycle actually takes place [96]. Under certain conditions, pyruvate (specifically $[2-^{13}\text{C}]$ pyruvate) may be used to image this cycle [97]. However, given the degree to which pyruvate is turned into lactate in many cancer cells, other agents are desirable.

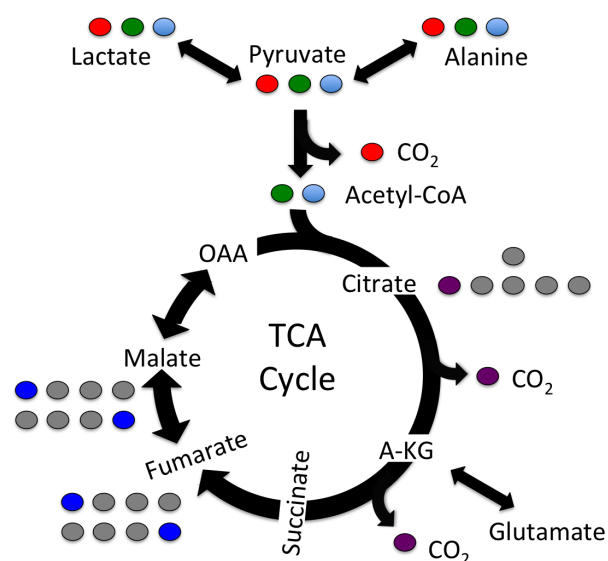


Figure 1.11. The TCA cycle, showing the substrates that are useful for hyperpolarized ^{13}C magnetic resonance. Circles denote carbon atoms and are color-coded to show the possible metabolic fate of ^{13}C atoms of a given location.

One such agent is $[1,4-^{13}\text{C}_2]$ fumarate [98, 99]. Fumarate is preferentially turned into malate by mitochondrial enzymes. However, fumarate is unable to pass through the mitochondrial membrane. For this reason, it is used primarily as a marker of cell death. At cell death, cell and mitochondrial membranes are compromised, releasing enzymes into the intercellular medium. Therefore, high malate production from hyperpolarized fumarate indicates high cell necrosis. This is of particular use for monitoring the effectiveness of cancer treatments [98].

Other substrates that have been used for studying TCA cycle metabolism are glutamine [34, 100, 101], glutamate [102], and α -ketoisocaproate [103].

While glycolysis and the TCA cycle are the main pathways currently being studied using hyperpolarized magnetic resonance, there are other pathways that may be explored. The Kennedy pathway, for instance, converts choline into lipids that are contributed to cell membranes (Figure 1.12) [104]. In cancer cells, malignant transformation of the cells leads causes upregulation of this pathway [105], so by tracking the uptake and metabolism of

choline, one can gather information concerning the location, size, and aggressiveness of a tumor.

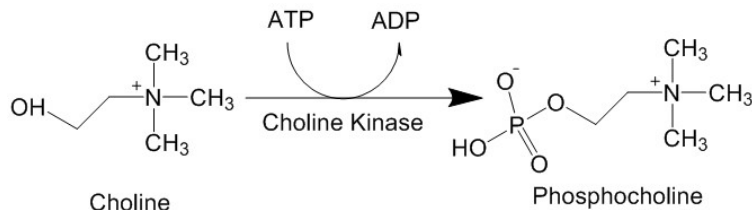


Figure 1.12. The first step of the Kennedy Pathway in which choline is converted to phosphocholine.

However, the hyperpolarization technique has not been exclusively used for tracking metabolic pathways. Another possible use is to monitor the pH of a microenvironment using ¹³C bicarbonate [106], ¹⁵N pyridine derivatives [58], or ⁸⁹Y complexes [60] based on their chemical shift.

Furthermore, there are some long-lived hyperpolarized substrates, particularly ¹⁵N-labeled molecules, that either are metabolized too slowly to be seen within the imaging window or have too little change in chemical shift with metabolism to directly track their metabolic fate. These may be useful despite their relatively inert behavior. For example, the perfusion of long-lived hyperpolarized compounds may be imaged. A long-lived ¹⁵N-labeled derivative of glutamine has been used for this purpose with some success [107].

With the recent advent of the clinical SPINLab polarizer (GE Healthcare), dissolution DNP is becoming more widely available to researchers studying metabolic processes. While there has been a great deal of progress in hyperpolarized ¹³C magnetic resonance since the invention of dissolution DNP, there is great promise of many advances in the near future.

CHAPTER 2

DYNAMIC NUCLEAR POLARIZATION MECHANISMS

2.1 DNP at Low Magnetic Fields

2.1.1 The Overhauser Effect

From the time that NMR and MRI were introduced, efforts have been made to increase the external magnetic field in order to increase the signal strength [108]. Recently, however, there have been renewed efforts at low magnetic fields ($< 1\text{T}$) due to the relative ease and reduced cost of creating low magnetic fields [109, 110, 111, 112]. Experiments done at low magnetic fields often are conducted at relatively high temperature as well, in which case the sample is in the liquid state. Of the DNP mechanisms, the Overhauser effect is the only one that works in the liquid state [37, 113]. For this reason, low field DNP is very commonly Overhauser DNP [113]. In addition, while the other DNP mechanisms are reduced in efficiency as the field is lowered, the Overhauser effect has greater efficiency at lower fields [37] making it very desirable in such experiments.

When Overhauser first proposed his theory, he posited, “If the electron spin resonance of the conduction electrons is saturated, the nuclei will be polarized to the same degree they would be if their gyromagnetic ratio were that of the electron spin” [24]. This hypothesis was verified by Carver and Slichter shortly after being proposed when they were able to enhance a ^7Li NMR signal by approximately one hundred-fold [25].

Though originally proposed for metals, the Overhauser effect was found also to be able to transfer polarization from free radicals to the nuclei of nonmetals [114, 115]. Therefore, it is possible to enhance the polarization of molecules in solution doped with paramagnetic ions. Today, the primary use of this DNP mechanism is to polarize ^1H and ^{13}C in solution rather than metals [37].

The most simple case of the Overhauser effect comes from a two-spin system in which both particles are spin-1/2. Considering higher spins leads to significant complications of the formalism without giving more insight into the Overhauser effect mechanism [115, 116]. One of the spins, denoted I , is coupled via the hyperfine interaction to another spin, denoted S . For the greatest enhancement, I should be a nuclear spin and S should be an electron spin, but the process works for two different types of nuclei as well, in which case it is known as the nuclear Overhauser effect (NOE) [116]. The Hamiltonian for this system is

$$\mathcal{H} = \gamma_S \hbar (\mathbf{S} \cdot \mathbf{H}_0) + \gamma_I \hbar (\mathbf{I} \cdot \mathbf{H}_0) + \gamma_S \gamma_I \hbar^2 \left[\frac{8\pi}{3} |\psi(0)|^2 (\mathbf{I} \cdot \mathbf{S}) + \frac{3(\mathbf{I} \cdot \mathbf{r})(\mathbf{S} \cdot \mathbf{r})}{r^5} - \frac{\mathbf{I} \cdot \mathbf{S}}{r^3} \right] \quad (2.1)$$

The first two terms are the Zeeman energies of the two spins, while the third term represents the interactions between the spins. This term is responsible for polarization-increasing relaxation processes and includes both scalar and dipolar components [117].

The four possible states of such a system are shown in Figure 2.1. When the system is irradiated at the electron Larmor frequency, the electron transitions are saturated leading to the populations of states 1 and 3 ($N_{++} = N_{+-}$) as well as the populations of states 2 and 4 ($N_{-+} = N_{--}$) to be equalized. From this point, the system will attempt to return to thermal equilibrium. However, if either of the transition probabilities W_0 and W_2 are non-zero, relaxation will occur via the “forbidden transitions” in which both I and S are flipped, causing a change in the population difference between the I spin states. These transitions are forbidden in the sense that they cannot be excited directly by an RF pulse and cannot cause directly detectable NMR signals, but they are permissible through relaxation [4]. The W_2 transition from $|++\rangle$ to $|--\rangle$ is known as the double quantum or flip-flip transition while the W_0 transition from $|+-\rangle$ to $|-+\rangle$ is known as the zero quantum or flip-flop transition. When the W_2 transition is dominant, there will be an increase in nuclear polarization, and when the W_0 transition is dominant, there will be a decrease [4].

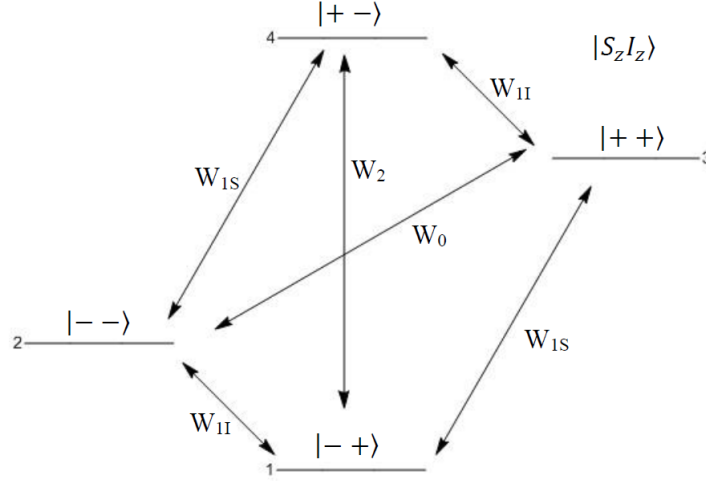


Figure 2.1. Possible states and transitions in a two-spin system

The amount of enhancement produced through these relaxation mechanisms is governed by the Solomon equation [12].

$$\frac{dI_z}{dt} = -\rho(I_z - I_0) - \sigma(S_z - S_0) \quad (2.2)$$

$$\rho = W_0 + 2W_{1I} + W_2 \quad (2.3)$$

$$\sigma = W_2 - W_0 \quad (2.4)$$

In the above equation, S_0 and I_0 are the thermal equilibrium values of S_z and I_z , respectively. The term σ is called the cross-relaxation rate constant and ρ is the dipolar longitudinal relaxation rate constant [4]. Using these values, three parameters useful in the description of the system may be defined: the coupling parameter ξ , the leakage factor f , and the saturation factor s [116].

$$\xi = \frac{\sigma}{\rho} = \frac{W_2 - W_0}{W_0 + 2W_{1I} + W_2} \quad (2.5)$$

$$f = \frac{\rho}{\rho + W_{1I}^0} \quad (2.6)$$

$$s = \frac{S_0 - S_z}{S_0} \quad (2.7)$$

The coupling parameter describes the efficiency of scalar or dipolar coupling between spins, and can range from 0.5 for pure dipolar coupling to -1 for pure scalar coupling [111] and is related to the relaxation rate of the nuclei [116]. The leakage factor is a measure of how the presence of the spin S affects the T_1 relaxation time of the nucleus being polarized and takes its maximum value when relaxation is completely controlled by the free radical [37]. In Equation 2.6, W_{1I}^0 is the longitudinal relaxation rate of the nucleus in the absence of the second spin and ranges between 0 and 1. The saturation factor is a measure of the degree to which the electron transitions are saturated, taking the value of 1 for complete saturation. Historically, there have been two common ways to theoretically describe the polarization achieved. The DNP enhancement [37] (which will be used in this work) is defined as

$$\epsilon = \frac{I_z}{I_0} = 1 - fs\xi \frac{|\gamma_e|}{\gamma_n}, \quad (2.8)$$

whereas the Dynamic Polarization or NOE factor [4, 116] is

$$P = \frac{I_z - I_0}{I_0} = fs\xi \frac{|\gamma_e|}{\gamma_n}. \quad (2.9)$$

The quantity $fs\xi$ takes the value of .5 for dipolar coupling and -1 for scalar coupling when the case of perfect saturation and a maximal leakage factor is considered, so if $|\gamma_e|/\gamma_n$ is known, the theoretical maximum of the enhancement is easily calculated. Clearly, then, weaker nuclear spins will experience a greater enhancement when using Overhauser DNP. For ^1H and ^{13}C the maxima may be found in Table 2.1.

Table 2.1. Maximum possible Overhauser enhancement for ^1H and ^{13}C

Coupling Type	^1H	^{13}C
Pure Scalar	658	2618
Pure Dipolar	-328	-1307

In practice, these maximum enhancements are not achievable, though they can be approached by optimizing certain factors. For free radicals commonly used in Overhauser

DNP, the leakage factor will typically be near to one [118]. However, the saturation factor and the coupling parameter are much more sensitive to experimental conditions. The value of the saturation factor depends both on the power of microwave irradiation used and the relaxation rate of electrons [37]. For a high level of saturation, high power must be used, but this can lead to sample heating, weakening the effect. Multiple EPR lines cannot be saturated simultaneously, so free radicals with more than a single EPR line can result in a lower saturation factor. The coupling parameter takes its maxima at low magnetic fields, and decreases as the magnetic field is increased [118]. It is for this reason that the Overhauser effect is best used at low magnetic fields and can be especially useful in ultra-low field NMR [111, 112, 119, 120]. Though it is most efficient at low field, it should be noted that because it is the only liquid state DNP mechanism, the Overhauser effect has also been used at high magnetic field strengths, yielding a small, but significant polarization enhancement [118, 121, 122, 123].

2.2 DNP at High Magnetic Fields

In liquid-state DNP, the polarization transfer from electrons to nuclei is accomplished through relaxation processes. In solid-state DNP, on the other hand, the transitions causing the polarization transfer are directly excited [26, 124, 125, 126]. This is possible because forbidden transitions become slightly allowed in solids where the dipole-dipole forces between nuclei and electrons do not average to zero [27]. In order for the saturation of forbidden transitions to be effective, the polarization of the electrons in a sample needs to approach 100% [22]. Thus, solid-state DNP generally takes place at low temperature and high magnetic field to ensure nearly complete electron polarization.

2.2.1 Solid Effect

The first DNP mechanism to be introduced after the Overhauser effect was found in an attempt to generalize the Overhauser effect to non-metallic solids and is known as the solid effect [115]. In the solid effect, an assembly of nuclear spins is embedded in a diamagnetic solid with paramagnetic impurities. In most cases, nuclear spins with $I = \frac{1}{2}$ are considered, while the impurities are electrons ($S = \frac{1}{2}$) that are dipolar coupled to nearby nuclear spins. At the conditions required (low temperature, high field), the primary relaxation method is a nuclear spin flip with no electron spin flip [6], so the method of irradiating the sample at the electron Larmor frequency as in the case of the Overhauser effect will not increase the nuclear polarization. However, because the spins are connected by a dipolar interaction, the forbidden flip-flop and flip-flip transitions (as defined above) may be driven by microwave irradiation if the frequency corresponds to the energy difference, $h(\nu_s \pm \nu_I)$, between the states [22, 124, 126]. By saturating this forbidden transition, an increase in polarization results. For the system to retain any polarization gain, the nuclear reaction rate must be much smaller than the electron relaxation rate, allowing for many reversals of electron spin for every reversal of a nuclear spin. Now, the temperature at which this occurs causes the electrons in all materials except metals to be essentially fixed in place, able only to polarize nearby nuclei. Though this at first seems to be an issue, spin diffusion carries the polarization to remote nuclei, allowing the entire sample to be polarized despite only a comparatively small number of nuclei directly being polarized [127].

The maximum polarization increase when using the solid effect occurs when irradiating the sample at a frequency equal to the sum of the nuclear and electron Larmor frequencies ($\nu_e + \nu_n$), while the maximum negative enhancement occurs at a frequency equal to the difference ($\nu_e - \nu_n$). This results in a well defined difference of $2\nu_n$ between the positive and negative polarization peaks (Figure 2.2).

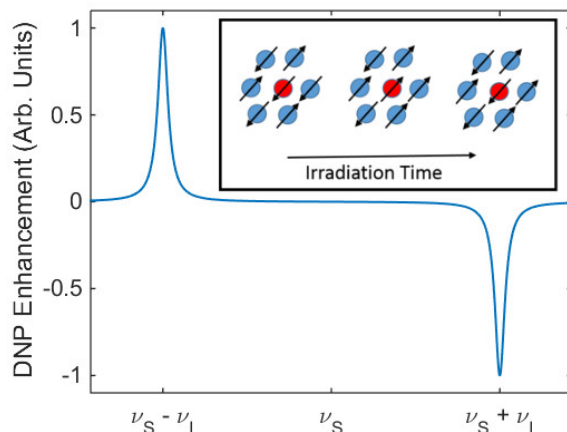


Figure 2.2. A visualization of the “well-resolved solid effect” showing the DNP enhancement as a function of irradiation frequency. The inset window is an illustration of the classic “flip-flip” view of the solid effect.

This polarization mechanism works only in the case where the concentration of the electron spins is low leading to negligible interaction between electrons [127]. In addition, the EPR line width of the free radical used should be much less than the nuclear Larmor frequency. Such a case is called by some the “well-resolved solid effect”. On the other hand, if the line-width is not much less than the nuclear Larmor frequency, the enhancement is decreased and is known as the “differential solid effect”. For this reason, high Larmor frequency nuclei such as ^1H doped with low concentrations of narrow-linewidth radicals such as Trityl OX063 and BDPA are used when the solid effect is the desired DNP mechanism [37, 127, 128].

2.2.2 Cross Effect

Shortly after the description of the solid effect, experiments were conducted in which the positive and negative polarization peaks were separated by less than what would be expected if the solid effect were taking place, possibly implying the discovery of a new DNP mechanism [129, 130]. This was found to be a distinct mechanism and termed the cross effect.

The cross effect is a three-spin process [128] which involves two dipolar coupled electrons interacting with a nucleus. Furthermore, the EPR frequencies of the two electrons must satisfy the condition

$$\nu_{S1} - \nu_{S2} = \nu_I. \quad (2.10)$$

In practice, this corresponds to a free radical with an EPR line that is inhomogeneously broadened so that the line width is larger than the nuclear Larmor frequency. In this case, there are at least two molecular orientations corresponding to different EPR frequencies which satisfy the above condition [128].

A general three spin system is shown in Figure 2.3(a). When the Cross Effect condition is met, a degeneracy is introduced in which the energies of states $| - + - \rangle$ and $| + - + \rangle$ (shown in 2.3(b) and (c)) are equal. Thus, the excitation energy for one of the electron's EPR transition is the same as the energy of the forbidden transition of the other electron and the nucleus [27]. Irradiation at the frequency corresponding to the larger electron frequency will lead to a negative polarization gain while irradiation at the smaller electron frequency will lead to positive polarization. Through this process, the DNP efficiency is greatly increased over the solid effect [131].

2.2.3 Thermal Mixing

Though effective under the right experimental conditions, the two and three-spin descriptions of the solid and cross effects have been found to be insufficient to describe experiments at common dissolution DNP conditions [27, 61]. In order to take into account all spin interactions, a many-particle theory is necessary. This is supplied by the statistical and thermodynamic picture of DNP known as Thermal Mixing [22, 132].

The Boltzmann law (Equation 1.6) gives the population of each of the energy states based on a parameter $\mathcal{T}$, which in typical cases is just the physical temperature. However, if the assumption is made that the system is in an internal equilibrium in a way that the

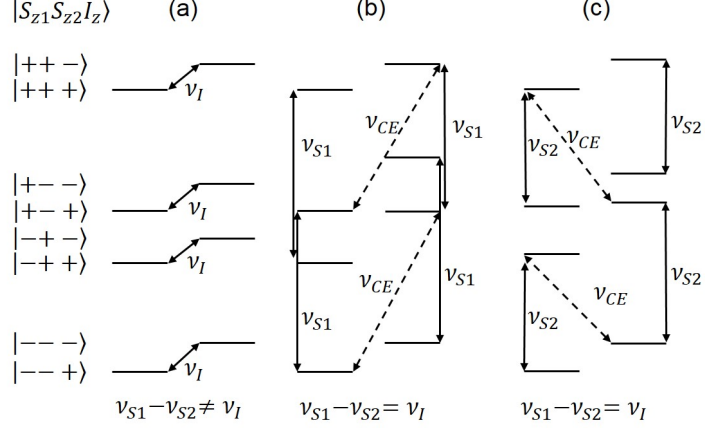


Figure 2.3. Available states and transitions for a system containing a nucleus and two electrons. The general system is shown in (a) while (b) and (c) depict the system when the electron resonances are separated by the the nuclear Larmor frequency. The transitions shown in (b) [(c)] correspond to irradiation at the first [second] electron's resonance, yielding negative [positive] enhancement

Boltzmann law is still valid, the parameter $\mathcal{T}$ describes the order present in the system and is referred to as the spin temperature [133]. In the thermal mixing mechanism, each particle interaction is considered as a thermodynamic system with an associated spin temperature. Specifically, there is the Nuclear Zeeman System (NZS), Electron Zeeman System (EZeS), and Electron Dipolar System (EDS) (Figure 2.4). When considering spin temperature, DNP consists of trying to reduce the $\mathcal{T}$ of the NZS below the temperature of the sample lattice.

If a sample is immersed in an external magnetic field B_0 , the orientation of a spin within the sample may be flipped by irradiation near the resonance frequency applied perpendicular to the z axis. From a reference frame that is rotating with the RF field, the effective magnetic field seen by the spin in the sample is

$$\vec{B}_{eff} = \left(B_0 - \frac{\omega}{\gamma} \right) \vec{e}_z + B_1 \vec{e}_x \quad (2.11)$$

The term in the z direction in this equation is the reduced external magnetic field, and the term in the x direction is the varying amplitude of the RF magnetic field [133]. ω and γ are

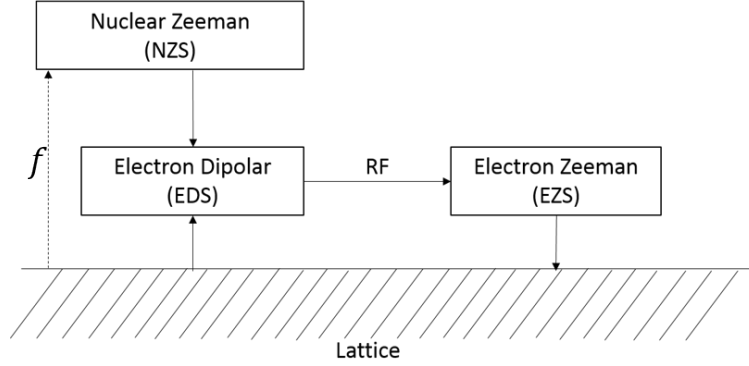


Figure 2.4. Schematic showing the heat transfer among the reservoirs in the Thermal Mixing process. By irradiating a sample with microwaves, the EDS is cooled through contact to the EVS, which allows for cooling of the NZS, and hence polarization enhancement.

the irradiation frequency and the gyromagnetic ratio of the particle, respectively. Through conservation of magnetization for a non-adiabatic change of magnetic field or conservation of entropy for an adiabatic change (if the local field at the spin's location is small compared to the RF field), the final spin temperature of the system is related to the initial spin temperature by [133]:

$$\mathcal{T}_f = \frac{B_f}{B_i} \mathcal{T}_i \quad (2.12)$$

which for the above effective field becomes

$$\mathcal{T}_f = \frac{B_{eff}}{B_0} \mathcal{T}_i. \quad (2.13)$$

By continuously irradiating the sample near the electron Larmor frequency, it is possible to greatly reduce the spin temperature of the EVS. In addition, the continuous irradiation causes thermal contact between the EVS and the EDS because it reduces the transition energies for the EVS to near those of the EDS, bringing them “on resonance” [133]. In addition, the EDS is in close thermal contact with the NZS because of their similar transition energies. Because the reservoirs are in thermal contact, it is important to define the heat capacity of each.

$$\mathcal{C}_{NZS} = N_I A_I \omega_I^2 \quad (2.14)$$

$$\mathcal{C}_{EVS} = N_S A_S \omega_S^2 \quad (2.15)$$

$$\mathcal{C}_{EDS} = N_S A_S D^2 \quad (2.16)$$

In the above equation, N is the number of spins, A is a constant depending on the spin quantum number of the spins, and $D = \gamma B_l$ is the frequency of the spins in the local magnetic field. For a system with no nuclear spins, it may be shown [133] that the inverse spin temperatures α and β of the EDS and EZS once they have reached equilibrium while being continuously irradiated is

$$\alpha = \beta = \frac{\mathcal{C}_{EVS}\alpha_L + (T_{EVS}/T_{EDS})\mathcal{C}_{EDS}\beta_L}{\mathcal{C}_{EVS} + (T_{EVS}/T_{EDS})\mathcal{C}_{EDS}} \simeq \frac{\mathcal{C}_{EVS}}{\mathcal{C}_{EVS} + (T_{EVS}/T_{EDS})\mathcal{C}_{EDS}}. \quad (2.17)$$

In the above equation, T_{EVS} and T_{EDS} are the longitudinal relaxation times for the EZS and EDS, and α_L and β_L are the inverse spin temperatures of the lattice in the rotating and laboratory frames, respectively. The approximation is only true when β_L is much less than α_L . In this situation, the EZS is being cooled by the RF irradiation while the EDS is being warmed by the lattice. When nuclear spins are included, the EZS is now cooling both the EDS and the NZS. The NZS may be approximated as being only coupled to the EDS since the nuclear relaxation time is very much longer than electron relaxation times. In reality, there is a reduction of the polarization gain from some coupling of the NZS to the lattice, described by the leakage factor f . In Equation 2.17, the heat capacity for the EDS must be replaced by the combined heat capacities of the NZS and EDS. However, because of the coupling of the EDS and NZS, the relaxation time of the EDS is affected as well, being slowed by the same factor as the heat capacity is increased from the case with no nuclei present [133]. Through this process, the spin temperature of the NZS can be greatly lowered, resulting in large polarization gains. The theoretical maximum polarization gain may be given by

$$P_{max} = \mathcal{B}_I \left(I \beta_L \omega_e \frac{\omega_I}{2D} \frac{1}{\sqrt{\eta(1+f)}} \right) \quad (2.18)$$

with $\mathcal{B}_J$ the Brillouin function, I the nuclear spin, $\beta_L = \frac{h}{kT_L}$ the inverse lattice temperature, ω_e and ω_n the electron and nuclear resonance frequencies, D the dipolar width of the free radical, η the ratio of electronic and dipolar relaxation times, and f the leakage factor [134].

This particular model provides good qualitative agreement with experiment, but fails to accurately predict experimental polarization values or the shape of DNP spectra. There are more recent, rigorous models of thermal mixing that are able to more accurately predict experimental results but have no readily solvable analytical solutions, so they are not ideal for qualitative discussion of results [135, 136, 137, 138]. One such model is discussed in more detail in Appendix B.

The thermal mixing mechanism is only dominant when the EPR linewidth is larger than or comparable to the nuclear Larmor frequency and the electron spin system is in a local equilibrium [37, 128, 133, 135]. This tends to be the case for low- γ nuclei at conditions common to dissolution DNP, so thermal mixing is often considered as the primary mechanism for such polarization experiments. Even very narrow-linewidth free radicals such as Trityl and BDPA have linewidths that are comparable to the Larmor frequency of commonly polarized nuclei such as ^2H , ^{13}C and ^{15}N . For the most part, the polarization experiments performed herein seem to be dominated by thermal mixing.

CHAPTER 3

INSTRUMENTATION

Over the course of the research conducted in this work, several significant instruments were constructed in order to take data and to more fully understand DNP and magnetic resonance. The most significant of these was a hyperpolarizer operating at 6.4 T with dissolution capability following the original design of Ardenkjær-Larsen [30] and other, more recently constructed systems [139, 140]. Additionally, for only solid state polarizations, a DNP probe compatible with a cryogen-free magnet system was constructed. At low magnetic fields, several systems were built, including a 0.35 T permanent magnet NMR system, and an Earth’s magnetic field NMR system based on the design of Michal [141].

3.1 6.4 T DNP Hyperpolarizer

Details describing the design and testing of this instrument are also published in [142]:

A. Kiswandhi, P. Niedbalski, C. Parish, S. Ferguson, D. Taylor, G. McDonald, and L. Lumata “Construction and ^{13}C Hyperpolarization Efficiency of a 180 GHz Dissolution Dynamic Nuclear Polarization System,” *Magn. Reson. Chem.*, vol. 55, pp. 828-836, Copyright © 2017 John Wiley & Sons, Ltd.

A. Kiswandhi was the primary builder of the instrument, with assistance from the author, C. Parish, and S. Ferguson. D. Taylor assisted with machining various parts for the probe, and G. McDonald energized the superconducting NMR magnet. The design for the system was the work of L. Lumata and A. Kiswandhi. Testing of the system was primarily done by A. Kiswandhi and the author.

3.1.1 Design and Construction

The design for the UTD hyperpolarizer may be found in Figure 3.1. It can loosely be considered to be composed of three systems. These will be referred to as the outer system, the DNP probe, and the dissolution system.

The outer system is composed of the magnet, cryostat, and vacuum pump. The main magnet is a Bruker 300 MHz superconducting magnet energized to 6.422 T (Proton Larmor frequency 273.45 MHz) to match the frequency range of the microwave source. In order to meet the temperature conditions necessary for high field DNP, a liquid helium cryostat is needed. For this polarizer, a Janis continuous-flow LHe NMR cryostat with a custom-built jacketed valve and needle valve control is used. This is inserted into the magnet bore with 3D-printed parts holding the cryostat such that the bottom of the cryostat is at the center of the magnetic field. Importantly, there is a narrow plastic collar, acrylic sheet, and rubber sheet on which the 3D printed parts rest. This serves keep weight off of the top O-ring and to dampen vibrations from the vacuum pump, both of which could otherwise damage or compromise the magnet. The cryostat is attached by a KF-50 vacuum hose to a Oerlikon Leybold WSU500/D65B rootsblower pump system.

At the connection of the vacuum hose and cryostat is a KF-50 cross (Figure 3.2). One end connects directly to the cryostat, while the opposite end goes through a KF-50 butterfly valve to the vacuum hose. One of the remaining arms of the cross has a KF-50 to Swagelok($\frac{1}{2}$ ") adaptor into which teflon tubing from a helium gas tank is led through a toggle valve. The final arm has a KF-50 to KF-25 adapter leading to an analogue pressure gauge and a KF-25 ball valve leading to a Pirani gauge. At the connection of the rootsblower and vacuum hose is a KF-50 ball valve.

The DNP probe is the heart of the hyperpolarizer. Its construction starts with a 3" ladish blank which allows for the probe to be inserted into the cryostat from the top while retaining a vacuum seal. In the center of the blank a .875" diameter hole is drilled with

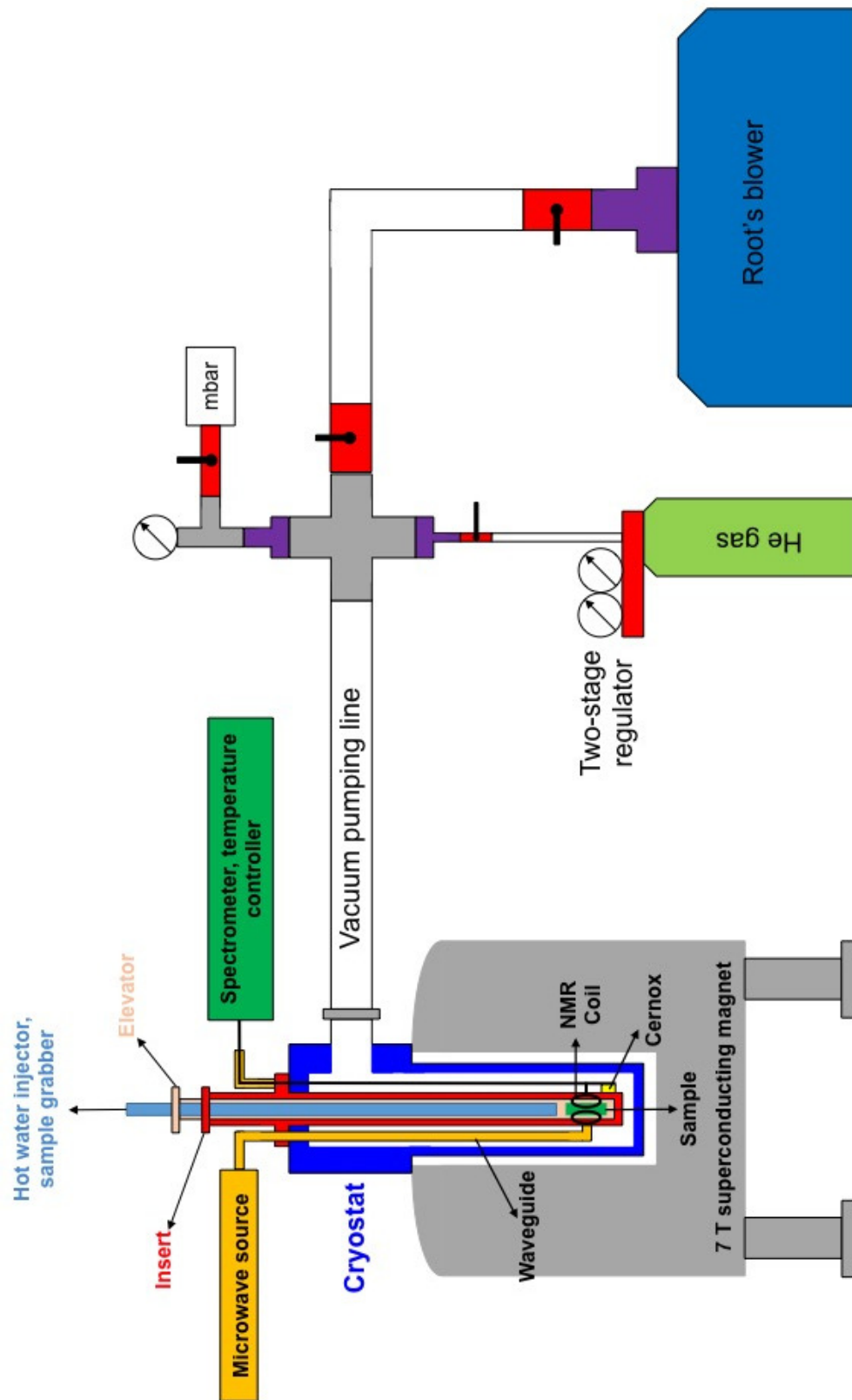


Figure 3.1. Design of the homebuilt UTD hyperpolarizer. Figure adapted with permission from A. Kiszandhi, P. Niedbalski, C. Parish, S. Ferguson, D. Taylor, G. McDonald, and L. Lumata "Construction and ^{13}C Hyperpolarization Efficiency of a 180 GHz Dissolution Dynamic Nuclear Polarization System," *Magn. Reson. Chem.*, vol. 55, pp. 828-836, Copyright © 2017 John Wiley & Sons, Ltd.

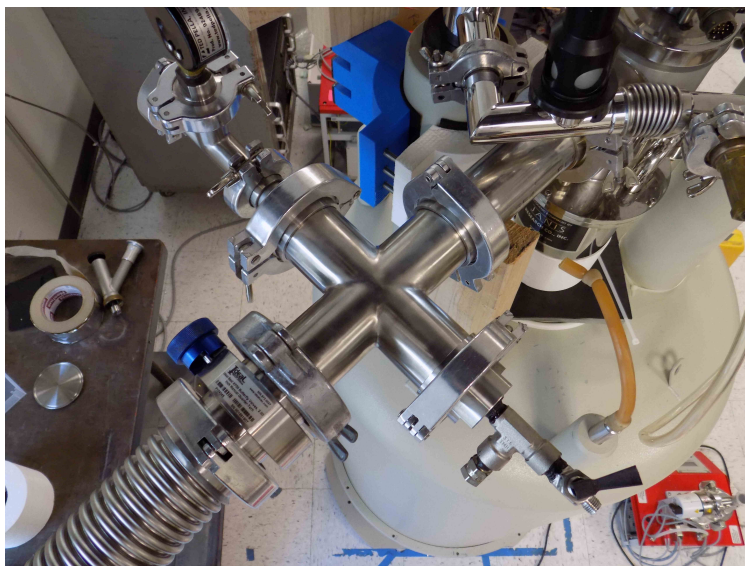


Figure 3.2. Top view of the connections of the KF-50 cross

four .25" diameter holes drilled around it. In the center hole, a .875" outer diameter (OD) stainless steel (SS) tube is welded so that 4.5" of tube will emerge from the cryostat while 10" penetrate into the cryostat. At the top end, the male part of a quick coupler is welded to the tube. At the bottom end, .875" OD G-10 tubing is affixed to the stainless steel, extending the probe to 44.75" from the bottom side of the ladish. Regularly spaced along the steel and G-10 are brass spacers that hold the probe in the center of the cryostat. In addition, small holes are drilled in the G-10 at regular intervals along its length to allow liquid helium to enter the probe. At the bottom, a teflon "chamber" 2" long with OD .875" is attached. This is the chamber in which the sample must be deposited to be irradiated and NMR signal monitored with the coil. It is made of teflon so that microwaves are able to penetrate into the sample. The teflon chamber rests at the bottom of the cryostat in the center of the magnetic field.

The four smaller holes drilled in the ladish are made to allow a liquid helium sensor, a waveguide, two NMR cables, and a temperature sensor to penetrate into the cryostat. The helium sensor is a 36" superconducting filament LHe sensor that connects to an American

Magnetics liquid helium level monitor. One NMR cable leads from a spectrometer through the ladish and to the bottom of the probe where it is connected to an NMR saddle coil wrapped around the teflon chamber. The other is a backup should the primary fail. The coil is made according to a design found in reference [143] and can be seen in Figure 3.3. The sample space is made so that the saddle coil can be replaced by a solenoid for solid-state DNP studies.

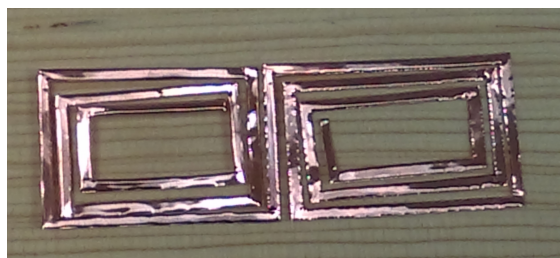


Figure 3.3. NMR saddle coil used in the UTDallas hyperpolarizer.

The temperature sensor is a Cernox RTD and it attaches to the outside of the microwave cavity to monitor the temperature at the location of the sample. This is attached to a Model 336 LakeShore temperature controller. The waveguide is a .25" OD thin-walled SS tube. At the bottom of the probe, the waveguide connects to a copper 90° bend into a copper chamber that is placed around the teflon chamber. Above the ladish, the waveguide is connected to a 90° bend waveguide which connects to a 180 GHz Virginia Diodes microwave source with WR5.1 rectangular E-plane output which can be swept from 178 to 182 GHz (Figure 3.4). To maintain the vacuum integrity of the ladish, each of the four small holes is sealed with Stycast 2850FT thermally conductive epoxy.

Into the top of the probe, a .75" OD SS tube may be inserted which can raise and lower the sample height within the cryostat. This "elevator system" has the female piece of a quick coupler around it and a male piece of a quick coupler at its top. The elevator has a length of $54\frac{1}{4}$ " and like the G-10, it has holes drilled into it that allow liquid helium access to the sample. At the bottom is a 2" long PEEK sample container in which the sample resides.



Figure 3.4. The 180 GHz sweepable microwave source used for polarizing samples.

This elevator may be closed at the top using a quick connect cap, or it may be open to allow access to a sample grabber or dissolution system. The completed system is shown in Figure 3.5.

The dissolution system is the component that allows for the rapid dissolution of the solid-state sample within the cryostat. It is assembled as shown in Figure 3.6. Not shown in the figure is the teflon tubing and stainless steel tube (dissolution stick) with a PEEK sample grabber at the end extending from the bottom of the dissolution system to the sample. Its construction allows for water to be inserted and held in the water container as it is being heated. Once it reaches the necessary temperature, the sample space may be pressurized with helium gas, forcing superheated water into the cryostat to dissolve the sample and remove it from the cryostat via a thin teflon tube. In order to do this without damaging the cryostat or causing excessive liquid helium boiloff, the elevator of the DNP probe is used to lift the sample above the liquid helium level, and the sample cup is tightly covered by the PEEK sample grabber.



Figure 3.5. The UTD Hyperpolarizer system. In the background, the roots blower vacuum pump may be seen, while the magnet and polarizer components are seen in the foreground. It should be noted that this picture was taken during a time when the magnet was temporarily de-energized, so the metal table and ladder near the magnet do not pose a danger to life or equipment. These objects are kept outside the 5 G limit during normal magnet operation.

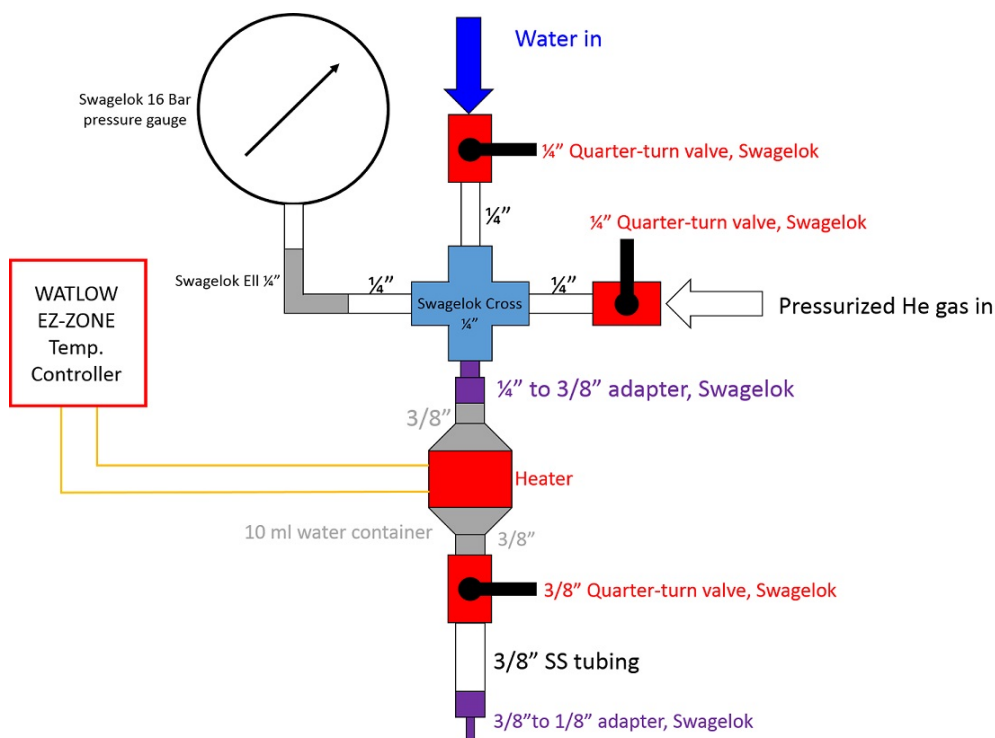


Figure 3.6. Schematic of the dissolution system used in the UTDallas Hyperpolarizer.

Once the sample has been dissolved, it is sent via teflon tubing to a 1 T SpinSolve NMR system (Magritek, New Zealand) to monitor the liquid-state NMR signal. This enables determining the signal enhancement, liquid-state T_1 , and ultimately will allow for the monitoring of cellular metabolism.

Detailed operating procedure for the polarizer is presented in Appendix C.

3.1.2 System Testing

A great deal of testing was required to ensure that the constructed hyperpolarizer worked efficiently. The first series of tests was to monitor the performance of the liquid helium (LHe) cryostat. This involved filling the cryostat with LHe and monitoring the LHe level and temperature over time with and without the rootsblower in operation. Without the rootsblower, the base temperature was about 2 K, with a LHe consumption of 0.26 L/hr. With the rootsblower, the base temperature was reduced to 1.4 K but the LHe consumption

increased to 0.69 L/hr (Figure 3.7). The cryostat holds just over 2 L, so a complete LHe fill allows about 3 hours of polarization time.

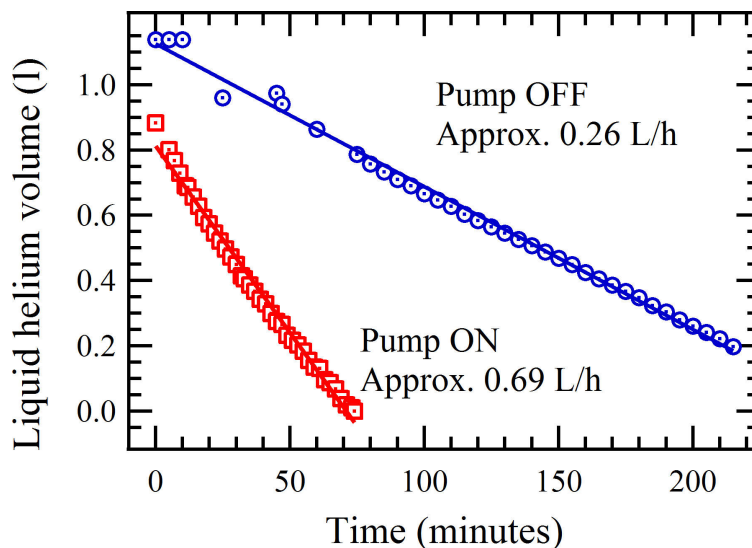


Figure 3.7. Liquid helium consumption of the Janis LHe cryostat both when the rootsblower pump is on and when it is off. Figure adapted with permission from A. Kiswandhi, P. Niedbalski, C. Parish, S. Ferguson, D. Taylor, G. McDonald, and L. Lumata “Construction and ^{13}C Hyperpolarization Efficiency of a 180 GHz Dissolution Dynamic Nuclear Polarization System,” *Magn. Reson. Chem.*, vol. 55, pp. 828-836, Copyright © 2017 John Wiley & Sons, Ltd.

Solid state polarization was then monitored to test the DNP efficiency of the polarizer. At first, this was tested using the saddle-coil configuration, but it was found that the saddle coil was not sensitive enough to measure the ^{13}C NMR signal early in the polarization time. Upon switching to a solenoid configuration, this problem was solved. Two different samples were prepared, both with 100 μL 3 M $[1-^{13}\text{C}]$ sodium acetate in 1:1 v/v glycerol:water. One of these was doped with 15 mM trityl OX063 and the other with TEMPONE. In turn, each sample was placed in a homemade sample cup and sealed within the NMR solenoid coil before placing the DNP probe into the cryostat. The cryostat was filled with LHe and polarization experiments performed. These included microwave frequency sweeps to determine the optimum irradiation frequencies and polarization buildup with irradiation at

the positive polarization peak. Additionally, a scan using the ^{13}C thermal polarization was taken by leaving the sample at the base temperature for several hours and taking a single scan. This allowed the quantification of the ^{13}C percent polarization able to be achieved by this hyperpolarizer in the solid state. Results are shown in Figure 3.8. The TEMPONE-doped sample reached $P(^{13}\text{C}) = 31\%$ and the trityl-doped sample reached $P(^{13}\text{C}) = 64\%$.

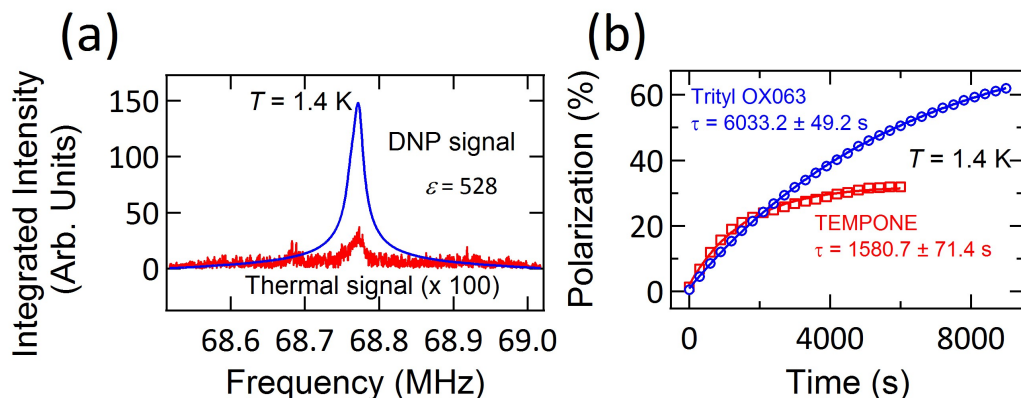


Figure 3.8. (a) Comparison of ^{13}C NMR signal of a sample of $[1-^{13}\text{C}]$ acetate doped with 15 mM trityl OX063 polarized at thermal equilibrium and after polarization for two hours. The center frequency is 68.768 MHz. (b) Polarization buildup curves for 3 M $[1-^{13}\text{C}]$ acetate doped with 15 mM trityl OX063 (blue circles) and 40 mM 4-oxo-TEMPO (red squares). Figure adapted with permission from A. Kiswandhi, P. Niedbalski, C. Parish, S. Ferguson, D. Taylor, G. McDonald, and L. Lumata “Construction and ^{13}C Hyperpolarization Efficiency of a 180 GHz Dissolution Dynamic Nuclear Polarization System,” *Magn. Reson. Chem.*, vol. 55, pp. 828-836, Copyright © 2017 John Wiley & Sons, Ltd.

Finally, dissolution DNP was tested. First, the solenoid was replaced by a saddle coil so that the sample cup could be removed from the top, a condition necessary for dissolution DNP. Next, samples of the same composition studied above were polarized in turn for several hours. At the end of the polarization time, the samples were rapidly dissolved and sent to a waiting SpinSolve 1 T NMR spectrometer where the polarization was monitored using 10° RF pulses every 4 s. Results are shown in Figure 3.9. Liquid-state ^{13}C NMR signal enhancements for both trityl and TEMPONE samples were over 100,000-fold.

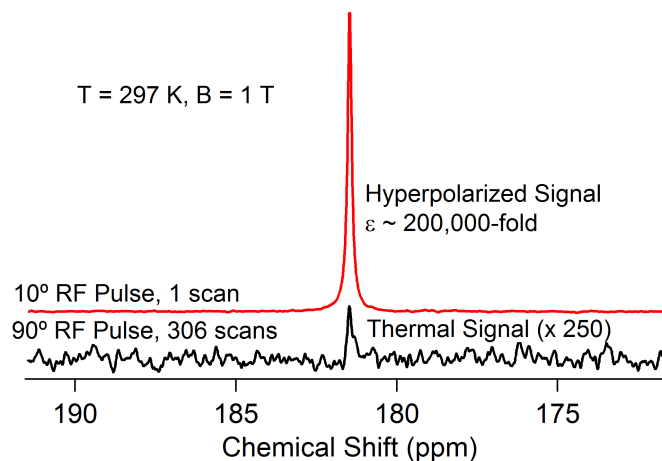


Figure 3.9. Hyperpolarized and thermal ^{13}C NMR signal taken using the SpinSolve NMR spectrometer following polarization by the home-built polarizer and dissolution. The initial sample was 3 M $[1-^{13}\text{C}]$ sodium acetate doped with 15 mM trityl OX063, which was reduced to about 37 mM $[1-^{13}\text{C}]$ sodium acetate post-dissolution. Figure adapted with permission from reference A. Kiswandhi, P. Niedbalski, C. Parish, S. Ferguson, D. Taylor, G. McDonald, and L. Lumata “Construction and ^{13}C Hyperpolarization Efficiency of a 180 GHz Dissolution Dynamic Nuclear Polarization System,” *Magn. Reson. Chem.*, vol. 55, pp. 828-836, Copyright © 2017 John Wiley & Sons, Ltd.

Overall, the system performs admirably and produces liquid-state hyperpolarized samples with more than adequate polarization to perform ^{13}C metabolic studies.

3.2 Cryogen-Free DNP System

Details surrounding the design, construction, and testing of the following system are also published in [144]:

A. Kiswandhi*, P. Niedbalski*, C. Parish, Q. Wang, and L. Lumata “Assembly and Performance of a 6.4 T Cryogen-Free Dynamic Nuclear Polarization System,” *Magn. Reson. Chem. Rapid Communication*, vol. 55, pp. 846-852, Copyright © 2017 John Wiley & Sons Ltd.

A. Kiswandhi, L. Lumata, and the author designed the system, and A. Kiswandhi did the machining and construction of the probe. The author performed the bulk of the testing of the system with some assistance from A. Kiswandhi, C. Parish, and Q. Wang.

3.2.1 Design and Construction

While a hyperpolarizer with dissolution capability is essential for applying DNP to biomedical research, a solid-state DNP system using a cryogen-free magnet is not without its advantages. Using such a system, free radicals may be studied in great detail without the financial burden associated with supplying a conventional DNP system with liquid helium. Furthermore, the magnet used is sweepable from 0 to 9 T, allowing for DNP at multiple field strengths, only limited by the frequency of the microwave sources available.

The magnet system used for this polarizer is a Model C-Mag Vari-9 Research System with a Model 90-275-010LCF Superconducting Magnet (Cryomagnetics Incorporated, Oak Ridge, TN) designed for electrical transport measurements. The author would like to offer thanks to Professor Mark Lee, who has made this magnet available. The system, as noted previously, is sweepable from 0 to 9 T and may reach sample-space temperatures below 2 K. In order to perform DNP, a probe with a sample chamber, NMR coil, and microwave waveguide must be constructed. A schematic for this is shown in Figure 3.10.

Because the DNP probe must maintain a vacuum seal with the sample space of the magnet system, and because, for this system, a good seal requires a specially machined G-10 sleeve in a non-standard size, the top flange for the probe was designed in AutoDesk Inventor (Autodesk Incorporated) and outsourced to Cryomagnetics Incorporated for production. In this design is a top flange that mates with a G-10 tube that forms the seal with the sample space. In the top flange, a 0.75" OD SS tube is welded through the center with about 5" extending above the sample space, and about 5" extending into the sample space. Around this tube are four 0.375" OD SS tubes evenly spaced around the center tube that extend 1" out of the sample space. The technical drawing for this is shown in Figure 3.11.

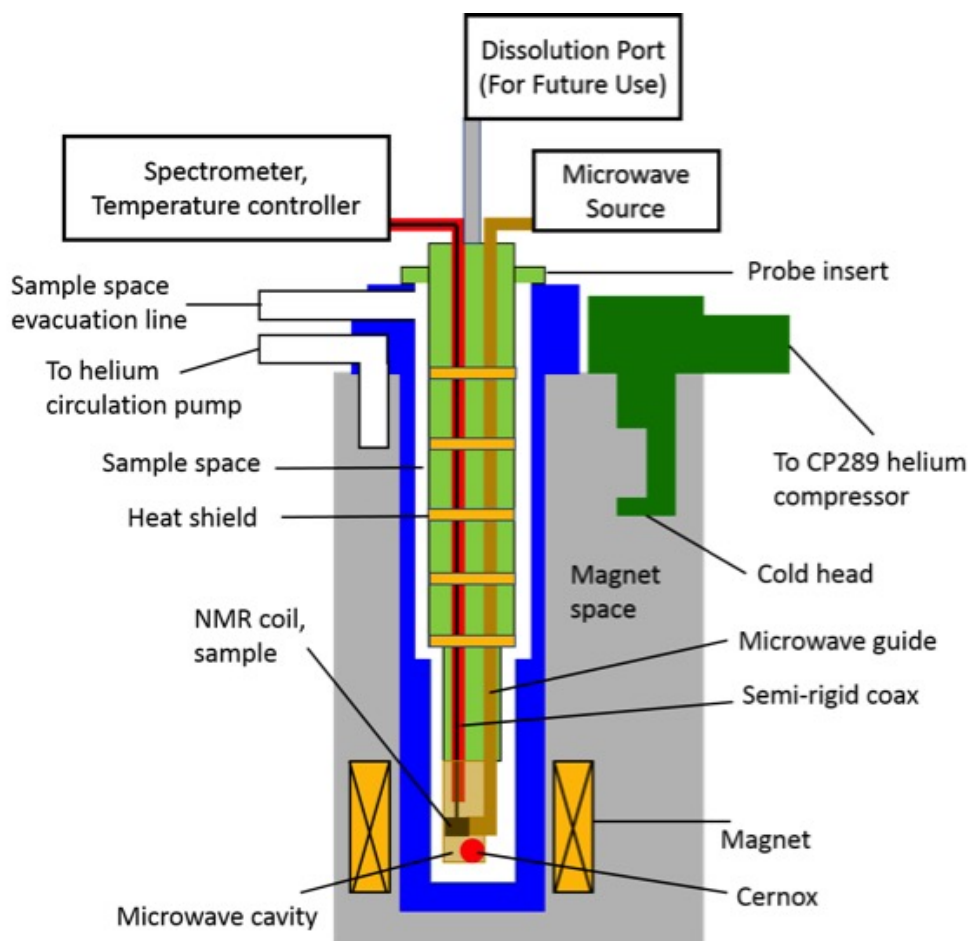


Figure 3.10. The schematic for the cryogen free DNP system that was constructed. Figure adapted with permission from A. Kiswandhi*, P. Niedbalski*, C. Parish, Q. Wang, and L. Lumata "Assembly and Performance of a 6.4 T Cryogen-Free Dynamic Nuclear Polarization System," *Magn. Reson. Chem. Rapid Communication*, vol. 55, pp. 846-852, Copyright © 2017 John Wiley & Sons Ltd.

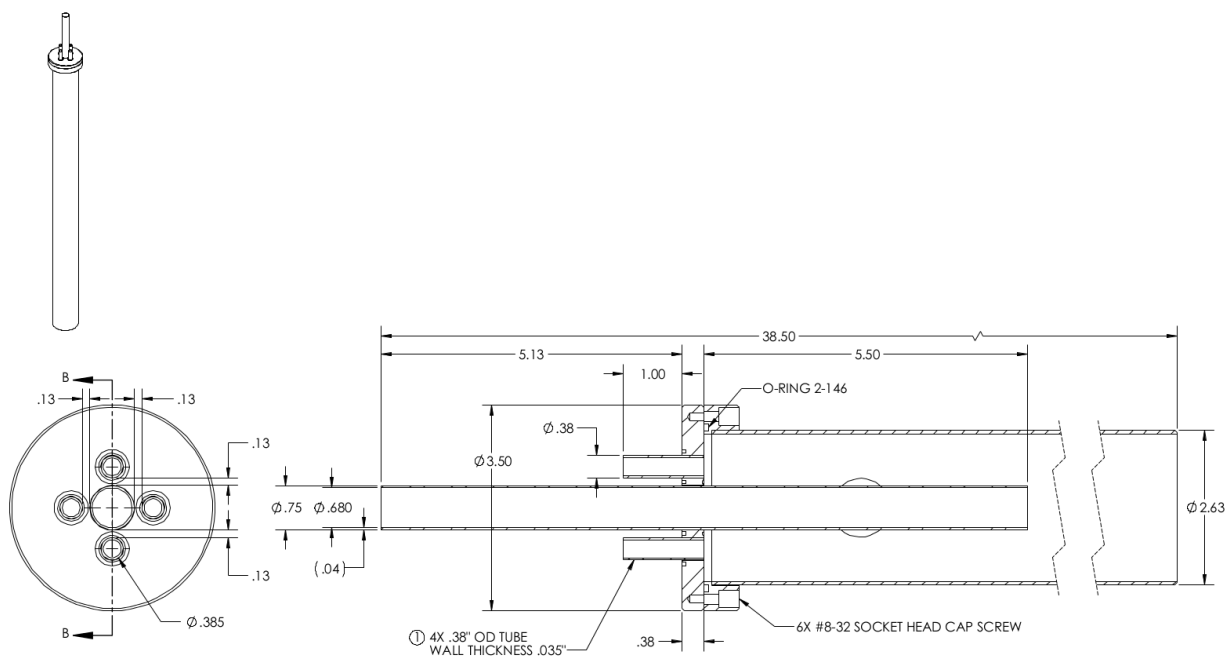


Figure 3.11. The technical drawing submitted to Cryomagnetics Incorporated for production

Originally, the desire was to create a probe that would accommodate four samples that could be rotated into a microwave chamber. This would eliminate the need to remove the probe from the magnet system to change samples. However, this necessitated the location of the microwave cavity to be offset from the center of the magnet. When a system was tested with a sample in this location, it was found that the magnetic field homogeneity was too poor to perform DNP. As a result, the design was changed so that the sample would be polarized at the center of the magnetic field. This design only allowed one sample to be in the probe at once but was necessary to ensure that DNP would take place efficiently. The ultimate probe design is shown in Figure 3.12.

From the bottom of the 0.75" OD SS tube through the top flange, a 33" long, 0.75" OD G-10 tube is extended. This leads down to the sample space at the center of the magnet. Starting 1" below the junction of SS and G-10, five brass heat shields are spaced at 6" intervals. Each heat shield has inner diameter 0.75" and outer diameter 2.5" with four 0.25"

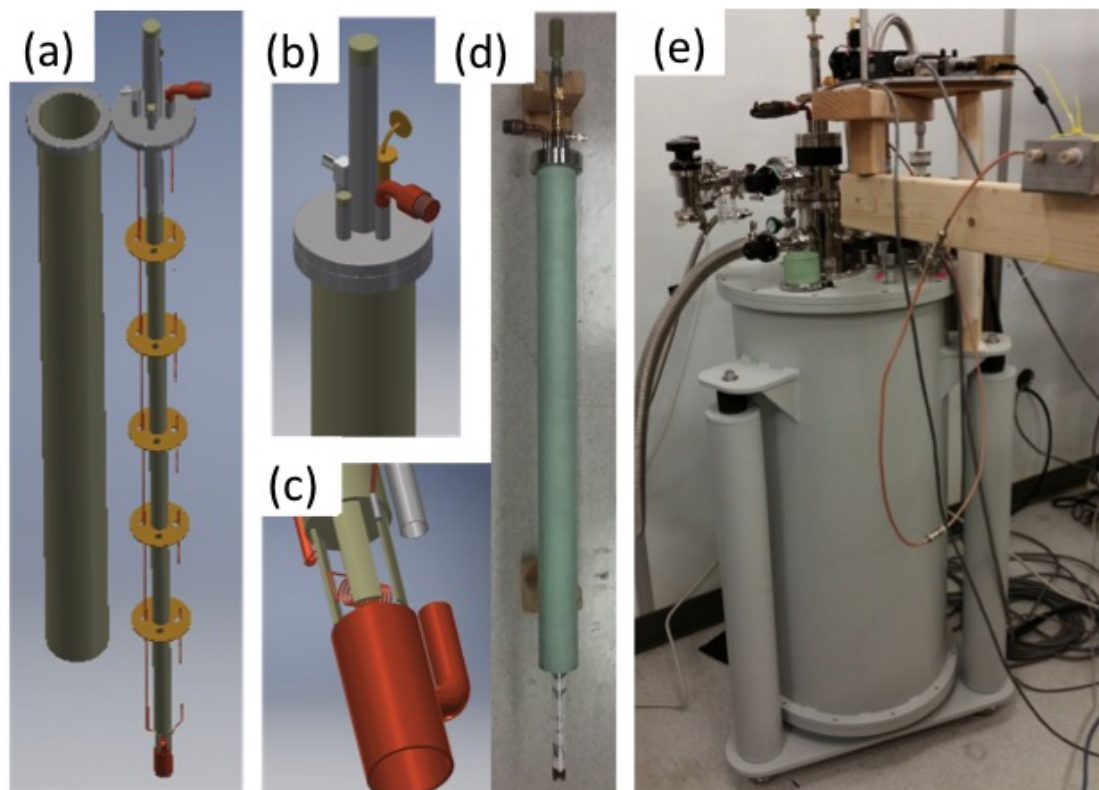


Figure 3.12. CAD drawings showing the various elements of the DNP probe that was built (a), (b), (c). Photographs of the completed probe (d) and the complete cryogen free DNP system (e). Figure adapted with permission from A. Kiswandhi*, P. Niedbalski*, C. Parish, Q. Wang, and L. Lumata “Assembly and Performance of a 6.4 T Cryogen-Free Dynamic Nuclear Polarization System,” *Magn. Reson. Chem. Rapid Communication*, vol. 55, pp. 846-852, Copyright © 2017 John Wiley & Sons Ltd.

holes drilled to line up with the 0.375” OD SS tubes at the top of the probe. At the bottom of the G-10 tube, there is a copper microwave chamber of the same type as made for the 6.4 T hyperpolarizer described in Section 3.1.

Three of the four 0.375”OD SS tubes are occupied, one with an NMR cable, one with a waveguide, and one with a temperature sensor. The NMR cable leads down to the center of the magnet where it is connected with a 15 turn solenoid that can accommodate a small section of a 5 mm NMR tube. The solenoid is a total of 10 mm long. The temperature sensor is a Cernox RTD and is connected to the outside of the microwave cavity. It is connected to the top of the probe using phosphor bronze twisted pairs. Phosphor bronze wires are used

because their thermal conductivity is significantly lower than that of copper which enables a lower base temperature for the sample space. The waveguide is a .25" OD thin-walled SS tube that connects at the bottom to a copper elbow leading into the microwave cavity. At the top, the waveguide is connected to a home-made brass tube, which connects to a 90° waveguide bend which connects to the microwave source. The microwave source is the same as used for the 6.4 T hyperpolarizer (Figure 3.4).

Once all of the pieces were in place, the four small holes in the flange are sealed using Stycast 2850FT thermally conductive epoxy. At the top of the center hole, a specially machined G-10 adapter is placed. This piece has a 0.325" hole passing through it and slides tightly into the 0.75" OD SS tube where it is sealed with epoxy. Inside this 0.325" hole is placed a 0.25" ID quick connect coupler (Kurt J. Lesker, Jefferson Hills, PA). This was originally intended to accommodate a SS tube leading to a rotating stage on which multiple samples could be mounted. As mentioned, the rotating stage is not feasible based on the homogeneity of the magnet, so a G-10 blank is placed in the quick coupler to seal the vacuum space.

Detailed operating procedure for this DNP system is provided in Appendix C.

3.2.2 System Testing

To test the system, 100 μ L samples containing 3 M $[1-^{13}\text{C}]$ sodium acetate and either 15 mM trityl or 40 mM TEMPONE were made in 1:1 v/v glycerol:water. Samples were placed in the probe solenoid and the probe situated within the magnet. The sample was brought down to the base temperature, which, if the setup was performed carefully, had extremely good stability. On multiple occasions, the sample temperature was observed to remain stable at 1.7 K for several weeks over the course of many experiments. When the magnet is energized, there is a magnetoresistive response within the Cernox temperature sensor, which brings the temperature reading up by about 0.1 K. Furthermore, the microwave irradiation causes

sample heating, increasing the temperature by another 0.15 K. Ultimately, polarization is performed at a temperature between 1.8 and 2.1 K, depending on the base temperature achieved (Figure 3.13).

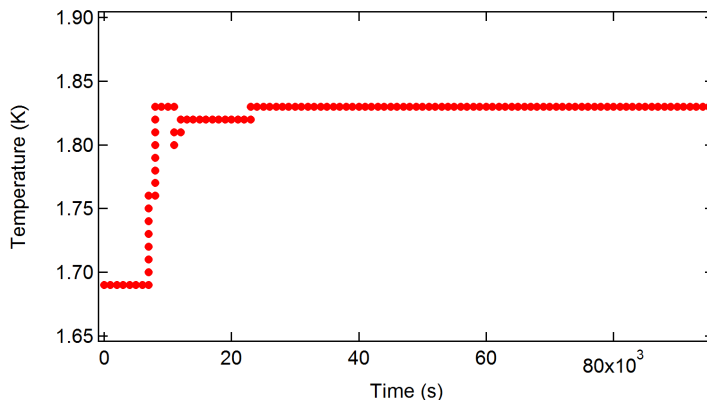


Figure 3.13. The sample temperature over time within the cryogen-free DNP sample space. This data was taken over 24 hours. Initially, magnet and microwave source are turned off. At 7,000 s, the ramp-up of the magnet to 6.423 T is begun, and halfway through the ramp-up at 8,000 s, the microwave is turned on. At 9,000 s the magnet reaches 6.423 T. At 11,000 s, the microwave is turned off and the magnet ramped further to 8.8 T, where it remains through the duration of displayed data

Polarization is performed at 6.423 T. Microwave frequency sweeps and polarization build-up curves are measured for both samples listed. DNP spectra showed that, unlike DNP in an NMR magnet, the polarization does not go to zero between the positive and negative polarization peaks (Figure 3.14). This is a result of the poor homogeneity of the magnet which is stated in the system’s documentation to have a homogeneity of 0.1% over a 1 cm³ volume at the center of the magnet. Because of this inhomogeneity, there are nuclear and electron spins in small regions of the magnetic field good for polarization even when the main field would preclude polarization at a certain irradiation frequency.

Polarization was built-up at the positive polarization peak until the polarization was nearly saturated. This took a long time, about 2.5 hrs for TEMPONE-doped samples and more than 6 hrs for trityl doped samples. For the trityl-doped sample, the magnetic field

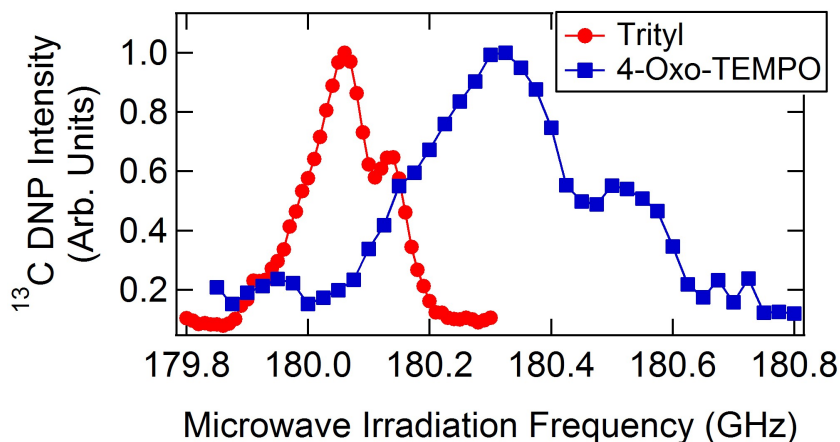


Figure 3.14. Microwave irradiation frequency sweeps for samples of 3 M $[1\text{-}^{13}\text{C}]$ sodium acetate doped with either 15 mM trityl OX063 or 40 mM 4-oxo-TEMPO. Figure adapted with permission from A. Kiswandhi*, P. Niedbalski*, C. Parish, Q. Wang, and L. Lumata “Assembly and Performance of a 6.4 T Cryogen-Free Dynamic Nuclear Polarization System,” *Magn. Reson. Chem. Rapid Communication*, vol. 55, pp. 846-852, Copyright © 2017 John Wiley & Sons Ltd.

was left on for 7 hrs, monitoring the ^{13}C NMR signal with small tip angle pulses every 30 min. By fitting an exponential decay to this data, the ^{13}C T_1 at 2 K and 6.4 T was estimated to be 24000 s. The trityl doped sample was also used to measure a thermal equilibrium signal by leaving the sample at 6.423 T and 2 K for 7 hrs. T_1 was used to extrapolate the acquired data to the theoretical maximum thermal signal. This was used to estimate the percent polarization achieved with DNP. It was calculated that $P(^{13}\text{C}) = 18\%$ for TEMPONE-doped samples and $P(^{13}\text{C}) = 58\%$ for trityl-doped samples (Figure 3.15).

The polarization measured is in relatively good agreement with the 6.4 T hyperpolarizer, which reached slightly higher percent polarizations. This is as expected since the hyperpolarizer operates at a lower base temperature, which should increase the polarization. However, the cryogen free system has greater stability, which seems to benefit the polarization and bring it close to that achieved by the hyperpolarizer despite the temperature difference.

This system offers several advantages over the hyperpolarizer. Most notably, it is essentially free to operate, only requiring a small amount helium gas once a month and needing no

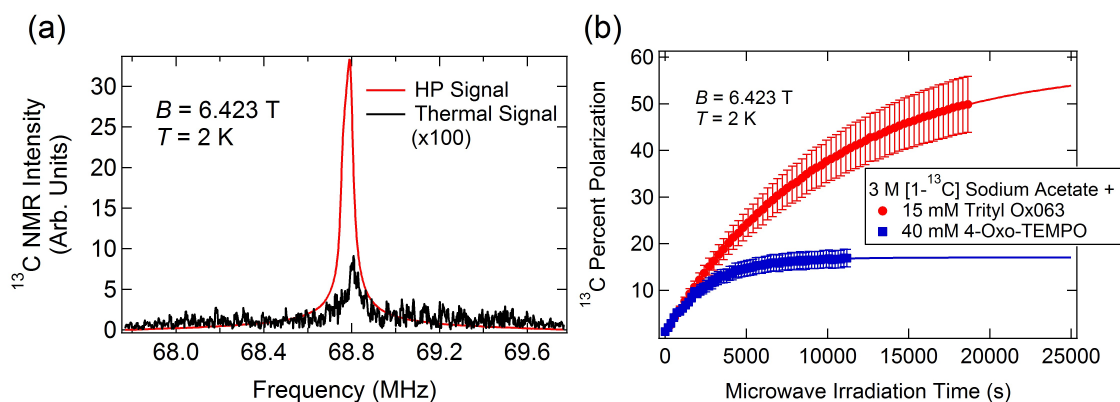


Figure 3.15. (a) Representative hyperpolarized and thermal ^{13}C NMR spectra in the cryogen free DNP system operating at 6.423 T. (b) Polarization buildup curves for samples of 3 M $[1-^{13}\text{C}]$ sodium acetate doped with either 15 mM trityl OX063 or 40 mM 4-oxo-TEMPO. Figure adapted with permission from A. Kiswandhi*, P. Niedbalski*, C. Parish, Q. Wang, and L. Lumata “Assembly and Performance of a 6.4 T Cryogen-Free Dynamic Nuclear Polarization System,” *Magn. Reson. Chem. Rapid Communication*, vol. 55, pp. 846-852, Copyright © 2017 John Wiley & Sons Ltd.

liquid helium. Furthermore, the magnetic field is sweepable, which allows study of ^{13}C NMR behavior at other field strengths, though polarization is limited to 6.4 T by the microwave source. Finally, this system has great stability, which allows for long polarization time unlike the hyperpolarizer which is limited by the amount of time it takes for the liquid helium to boil off (about 3 hrs).

The one area where this system does not improve upon the standard hyperpolarizer is dissolution. Because the sample space and the magnet space are cooled by the same cryocooler, the temperature of the two are closely linked. A rapid increase in the sample space temperature brought on by dissolution could lead to heating of the magnet space and magnet quenching. It may be possible to overcome this difficulty, but as of now, this has not been explored.

3.3 Construction of a 0.35 T NMR system

While the two instruments described above have become the workhorse instruments of the lab, they require a great deal of preparation, time, and, in the case of the conventional polarizer, expense to operate. In order to have a simpler, more easily used system, a small permanent magnet NMR system was constructed. The magnetic field used in this system is provided by two 3" diameter neodymium disc permanent magnets, one $\frac{1}{4}$ " thick and one $\frac{1}{8}$ " thick. While there are a number of methods that were employed to separate these magnets, the method that proved the most effective was attaching each magnet to a steel plate and holding the plates apart using $\frac{1}{4}$ " zinc bolts (Figure 3.16). Other methods attempted included 3D printing a plastic platform, and constructing a spinning rig. The 3D printed platform suffered from weak field and lack of homogeneity. To reach the desired field of 0.35 T, two 3" diameter and $\frac{1}{2}$ " thick magnets were required. While this setup gave the correct field, the lack of field homogeneity led to extreme line-broadening of the NMR signal. The spinning rig used the same two magnets as the 3D-printed platform, but because of the materials out of which it was made, it required a great deal of torque to spin making it impractical for use.

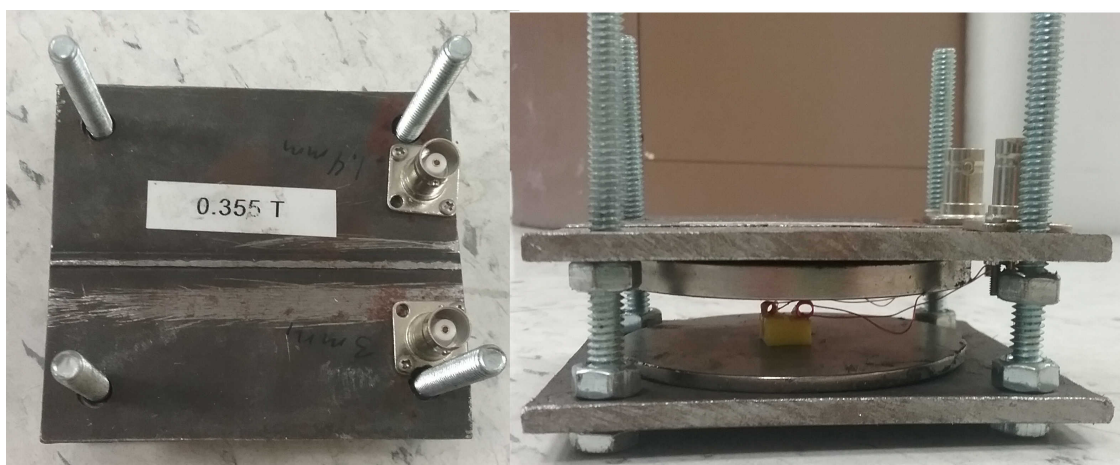


Figure 3.16. Photographs of the top (left) and side (right) views of the permanent magnet NMR system constructed

The primary benefit of the steel plate setup is that the magnetic flux is able to be channeled from the magnets' opposite poles, leading to automatic shielding of the magnets, greater field strength, and greater field homogeneity. In fact, holding $\frac{1}{2}$ " thick magnets at the same distance apart as in the plastic platform led to a magnetic field strength of 0.6 T, almost twice that of the plastic platform setup. Two other sizes of magnets were tested, $\frac{1}{4}$ " and $\frac{1}{8}$ " thick, which led to field strengths of 0.46 T and 0.26 T respectively when the magnets were held the desired 0.5" apart. Ultimately, it was found that one $\frac{1}{4}$ " thick and one $\frac{1}{8}$ " thick magnet resulted in a center field of almost exactly 0.35 T.

The magnets are held 0.5" apart in order to accommodate a WR-90 waveguide and two NMR solenoids in the sample area. One coil was wrapped to accommodate 1.4 mm thin-walled test tubes while the other accommodates 3 mm NMR tubes. The two coils are connected to two different coaxial BNC cable connections mounted on the external steel frame so that the platform is grounded to the spectrometer. This greatly improves the signal-to-noise ratio of NMR scans taken using this system.

A simple tuning and matching system was constructed by mounting two glass trimmer capacitors in an aluminum box in the most basic NMR circuit configuration (Figure 3.17). The box also had two BNC connectors connected so that a cable could be attached to one of the coils between the magnets and a cable attached to a spectrometer. The two coils have similar inductance, so the same capacitor box can be used for both coils with only small adjustments necessary in order to tune and match the circuit.

With the small sample volume and the weak magnetic field, it is difficult at first to find a proton NMR signal from a water sample. Additionally, the inhomogeneity of the magnetic field given off by the permanent magnets leads to very short T_2^* which gives a wide spectral peak, further increasing the difficulty of finding a signal. Using a gaussmeter (Lakeshore), the field at the location of each coil can be estimated and the NMR circuit tuned to the corresponding proton Larmor frequency. Then, by signal averaging a large number of scans

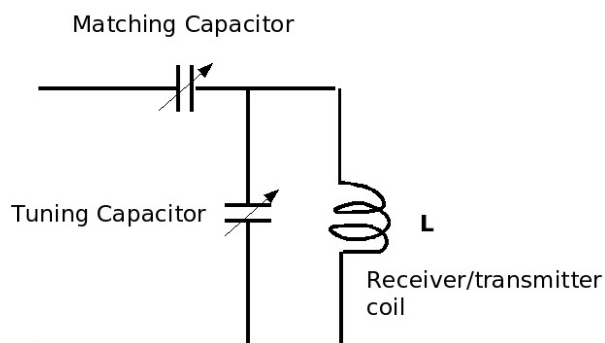


Figure 3.17. The most basic NMR circuit made with two variable capacitors and a coil. One capacitor is used to tune the circuit resonance while the other is used for narrowing the range of frequencies over which the circuit is resonant, also known as matching.

(>100), an initial signal may be found. Once the signal is found, the NMR circuit is then fine-tuned so that its resonance is precisely the same as the frequency at which the signal is observed. After this has been done, a reasonably strong signal may be found with only one scan (Figure 3.18).

Though the steel platform improves the homogeneity of the magnetic field between the two magnets, the NMR line is still broadened to many kilohertz, making high resolution NMR impossible without significant efforts to shim the magnet. However, relaxation experiments may be performed even in fields with low homogeneity. Additionally, it may be possible to perform DNP in this system using the Overhauser Effect.

In order to conduct DNP via the Overhauser effect, a microwave source operating near 10 GHz is needed. For this, an Avantek YIG (Yttrium Iron Garnet) tuned oscillator that is sweepable between 8.0 and 13.4 GHz is used. In order to have the high power necessary for large Overhauser enhancement, the oscillator is connected to a CTT broadband amplifier by a HS-MF086 coaxial cable. The amplifier then connects to a WR90 waveguide adapter, which connects to a 9 inch WR90 waveguide leading to the magnet setup and directs irradiation at the sample.

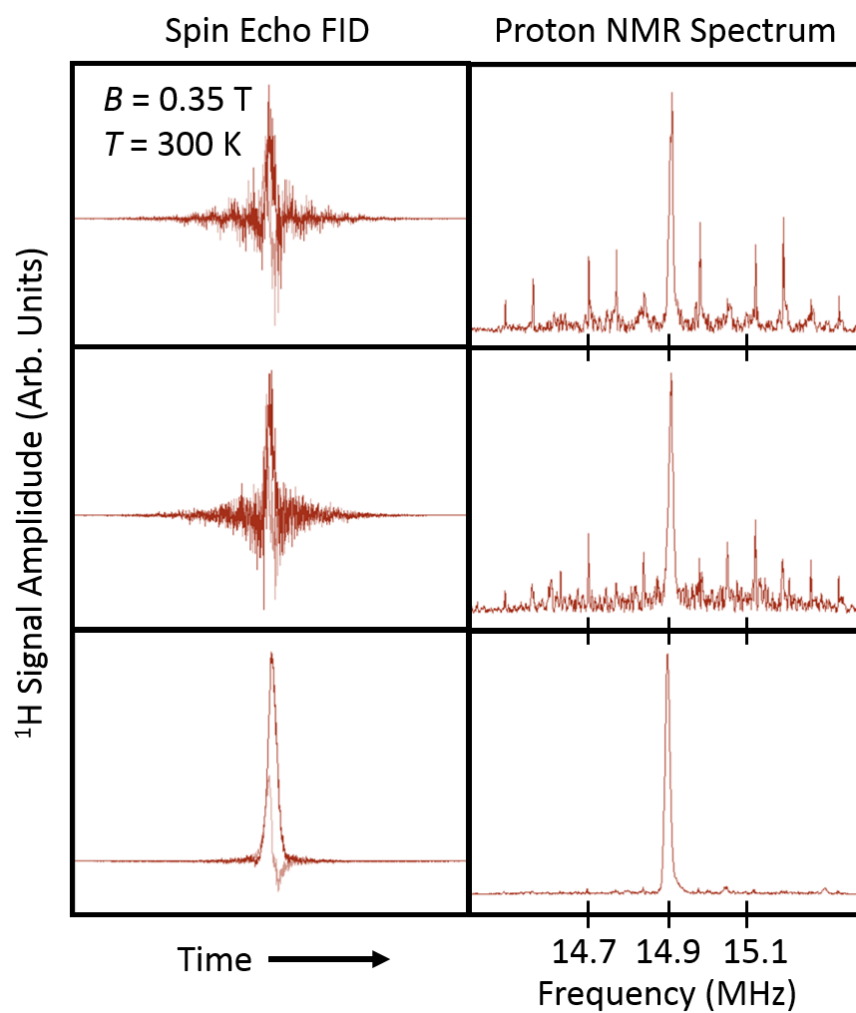


Figure 3.18. Spin echo FID (left) and NMR spectrum (right) for a water sample in the 0.35 T permanent magnet NMR system using 1 (top), 10 (middle), and 1000 (bottom) scans

In order to operate the YIG oscillator, several different power sources were needed (Figure 3.19). One power source supplied the 15 V necessary for the oscillator to operate. A second source turned on the heater, which keeps the YIG resonator at a consistent temperature. A third source supplied a current to the frequency tuning coils, which modulate the frequency of the wave produced by creating a magnetic field at the site of the YIG resonator. In order to know the frequency of waves produced at a given current, a spectrum analyzer was used to calibrate the YIG oscillator (Figure 3.20). Finally, because high power is needed for Overhauser DNP, a 15 V DC source is required to power the amplifier.

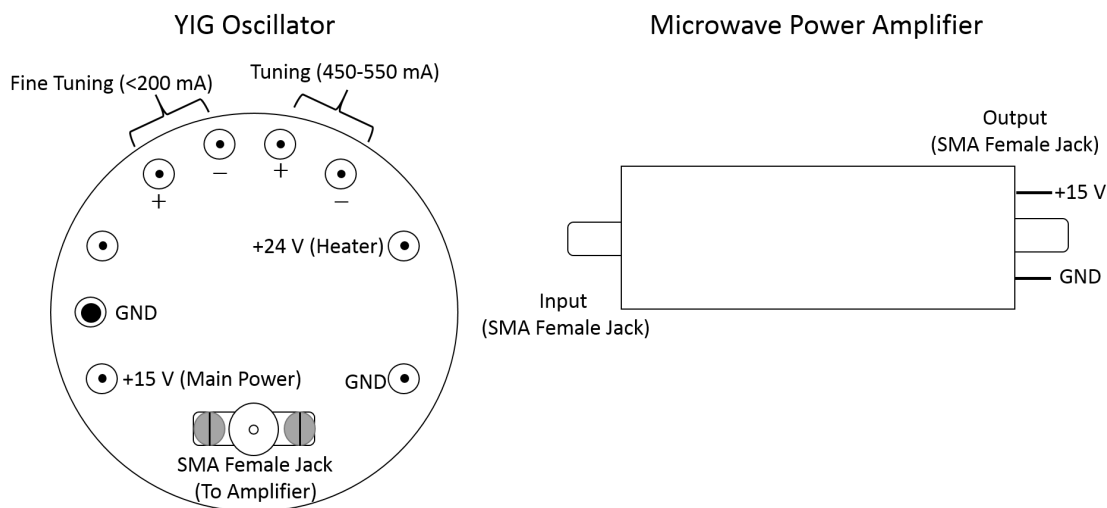


Figure 3.19. Diagram showing the proper pinning for operation of the YIG oscillator and associated amplifier

Based on this plot, a current of between 450 and 500 mA will be necessary for the proper irradiation frequency to induce Overhauser Effect DNP. However, to this point, no signal enhancement has been observed. There are a number of possible reasons for this. Most likely, the problem is that the power reaching the sample is too low to have a significant effect. In order to fix this, it may be necessary to construct a chamber in which microwaves will interfere constructively at the location of the sample, creating a standing wave at the proper irradiation frequency.

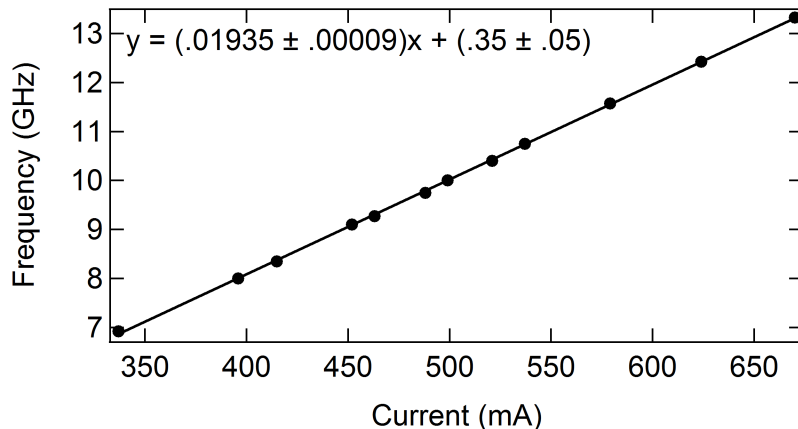


Figure 3.20. The calibration data for the YIG oscillator used in this work. Data was obtained by supplying a current to the oscillator and recording the frequency displayed on the spectrum analyzer.

3.4 Construction of an Earth's Magnetic Field NMR System

Given the importance of NMR as a spectroscopic method in many branches of science, it is useful for students to be exposed to it. However, given the expense of the technology used in NMR, it is often difficult for students to get time working with machines and getting hands-on experience. Earth's magnetic field NMR is a possible way to fix this problem. Systems can be made relatively cheaply and operated almost anywhere. Based on a previous design [141], an earth's magnetic field NMR system was constructed. The design is based off of a small, inexpensive Arduino microcontroller board and uses several amplifying circuits to transmit pulses and receive an NMR signal. There are two main pieces to the system: the transmitter/receiver, and the polarizing coil. The schematic for the circuits are adapted from Michal [141] and shown in Figure 3.21. The transmitter/receiver uses op amps to combine, smooth, and amplify or attenuate pulses from the arduino into an RF pulse, which is then sent to a transmit/receive coil. This coil is made from 3000 turns of thin (30 AWG) copper magnet wire around a 3" diameter pvc pipe. The coil is 10 cm long and has 10 layers of 300 turns each.

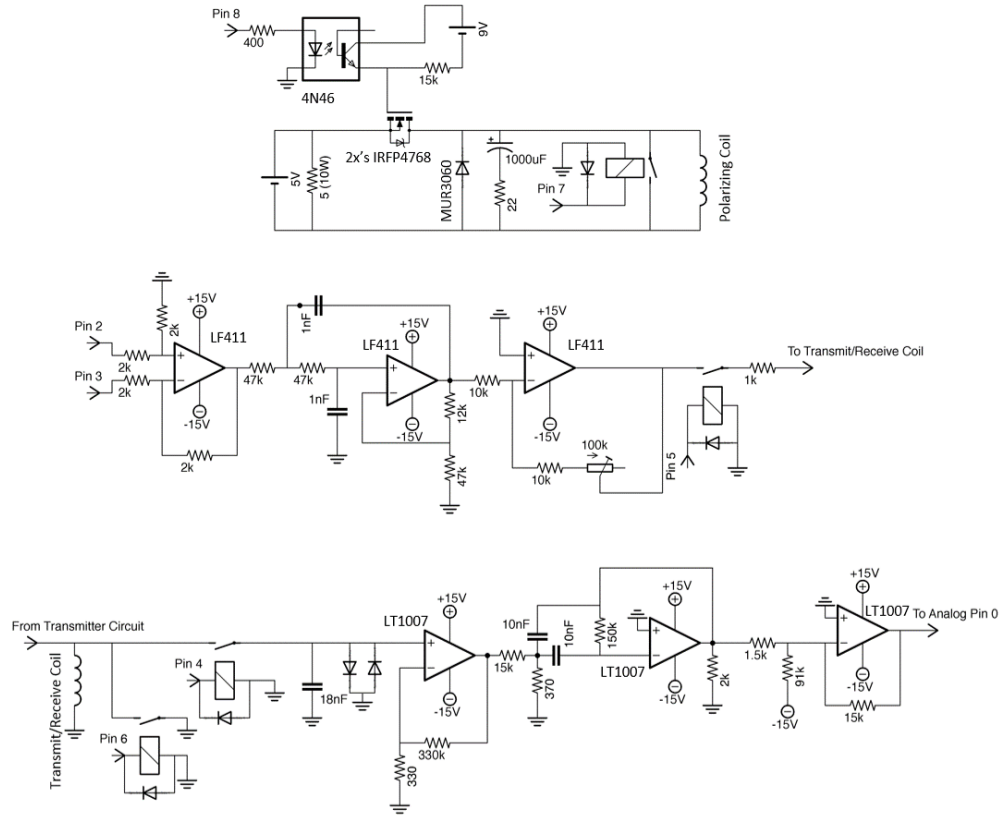


Figure 3.21. Circuit schematics for the homebuilt EFNMR system. Top: Polarizing circuit, Middle: Transmitter circuit, Bottom: Receiver circuit. All relays are HE3351A0500 and all diodes are 1N4148 unless otherwise specified.

After the pulse has been transmitted to the coil, the signal from the sample is received by the same coil and sent through a series of op amps to amplify the signal so that it may be detected by the analog to digital converter (ADC) on the Arduino. Both the transmitter and receiver circuits are constructed on the same 5 cm x 16 cm breadboard.

Because the NMR signal at Earth's magnetic field is extremely weak, a prepolarizing coil must be used to increase the signal to the point where it is visible in a reasonable time frame. This coil is 15 cm long and consists of 3 layers of about 150 turns of 18 AWG copper magnet wire. The 3 layers are connected in parallel, i.e. there are three separate coils one on top of another. By sending a strong current through this coil, a magnetic field much stronger than

the earth's magnetic field is created, which greatly increases the NMR signal. The current is supplied by a 5 V repurposed computer power supply and is controlled by the Arduino via a reed relay which allows current to flow through the coil when needed and shuts off the current otherwise. In the coil constructed by the author, the prepolarizing coil creates a magnetic field of about 8 mT. The circuit for the polarizing coil is soldered to a printed circuit board (PCB) designed and etched by the author.

The system is controlled by open source software that has been written and made available by Carl Michal of the University of British Columbia.

Though the design presented is quite straightforward, there are a few subtleties that led to difficulties in acquiring a signal for the first time. The first is tuning the coil to the resonance frequency of a proton in earth's magnetic field (1600 - 2200 Hz). Because of the low frequency needed, the available network analyzer was not adequate for this tuning. Instead, this was done by applying a series of audio pulses through the coil and monitoring the response using the spectrometer software. In this manner, the coil and bandpass filter were tuned to the proper resonance.

The second difficulty is in monitoring the pulse transmitted to the sample. The pulse must be sinusoidal and at a relatively low power in order to lead to signal acquisition. This may be checked using a standard oscilloscope. In the case of the system built, the power in the initial configuration was far too high and needed to be attenuated significantly. This was done by adjusting a variable resistor connected to the third op amp in the transmitter circuit until the maximum voltage of the signal was about five volts. In the final configuration, the power was such that an applied pulse of 3 half-cycles led to maximum SNR.

The system was found to perform as expected, and an NMR signal of a sample of water was able to be measured. The observed signal was significantly broadened (Figure 3.22), owing to inadequate homogeneity in Earth's magnetic field. The next step in optimizing this system is to add a set of gradient coils which can be used to shim the magnetic field. At the time of writing, this had not yet been completed.

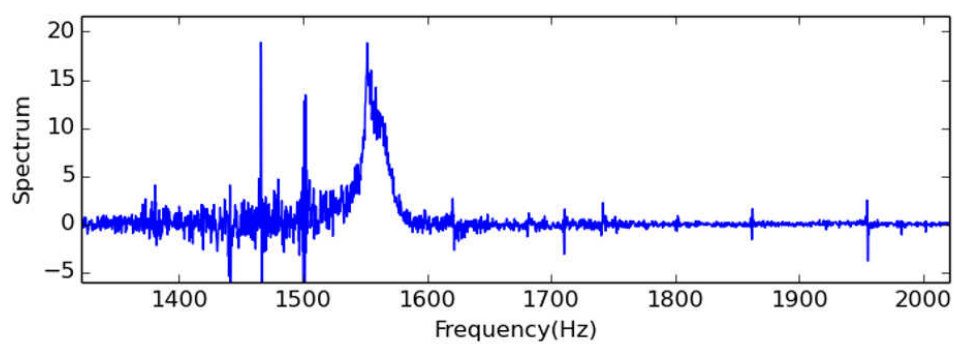
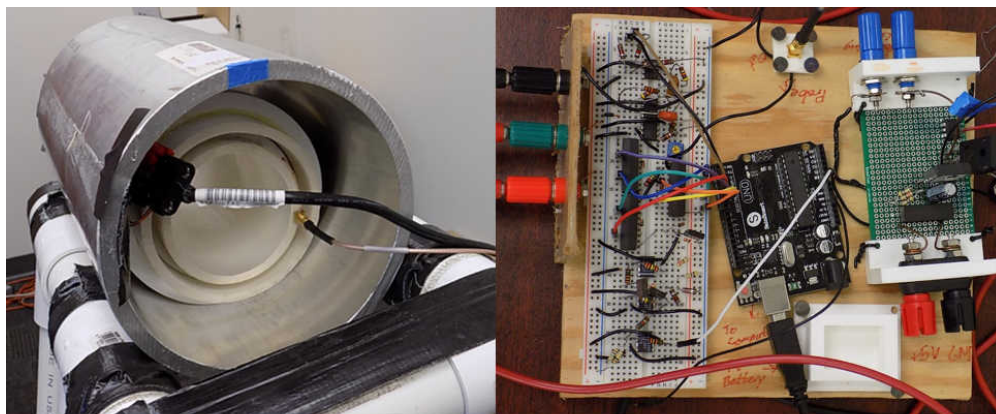


Figure 3.22. The EFNMR system built by the author with a example spectrum of a bulk water sample taken using the system

CHAPTER 4

OPTIMIZATION OF DISSOLUTION DNP

Though research into DNP applications is ongoing, there is a great deal of work left to be done in the optimization of the process. While conventional DNP methods are able to result in greater than 10,000-fold liquid state polarization enhancement, alternative instrumentation and sample presentation methods may be able to increase total enhancement, decrease build-up time, and increase liquid-state T_1 . In this chapter, a series of experiments designed to explore optimization methods are described.

Unless otherwise specified, DNP experiments were performed in a HyperSense polarizer (Oxford, UK) operating at 3.35 T and 1.2 K.

4.1 Free Radicals

Of the eleven free radicals in use in this work, only two, trityl OX063 and 4-oxo-TEMPO have seen significant usage in dissolution DNP. Trityl was the radical used in the first dissolution DNP experiment [30], and because of its efficiency as a polarizing agent, water solubility, and narrow EPR linewidth has consistently been the preferred radical for ^{13}C DNP [34, 37, 43, 66, 71]. 4-Oxo-TEMPO, though not as efficient for ^{13}C DNP, is commonly used because of its water solubility, ability to be scavenged easily, and, perhaps most importantly, very low price [37, 41, 145]. Following the bulk of DNP research, the emphasis in this work is placed on these two classes. Other radicals, including BDPA [35], a water soluble BDPA - SA-BDPA [146], DPPH [47], O-DPPH [47], and galvinoxyl [48] have been used for polarization but are not in common use. The other free radicals listed in Table 1.2 have not appeared in the literature as polarizing agents for dissolution DNP.

Common practice is to use narrow-linewidth radicals like trityl and BDPA for polarization of low- γ nuclei and wide-linewidth radicals for high- γ nuclei [33, 39]. When this practice is

followed, it is largely thought that thermal mixing is the dominant polarization mechanism at the intermediate magnetic fields (3.35-5 T) and low temperatures (1-2 K) at which dissolution DNP is most commonly performed. Recently, there have been several modified Thermal Mixing models published [135, 136, 137, 138] that are able to predict fairly accurately the results under these conditions, suggesting that a thermal mixing description is appropriate for the free radicals examined herein. More recently, it has been suggested that polarization at these conditions could be described using a combination of the cross effect and solid effect [147, 148, 149, 150], but this description fails to adequately explain some of the data collected herein. This will be emphasized as appropriate.

4.1.1 Electron Paramagnetic Resonance

One of the first steps of a complete understanding of DNP free radicals is a study of some of their electronic properties by way of EPR. Room temperature X-band EPR spectra were found for each of the free radicals of interest using a Bruker EMX spectrometer in the NanoTech Institute at The University of Texas at Dallas (Figures 4.1 and 4.2).

While the EPR spectra can provide insight into DNP mechanisms for individual polarizing agents, there is much more to be learned. In particular, the low temperature spectra and spin lattice relaxation are important in determining the particulars of the DNP process [44]. Further experiments will be described that detail efforts to more fully understand DNP free radicals and their electronic properties.

4.1.2 UV-Vis Spectrophotometry

One useful property of the free radicals used in dissolution DNP is that when dissolved in a solvent each radical exhibits a particular color. For example, trityl gives a dark green solution while TEMPONE gives a light yellow. Because these colors are unique to a given radical, the free radical may be further characterized using UV-Vis spectrophotometry.

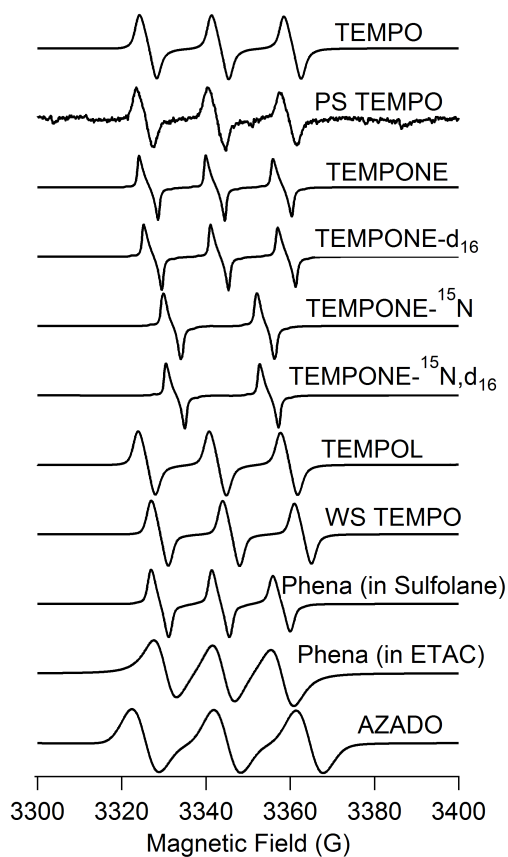


Figure 4.1. X-band EPR Spectra at room temperature for the nitroxide-based radicals studied in this work.

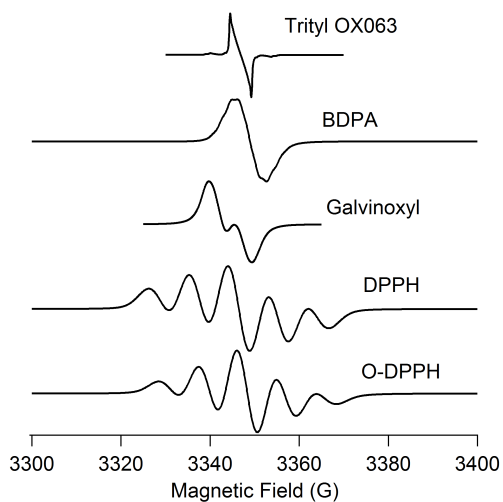


Figure 4.2. X-band EPR Spectra at room temperature for the radicals studied in this work that are not nitroxide based.

UV-Vis (Ultraviolet-Visible) spectrophotometry refers to spectroscopy performed in the ultraviolet and visible wavelengths. Typically, the range of wavelengths studied by this technique is between 200 and 800 nm, though it can extend beyond those boundaries if the instrument allows [151]. The goal of this form of spectroscopy is to measure the absorbance (A) of a given substance defined as

$$A = \log \left(\frac{I_0}{I} \right) \quad (4.1)$$

where I_0 and I are the intensity of light measured when shone through the pure solvent and the solvent with substance of interest dissolved, respectively. Physically speaking, this absorbance occurs when an electron is excited from its ground state to an excited state by absorbing a photon in the specified wavelength range. The excited state may be vibrational or rotational, causing UV-Vis spectral lines to be quite broad.

While these transitions can occur, they are not guaranteed, and the rate at which they occur is governed by the Beer-Lambert Law. This gives a relation between the absorbance and the composition and size of a given sample. Namely,

$$A = \epsilon cl \quad (4.2)$$

where ϵ is the molar absorptivity of the molecule in question, c is the concentration in solution, and l is the path length of light through the sample.

Because the UV-Vis technique is focused on measuring absorbance of light in the ultraviolet and visible range, it is an ideal tool for classifying and detecting free radicals based on the color of a solution. Not only is it effective, but it is very sensitive, able to detect concentrations of free radicals on the order of hundreds of micromolar, and it is both fast and inexpensive when compared to other techniques of classifying free radicals such as EPR. A particularly useful application of UV-Vis spectrometry to DNP is to test the absorbance of the post-dissolution sample. This allows for a very fast and reasonably accurate estimate of

free radical concentration, which is essential when applying dissolution DNP to *in vivo* human studies. In fact, a UV-Vis spectrophotometer is included in the quality control system of the commercial clinical polarizer, SPINLab (GE Healthcare, UK).

In order to characterize the free radicals studied in this work, several different UV-Vis techniques were employed. Each of these was based on a simple scan in which the instrument scanned through a large range of wavelengths, measuring the absorbance of a given sample throughout the range. The spectrometer used for all measurements was an Evolution 220 (ThermoFisher Scientific) (Figure 4.3) which can measure absorbance between 190 and 1100 nm. A scan over the maximum range was used to find the characteristic absorbance spectrum for each free radical. Additionally, scans were taken at regular intervals in order to assess the stability of radicals. For the less stable radicals, such as Blue, a single wavelength known to be an absorbance peak was scanned at regular time intervals. Samples at different concentrations were also studied in order to establish a calibration by which concentration could be estimated based on absorbance.



Figure 4.3. Evolution 220 Spectrophotometer with Peltier temperature control unit

TEMPONE and Isotopically Labeled Variants A stock solution of 2 mM TEMPONE variant in water was made. This sample was then diluted to 800 μL samples with concentrations of 1 mM, 500 μM , 250 μM , 125 μM , 62.5 μM , 31.25 μM , and 15.625 μM . Absorbance scans were taken for each sample at room temperature between 190 and 1100 nm. One broad peak was visible between 210 and 280 nm (Figures 4.4, 4.5, 4.6, and 4.7). The maximum

absorbance of this peak was recorded and found to vary linearly with the concentration of TEMPONE variant in solution. The decay of non-enriched TEMPONE over time was also measured, though the radical showed remarkable stability with only a slight reduction in intensity after the maximum time scan of the instrument (5 days).

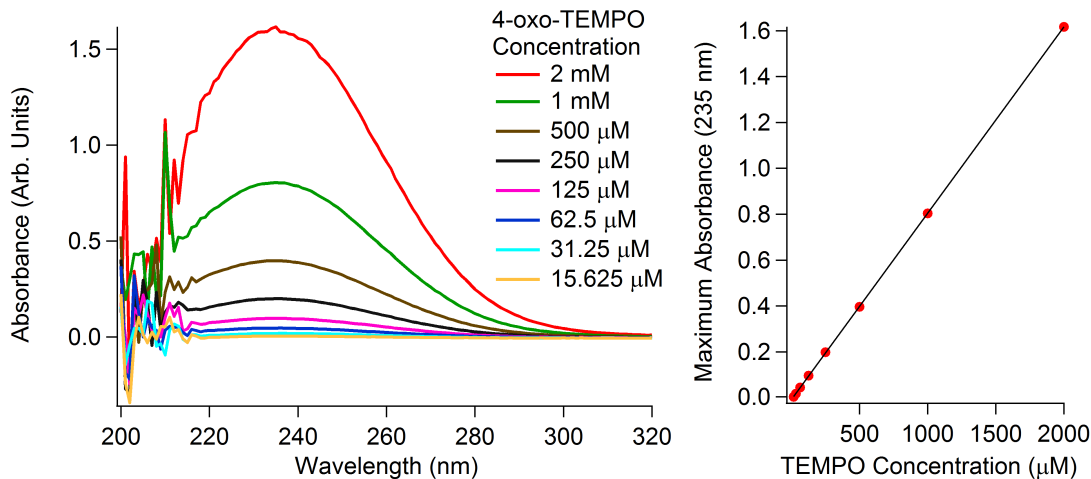


Figure 4.4. UV-Vis spectra of 4-oxo-TEMPO at room temperature at various concentrations (left). Maximum absorbance of peak vs radical concentration (right).

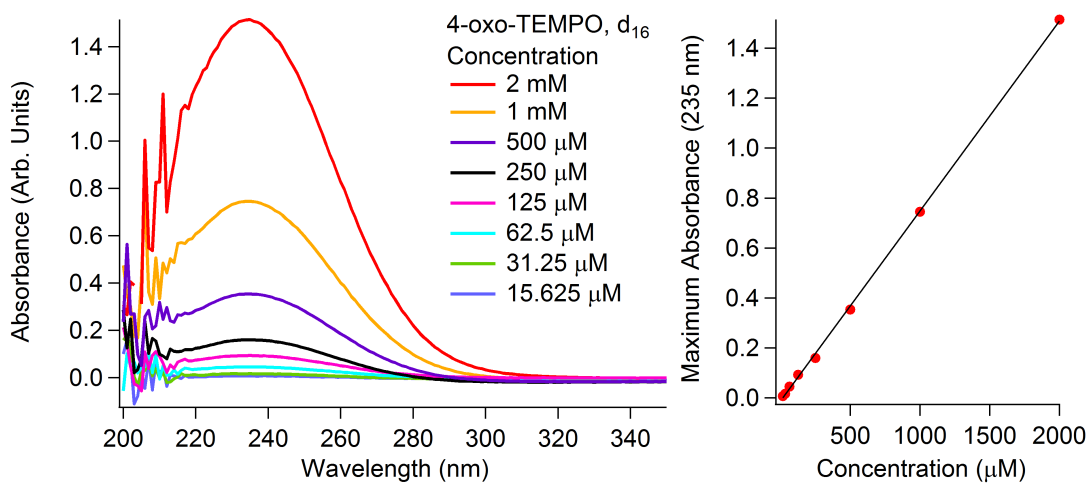


Figure 4.5. UV-Vis spectra of 4-oxo-TEMPO, d_{16} at room temperature at various concentrations (left). Maximum absorbance of peak vs radical concentration (right).

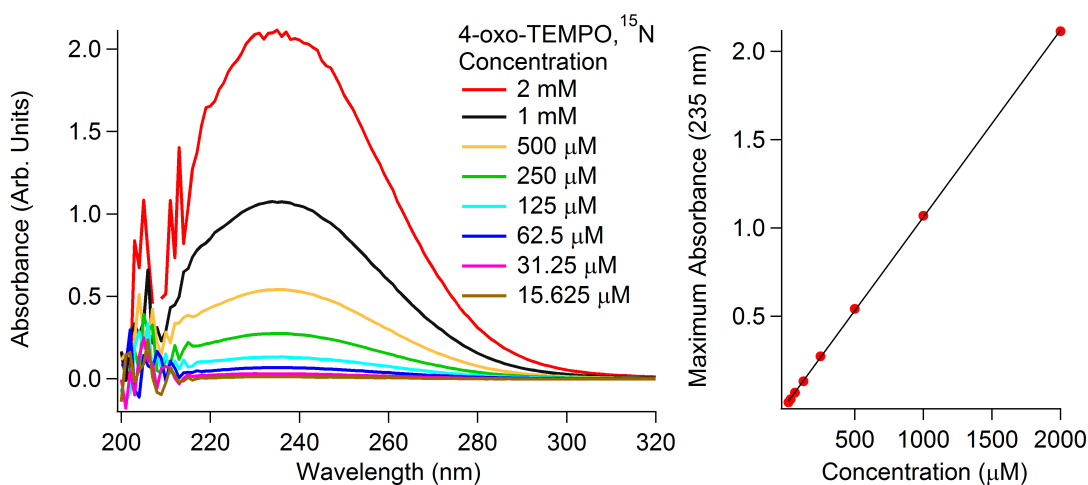


Figure 4.6. UV-Vis spectra of 4-oxo-TEMPO, ^{15}N at room temperature at various concentrations (left). Maximum absorbance of peak vs radical concentration (right).

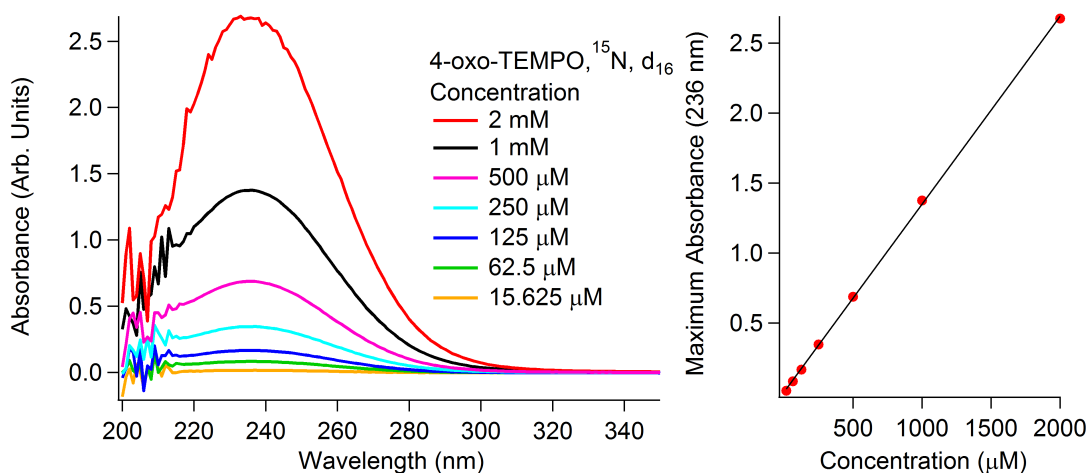


Figure 4.7. UV-Vis spectra of 4-oxo-TEMPO, $^{15}\text{N}, \text{d}_{16}$ at room temperature at various concentrations (left). Spectra at $31.25 \mu\text{M}$ was not obtained due to spectrometer malfunction. However, because the trend is clearly visible, this was not reproduced. Maximum absorbance of peak vs radical concentration (right).

BDPA A stock solution of 1 mM BDPA in 1:1 v/v sulfolane:DMSO was made. This sample was then diluted to 800 μL samples with concentrations of 750 μM , 500 μM , 250 μM , 125 μM , 62.5 μM , and 31.25 μM . Absorbance scans were taken for each sample at room temperature between 190 and 1100 nm. One peak was visible between 300 and 400 nm, and a second, much smaller, peak was visible between 700 and 800 nm (Figure 4.8). For both peaks, the maximum absorbance varied linearly with the concentration. Subsequent to concentration dependence studies, a 500 μM sample was placed in the spectrometer and absorbance scans taken every 3 hours for 144 hours. BDPA was found to decay significantly, with greatly reduced absorbance over the course of this time period (Figure 4.9).

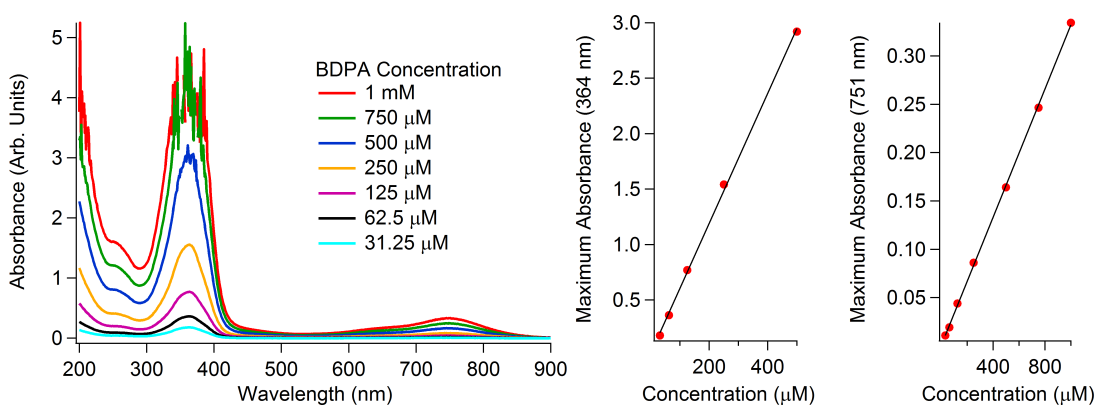


Figure 4.8. UV-Vis Spectra of BDPA at room temperature at various concentrations (left). Maximum absorbance of low wavelength peak (middle) and high wavelength peak (right) vs. BDPA concentration.

DPPH A stock solution of 697 μM DPPH in 1:1 v/v sulfolane:DMSO was made. This sample was then diluted to 800 μL samples with concentrations of 500 μM , 250 μM , 125 μM , 60 μM , 30 μM , 15 μM , and 7.5 μM . Absorbance scans were taken for each sample at room temperature between 190 and 1100 nm. Two absorbance peaks were visible, one near 300 nm and one near 500 nm (Figure 4.10). The maximum absorbance was roughly linear with concentration. The 697 μM sample was then placed in the spectrometer and absorbance

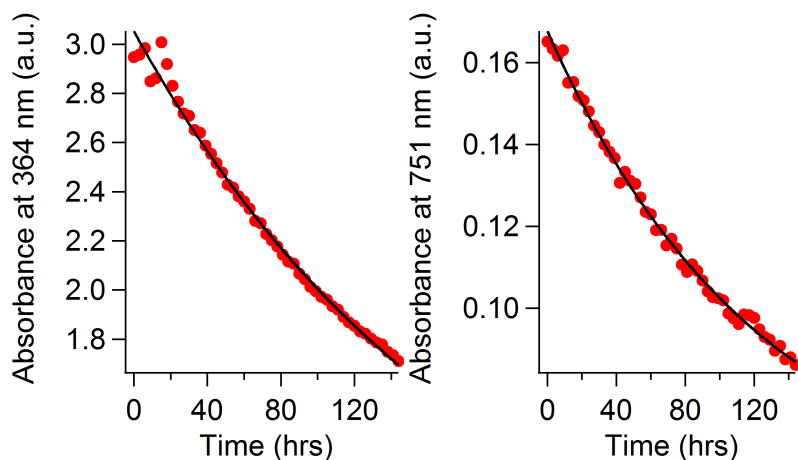


Figure 4.9. Maximum absorbance of large peak (left) and small peak(right) of BDPA at 3 hour intervals. Decays are fit with a single exponential fitting.

scans were taken every 30 minutes for 1410 minutes. The maximum absorbance for the peak near 500 nm was plotted as a function of time and a single exponential fitting applied to the plot (Figure 4.11). Compared to BDPA, DPPH decayed much more rapidly, giving a similar reduction in absorbance over a much shorter time period.

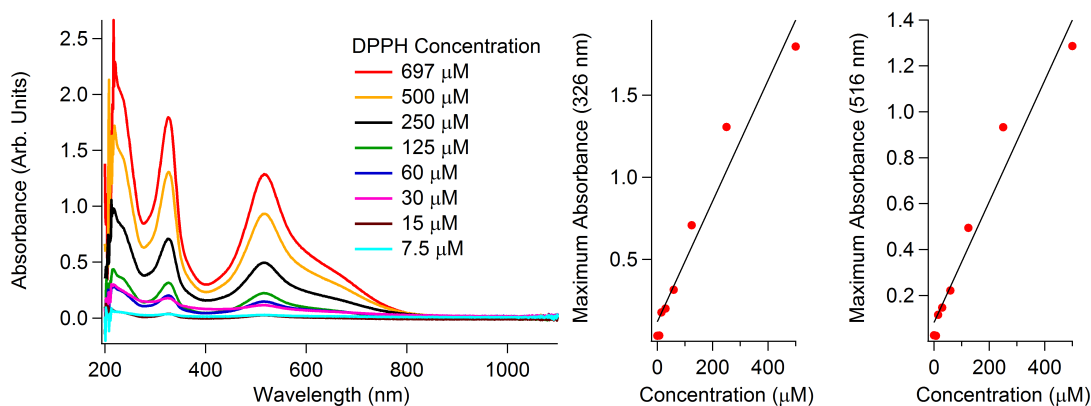


Figure 4.10. UV-Vis Spectra of DPPH at room temperature at various concentrations (left). Maximum absorbance of low wavelength peak (middle) and high wavelength peak (right) vs. DPPH concentration.

Galvinoxyl A sample with 1 mM galvinoxyl in ethyl acetate was placed in the spectrometer and absorbance scans taken every 30 seconds for 1470 seconds (≈ 25 minutes). Initially,

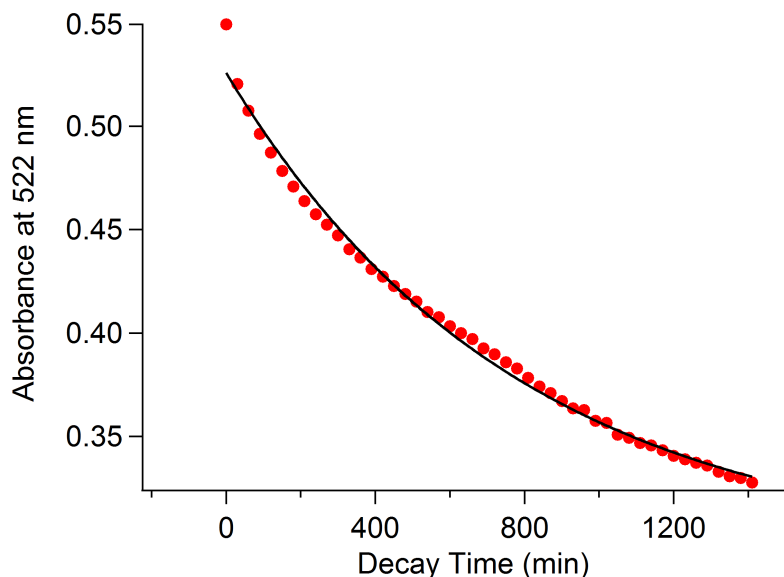


Figure 4.11. Absorbance of DPPH at 522 nm over time in 30 minute intervals. Data are fit with a single exponential.

the absorbance of the maximum peak was too great for the spectrometer to read accurately. However, starting near 800 seconds, a decay was clearly visible and was fit using a single exponential function (Figure 4.12).

Blue The Blue radical posed significant difficulties in acquiring UV-Vis spectra. The radical degrades very rapidly, so in many cases, by the time the scan was acquired, the radical had completely degraded. Additionally, the radical degraded a significant amount over the course of a scan over the whole range of wavelengths (≈ 30 seconds). For this reason, once a peak was found, only the small range of wavelengths corresponding to a peak was scanned. In order to further combat the fast degradation of the radical, the solvent (1:1 v/v sulfolane:DMSO) was chilled in a lab freezer (-10°C) for 30 minutes prior to the addition of Blue. A sample was made with 2 mM Blue radical in the chilled solvent and placed in the spectrometer. Scans were taken every 15 seconds from 580 to 790 nm. With this shortened range, the scan took a very short time (≈ 2 seconds) and encompassed the point of interest

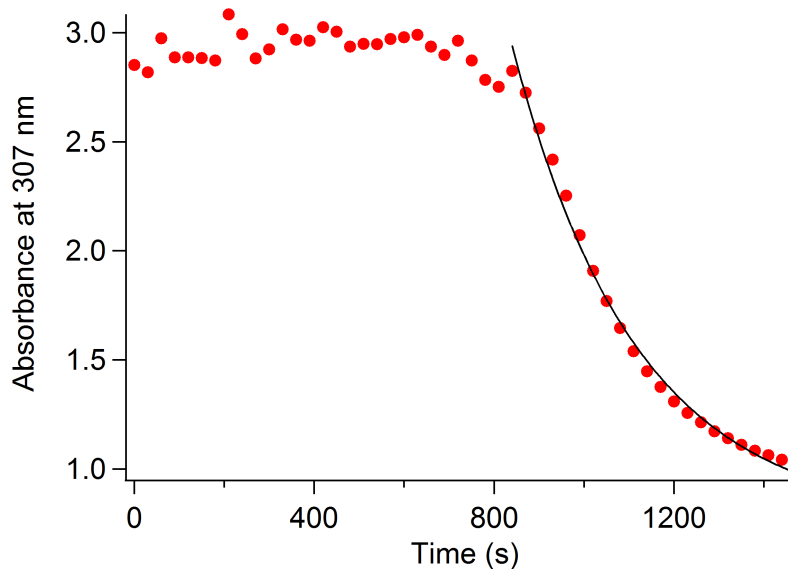


Figure 4.12. Absorbance of galvinoxyl at 307 nm over time in 30 second intervals. Data are fit with a single exponential.

for the blue radical. The absorbance at 715 nm was plotted vs time and a single exponential fit to the plot (Figure 4.13).

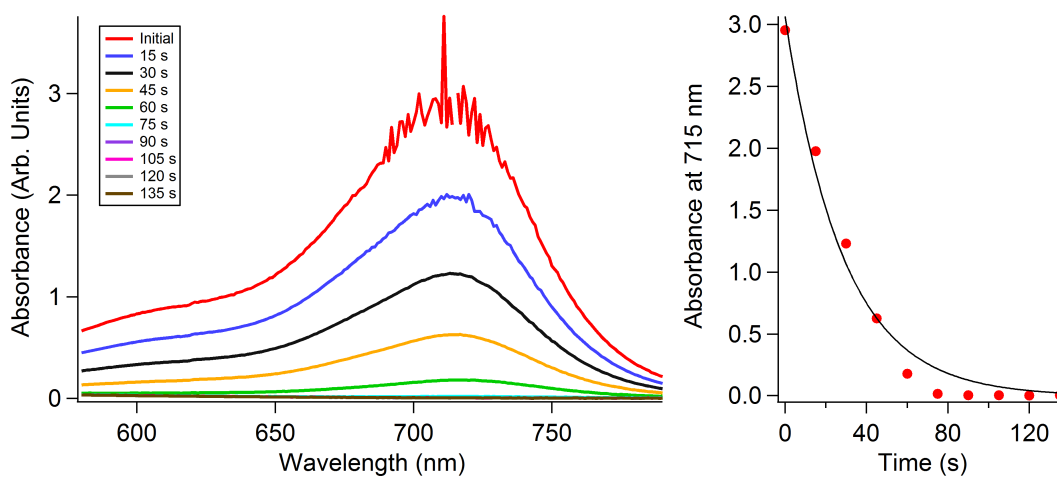


Figure 4.13. Absorbance of blue radical between 580 and 790 nm (left). Absorbance at 715 nm as a function of time. Fitting was done using a single exponential.

A summary of the information gleaned from UV-Vis experiments is shown in Table 4.1.

Table 4.1. UV-Vis Spectrophotometry Results showing the primary UV-Vis peaks for each radical, as well as the dependence of absorbance (A) on concentration (C) and the decay time constant, determined by fitting a single exponential decay to time course data.

Free Radical	Peaks (nm)	Concentration Dependence	Decay Time Constant
TEMPONE	235	$A = 0.000812C - 0.0067$	—
TEMPONE, d ₁₆	235	$A = 0.000760C - 0.0116$	—
TEMPONE, ¹⁵ N	235	$A = 0.00106C + 0.0028$	—
TEMPONE, ¹⁵ N, d ₁₆	236	$A = 0.00134C + 0.0054$	—
BDPA	364	$A = 0.00586C + 0.019$	185 hrs
	751	$A = 0.000331C + 0.00059$	123 hrs
DPPH	326	$A = 0.00253C + 0.039$	—
	516	$A = 0.00182C + 0.024$	775 min
Galvinoxyl	307	—	275 s
	750	—	—
Blue	715	—	28 s

4.1.3 Free Radical Concentration

At this point the discussion is brought back to the subject of dissolution DNP. Due to the rapid relaxation rate of electrons, small quantities of electrons are able to polarize a large number of nuclei [22]. For this reason, only trace amounts of electrons are needed to effectively polarize the nuclear spins in a sample. Too few electrons and the lack of polarizing centers results in very slow polarization and a lesser ultimate signal enhancement. Too many electrons and there is a greater effect on nuclear relaxation which also reduces the total achievable polarization enhancement. For this reason, it is important to optimize the concentration of free radical. For most of the free radicals studied, the optimal concentration has been previously determined (Table 4.2).

It is expected that SA-BDPA would have a similar optimum concentration to BDPA. However, this was unable to be tested given that only a limited quantity of SA-BDPA was able to be procured. When tested in a fairly standard DNP sample (3 M [1-¹³C]sodium acetate in 1:1 v/v glycerol:water), a chemical reaction occurred which neutralized the free

Table 4.2. Optimal concentrations of free radicals for dissolution DNP

Free Radical	Optimal Concentration
Trityl	15 mM
TEMPO derivatives	40 mM
BDPA	40 mM
DPPH and O-DPPH	40 mM
Galvinoxyl	40 mM

radical. This was evidenced by a dramatic change in color upon mixing. SA-BDPA in glycerol:water had a dark brown color, but the addition of sodium acetate immediately changed the color to a light blue. EPR studies of these samples after mixing further indicated that the radical had been effectively neutralized. Clearly, if SA-BDPA is to be used in DNP studies, care must be taken to ensure that the substrate being polarized is chemically compatible with the radical.

4.2 Isotopic Labeling of the 4-Oxo-TEMPO Free Radical

The details of the work and results described in this section may also be found in [152]:

P. Niedbalski, C. Parish, A. Kiswandhi, and L. Lumata, “ ^{13}C Dynamic Nuclear Polarization Using Isotopically Enriched 4-Oxo-TEMPO Free Radicals,” *Magn. Reson. Chem.* vol. 54, pp. 962-967, Copyright © 2016 John Wiley & Sons Ltd.

C. Parish and A. Kiswandhi assisted with sample preparation. All DNP studies and data analysis were performed by the author.

^2H -labeling of solvents and substrates is a common method of increasing polarization and decreasing build-up time [36] for ^1H and ^{13}C DNP studies using nitroxide radicals. Because of the sensitivity of DNP efficiency to the electronic properties of the free radical, isotopic enrichment of the free radical may also lead to some effect. Deuterated and ^{15}N -doped variants of 4-Oxo-TEMPO are readily available, providing an excellent opportunity to test this hypothesis. Specifically, 4-Oxo-TEMPO, 4-Oxo-TEMPO- d_{16} , 4-Oxo-TEMPO- ^{15}N , and

4-Oxo-TEMPO- $^{15}\text{N}, \text{d}_{16}$ were used as polarizing agents for DNP of $[1-^{13}\text{C}]$ acetate and the results compared against each other.

Looking back to Figure 4.1, deuteration had little effect on the EPR spectrum of the radicals, but ^{15}N -doping had a significant effect, reducing the number of hyperfine peaks from three to two. Because of this difference, it was thought that a greater number of electron spins could be excited within the narrow irradiation frequency range used in DNP. This would result in more efficient DNP, perhaps improving the achieved polarization or the buildup time.

Samples were prepared with 3 M of $[1-^{13}\text{C}]$ and 40 mM of one of the isotopically enriched TEMPONE variants in a 1:1 glycerol: H_2O glassing solvent. Samples were made and measured in triplicate. An additional set of samples were made in a fully ^2H -labeled glassing solvent of glycerol, d_8 : D_2O .

DNP frequency sweeps were run on each of the samples with non-deuterated solvents. Prior work has shown that deuteration of solvents has a negligible effect on the DNP spectra [36], but one sample (TEMPONE- $^{15}\text{N}, \text{d}_{16}$) was chosen for a frequency sweep to confirm this expectation. Due to the similarity of the radicals and the wide linewidth of TEMPONE, the frequency sweeps had nearly identical results, so 94.15 GHz was chosen as the irradiation frequency for all eight samples.

Subsequent to the frequency sweeps, samples were polarized at 3.35 T and 1.2 K until the ^{13}C polarization had approximately reached saturation which took about an hour for each sample. A comparison of each build-up curve in like solvents shows nearly identical polarization enhancement (Figure 4.14). Deuteration of solvents led to a near doubling of final polarization and a halving of the build-up time. This was in agreement with prior work that had seen improvements in DNP efficiency when using deuterated solvents [36, 153].

Contrary to the expectation, there was nearly no difference in polarization efficiency with isotopic enrichment of TEMPONE. In the thermal mixing model, the specific heat of the

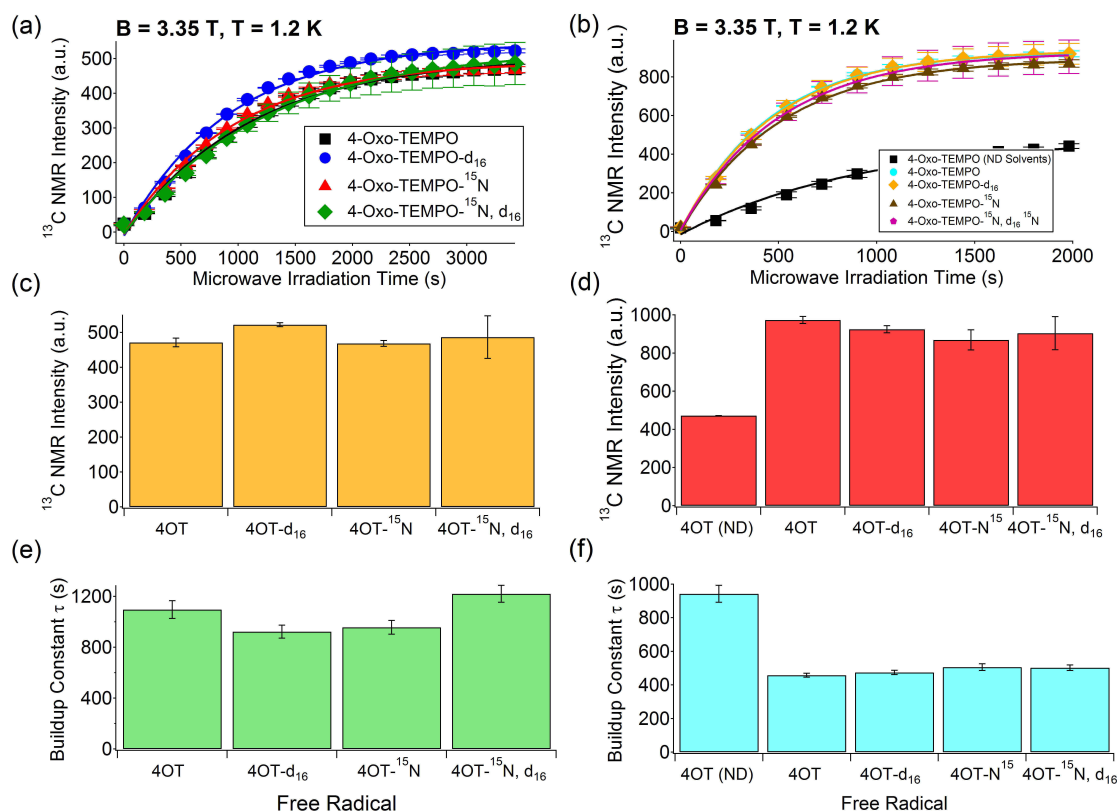


Figure 4.14. (a) and (b) show DNP Buildups for $[1-^{13}\text{C}]$ samples doped with the 4-Oxo-TEMPO Variants in non-deuterated and deuterated solvents, respectively. (c) through (f) show a comparison of final ^{13}C NMR intensity (c and d) and build-up time constants (e and f) for non-deuterated and deuterated solvents. Figure adapted with permission from P. Niedbalski, C. Parish, A. Kiswandi, and L. Lumata, “ ^{13}C Dynamic Nuclear Polarization Using Isotopically Enriched 4-Oxo-TEMPO Free Radicals,” *Magn. Reson. Chem.* vol. 54, pp. 962-967, Copyright © 2016 John Wiley & Sons Ltd.

NZS is proportional to the number of spins multiplied by the Larmor frequency squared (2.14) [133]. Although the change in Larmor frequency when doping with deuterons and ^{15}N would lead to some theoretical change in efficiency, the small number of TEMPO molecules (40mM) as compared to water (55 M), glycerol (13 M), or acetate (3M) causes the effect to be negligible.

Because of the similarity in DNP efficiency when using the 4-Oxo-TEMPO variants, low temperature EPR of the variants was performed. The differences in hyperfine coupling are mitigated by the low temperatures (1.2 K) at which DNP takes place. At such temperatures,

dipolar interactions dominate the EPR spectra. As a result, the EPR spectra of the four different radicals lose their distinct characteristics and are largely identical. Hence, DNP efficiency for each of the radicals is approximately the same. Based on these findings, it is expected that these results may be generalized and that isotopic enrichment of any DNP free radical will yield little to no benefit in overall polarization.

4.3 DNP Using TEMPO Derivatives

The details of the work and results described in this section may also be found in [154]:

“¹³C Dynamic Nuclear Polarization Using Derivatives of TEMPO Free Radical,” *App. Magn. Reson.* vol. 48, 2017, pp. 933-942, P. Niedbalski, C. Parish, Q. Wang, A. Kiswandhi, and L. Lumata, Copyright © Springer-Verlag GmbH Austria 2017 With permission of Springer

C. Parish, Q. Wang, and A. Kiswandhi assisted with sample preparation, and Q. Wang assisted the author with DNP experiments. All data analyses were performed by the author.

Of the nitroxide radicals that have been used in DNP, TEMPO and its derivatives are most commonly utilized. This is in part due to the wide commercial availability of these radicals. However, of the many TEMPO derivatives available, only TEMPO [155], 4-amino-TEMPO [31, 156], 4-hydroxy-TEMPO [157], and 4-oxo-TEMPO [41, 155] have prior evidence of use in DNP studies. Despite widespread use, these four radicals have never been directly compared as polarizing agents. To fill this void, the four previously named TEMPO derivatives as well as 4-methoxy-TEMPO, 4-maleimido-TEMPO, and 4-acetamido-TEMPO were obtained and studied as agents for ¹³C polarization. One other nitroxide radical was also studied, namely AZADO, but this radical did not have the base TEMPO structure. The structure of each of these radicals is shown in Figure 1.8.

These particular radicals were chosen for their inexpensiveness, closeness in structure to TEMPO, and solubility. Originally, data was taken using samples made with a glassing

solvent of 1:1 v/v glycerol:water using the free radicals TEMPO, TEMPONE, TEMPOL, 4-Amino-TEMPO and WS-TEMPO. However, it was found that the other TEMPO radicals were insoluble in this solvent. In fact, TEMPO itself dissolved very poorly in this mixture. To directly compare all of these radicals, a solvent in which all were soluble is desired, so a number of different options were tested, finally settling on a mixture of 1:1 v/v ethanol:water which is known to form a glass at the cryogenic temperatures used for DNP [36] and proved to dissolve all nine of the nitroxide radicals that were studied.

Samples were prepared with 3 M [1- ^{13}C] sodium acetate and 40 mM of one of the radicals mentioned above in the 1:1 v/v ethanol:water mixture. 40 mM was chosen because it is well established as the optimum concentration for 4-oxo-TEMPO [41], so it was assumed that the other very similar radicals would behave similarly. Samples with identical concentrations of ^{13}C substrate and free radical were also made using fully ^2H -labeled solvents.

Samples were polarized at 3.35 T and 1.2 K using the HyperSense polarizer. The first step taken was to perform microwave frequency sweeps on all samples. It was found that the combination of nitroxide free radicals and the ethanol:water glassing matrix led to very fast polarization, so samples only needed to be polarized for about 30 minutes for saturation to occur.

It was found that variations in ^{13}C polarization efficiency among the derivatives were minimal (Figure 4.15). TEMPONE and 4-Amino-TEMPO led to the greatest ^{13}C polarization by a small margin, but this was accentuated by the deuteration of solvents. By ^2H -labeling the solvent, polarization was increased between 2 and 2.5-fold for each of the TEMPO derivatives (Figure 4.16). Additionally, the solvent composition had a significant effect on the achievable polarization, with samples in glycerol:water reaching higher ^{13}C polarization than samples in ethanol:water (Figure 4.17). Furthermore, the polarization efficiency of TEMPOL was reduced to a greater degree than TEMPONE and 4-amino-TEMPO with the solvent change, an effect for which the explanation is unclear.

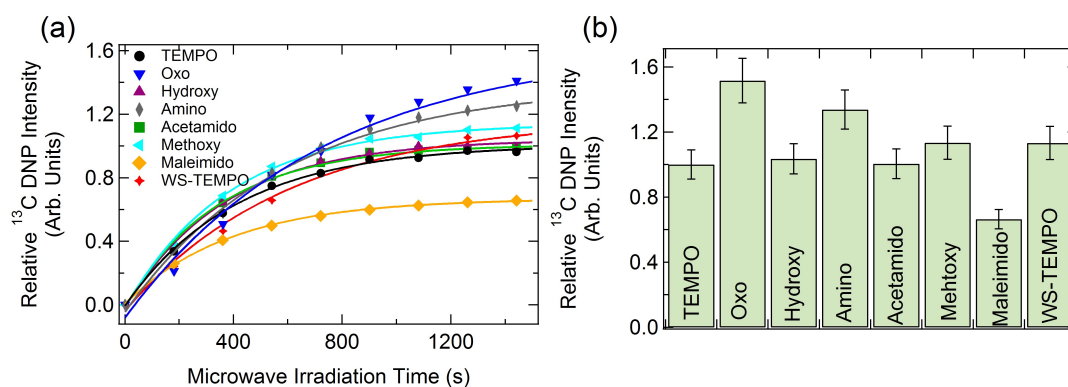


Figure 4.15. (a) Polarization buildup curves for ^{13}C -labeled acetate doped with different derivatives of the TEMPO radical in a glassing matrix of 1:1 v/v ethanol:water. (b) Comparison of maximum polarization achieved by using each of the TEMPO derivatives. Figure adapted from “ ^{13}C Dynamic Nuclear Polarization Using Derivatives of TEMPO Free Radical,” *App. Magn. Reson.* vol. 48, 2017, pp. 933-942, P. Niedbalski, C. Parish, Q. Wang, A. Kiswandhi, and L. Lumata, Copyright © Springer-Verlag GmbH Austria 2017 With permission of Springer

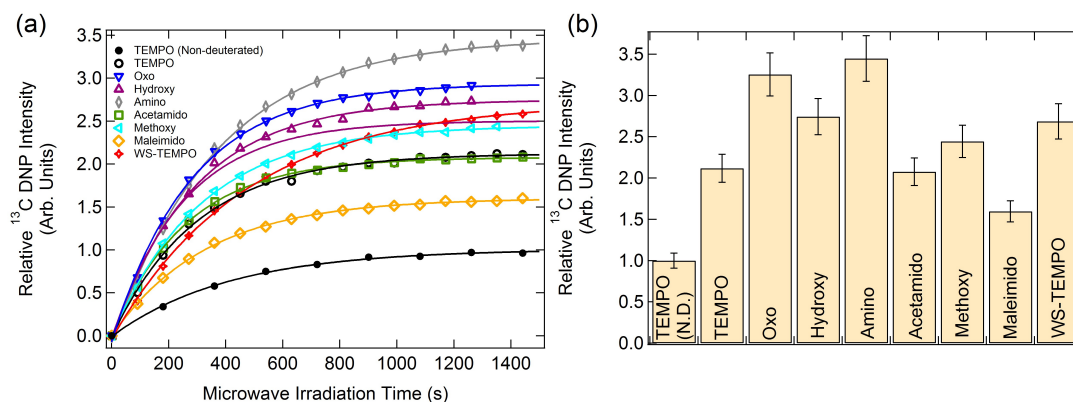


Figure 4.16. (a) Polarization buildup curves for ^{13}C -labeled acetate doped with different derivatives of the TEMPO radical in 1:1 v/v ethanol, d_6 : D_2O . (b) Comparison of maximum polarization achieved by using each of the TEMPO derivatives in deuterated solvents. Polarization using TEMPO in non-deuterated solvents is shown for comparison. Figure adapted from “ ^{13}C Dynamic Nuclear Polarization Using Derivatives of TEMPO Free Radical,” *App. Magn. Reson.* vol. 48, 2017, pp. 933-942, P. Niedbalski, C. Parish, Q. Wang, A. Kiswandhi, and L. Lumata, Copyright © Springer-Verlag GmbH Austria 2017 With permission of Springer

Minor differences in ^{13}C polarization efficiency achieved with different TEMPO derivatives are likely a result of different electronic T_1 relaxation times among the radicals. The different shapes of the molecules lead to different rates of molecular tumbling, which would have an effect on electron relaxation. Unfortunately, this is unable to be confirmed given the instrumentation available.

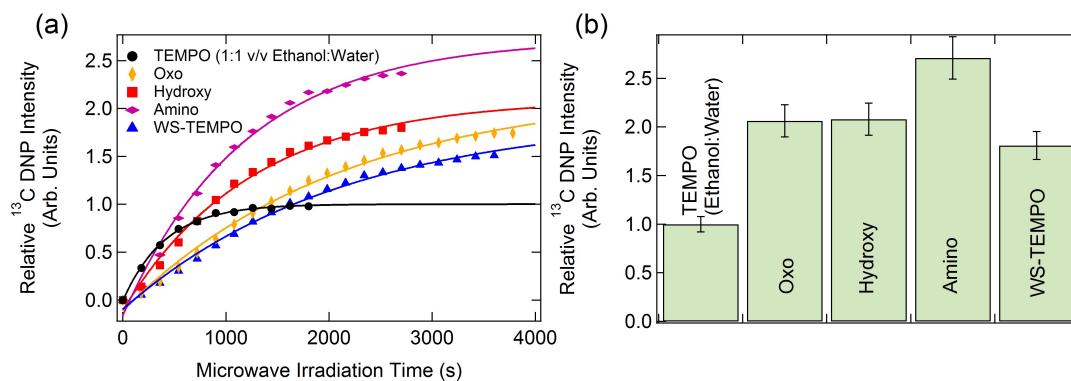


Figure 4.17. (a) Polarization buildup curves for ^{13}C -labeled acetate doped with different water soluble derivatives of the TEMPO radical in 1:1 v/v glycerol:water. (b) Comparison of maximum polarization achieved by using these select radicals. Polarization using TEMPO in non-deuterated 1:1 v/v ethanol:water is shown for comparison.

Overall, regardless of the solvent used, 4-Amino-TEMPO or TEMPONE provide the largest ^{13}C polarization enhancements, though there is only a small reduction in efficiency with other TEMPO derivatives.

4.4 The Effect of Isotopic Labeling on Solid-State Polarization Enhancement and Relaxation in Sodium Acetate

The details of the work and results described in this section may also be found in [158]:

P. Niedbalski, C. Parish, A. Kiswandhi, Z. Kovacs, and L. Lumata, "Influence of ^{13}C Isotopic Labeling Location on Dynamic Nuclear Polarization of Acetate," *J. Phys. Chem. A* vol. 121, pp. 3227-3233, 2017 Copyright 2017 American Chemical Society.

A portion of the experimental work was performed by L. Lumata under the supervision of Z. Kovacs. This project was completed by the author under the supervision of L. Lumata. C. Parish and A. Kiswandhi assisted with sample preparation. Data analyses were performed by the author.

In order to come to a more complete understanding of the dissolution DNP process, more information into solid state polarization and relaxation mechanisms is essential. To that end, the effect of ^{13}C and ^2H labeling on polarization and relaxation in the solid state was studied by looking at the common and metabolically relevant substrate acetate.

This substrate is composed of a central carbon doubly bonded to an oxygen and singly bonded to an oxygen ion and a methyl group. This leads to six different isotopical labelings of interest: (1) ^{13}C labeling in the carboxyl location, (2) ^{13}C labeling in the methyl location, (3) ^{13}C labeling in both locations, and (4), (5), and (6) deuteration of the methyl group for the ^{13}C labelings of (1), (2), and (3).

Samples containing 3 M of each of these substrates were made and doped with 15 mM of trityl. Following the sample preparation, samples were polarized in the HyperSense polarizer (Oxford, UK) at 3.35 T and 1.4 K for three hours, at which point the microwave irradiation was turned off but the sample left in the polarizer. ^{13}C polarization was monitored both as the polarization was taking place (every 5 min) and as the ^{13}C relaxed back to equilibrium after polarization was complete (every 20 min). The ^{13}C T_1 is quite long in the solid state, so samples were monitored overnight in order that a complete relaxation curve may be obtained.

It was found, as was expected, that $[1-^{13}\text{C}]$ acetate was able to be polarized to the highest level (Figure 4.18). $[1-^{13}\text{C},\text{d}_3]$ acetate polarized to nearly the same level. The other isotopically labeled substrates all resulted similar polarizations much weaker than that of the two carboxyl-labeled acetates. Clearly, then, a methyl ^{13}C labeling has a profound effect on polarization. This is caused by the introduction of a nuclear relaxation leakage factor owing to close proximity to nuclear spins. However, the leakage factor is thought to be proportional

to the square of the gyromagnetic ratio to which a spin is coupled [159]. Therefore, it would be expected that ^1H in the methyl group would lead to a large leakage factor and greatly lower polarization, while ^2H in the methyl group would have a much smaller effect. The data show, though, that deuteration of the methyl group reduces the overall polarization that is achieved even for carboxyl ^{13}C labeling. This suggests that there is some other factor at play.

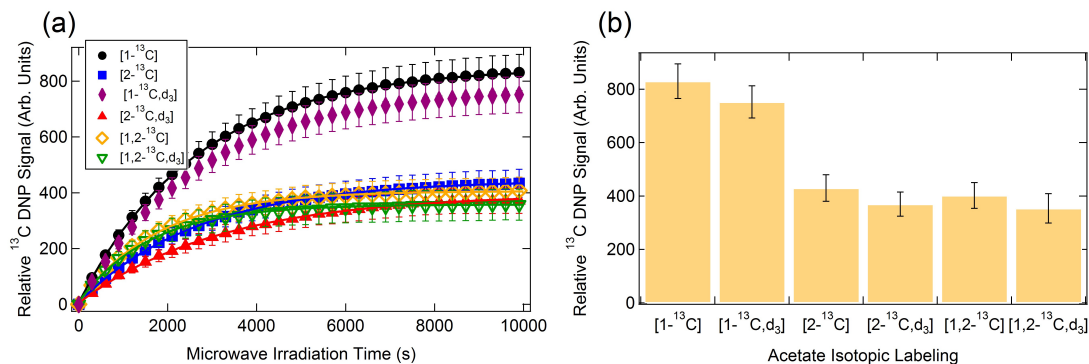


Figure 4.18. (a) ^{13}C polarization buildup curves for the differently isotopically labeled acetate molecules studied in this work. (b) Comparison of maximum polarization for these substrates. Figure adapted with permission from P. Niedbalski, C. Parish, A. Kiswandhi, Z. Kovacs, and L. Lumata, “Influence of ^{13}C Isotopic Labeling Location on Dynamic Nuclear Polarization of Acetate,” *J. Phys. Chem. A* vol. 121, pp. 3227-3233, 2017 Copyright 2017 American Chemical Society

It is expected by the thermal mixing model that deuteration of the methyl group would have a detrimental effect on polarization for the trityl radical. Because of the narrow EPR line of trityl, the Larmor frequency of the proton is well outside of the EPR line and thus the proton Zeeman system would not be in thermal contact with the EDS during irradiation. However, the Larmor frequency of deuterons is well within the trityl EPR line, so the deuteron Zeeman system is brought into contact with the EDS during irradiation, increasing the heat load, and increasing the minimum spin temperature to which the NZS may be cooled [133]. This has been witnessed previously with deuteration of the glassing solvent reducing the efficacy of polarization using trityl [153].

However, the concentration of ^1H is about 55 M in water and 13 M in glycerol, whereas the acetate concentration is 3 M. Therefore, the observed reduction in polarization brought on by ^2H labeling is small compared to what is observed when the same is done for the glassing matrix.

Combining these considerations, a plausible explanation for the observed effects is that the leakage factor leads to reduction in polarization any time there is ^{13}C labeling in the methyl group. Replacing ^1H with ^2H decreases the leakage factor, but increases the heat load of the system, ultimately resulting in a slight overall decrease of ^{13}C polarization.

Monitoring the relaxation of ^{13}C gave unexpected results. Once again, $[1-^{13}\text{C}]$ acetate gave optimal results, $[1-^{13}\text{C},\text{d}_3]$ had slightly shorter T_1 , and the other substrates all had similar, much shorter T_1 (Figure 4.19). However, there was more variation in the T_1 values measured than with polarization enhancement, with the deuterated substrates exhibiting the shortest relaxation times. This is the opposite effect of what is expected in the liquid state where deuteration is a common method of lengthening T_1 [34].

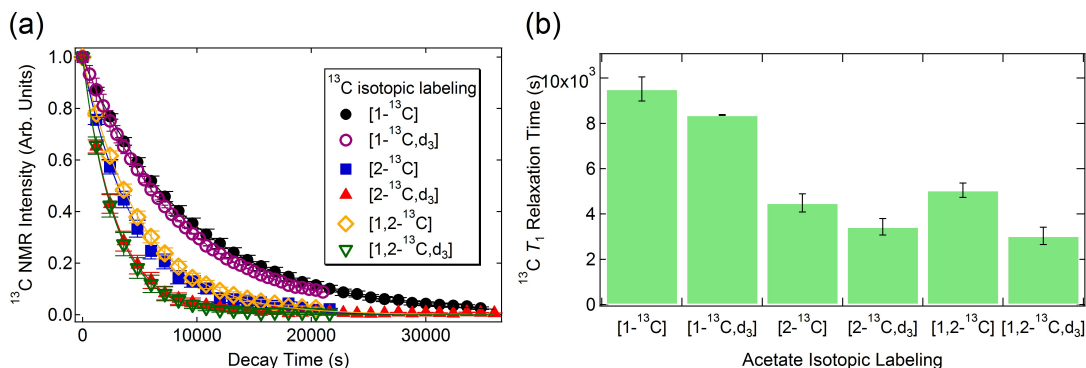


Figure 4.19. (a) ^{13}C polarization decay monitored at 3.35 T and 1.4 K for different isotopically labeled versions of acetate. (b) Comparison of T_1 relaxation time for these substrates. Figure adapted with permission from P. Niedbalski, C. Parish, A. Kiswandhi, Z. Kovacs, and L. Lumata, "Influence of ^{13}C Isotopic Labeling Location on Dynamic Nuclear Polarization of Acetate," *J. Phys. Chem. A* vol. 121, pp. 3227-3233, 2017 Copyright 2017 American Chemical Society

This counterintuitive result seems to correspond to heteronuclear “cross-talk” between ^{13}C and ^2H nuclei [160]. This results in polarization exchange between the two spin species. Due to its quadrupolar nature, ^2H relaxes much more rapidly than does ^{13}C , so as it relaxes, it draws polarization from ^{13}C spins causing ^2H to act as a “sink” for ^{13}C polarization. This results in more rapid decay of ^{13}C polarization than would otherwise be expected. This could be tested by applying a series of hard RF pulses to saturate the ^2H resonance during the decay of the ^{13}C signal, but unfortunately the HyperSense polarizer in which these data were taken does not have a two-coil probe, so this experiment is not possible with the available instrumentation.

4.5 Additives to DNP using Trityl

Gadolinium and other paramagnetic ion contrast agents have long been used for contrast-enhanced MRI. It has also been found that these same agents can further enhance polarization when added in small concentrations to DNP samples [41, 43, 46]. Because of the prevalence of gadolinium-based contrast agents and their effectiveness as DNP additives, little exploration has been done with other chemicals that have similar properties to gadolinium. One of the largest portions of optimization performed in this work has been to investigate lanthanide ions, transition metals, and novel complexes as additives in ^{13}C dissolution DNP.

In each of the following experiments, microwave frequency sweeps are taken in order to more explicitly understand the underlying polarization mechanisms and to estimate the positive polarization peak ($P(+)$). Because of limited access to the HyperSense polarizer, abbreviated sweeps were performed, only irradiating samples for a short time rather than allowing polarization to saturate at each frequency point. While this could lead to an erroneous estimate of $P(+)$ because of differing buildup time constants, previous work has shown that the $P(+)$ obtained with short irradiation time is nearly identical to that obtained with longer irradiation time [41]. Therefore, it is expected that irradiation at the measured

P(+) in each of the following experiments leads to negligible error in ultimate polarization measurements.

4.5.1 Ln(III)-DOTA Complexes

The details of the work and results described in this section may also be found in [161, 162]:

A. Kiswandhi, P. Niedbalski, C. Parish, P. Kaur, A. Martins, L. Fidelino, C. Khemtong, L. Song, A. D. Sherry, and L. Lumata, "Impact of Ho³⁺-Doping on ¹³C Dynamic Nuclear Polarization Using Trityl OX063 Free Radical," *Phys. Chem. Chem. Phys.*, vol. 18, pp 21351 - 21359, 2016 with permission from the PCCP Owner Societies.

P. Niedbalski, C. Parish, A. Kiswandhi, L. Fidelino, C. Khemtong, Z. Hayati, L. Song, A. Martins, A. D. Sherry, and L. Lumata, "Influence of Dy³⁺ and Tb³⁺ Doping on ¹³C Dynamic Nuclear Polarization," *J. Chem. Phys.*, vol. 146, pp. 014303, 2017 with the permission of AIP Publishing.

Data taken for holmium was measured primarily by A. Kiswandhi with the assistance of the author. Data for terbium, dysprosium, and gadolinium was measured by the author. L. Fidelino and C. Khemtong performed DNP on one of the Tb samples during a time when the author was unable to be present. Z. Hayati and L. Song performed the electron paramagnetic resonance studies used to support DNP results. A. Martins and A. D. Sherry provided the Ln(III)-DOTA complexes used for this study. C. Parish and A. Kiswandhi assisted with sample preparation and running DNP experiments.

Four different lanthanides caged in the DOTA complex were obtained with the intention of testing their efficacy as additional DNP doping agents. Namely, gadolinium, holmium, dysprosium, and terbium were used in conjunction with trityl OX063 for polarization of ¹³C labeled acetate.

For Gd³⁺, the optimal doping concentration has been found to be near 2 mM [41] so this was chosen as a starting point for this study. The first step was to examine the effect that

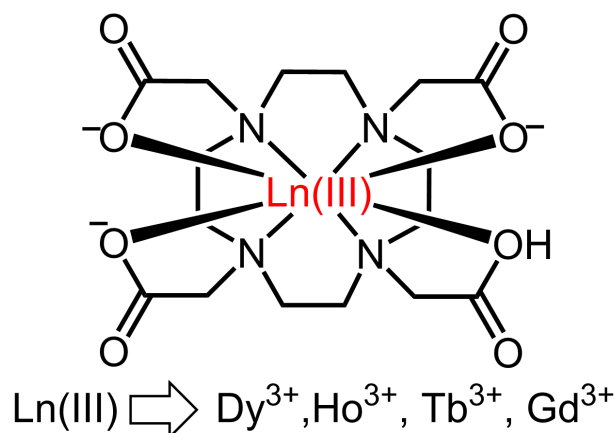


Figure 4.20. The DOTA ligand in which Dy^{3+} , Gd^{3+} , Ho^{3+} , and Tb^{3+} were chelated.

lanthanide doping had on EPR of the trityl free radical. There were 5 total samples made, each with 15 mM trityl and one for each of the Lanthanides and a control. Namely then, the five samples were 2 mM Gd^{3+} -DOTA, 2 mM Ho^{3+} -DOTA, 2 mM Tb^{3+} -DOTA, 2 mM Dy^{3+} -DOTA, and undoped.

These samples were taken to the National High Magnetic Field Laboratory (NHMFL) at Florida State University for W-band EPR. The temperature dependence of electronic T_1 was measured by finding the T_1 of trityl at various temperatures between 5 and 200 K using a Bruker Elexsys 680 spectrometer. Additionally, a field swept EPR spectrum was measured for each sample at every temperature at which T_1 was measured (Figure 4.21). It was found that there were no significant differences in the field swept spectra of trityl doped with the different lanthanides. Additionally, no EPR signal for the lanthanide ion complexes were visible.

Previous research [41] has shown that for the T_1 inversion recovery data gathered, a double exponential fitting (Equation 4.3) provides the best fit at all temperatures. This was verified by applying various fittings to the data taken. It was found that although single exponential fittings are adequate at high temperatures, the fit becomes poor at temperatures under about 50 K. A stretched exponential fitting (Equation 4.4) performs better, but still

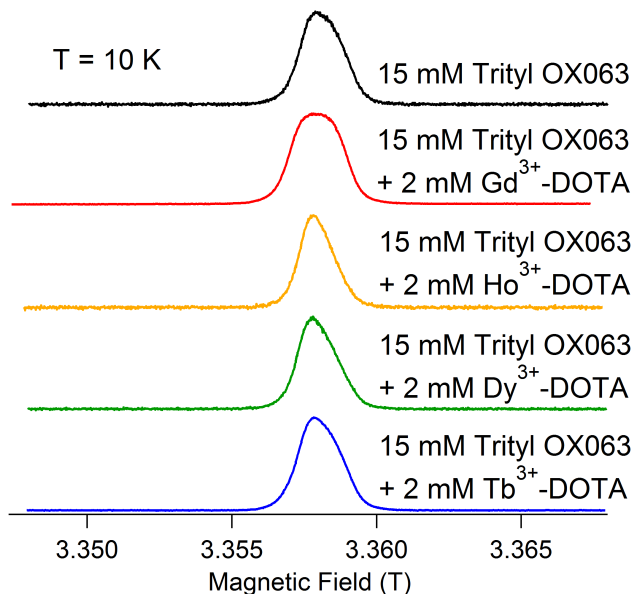


Figure 4.21. Field swept EPR spectra for trityl doped with each of the Ln(III)-DOTA complexes studied in this work. Spectra were measured at $T = 10$ K. Figure adapted from A. Kiswandhi, P. Niedbalski, C. Parish, P. Kaur, A. Martins, L. Fidelino, C. Khemtong, L. Song, A. D. Sherry, and L. Lumata, “Impact of Ho^{3+} -Doping on ^{13}C Dynamic Nuclear Polarization Using Trityl OX063 Free Radical,” *Phys. Chem. Chem. Phys.*, vol. 18, pp 21351 - 21359, 2016 with permission from the PCCP Owner Societies and from P. Niedbalski, C. Parish, A. Kiswandhi, L. Fidelino, C. Khemtong, Z. Hayati, L. Song, A. Martins, A. D. Sherry, and L. Lumata, “Influence of Dy^{3+} and Tb^{3+} Doping on ^{13}C Dynamic Nuclear Polarization,” *J. Chem. Phys.*, vol. 146, pp. 014303, 2017 with the permission of AIP Publishing.

converges poorly at temperatures below 20 K. The double exponential fitting was able to fit the data well at all temperatures (Figure 4.22).

$$f(t) = A - Be^{-t/T_{1a}} - Ce^{-t/T_{1b}} \quad (4.3)$$

$$f(x) = A - Be^{-(t/T_1)^\beta} \quad (4.4)$$

In the equation for the double exponential fit (Equation 4.3), two time constants are found. The longer of the two corresponds to the electron longitudinal relaxation time constant (T_1), while the shorter describes lesser electron-electron cross relaxation effects [163, 164].

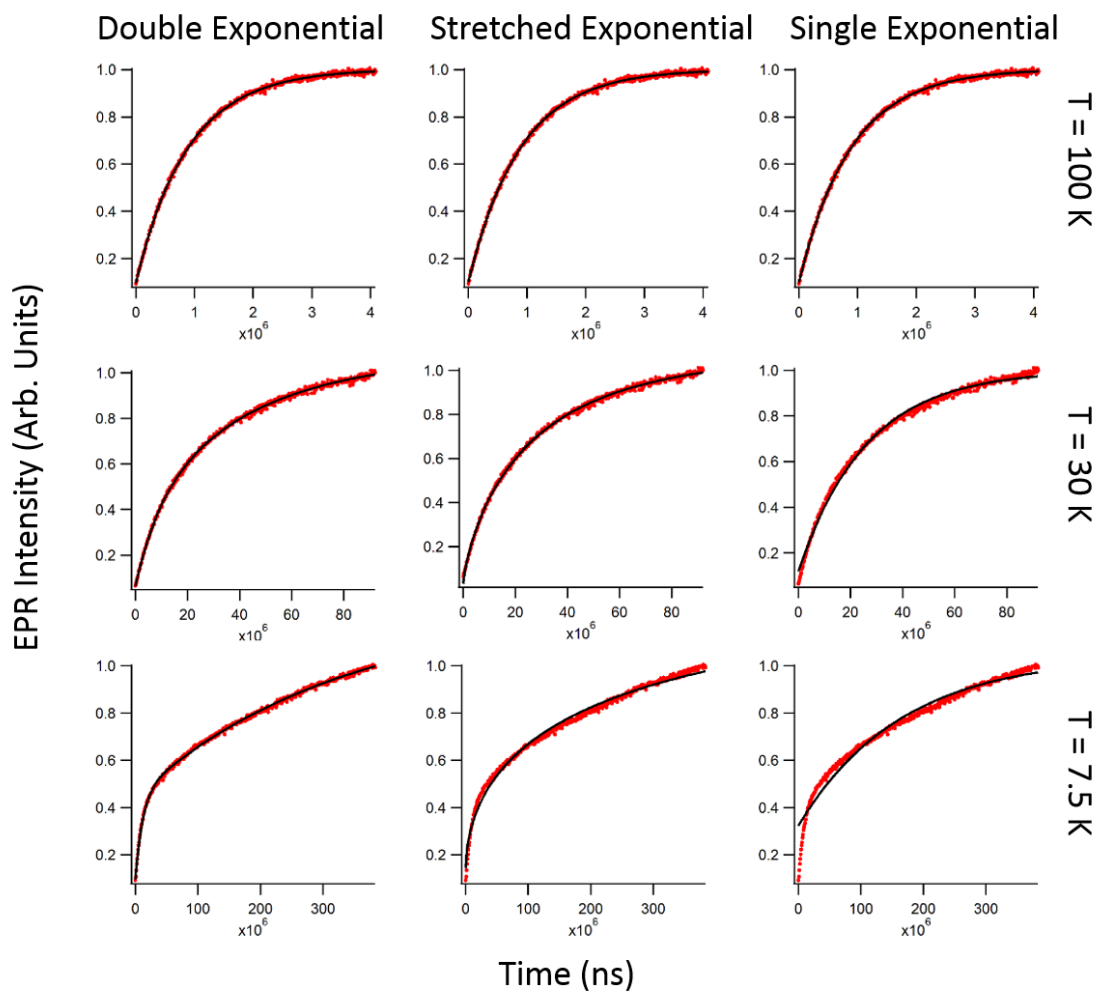


Figure 4.22. Double exponential, stretched exponential, and single exponential fitting is shown for the collected inversion recovery data for the reference sample (15 mM trityl OX063) at temperatures of 100 K, 30 K, and 7.5 K. From this it is clear that the double exponential fitting results in the most consistent fit.

From the data collected, it is apparent that each of the Ln(III)-DOTA complexes studied has a similar effect on the electron relaxation of trityl, showing very nearly identical temperature dependence. By fitting segments of the data to a power law fit, $T_1^{-1} = AT^\alpha$, the dominant electron relaxation mechanism may be inferred (Figure 4.23).

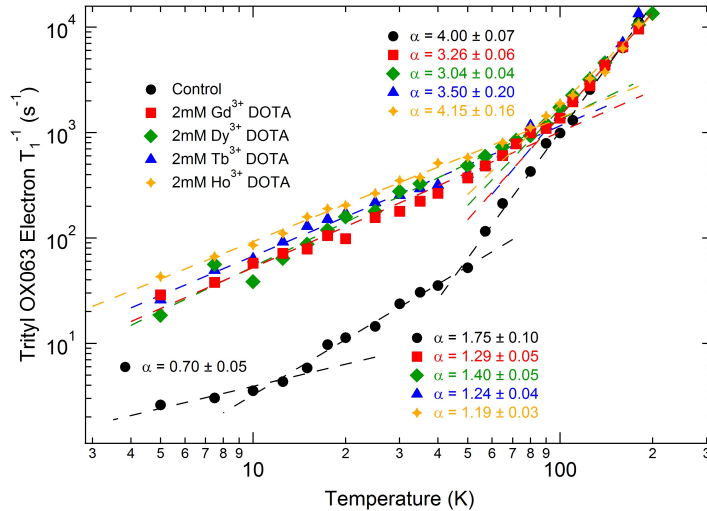


Figure 4.23. T_1^{-1} data obtained using double exponential fitting plotted versus temperature on a log-log scale. Power law fitting has been performed on segments of the plot and α is shown for each region and sample. Figure adapted from rA. Kiswandhi, P. Niedbalski, C. Parish, P. Kaur, A. Martins, L. Fidelino, C. Khemtong, L. Song, A. D. Sherry, and L. Lumata, “Impact of Ho^{3+} -Doping on ^{13}C Dynamic Nuclear Polarization Using Trityl OX063 Free Radical,” *Phys. Chem. Chem. Phys.*, vol. 18, pp 21351 - 21359, 2016 with permission from the PCCP Owner Societies and from P. Niedbalski, C. Parish, A. Kiswandhi, L. Fidelino, C. Khemtong, Z. Hayati, L. Song, A. Martins, A. D. Sherry, and L. Lumata, “Influence of Dy^{3+} and Tb^{3+} Doping on ^{13}C Dynamic Nuclear Polarization,” *J. Chem. Phys.*, vol. 146, pp. 014303, 2017 with the permission of AIP Publishing.

For temperatures above about 70 K, all of the Ln(III)-DOTA doped samples had $\alpha \approx 3$. This suggests that electron relaxation is dominated by multiple-phonon Orbach processes [165]. Likewise, in the reference sample at temperatures above 50 K, Orbach processes dominate. Below 70 K in Ln(III)-doped samples, α is much closer to one, implying that relaxation is mostly dominated by the single phonon direct process, with perhaps a small contribution from the two phonon Raman process. In the reference sample, $\alpha \approx 2$ for

temperatures between 12.5 and 50 K, pointing to the dominance of the Raman process in this range. Finally, below 10 K, the direct process dominates relaxation in the reference sample, with $\alpha \approx 1$. Though the instrumentation used to gather this data is incapable of reaching 1 K, it is entirely likely that these trends will continue down to the temperature regime in which DNP takes place. Therefore, the direct process very likely is the dominant electron relaxation process for trityl in all five samples at DNP temperatures of 1 - 2 K.

Despite very similar EPR results for electron relaxation of trityl doped with terbium, dysprosium, gadolinium, and holmium, ^{13}C DNP yielded more varied results. Samples were prepared with 3 M $[1-^{13}\text{C}]$ sodium acetate and 15 mM trityl in 1:1 v/v glycerol:water and doped with varying concentrations of the Ln(III)-DOTA complexes in order to determine the optimum concentration for each. The optimum concentration for gadolinium is well established as 2 mM [41, 42, 43], so the only Gd^{3+} sample studied was at that concentration. Holmium was studied at concentrations of 0.5, 1, 2, 3, 4, 6, 8, and 10 mM. Dysprosium samples were made at concentrations of 1, 1.5, 2, 3, and 4 mM. Terbium samples were made at concentrations of 0.125, 0.25, 0.5, 1, 1.5, 2, 3, and 4 mM. In addition to each of these samples, a reference sample with no added lanthanide was also prepared.

For each sample mentioned above, DNP microwave sweeps were measured so that the optimum irradiation frequency could be estimated. Samples were irradiated for three minutes in 5 MHz intervals in the HyperSense polarizer at 3.35 T and 1.2 K. The results of these sweeps are shown in Figure 4.24. The addition of lanthanide, even in low concentrations, has a significant effect on the frequency sweep. The overall spectrum is narrowed with the positive polarization peak ($\text{P}(+)$) shifted to higher frequency and the negative polarization peak ($\text{P}(-)$) shifted to lower frequency. Of note is the Tb^{3+} -doped sample, which requires a significantly lower concentration (<0.5 mM) to yield the same spectrum alteration as the other lanthanides.

After DNP sweeps had been conducted, samples were irradiated at the positive polarization peak ($\text{P}(+)$) and the ^{13}C polarization buildup monitored every three minutes (Figure

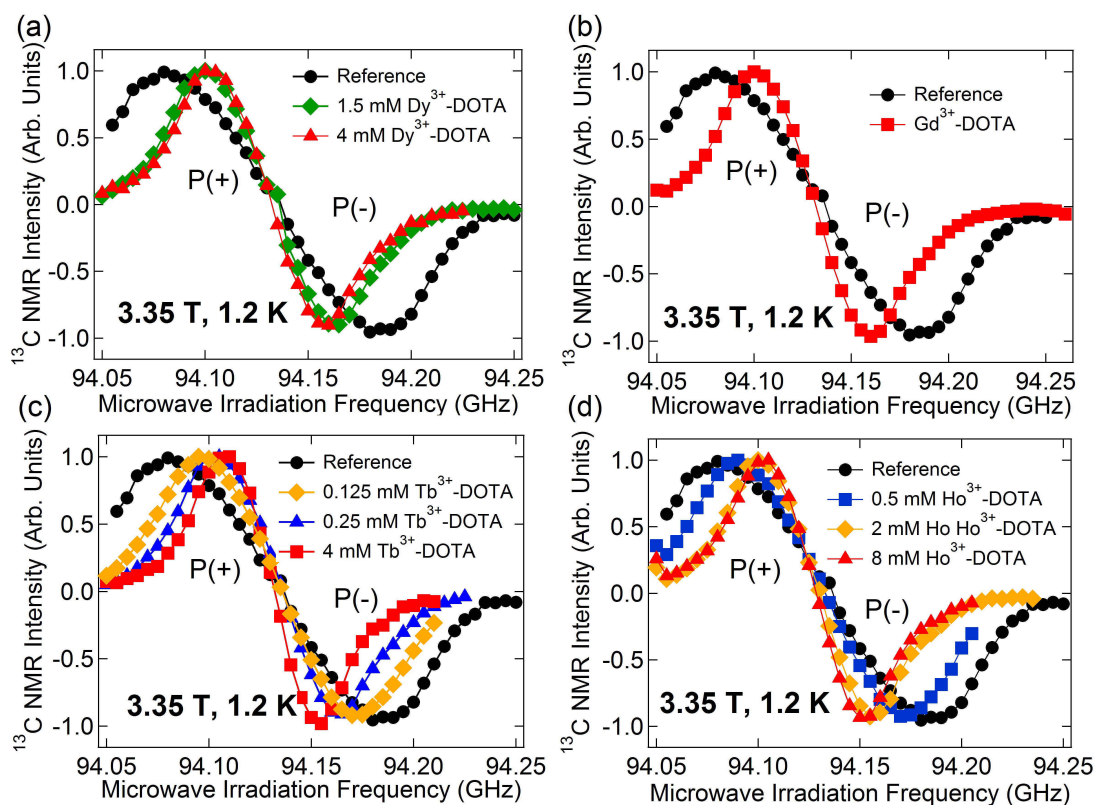


Figure 4.24. DNP microwave sweeps for samples doped with (a) gadolinium, (b) holmium, (c) dysprosium, and (d) terbium. The sweep of a control sample with no lanthanide is shown in each plot for reference. Figure adapted from A. Kiswandhi, P. Niedbalski, C. Parish, P. Kaur, A. Martins, L. Fidelino, C. Khemtong, L. Song, A. D. Sherry, and L. Lumata, "Impact of Ho^{3+} -Doping on ^{13}C Dynamic Nuclear Polarization Using Trityl OX063 Free Radical," *Phys. Chem. Chem. Phys.*, vol. 18, pp 21351 - 21359, 2016 with permission from the PCCP Owner Societies and from P. Niedbalski, C. Parish, A. Kiswandhi, L. Fidelino, C. Khemtong, Z. Hayati, L. Song, A. Martins, A. D. Sherry, and L. Lumata, "Influence of Dy^{3+} and Tb^{3+} Doping on ^{13}C Dynamic Nuclear Polarization," *J. Chem. Phys.*, vol. 146, pp. 014303, 2017 with the permission of AIP Publishing.

4.25). Microwave irradiation of the sample was allowed to continue until the ^{13}C polarization had approximately reached a plateau. In this manner, the optimum concentration was able to be determined for each Ln(III) complex (Figure 4.26). Holmium, like gadolinium, had an optimum concentration of 2 mM. Dysprosium was near to this value at 1.5 mM. Terbium, on the other hand, only required 0.25 mM to optimize the buildup. Using these concentrations of Ln(III), ^{13}C polarizations were able to be reached that were about 3-fold (gadolinium), 2.5-fold (terbium and holmium) and 1.5-fold (dysprosium) over the undoped reference sample .

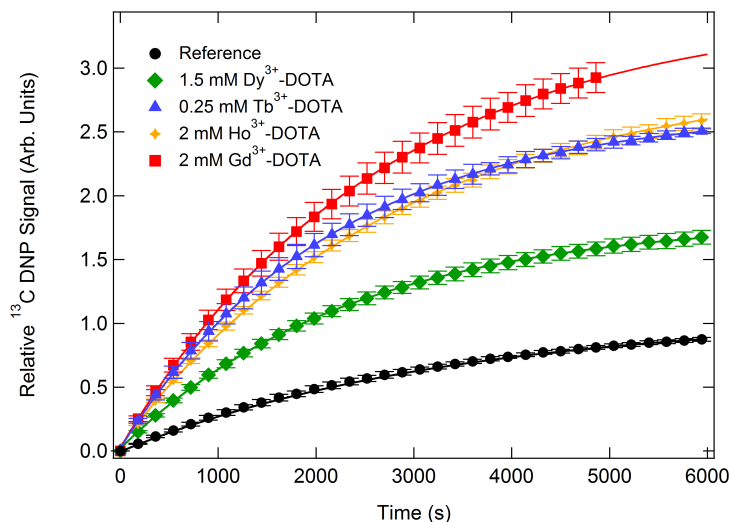


Figure 4.25. Buildup curves for ^{13}C sodium acetate doped with 15 mM trityl OX063 and further doped with the optimal concentration of an Ln(III)-DOTA complex. Figure adapted from reference [161] with permission from PCCP Owner Societies and from P. Niedbalski, C. Parish, A. Kiswandhi, L. Fidelino, C. Khemtong, Z. Hayati, L. Song, A. Martins, A. D. Sherry, and L. Lumata, “Influence of Dy³⁺ and Tb³⁺ Doping on ^{13}C Dynamic Nuclear Polarization,” *J. Chem. Phys.*, vol. 146, pp. 014303, 2017 with the permission of AIP Publishing.

These results for the solid state polarization of ^{13}C are in conflict with a previous report which saw polarization improvement when using gadolinium and holmium but not other lanthanide ions [46]. The most probable explanation for this discrepancy is that the previous report used lanthanide chlorides, while the present work used lanthanides chelated within

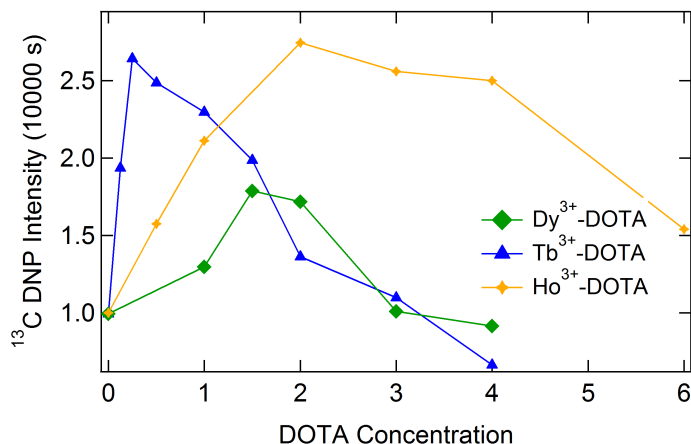


Figure 4.26. The ^{13}C DNP intensity achieved at 10,000 s for samples doped with varying concentrations of holmium, dysprosium, and terbium. For some samples, the build up curve was stopped prior to 10,000 s and the curve extrapolated to that time using a single exponential fit. Figure adapted from A. Kiswandhi, P. Niedbalski, C. Parish, P. Kaur, A. Martins, L. Fidelino, C. Khemtong, L. Song, A. D. Sherry, and L. Lumata, “Impact of Ho^{3+} -Doping on ^{13}C Dynamic Nuclear Polarization Using Trityl OX063 Free Radical,” *Phys. Chem. Chem. Phys.*, vol. 18, pp 21351 - 21359, 2016 with permission from the PCCP Owner Societies and from P. Niedbalski, C. Parish, A. Kiswandhi, L. Fidelino, C. Khemtong, Z. Hayati, L. Song, A. Martins, A. D. Sherry, and L. Lumata, “Influence of Dy^{3+} and Tb^{3+} Doping on ^{13}C Dynamic Nuclear Polarization,” *J. Chem. Phys.*, vol. 146, pp. 014303, 2017 with the permission of AIP Publishing.

the DOTA complex, which could have a significant effect on the magnetic and relaxation properties of the $\text{Ln}(\text{III})$ ions [166, 167, 168]. In particular, the ligand could change the relaxation rate, and thus relaxivity, of the lanthanide ion caged within it.

The polarization increase may be explained in terms of thermal mixing by considering electronic T_1 [41, 46, 137, 138]. Using the older, more qualitative picture of thermal mixing (Equation 2.18), it can be seen that shortening the electronic T_1 reduces the parameter η , which increases the theoretical maximum polarization. However, the sweep results are perhaps better explained using the model discussed in Appendix B. As can be seen in Figure 4.21, the EPR spectra for each of the five samples studied using EPR remain approximately consistent despite the differing dopants used. Because of this as well as the relatively symmetric spectra observed, the trityl EPR spectra can be approximated by a Gaussian function

for all samples. Then, holding all other parameters constant and shortening electronic T_1 results in increased polarization concurrent with narrowing of the DNP spectra which agrees well with the results presented in Figure 4.24. Of course, not all of the samples gave equivalent polarization which suggests that there are effects that are not being taken into account. Most likely, there are differences in nuclear relaxation brought on by T_1 relaxation and spin diffusion that lead to differences in polarization efficiency. These particular interactions are difficult to quantify, but could be taken into account as part of the leakage factor f .

It should be noted that a purely quantum mechanical model combining contributions from the cross and solid effects could also be used to describe the observed effects [147, 148]. Specifically, as electronic T_1 is shortened, there is a greater contribution from the cross effect, which serves to narrow the DNP spectra and increase polarization. However, it was decided that this is not the ideal description of the collected data because of the features of the control sample's DNP spectrum. Specifically, the positive and negative polarization peaks for this sample are separated by 110 MHz. Polarization via a pure solid effect would result in a peak separation of twice the ^{13}C Larmor frequency (70 MHz), and cross effect contributions would narrow the peak separation. Because this model is inadequate to predict the control DNP spectrum, thermal mixing seems to be the more appropriate model.

It is not entirely clear why samples required comparatively small concentrations of terbium. One possible explanation is that terbium and holmium are non-Kramers ions, while dysprosium and gadolinium are Kramers ions. This is significant because it has been proposed that a quadrupolar cross relaxation mechanism may be active between Kramers and non-Kramers ions [169]. Trityl, having a single free electron, would be considered Kramers, so the quadrupolar cross relaxation would be present between it and terbium, causing greater relaxation with less paramagnetic dopant. However, by this explanation, it would be expected that holmium would require a very small concentration as well, which was not borne out experimentally. More work is necessary to fully understand the interactions between lanthanide ions and the trityl free radical.

Once the optimum concentration of each of the lanthanides was determined, samples were prepared at those concentrations for polarization and subsequent dissolution. Samples were polarized for two hours, dissolved in 4 mL deionized water, and sent to a 10 mm NMR tube in an adjacent 9.4 T high resolution NMR magnet. The shuttling time was approximately 8 s, and, once in the spectrometer, scans were taken using 5° RF pulses every 2 s. This enabled the calculation of the liquid-state ^{13}C polarization enhancement and liquid-state T_1 . The polarization enhancement was calculated by measuring the integrated intensity of the first hyperpolarized signal measured and the thermal equilibrium NMR scan. These values were then plugged into the equation

$$\varepsilon = \frac{I_{HP}}{I_{Th}} \frac{1}{\sin \theta}. \quad (4.5)$$

In the above equation, θ is the tip angle of the RF pulse used to monitor the hyperpolarized signal. Liquid state T_1 is calculated by fitting to the integrated intensities of the hyperpolarized ^{13}C NMR signals vs. time an adapted exponential which takes into account the effect of RF pulsing.

$$M_z(t) = M_0 \sin \theta (\cos \theta)^{t/TR} e^{-t/T_1} \quad (4.6)$$

In this equation, M_z is the intensity of the ^{13}C NMR signal at time t , M_0 is the initial intensity, θ is the tip angle of the RF pulse, and TR is the time delay between scans.

Of the lanthanides studied, only gadolinium has a significant effect on the liquid-state relaxation time, greatly shortening T_1 (Figure 4.27). None of the other three has a noticeable effect on the relaxation of the NMR signal. This is due to the relaxation rate of the electrons in the outermost f orbital of the ions [165]. In gadolinium, these electrons relax slowly, leading to a strong paramagnetic effect and greatly increasing the relaxation rate of adjacent nuclei. In the other lanthanides, these outermost electrons relax very rapidly, leading to a weak magnetic effect on adjacent nuclei.

This directly correlates to the enhancement of liquid-state NMR signals. While gadolinium-doped samples had the largest solid-state polarization, their liquid-state polarization enhancement is comparable to holmium and terbium samples. The greater rate of relaxation of nuclear spins in these samples during the 8 s shuttling time from the polarizer to the NMR magnet reduces the polarization to a level similar to that of holmium and terbium-doped samples. Dysprosium, while it does increase the liquid-state polarization, performs significantly worse than the other three lanthanides studied, a direct result of the lower solid state polarization achieved when using Dy^{3+} -DOTA.

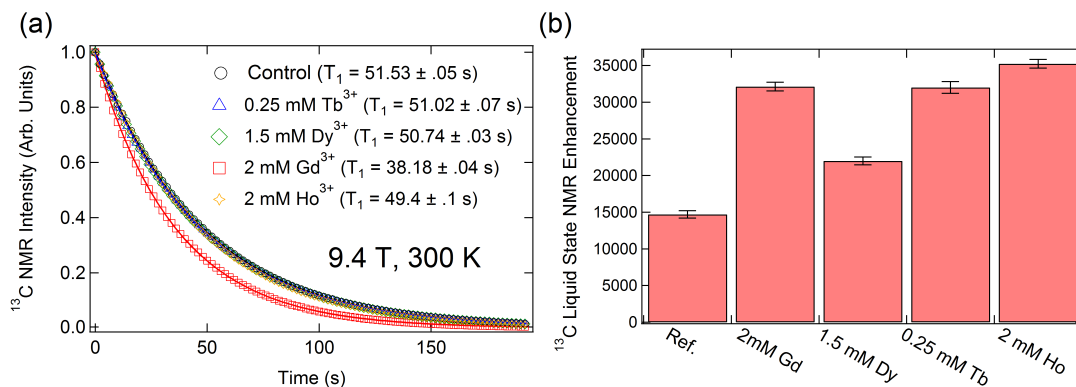


Figure 4.27. (a) Decay of hyperpolarized ^{13}C signal after dissolution at 9.4 T and room temperature. Data are fit using Equation 4.6 and T_1 displayed for each sample. (b) Comparison of liquid-state enhancement when using each of the Ln(III) complexes.

4.5.2 Trimeric Gadolinium Complex

The details of the work and results described in this section may also be found in [170]:

P. Niedbalski, C. Parish, Q. Wang, A. Kiswandhi, Z. Hayati, L. Song, and L. Lumata, “ ^{13}C Dynamic Nuclear Polarization Using a Trimeric Gd^{3+} Complex as an Additive,” *J. Phys. Chem. A*, vol. 121, pp.5127-5135, 2017, Copyright 2017 American Chemical Society

C. Parish, Q. Wang, and A. Kiswandhi assisted the author with monitoring polarization experiments. Z. Hayati and L. Song performed the EPR measurements used to support polarization data. All sample preparation and data analysis was performed by the author.

While there are a wealth of gadolinium complexes of all shapes and sizes, the gadolinium complexes that have been heavily studied in DNP have been monomeric [42, 43]. That is, there is a single Gd^{3+} ion chelated within a stable ligand. However, there are a multitude of gadolinium contrast agents that use multiple Gd^{3+} ions within a single molecule, some of which have been used in DNP studies [67, 98, 102]. However, the performance of monomeric and multimeric gadolinium complexes has not been directly compared. In order to evaluate similarities and differences between such ligands, a trimeric gadolinium complex (3-Gd) was obtained from collaborators at the Technical University of Denmark and Albeda Research (Copenhagen, Denmark). The complex consists of three Gd^{3+} -DOTA's bonded together (Figure 4.28).

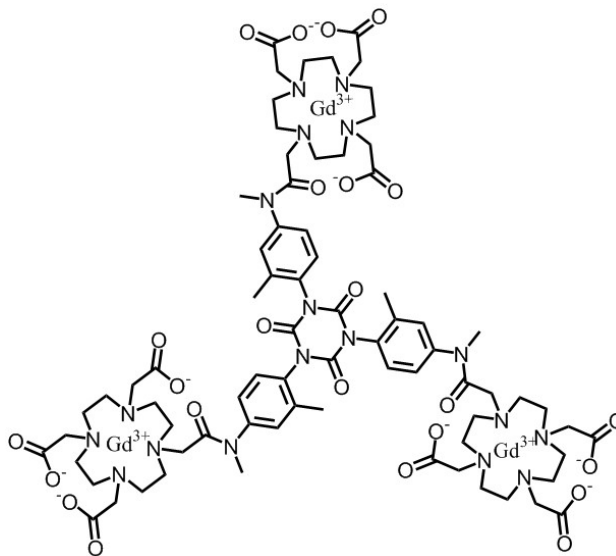


Figure 4.28. The trimeric gadolinium complex studied as a DNP additive

Similar to the manner described above, 3-Gd was studied extensively for use in DNP. In this case, however, DNP was performed prior to EPR studies so that the optimum concentration of 3-Gd could be used in preparing EPR samples. Samples of 100 μL were made with $[1\text{-}^{13}\text{C}]$ sodium acetate and 15 mM trityl OX063 in a glassing matrix of 1:1 v/v glycerol:water. These were further doped with a concentration of 3-Gd between 0 and 2 mM.

Specifically, the concentrations studied were 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1, 1.5, and 2 mM. Lower concentrations were studied from the start due to the expectation that, with three Gd^{3+} ions for every 3-Gd molecule, only one third of the amount used for monomeric gadolinium complexes would be needed.

Samples were polarized at 3.35 T and 1.2 K using the HyperSense polarizer. To begin with, microwave frequency sweeps were measured starting at 94.05 GHz and swept in steps of 5 Mhz until the complete DNP spectrum profile was complete. Polarization was reasonably fast, so the duration of each step was set at 90 s and resulted in smooth data.

After the DNP spectra were obtained, samples were irradiated at the positive polarization peak and the ^{13}C polarization buildup monitored. The majority of samples were polarized for two hours, but samples at less significant concentrations were only studied for 1.5 hours.

The buildup results showed that ^{13}C polarization reached a maximum when 0.4 mM 3-Gd was used. This optimum concentration yielded a solid-state ^{13}C polarization approximately equal to that achieved with monomeric Gd^{3+} -DOTA-doping. At concentrations higher than this, there was a slight reduction in polarization enhancement up to 0.8 mM, and a significant dropoff at 2 mM.

Once solid state DNP optimization conditions were determined, dissolution experiments were performed for three different types of samples: Control, 2 mM Gd^{3+} -DOTA, and 0.4 mM 3-Gd. Additionally, the amount of sample polarized was varied as well, ultimately running dissolutions on 20 μL , 100 μL , and a simulated 200 μL sample for each of the three sample types. The 200 μL sample is simulated by polarizing 100 μL of sample and mixing with 100 μL of de-ionized water, 0.8 mM 3-Gd, or 4 mM Gd^{3+} -DOTA.

It was found that, regardless of the initial sample volume, the liquid state ^{13}C NMR enhancements were approximately equal for samples with 3-Gd and Gd^{3+} -DOTA. Furthermore, the ^{13}C relaxation times for the two different types of gadolinium-doped samples were approximately the same regardless of the initial sample volume.

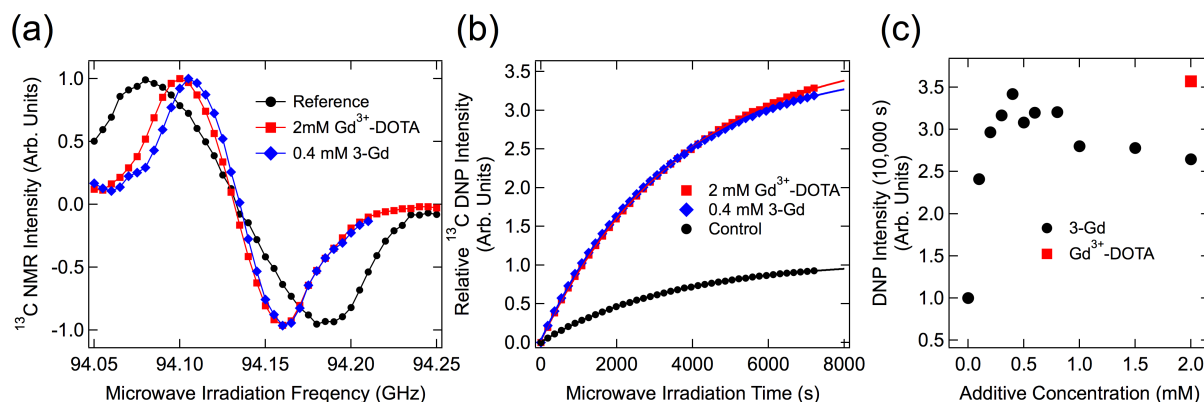


Figure 4.29. (a) Microwave frequency sweeps for samples of 3 M $[1-^{13}\text{C}]$ sodium acetate in 1:1 v/v glycerol:water doped with 15 mM trityl OX063 and additionally doped with either no additive, 2 mM Gd^{3+} -DOTA, or 0.4 mM 3-Gd. (b) ^{13}C polarization buildup curves for the three samples described in (a). (c) 3-Gd Concentration dependence of the ^{13}C DNP intensity. ^{13}C DNP intensity achieved with 2 mM Gd^{3+} -DOTA-doping is shown for reference.

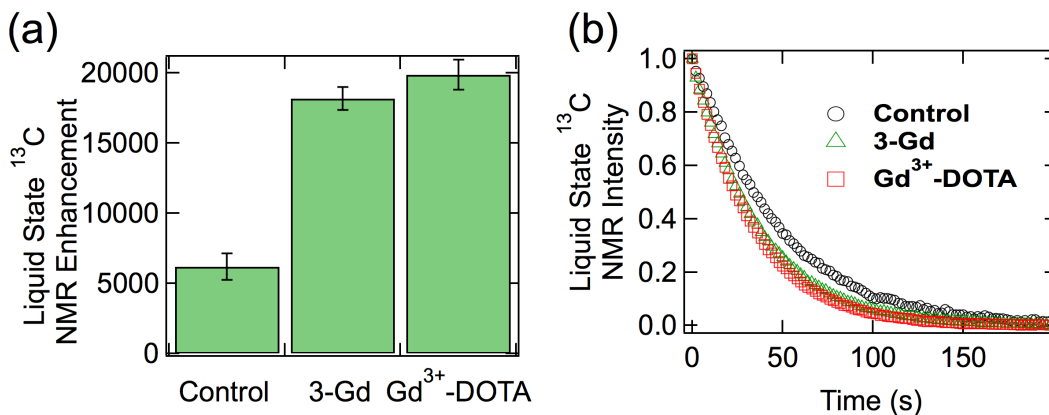


Figure 4.30. (a) Comparison of liquid state ^{13}C NMR enhancement for control, 3-Gd, and Gd^{3+} -DOTA samples. (b) Liquid state ^{13}C NMR signal relaxation for dissolutions of a 100 μL samples for the three aforementioned samples. Figure adapted with permission from P. Niedbalski, C. Parish, Q. Wang, A. Kiswandhi, Z. Hayati, L. Song, and L. Lumata, “ ^{13}C Dynamic Nuclear Polarization Using a Trimeric Gd^{3+} Complex as an Additive,” *J. Phys. Chem. A*, vol. 121, pp.5127-5135, 2017, Copyright 2017 American Chemical Society

DNP results were supported by W-band EPR studies in which field swept EPR spectra and inversion recovery T_1 data was collected at a series of temperatures between 5 and 200 K. EPR spectra were fit using Voigt profile fitting, which showed that each spectrum was dominated by a Gaussian lineshape, suggesting that the spectra can be roughly approximated by a Gaussian function.

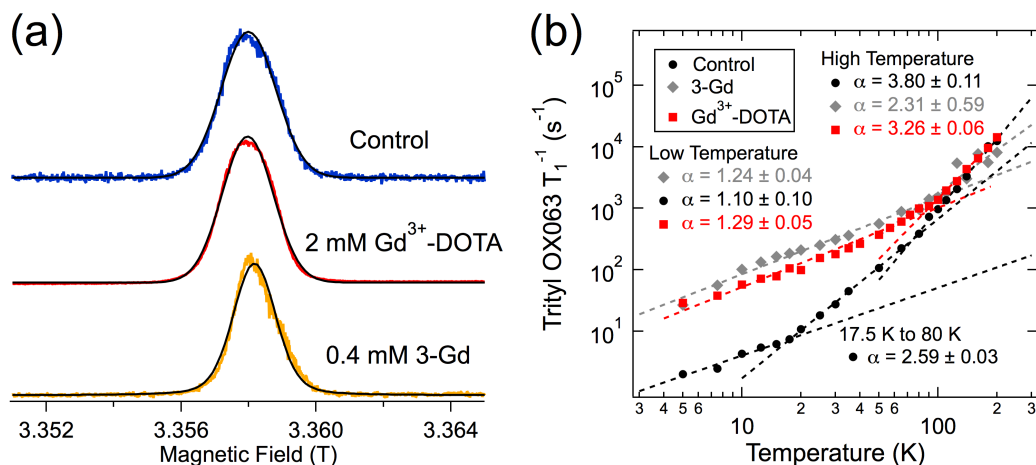


Figure 4.31. (a) Field Swept EPR spectra for samples of 1:1 v/v glycerol:water containing 3 M [1-¹³C] sodium acetate, 15 mM trityl OX063, and either no additive, 2 mM Gd³⁺-DOTA, or 0.4 mM 3-Gd (b) Temperature dependence of the trityl OX063 electronic relaxation rate for the aforementioned three samples. Figure adapted with permission from P. Niedbalski, C. Parish, Q. Wang, A. Kiswandhi, Z. Hayati, L. Song, and L. Lumata, “¹³C Dynamic Nuclear Polarization Using a Trimeric Gd³⁺ Complex as an Additive,” *J. Phys. Chem. A*, vol. 121, pp.5127-5135, 2017 Copyright 2017 American Chemical Society

As seen with monomeric lanthanide-ion complexes, the electronic T_1 of trityl is drastically shortened with the addition of 3-Gd which agrees with the explanation for the increase in polarization presented in the previous section.

Overall, the performance of 3-Gd as a DNP additive was essentially identical to Gd³⁺-DOTA. However, this is achieved with a lesser concentration of Gd³⁺ ions in the 3-Gd case, as only 0.4 mM 3-Gd is required for optimum effect as compared to 2 mM for Gd³⁺-DOTA. This is a result of the increased relaxivity of the 3-Gd molecule. Because of its large size, the molecular tumbling of 3-Gd is slow when compared to the smaller DOTA complex

[171, 172]. This enables a longer relaxation time for the electrons of the Gd^{3+} ions, which increases their effect on other spin species within the sample. A similar effect is witnessed in other multimeric gadolinium complexes used as contrast agents [171, 172].

4.5.3 Transition Metals

The details of the work and results described in this section may also be found in [173]:

P. Niedbalski, C. Parish, Q. Wang, Z. Hayati, L. Song, Zackary I. Cleveland and L. Lumata, “Enhanced Efficiency of ^{13}C Dynamic Nuclear Polarization by Superparamagnetic Iron Oxide Nanoparticle,” *J. Phys. Chem. C*, vol. 121, pp. 19505-19511, 2017, Copyright 2017 American Chemical Society.

and

P. Niedbalski, C. Parish, Q. Wang, Z. Hayati, L. Song, A. Martins, A. D. Sherry, and L. Lumata, “Transition Metal Doping Reveals Link between Electron T_1 Reduction and ^{13}C Dynamic Nuclear Polarization Efficiency,” Manuscript in Preparation

C. Parish and Q. Wang assisted in monitoring polarization experiments. Z. Hayati and L. Song performed the supporting EPR measurements. Z. I. Cleveland provided Feraheme which was the subject of the former paper while A. Martins and A. D. Sherry provided transition metal ion complexes which were the subject of the latter.

Though lanthanides have been the most heavily used as additives for DNP, there are many elements with similarly paramagnetic properties. Most notably, there are many transition metal ions with high magnetic moments that could lead to similar DNP behavior as seen with lanthanide ions. Metals with previous use as contrast agents in MRI (iron, manganese, copper, and cobalt) were chosen as promising additives [174, 175, 176, 177, 178, 179, 180, 181]. Because of possible biomedical implications, the metals were chelated within complexes for this study. In particular, manganese, copper, and cobalt were caged within the NOTA complex (Figure 4.32). Iron, on the other hand, was studied in the form of the superparamagnetic iron oxide nanoparticle ferumoxytol, an FDA approved treatment for iron-deficiency anemia.

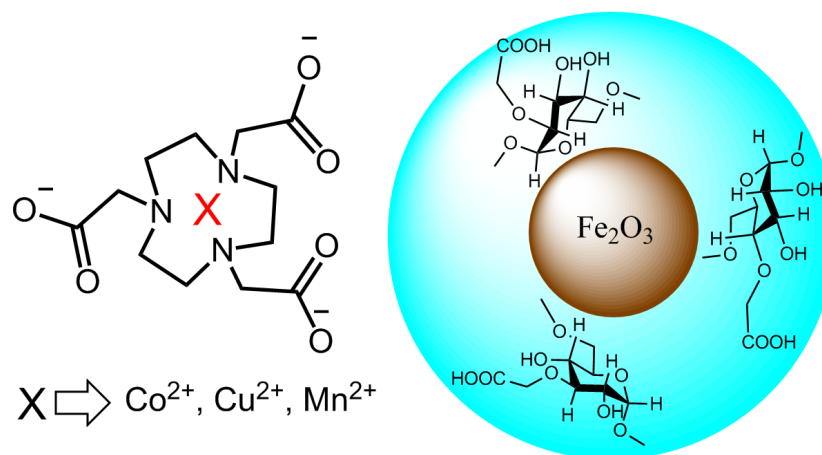


Figure 4.32. The NOTA ligand in which Co^{2+} , Cu^{2+} , and Mn^{2+} were chelated (left) and the ferumoxytol nanoparticle (right).

In the same manner as above, different concentrations of the metals were added to samples containing 3 M $[1\text{-}^{13}\text{C}]$ sodium acetate and 15 mM trityl in 1:1 v/v glycerol:water. The concentrations studied are shown in Table 4.3. It should be noted that the concentration of interest is the concentration of magnetic centers, which for Cobalt, Copper, and Manganese is simply the concentration of metal complex. Feraheme on the other hand consists of a large (≈ 5 nm) iron oxide core surrounded by dextrose [182, 183, 184]. Each nanoparticle acts as a single magnetic center despite having a very large number of iron atoms in each. Unfortunately, because of nanoparticle polydispersity, it is difficult to accurately calculate the concentration of nanoparticles in a given sample. Therefore, the concentration of elemental iron is used to describe Feraheme samples because it is more easily and accurately determined.

Samples were studied in the same manner as described above. Microwave frequency sweeps were performed for samples except where deemed unnecessary. This was the case for samples where the optimum irradiation frequency was equal at concentrations above and below the concentration in question. For all samples, the frequency was swept in intervals

Table 4.3. Concentrations of metals added to DNP samples. Optimum concentrations are shown in bold.

Metal	Concentrations (mM)
Cobalt	0.5, 1 , 2, 8, 14, 20
Copper	2, 4 , 6, 10
Manganese	1, 2, 3 , 4, 6
Iron (Feraheme)	0.2, 2, 3.5, 5, 6.5, 8, 9.5, 11 , 12.5, 14, 15.5, 17, 20, 24.5, 29, 40, 60, 80, 100

of 5 MHz and each step measured for 90 or 120 s depending on the speed and intensity of the polarization buildup.

From the DNP spectra (Figure 4.33), P(+) was estimated and samples irradiated at that frequency for 1-2 hours, monitoring the ^{13}C NMR signal every 3 minutes using a small tip angle pulse. These data were extrapolated to 10,000 s using a single exponential fit and the extrapolated intensity plotted vs metal concentration.

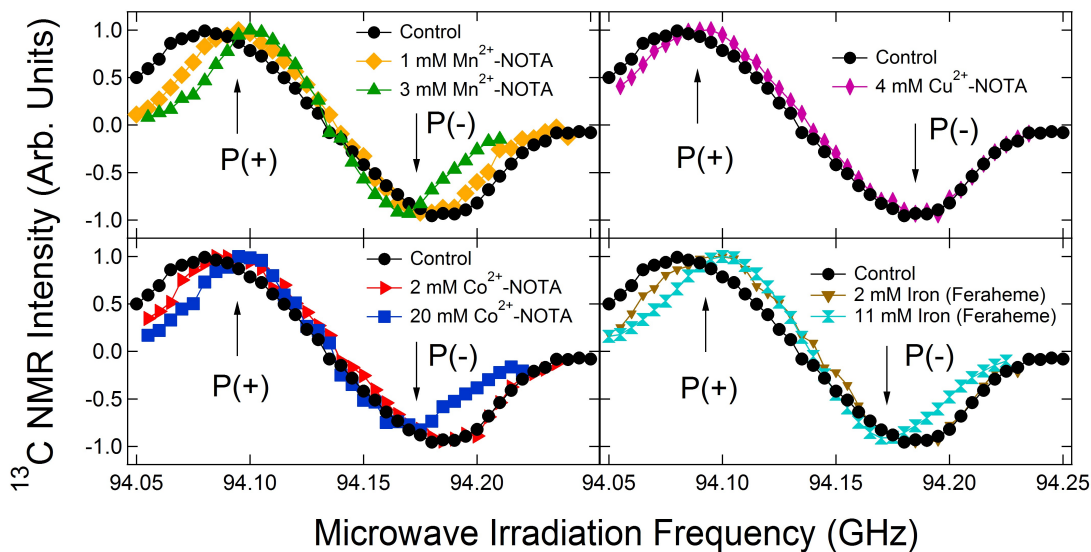


Figure 4.33. Normalized microwave irradiation frequency sweeps for samples of 3 M $[1-^{13}\text{C}]$ sodium acetate and 15 mM trityl OX063 doped with different concentration of the four different transition metals that were studied.

Feraheme and manganese produced the familiar narrowed DNP spectra as was observed with the addition of lanthanide ions. Copper and cobalt, on the other hand, had very little

effect on the DNP spectra at low concentrations. At high concentrations, cobalt led to narrowing of the spectrum to a similar degree as achieved with the other additives.

The degree to which ^{13}C polarization was enhanced correlated with the narrowing of DNP spectra. Feraheme and manganese, with their significant narrowing effect were very effective as additives, leading to a maximum increase in ^{13}C polarization of nearly 3 and 2.5-fold over the reference sample, respectively (Figures 4.34 and 4.35). Copper and cobalt had very little effect on ^{13}C polarization, only increasing it to less than 1.2-fold over reference. From these data, the optimum concentration of each metal dopant was determined as being the lowest concentration that produces at least 90% of the highest polarization achieved (Table 4.3).

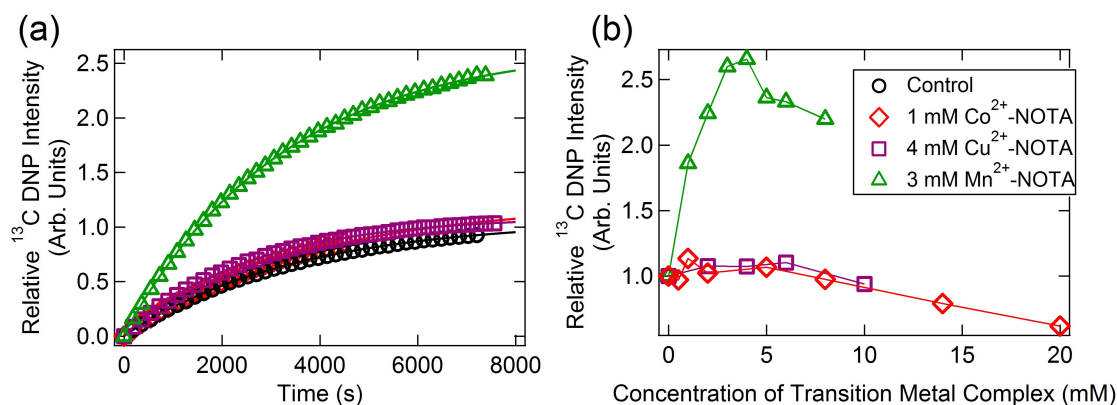


Figure 4.34. (a) ^{13}C polarization buildup curves for samples of 3 M $[1-^{13}\text{C}]$ sodium acetate and 15 mM trityl OX063 doped with different concentrations of the 3 metals chelated within NOTA. Data are normalized such that the control builds up to unity. (b) Dependence of ^{13}C DNP intensity on the concentration of transition metal ion complex.

In order to understand these results, W-band EPR was performed on samples containing 3 M $[1-^{13}\text{C}]$ sodium acetate, 15 mM trityl OX063, and the optimum concentration of transition metal in 1:1 v/v glycerol:water. As done previously, trityl's electronic T_1 and field swept EPR spectrum were measured at a series of temperatures between 5 and 200 K. These experimental data were analyzed in the same manner as described in Section 4.5.1 (Figure 4.36).

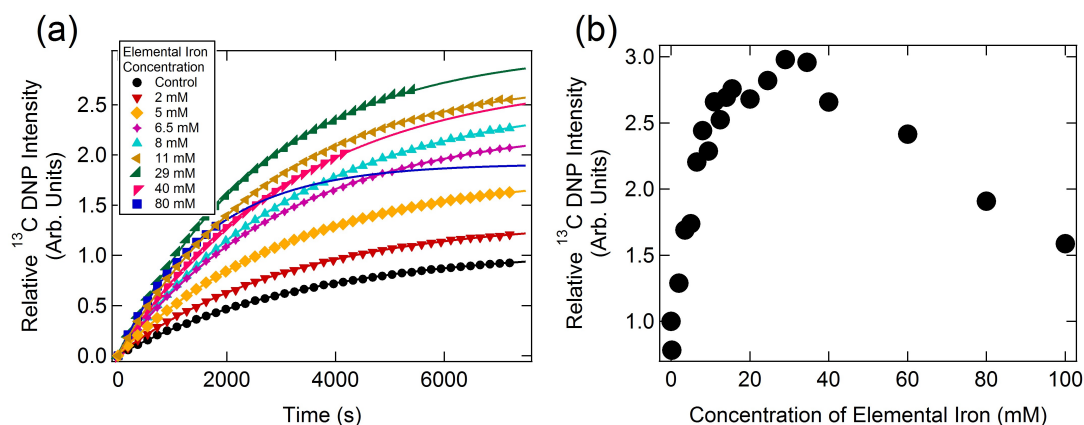


Figure 4.35. (a) ^{13}C polarization buildup curves for samples of 3 M $[1-^{13}\text{C}]$ sodium acetate and 15 mM trityl OX063 doped with different concentrations elemental iron in the form of Feraheme. Data are normalized such that the control builds up to unity. (b) Dependence of ^{13}C DNP intensity on the iron concentration. Figure adapted with permission from P. Niedbalski, C. Parish, Q. Wang, Z. Hayati, L. Song, Zackary I. Cleveland and L. Lumata, “Enhanced Efficiency of ^{13}C Dynamic Nuclear Polarization by Superparamagnetic Iron Oxide Nanoparticle,” *J. Phys. Chem. C*, vol. 121, pp. 19505-19511, 2017, Copyright 2017 American Chemical Society.

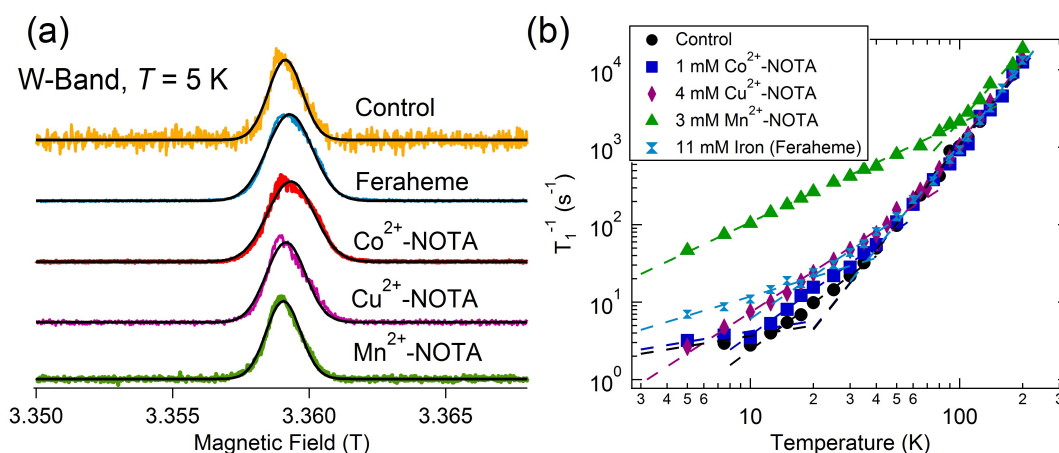


Figure 4.36. (a) Field swept EPR spectra of the trityl radical when doped with transition metals. (b) Temperature dependence of electron T_1 for the 5 samples studied.

The results obtained through EPR agreed well with DNP experiments. Feraheme and manganese, which significantly enhanced ^{13}C DNP also shortened trityl's electronic T_1 . Copper and Cobalt had essentially no effect on T_1 , agreeing well with the minimal enhancement of ^{13}C DNP. Copper did seem to have a small effect on the temperature dependence of T_1 , slightly altering the change in T_1 with temperature but having little effect on low-temperature electronic T_1 . With the intimate relationship observed between electronic T_1 and ^{13}C DNP efficiency, these EPR results confirm the popular perception that a shortened electronic T_1 leads to enhanced DNP.

The reason for the observed changes in electronic T_1 with transition metal ion doping are best understood through the lens of paramagnetic relaxation. A theory of relaxation in the presence of paramagnetic agents has long been well understood in the NMR context [185, 186, 187]. This has been adapted for two electrons, one of which is fast relaxing and the other being slow-relaxing [188]. In this adaptation, the relaxation rate of the slow-relaxing electron (in this case, the trityl free electron) is given by:

$$\begin{aligned}\frac{1}{T_{1s}} &= \frac{1}{T_{1s}^0} + S(S+1)[B + C + E] \\ B &= \frac{b^2 T_{2f}}{1 + (\omega_f - \omega_s)^2 T_{2f}^2 + b^2 T_{1f} T_{2f}} \\ C &= \frac{c^2 T_{1f}}{1 + \omega_s^2 T_{1f}^2} \\ E &= \frac{e^2 T_{2f}}{1 + (\omega_f + \omega_s)^2 T_{2f}^2}\end{aligned}\tag{4.7}$$

In the above equation, s and f subscripts denote quantities related to the slow and fast relaxing spins respectively, T_{1s}^0 is the spin-lattice relaxation time of an undoped trityl electron, S is the electron spin, b^2 , c^2 , and e^2 are constants depending on inter-spin distance and orientation, and ω is the resonant frequency of the specified spins. Though it was not possible to measure T_1 and T_2 of the transition metal complexes (the fast-relaxing spins) with the available instrumentation, it is reasonable to assume that these time constants are

similar among the different ions. Because of this, the parameter C is thought to be relatively constant among the different metal ion samples. Furthermore, with very large frequencies being studied (near 100 GHz), it is expected that the parameter E will be negligible in all cases. However, B is going to depend highly on the difference between resonant frequencies of slow and fast-relaxing spins. In order to evaluate this, the EPR spectra of the transition metal ion complexes must be known. For this, a W-band quasi-optical EPR spectrometer (HiPER) at the National High Magnetic Field Laboratory was used to obtain EPR spectra at 30 K for the transition metals caged in the NOTA complex.

The electron resonance spectra of Mn^{2+} -NOTA and trityl are centered at approximately the same magnetic field strength, and the spectrum of Mn^{2+} -NOTA is comparatively narrow, suggesting that the average value of $(\omega_f - \omega_s)^2$ will be comparatively small (Figure 4.37). This in turn leads to a large parameter B . For Cu^{2+} -NOTA, the spectrum is still relatively narrow, but has a large offset from the trityl resonance, indicating that $(\omega_f - \omega_s)^2$ will be large and hence B small. Similarly, the spectrum of Co^{2+} -NOTA is extremely wide, leading to large $(\omega_f - \omega_s)^2$ and again small B . As can be seen in Equation 4.7, small B leads to small change in trityl's electron relaxation rate. This is further strengthened by the multiplicative factor $S(S + 1)$. Manganese has spin 5/2, increasing the impact of the large B , while cobalt has spin 3/2 and copper has spin 1/2 so $S(S + 1)$ has a lesser impact.

Upon dissolution, liquid state ^{13}C NMR enhancements mirrored the results from solid state polarization buildup (Figure 4.38). Manganese led to a large enhancement compared to the control, while copper and cobalt led to similar enhancement as seen with the control sample. Manganese and cobalt shortened ^{13}C T_1 , with manganese having the greater effect. Copper had no observed effect on ^{13}C T_1 .

Feraheme, however, proved to be extremely paramagnetic at room temperature, shortening the ^{13}C T_1 to about 15 s as opposed to the 47 s T_1 seen in the control sample. As a result of this shortening and the 8 s shuttling time from polarizer to magnet, the liquid state

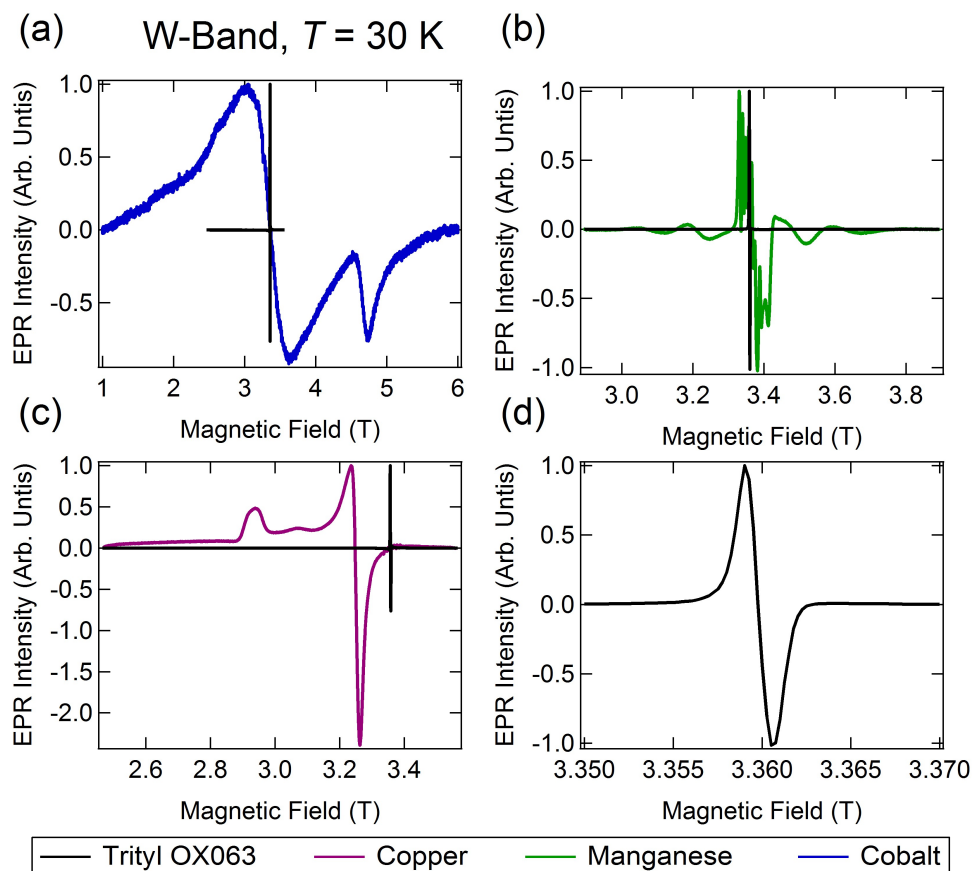


Figure 4.37. (a) Comparison of continuous wave EPR spectra of trityl OX063 and each of the transition metal ion complexes studied. (a) Co^{2+} -NOTA, (b) Mn^{2+} -NOTA, (c) Cu^{2+} -NOTA. In (d), the continuous wave EPR spectrum of trityl is shown with adequate resolution in the x axis to discern its features. All spectra are normalized.

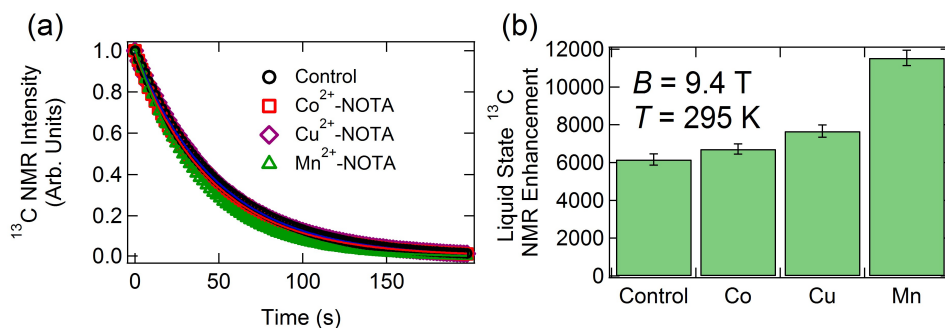


Figure 4.38. (a) Relaxation of hyperpolarized ^{13}C NMR signal for transition metal ion complex-doped samples after dissolution. (b) Comparison of liquid-state ^{13}C NMR signal enhancement 8 s following dissolution for samples optimally doped with transition metal ion complexes

^{13}C NMR signal enhancement for Feraheme-doped samples is nearly equal to the control sample, a poor result considering the nearly 3-fold improvement in the solid state. This can be alleviated by polarizing small sample volumes. Using a 50 μL sample instead of 100 μL improves the ^{13}C relaxation time from 15 to 20 s. A 10 μL sample gives an even better result, increasing the relaxation time to 44 s which consequently allows for a very high signal enhancement of nearly 20,000-fold over thermal equilibrium, almost a 4-fold improvement over the control sample (Figure 4.39).

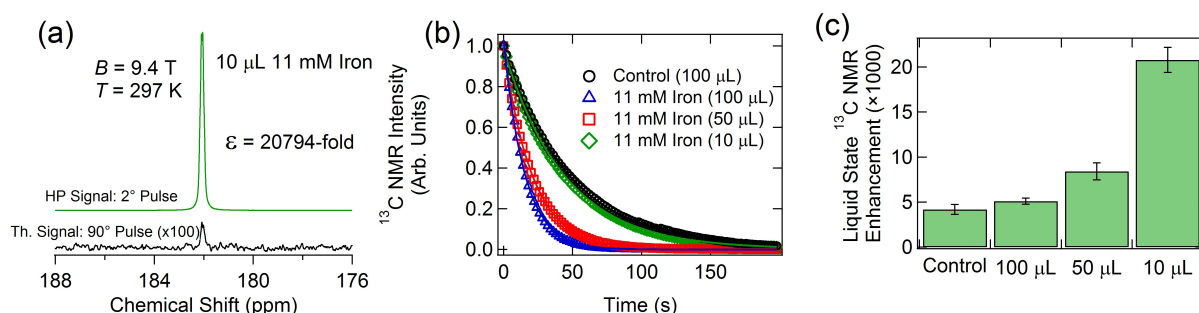


Figure 4.39. (a) Representative hyperpolarized and thermal signals for the dissolution of the 10 μL sample containing 3 M $[1\text{-}^{13}\text{C}]$ sodium acetate, 15 mM trityl, and 11 mM elemental iron post dissolution. (b) Decay of the hyperpolarized ^{13}C signal for control and different volumes of polarized sample containing the optimum concentration of iron. (c) Comparison of liquid state ^{13}C NMR signal enhancement for the different volume samples containing an optimum concentration of iron. Figure adapted with permission from P. Niedbalski, C. Parish, Q. Wang, Z. Hayati, L. Song, Zackary I. Cleveland and L. Lumata, “Enhanced Efficiency of ^{13}C Dynamic Nuclear Polarization by Superparamagnetic Iron Oxide Nanoparticle,” *J. Phys. Chem. C*, vol. 121, pp. 19505-19511, 2017, Copyright 2017 American Chemical Society.

While this improvement with small sample sizes could be beneficial for small animal, *in vitro*, or perfusion samples, the small sample would be of limited utility for human studies, which require large volumes of polarized liquid. However, if the Feraheme were to be removed from solution immediately following dissolution, the benefit to polarization could be retained with little to no adverse affect on T_1 . As a nanoparticle, Feraheme has a comparatively large diameter ($\approx 30 \text{ nm}$), allowing it to be filtered from solution using commercially available filters. To demonstrate this, syringe filters with 0.02 μm pores (Whatman, GE Healthcare,

UK) were used to filter two different samples containing Feraheme. The first was a mock dissolution sample with 0.268 mM elemental iron (Feraheme) to mimic a 100 μL sample doped with 11 mM Feraheme. Prior to filtration, a series of samples containing different concentrations of Feraheme were studied using UV-Vis spectrophotometry and proton T_1 measurements. The mock dissolution sample was studied in the same manner both before and after filtration (Figure 4.40). Prior to filtration, the mock sample had a proton T_1 of 0.15 s and absorbance at 306 nm of 0.37. After filtration, proton T_1 was increased to 2.82 s, nearly equal to a control sample ($T_1 = 3.13$ s), and the absorbance was reduced to 0.007, again approaching the value of the control sample (0.001). This suggests that such simple filtration is able to remove up to 98% of Feraheme from solution.

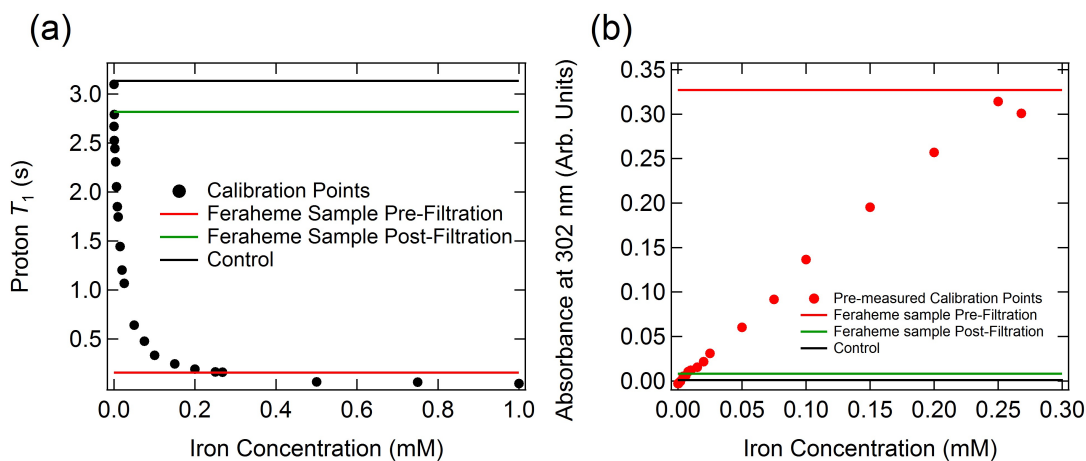


Figure 4.40. (a) Dependence of proton T_1 on iron concentration in water. Horizontal lines denote measured proton T_1 of a pure water control and a mock dissolution sample before and after filtration. (b) Dependence of absorbance at 302 nm on iron concentration in water. Horizontal lines denote absorbance of control and a mock dissolution sample before and after filtration.

To study the effect of filtration on an actual dissolution sample, the ^{13}C T_1 of a hyperpolarized sample was measured by monitoring the decay of hyperpolarized signal in a 9.4 T high resolution NMR magnet. Then, the sample was filtered by forcing the solution through the filters mentioned above. Finally, ^{13}C T_1 of the post-filtration sample was measured in the

same magnet using the inversion recovery method. Prior to filtration, the ^{13}C T_1 was about 15 s, and filtration increased this value to over 30 s (Figure 4.41). While this does not reach back to the control value of 47 s, it is a drastic improvement and more rigorous methods of filtration could remove more of the Feraheme and result in further improvement. This filtration was not implemented for an actual dissolution experiment due to the instrumentation challenges in adding a filter to the HyperSense dissolution line. However, in the more modern SPINlab polarizer, filtration of the solution would be comparatively easily added to the sterile fluid path, suggesting that this could be potentially useful clinically. There is further advantage in the fact that Feraheme is FDA approved with little toxicity concern. This is in stark contrast to gadolinium contrast agents, which are increasingly the source of toxicity concerns [189]. For this reason, gadolinium has not been used in the initial clinical hyperpolarization studies [73], but Ferheme would not have this limitation.

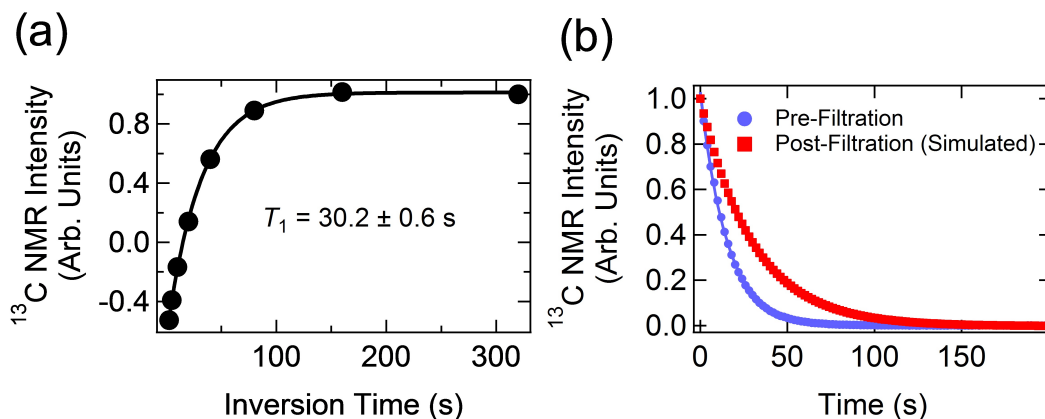


Figure 4.41. (a) Inversion recovery data for a dissolution sample containing approximately 0.268 mM elemental iron (prior to filtration) that had been forced through a syringe filter. (b) Comparison of ^{13}C NMR signal decay after dissolution of a sample containing 11 mM (0.268 mM after dissolution) elemental iron before and after filtration. Post filtration data is simulated based on the T_1 found using inversion recovery shown in (a).

4.6 Measuring ^{13}C Spin Lattice Relaxation Time at Cryogenic Temperatures Using a Sweepable Magnet

At the time of the writing of this dissertation, a manuscript was in preparation for the experiment described in this section. The tentative authorship and title are:

P. Niedbalski, Q. Wang, C. Parish, F. Khashami, A. Kiswandhi, and L. Lumata, “Magnetic Field Dependent Lifetimes of Hyperpolarized Carboxyl ^{13}C at Cryogenic Temperatures.”

Co-authors assisted the author in monitoring the magnet system during experiments.

At cryogenic temperatures, ^{13}C is known to have very long relaxation times, but T_1 is difficult to measure because of the combination of low ^{13}C NMR signal strength and because conventional methods of measuring T_1 require a prohibitively large amount of time. For example, using the inversion recovery pulse sequence (Appendix A) to measure the T_1 of $[1-^{13}\text{C}]$ pyruvate at 2 K and 3.35 T would require more than 2 weeks of experiment time. However, as an intrinsic property, the T_1 measured using conventional methods (such as inversion recovery) and by monitoring the hyperpolarized signal decay are equivalent. The sweepable magnet used to build the cryogen-free DNP system described in Chapter 3 provides a unique opportunity to measure ^{13}C relaxation times at cryogenic temperatures over a wide range of magnetic fields. Samples may be polarized at the polarization field (6.423 T) and 2 K and then the magnetic field swept to a monitoring field where the ^{13}C NMR signal is monitored using a small tip angle pulse in time intervals of about 1/10 the expected T_1 (Figure 4.42). In this manner, ^{13}C T_1 can be measured at a large number of magnetic fields in a fraction of the time that would be required to obtain the same data conventionally.

For dissolution DNP, ^{13}C labeling within carboxyl groups is common practice as these nuclei tend to have long relaxation time. Examples include $[1-^{13}\text{C}]$ pyruvate, $[1-^{13}\text{C}]$ acetate, and $[1-^{13}\text{C}]$ glycine. Because these substrates are commonly used, knowledge of their

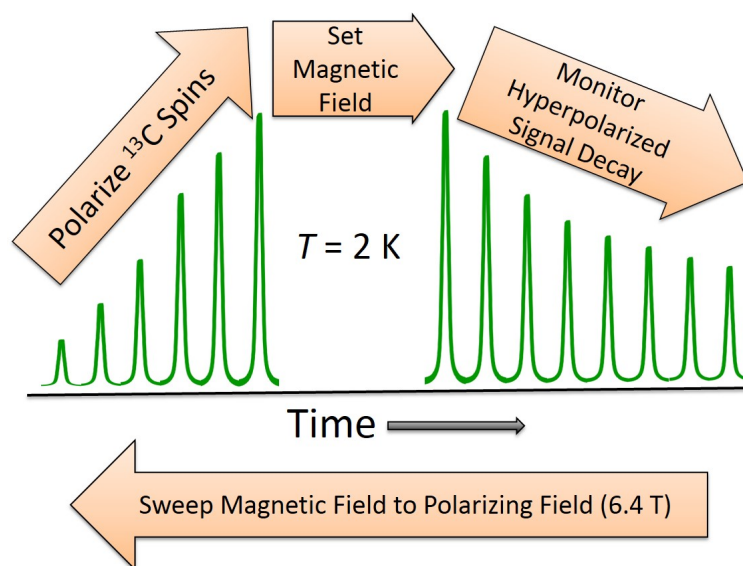


Figure 4.42. Schematic showing the experimental procedure used to measure ^{13}C spin lattice relaxation times at a large number of magnetic fields

relaxation at cryogenic temperatures would be beneficial to the overall understanding of dissolution DNP. To achieve this end, the magnetic field dependence of four different samples were measured - 1.4 M $[1-^{13}\text{C}]$ sodium pyruvate, 1.5 M $[1-^{13}\text{C}]$ glycine, 3 M $[1-^{13}\text{C}]$ sodium acetate, and $[1-^{13}\text{C}]$ pyruvic acid (14 M), each doped with 15 mM trityl OX063 (Figure 4.43). Pyruvic acid is self-glassing, so no further glassing agent was necessary. The other three substrates were dissolved in a glassing matrix of 1:1 v/v glycerol:water. In turn, samples were placed in the cryogen-free polarizer and the temperature brought down to the operating temperature of approximately 2 K. The NMR circuit was tuned to the ^{13}C resonance corresponding to the desired magnetic field, and then the magnetic field was ramped to the polarization field of 6.4 T. The sample was polarized for between 20 min (for pyruvic acid, which polarized quickly) and 90 min (for glycine and sodium pyruvate, which polarized slowly). Next, the microwave source was turned off and the magnetic field ramped up or down to the desired magnetic field. The ramp rate was about 0.23 T/min, for a maximum time delay of 24 min between the end of polarization and the beginning of the acquisition of relaxation data points. ^{13}C NMR signal was monitored using small tip angle pulses with

a time delay of about 1/10 the estimated T_1 until between 1 and 3 T_1 had passed. At this point, a series of NMR scans were taken rapidly (1 s repetition time) in order to calibrate the precise tip angle used for data acquisition. This process was repeated until a large number of magnetic field strengths had been studied. Samples were left in the system at 2 K in between scans.

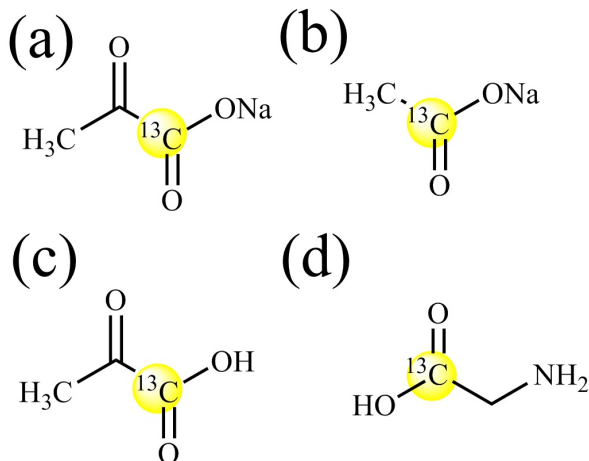


Figure 4.43. The ^{13}C -labeled carboxyl molecules whose field dependence of ^{13}C relaxation was studied (a) Sodium Pyruvate, (b) Sodium Acetate, (c) Pyruvic Acid, and (d) Glycine

All data was fit using Equation 4.6. The pulse calibration data was fit first by setting the T_1 value to an order of magnitude estimate and using θ as a fitting parameter. Once the tip angle of the monitoring pulse was found, the same equation was used to fit the relaxation data, this time setting θ to the value found in the pulse calibration and using T_1 as a fitting parameter. Sample relaxation data and fittings are shown for the sodium pyruvate sample in Figure 4.44.

For each of the four substrates studied, T_1 was shortest at low magnetic field and increased roughly with a power-law dependence with increasing magnetic field (Figure 4.45). For the two pyruvate samples, the magnet field dependence of T_1 was approximately cubic ($T_1 \propto B^3$). Glycine also had nearly cubic dependence, with $T_1 \propto B^{2.9}$. Acetate was closer to having quadratic field dependence, with $T_1 \propto B^{2.4}$. These magnetic field dependencies give insight

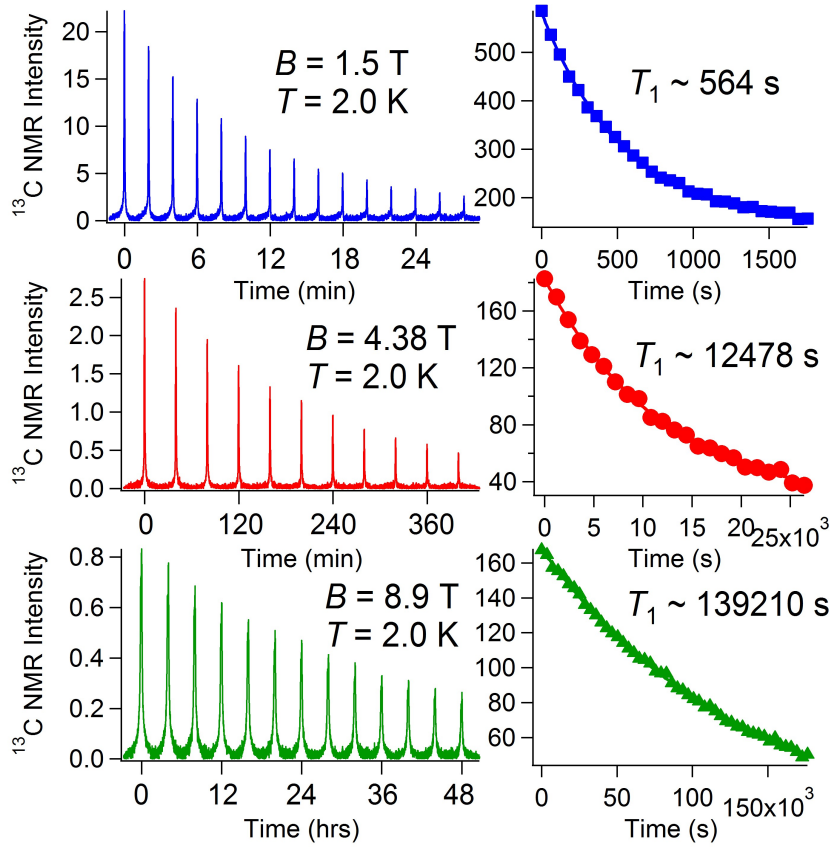


Figure 4.44. Sample spectra (left) and integrated intensity (right) for the decay of the hyperpolarized ^{13}C signal of sodium pyruvate at three different magnetic field strengths.

into the dominant relaxation mechanism in each case. It is expected that relaxation will be driven by dipolar interactions between ^{13}C and the different spin species present in each sample (trityl free electron, ^1H , and ^{13}C). It is expected that, because of the strength of their magnetic moments, the free radical and ^1H will have the largest contributions to relaxation.

Relaxation induced by dipolar interactions between ^{13}C and ^1H can be described by [190]:

$$\frac{1}{T_1^H} = C_H r^{-6} \gamma_C^2 \gamma_H^2 \hbar^2 S(S+1) \left(\frac{\tau_C}{1 + \omega_C^2 \tau_C^2} + \frac{6\tau_C}{1 + (\omega_C + \omega_H)^2 \tau_C^2} + \frac{\tau_C}{1 + (\omega_C - \omega_H)^2 \tau_C^2} \right) \quad (4.8)$$

In this equation C is a constant, r is the distance between ^{13}C and ^1H spins, and τ_C is the correlation time of the molecule. Similarly, the relaxation induced by the paramagnetic

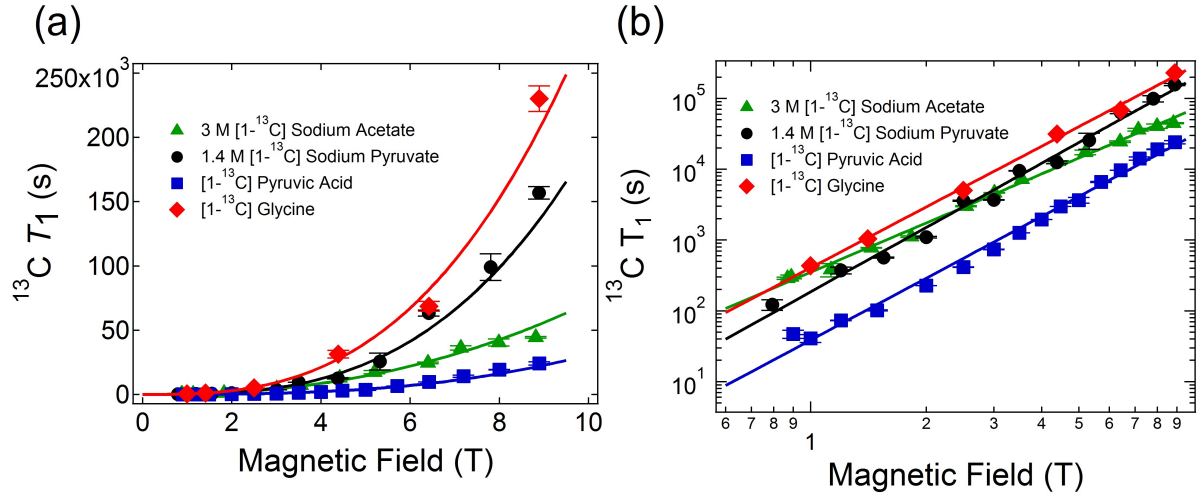


Figure 4.45. ^{13}C T_1 shown vs. magnetic field in both (a) linear and (b) logarithmic scales. This is to emphasize both the vast difference between ^{13}C relaxation times and the power law field dependence observed ($T_1 = CB^\alpha$). Sodium pyruvate - $\alpha = 3.01 \pm 0.05$, Pyruvic acid - $\alpha = 3.1 \pm 0.1$, Glycine - $\alpha = 2.85 \pm 0.07$, Sodium acetate - $\alpha = 2.35 \pm 0.05$

impurities can be described by [22, 41]:

$$\frac{1}{T_1^e} = C_e \frac{N_s \gamma_C^2 \gamma_e^2 \hbar^2 S(S+1)}{b^3} \frac{\tau_C}{1 + \omega_C^2 \tau_C^2} (1 - P_e^2) \quad (4.9)$$

In this equation, N_s is the concentration of paramagnetic impurities, and P_e is the electron polarization.

The temperature regime in which data was taken is one in which molecular motions are very slow, i.e. $\omega_C \tau_C \gg 1$. Using this and the fact that $\omega_C = \gamma_C B_0$, the approximate field dependence can be extracted from Equations 4.8 and 4.9.

$$\frac{1}{T_1^H} \propto \frac{1}{B^2} \quad (4.10)$$

$$\frac{1}{T_1^e} \propto \frac{1}{B^2 \exp\left(\frac{\hbar \gamma_e B}{kT}\right) + B^2} \quad (4.11)$$

Considering, then, the structures of the substrates examined, it can be seen that the carboxyl ^{13}C of sodium pyruvate and pyruvic acid are three bond lengths away from methyl ^1H spins. Given the r^{-6} dependence of the dipolar interaction, the dipolar interaction between ^{13}C

and ^1H has a comparatively weak impact on the relaxation of ^{13}C in these substrates. Thus, the contribution from the paramagnetic impurities has a greater impact which, with its exponential field dependence, strengthens the B dependency to approximately cubic. Of note is that pyruvic acid has significantly faster relaxation than does sodium pyruvate despite nearly identical magnetic field dependence. This is because of the much higher concentration of spins in pyruvic acid (14 M vs 1.4 M) that enables a much greater rate of ^{13}C spin diffusion and thus overall relaxation. In contrast to pyruvate, acetate has its carboxyl ^{13}C only two bonds from methyl ^1H which increases the contribution from the ^1H - ^{13}C interaction. This brings the field dependence closer to B^2 as would be expected for relaxation purely driven by protons. Glycine is intermediate between the two, despite having three ^1H spins within two bond lengths of the carboxyl ^{13}C . Most likely, the difference in field dependence has to do with the lack of methyl ^1H spins in glycine. Methyl groups tend to be very mobile, so even at low temperature, it would be expected that methyl spins would lead to greater magnetic field fluctuations and thus relaxation. Because there is no methyl group in glycine, the protons causing relaxation have a weaker impact.

These measurements could be of use for the future of dissolution DNP instrumentation. Though it has been shown that DNP enhancements are weaker at high magnetic field in MAS DNP [128], this has not as yet been observed for dissolution DNP [39, 191, 192]. The reason for this could be the drastic increase in nuclear relaxation time with increased magnetic field as observed herein. The maximum DNP enhancement in a given experiment occurs when the system comes to a steady state in which polarization processes are matched by nuclear relaxation processes [135, 159]. Because relaxation is so slow at high magnetic field, polarization may be brought to a higher level before relaxation processes match polarization.

The results described above are believed by the author to be the only of their kind. The cryogen free polarizer using a sweepable magnet uniquely allows for experiments of this type to be performed. As more systems follow this design, further investigation into the field

dependence of nuclear T_1 will be possible and will accelerate the understanding of higher field dissolution DNP.

4.7 Summary of DNP Optimization Studies

Overall, it was found, as expected, that TEMPO and its derivatives were only marginally effective at directly polarizing ^{13}C , when compared to polarization using trityl. Deuteration of solvents led to an approximate doubling of the polarization when using TEMPO, which brought the ^{13}C polarization to near to that achieved using trityl with no other additives. However, polarization when using trityl could be significantly improved through the addition of paramagnetic additives. Gadolinium has long been the standard for enhancing polarization, though it is known to have downsides including its shortening of ^{13}C T_1 and growing toxicity concerns when used in clinical studies. Of the paramagnetic additives studied, Ho^{3+} -DOTA, Tb^{3+} -DOTA, and 3-Gd led to liquid state polarization enhancements on par with those achieved by Gd^{3+} -DOTA. Ho^{3+} -DOTA and Tb^{3+} -DOTA had the added benefit of having no effect on ^{13}C relaxation in the liquid state, and Tb^{3+} -DOTA only required a very small concentration. The addition of Feraheme was beneficial in the solid state, but caused extremely fast ^{13}C relaxation in the liquid state due to its strong paramagnetism. This can be alleviated by removing the nanoparticle from solution by a relatively simple filtration process, suggesting that Feraheme may still have utility if filtration is used immediately following dissolution. Mn^{2+} -NOTA improved ^{13}C polarization, but Co^{2+} -NOTA and Cu^{2+} -NOTA gave no benefit. The associated W-band EPR experiments confirm the link between ^{13}C polarization efficiency and electronic T_1 . Overall, the results obtained show that holmium and terbium are preferable to gadolinium-based contrast agents for pre-clinical studies as they result in similar liquid-state ^{13}C NMR signal enhancement to gadolinium with no adverse effect on ^{13}C T_1 . Feraheme has potential as an additive for clinical studies, but would necessitate filtration to be useful.

CHAPTER 5

METABOLIC STUDIES USING DISSOLUTION DNP

As discussed in Chapter 1, one of the most promising applications of dissolution DNP is the tracking of metabolic activity using hyperpolarized substrates [34, 55, 74, 75, 76, 193, 194, 195]. By polarizing important substrates and administering to *in vitro* cells, such as human cancer cells, metabolic processes may be monitored. While DNP provides a method of monitoring metabolism in real time and non-destructively, other imaging and spectroscopic modalities can provide useful supplementary information. In this chapter, studies involving hyperpolarized ^{13}C and ^{15}N magnetic resonance are presented with supporting information gleaned from conventional NMR.

5.1 Hyperpolarization of ^{13}C and ^{15}N -labeled Substrates

In addition to the standard in the field (pyruvate), several other substrates were used to study metabolism using hyperpolarized magnetic resonance. Namely, the molecules used were [^{13}C ,U- ^2H] glucose, [2- ^{13}C] fructose, and ^{15}N choline. Glucose and fructose, as mentioned previously are essential to the body for energy production, making them highly desired agents for tracking metabolism. Glucose [66, 70, 196] and fructose [71] isotopomers have been used in previous hyperpolarization studies, showing their potential utility. ^{15}N choline is somewhat more novel. Choline is essential for production of cell membranes, but ^{13}C choline has a very short T_1 , making it ineffective as a metabolic tracer [193]. ^{15}N , having a weak gyromagnetic ratio and spin $\frac{1}{2}$, is expected to have longer T_1 than does ^{13}C . The first step was to hyperpolarize the substrates to verify in the case of glucose and fructose or determine in the case of choline whether their polarization enhancement and liquid-state T_1 are adequate for *in vitro* and *in vivo* studies.

To that end, samples containing a high concentration of substrate (3 M) were doped with 15 mM trityl and polarized for one hour. Then, dissolutions were performed using 4

mL deionized water as the dissolution solvent. Samples were sent to a 10 mm NMR tube in a 400 MHz high resolution Varian NMR magnet (Agilent Technologies, Palo Alto, CA). The probe in the magnet was pre-tuned either to the ^{15}N or ^{13}C resonance as appropriate. The ^{13}C signal was monitored using 100 2° RF pulses with 2 s between each scan, while ^{15}N was monitored using 10° RF pulses every 5 s.

Results for glucose polarization are shown in Figure 5.1. Though there are only six carbons in glucose, eight peaks are clearly visible in the hyperpolarized spectra, which correspond to the different anomers of glucose (α and β) as described in Table 5.1. Each of the eight peaks had a different T_1 , though most were about 9 s, suggesting that there would be about a 30 s imaging window for hyperpolarized glucose. Glucose metabolism proceeds very rapidly, so this time should, in theory, be adequate for metabolic imaging.

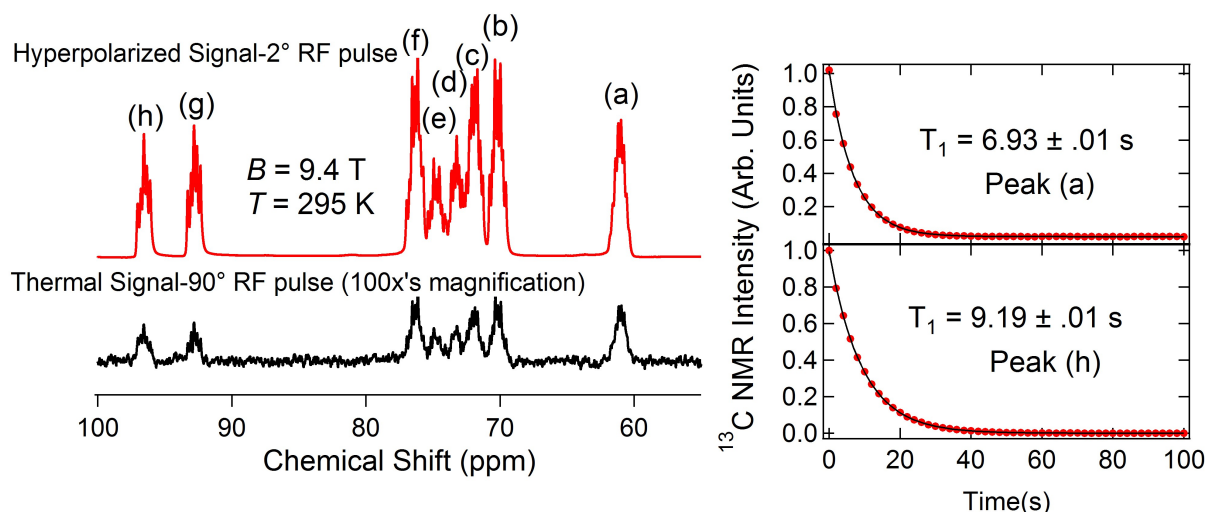


Figure 5.1. A comparison of the ^{13}C NMR signal for hyperpolarized and thermal $[\text{U-}^{13}\text{C}_{6,1,2,3,4,5,6,6-\text{d}_7}]$ glucose (left). Representative decay of the hyperpolarized ^{13}C NMR signal for the fastest relaxing peak (Peak (a)) and the slowest relaxing peak (Peak (h)) of the uniformly ^{13}C labeled glucose (right)

Fructose hyperpolarization data is shown in Figure 5.2. Despite only a single ^{13}C label, three different peaks are clearly visible. These peaks correspond to the three different fructose anomers as described in Table 5.1. As claimed in the literature [71], the ^{13}C relaxation time for

Table 5.1. ^{13}C location and anomer corresponding to NMR peak

Figure	Peak Label	Anomer and ^{13}C Location
Figure 5.1	(a)	$\alpha\text{C}_6 + \beta\text{C}_6$
	(b)	$\alpha\text{C}_4 + \beta\text{C}_4$
	(c)	$\alpha\text{C}_2 + \alpha\text{C}_5$
	(d)	αC_3
	(e)	βC_2
	(f)	$\beta\text{C}_3 + \beta\text{C}_5$
	(g)	αC_1
	(h)	βC_1
Figure 5.2	a	$\beta\text{-fp C}_2$
	b	$\beta\text{-ff C}_2$
	c	$\alpha\text{-ff C}_2$

$[2\text{-}^{13}\text{C}]$ fructose surpasses the glucose sample studied. This affords a slightly longer imaging window while still allowing for the possibility of monitoring important energy producing metabolic reactions.

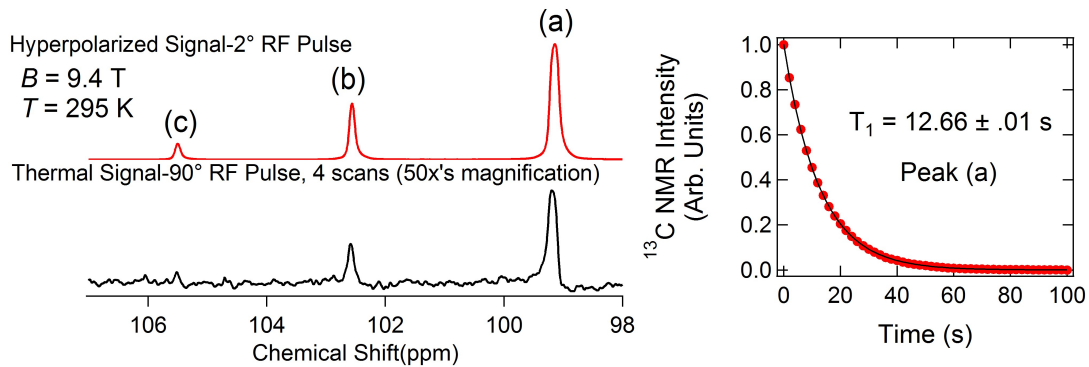


Figure 5.2. A comparison of the ^{13}C NMR signal for hyperpolarized and thermal $[2\text{-}^{13}\text{C}]$ fructose (left). Representative decay of the hyperpolarized ^{13}C NMR signal for peak (a) (right). All three peaks had approximately the same relaxation time.

Of the substrates tested, ^{15}N choline had the longest relaxation time by far (Figure 5.3), having T_1 longer than two minutes. This suggests that the imaging window for this substrate is very long, which shows a great deal of promise for metabolic imaging studies.

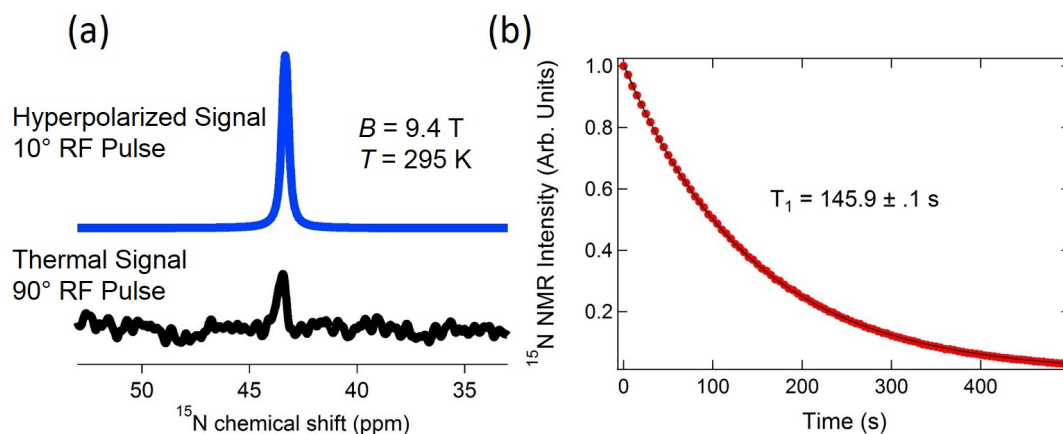


Figure 5.3. A comparison of the ^{15}N NMR signal for hyperpolarized and thermal ^{15}N labeled choline (left). Decay of the hyperpolarized ^{15}N NMR signal and liquid state T_1 .

5.2 Yeast Metabolism

Yeast offers an interesting alternative to cancer cells for metabolic studies using DNP and NMR. It has the advantages of being safe, simple, and inexpensive to procure and culture. In addition, while the metabolic pathways present in yeast are different than in human cells, there are many similarities in its uptake and metabolism of substrates, making it useful as a safe, inexpensive model [67, 68]. One of the most prominent similarities is that glycolysis is a primary method of energy production. An important difference, however is that the products of mammalian glycolysis enter the Krebs cycle and are turned into energy and lactate while the products of yeast glycolysis undergo fermentation and are turned into ethanol.

5.2.1 Conventional NMR of Living Yeast

The wide availability of yeast makes it a good candidate not only for DNP NMR, but also for conventional NMR. By applying ^{13}C labeled substrates to yeast and measuring the ^{13}C NMR signal at regular intervals, the flux through a metabolic pathway may be monitored. However, because of the weakness of the ^{13}C thermal signal, the substrate must be chosen carefully. Specifically, it is necessary to choose a substrate that may be metabolized readily by

yeast and it is preferable to have a relatively short T_1 relaxation time. This is of particular importance for studies in low field NMR spectrometers in which adequate ^{13}C signal-to-noise is impossible in only one scan when using low concentration as is necessary for this experiment.

Following the example of previous studies [197], $[1-^{13}\text{C}]$ glucose was chosen as the primary substrate for monitoring yeast metabolism. However, other substrates, including $[\text{U}-^{13}\text{C}]$ glucose, $[2-^{13}\text{C}]$ fructose, $[\text{U}-^{13}\text{C}]$ fructose, $[3-^{13}\text{C}]$ pyruvate, $[1-^{13}\text{C}]$ choline, and $[2-^{13}\text{C}]$ acetate.

Fleischmanns Active Dry yeast (*Saccharomyces cerevisiae*) was obtained and used for ^{13}C NMR studies. A yeast suspension was made using 2 g yeast in 20 mL of a pH 6.0 buffer. The buffer was homemade using a mixture of potassium phosphate dibasic (K_2HPO_4) and potassium phosphate monobasic (KH_2PO_4) and the pH was tested using a ecoTestrpH2 (Eutech Instruments) pH sensor. The yeast mixture was constantly stirred for one hour using a magnetic stirrer. Once the suspension was fully prepared, 400 μL was added to a 5 mm NMR tube. Then, a solution containing 20 mg of substrate in 50 μL water was added to the tube. Finally, 400 μL more of the yeast suspension was added and the tube placed on a vortex mixer for 30 seconds. Then, the tube was capped and placed in the NMR spectrometer. These experiments were conducted in both the SpinSolve NMR spectrometer at 1 T and a 400 MHz NMR spectrometer (Figure 5.4). For most of the substrates used, four scans of a simple 90° pulse were necessary to have adequate signal-to-noise, even in the 400 MHz magnet. Of course, the signal-to-noise is much better using 400 MHz NMR, but using the SpinSolve is adequate. The time between scans depended on the substrate used, being as short as 15 s for $[1-^{13}\text{C}]$ glucose, $[3-^{13}\text{C}]$ pyruvate, and $[2-^{13}\text{C}]$ acetate or as long as 60 s for $[2-^{13}\text{C}]$ fructose.

As would be expected, different substrates led to different speeds of metabolic flux and different metabolites. The most successful substrates were those that are metabolized via glycolysis. Of those, $[\text{U}-^{13}\text{C}]$ and $[1-^{13}\text{C}]$ glucose gave the best results, showing a clear production of ethanol in a relatively short time (~ 30 min). Fructose led to ethanol production, but

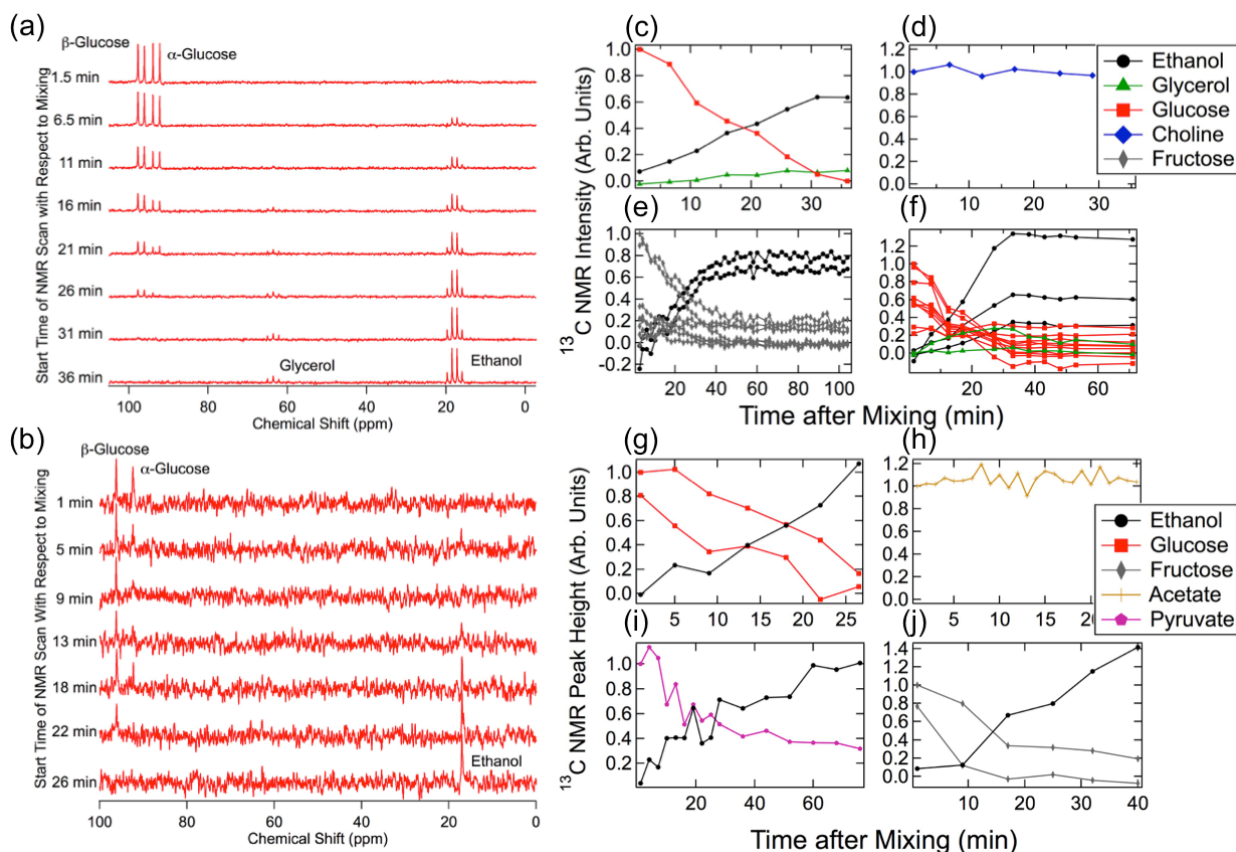


Figure 5.4. Sample NMR spectra for metabolic studies in yeast using $[1-^{13}\text{C}]$ glucose at 9.4 T (a) and 1 T (b). Plots showing the integrated intensity of the NMR spectra of metabolic reactants and products for different administered substrates at 9.4 T ((c)-(f)) and 1 T ((g) - (j)).

the resonances for ethanol and fructose have similar chemical shift, causing difficulty in peak identification. In addition, fructose has a comparatively long relaxation time, which leads to greater acquisition time, a detriment for this experiment. Pyruvate, while it was eventually metabolized into ethanol, required greater time spans, likely due to slow transport into the cell rather than slow metabolism. Acetate and choline, being the only substrates tested that are not used for energy production, showed no evidence of metabolism taking place even after a rather long wait time of over one hour, showing that yeast does not metabolize these substrates in a reasonable time frame for this particular experiment.

5.2.2 Hyperpolarized ^{13}C Studies of Living Yeast

A similar experiment may be conducted using hyperpolarized ^{13}C to monitor yeast metabolism. From conventional NMR, it was learned that glucose and fructose are the best of the tested substrates to monitor glycolytic activity of yeast, particularly over the short time span in which the hyperpolarized signal persists. Other substrates take much longer to be metabolized, suggesting that metabolic products will not be visible within the hyperpolarized lifetime.

When using conventional NMR, long T_1 was a liability, but for hyperpolarized NMR, it is a necessity in order to extend the imaging time as long as possible. As discussed previously, $[\text{U-}^{13}\text{C}, \text{U-}^2\text{H}]$ Glucose and $[2\text{-}^{13}\text{C}]$ fructose are the substrates most likely to be well-suited for hyperpolarization studies of yeast.

However, given the ubiquity of $[1\text{-}^{13}\text{C}]$ and $[2\text{-}^{13}\text{C}]$ pyruvate in DNP metabolic studies, these were also tested in yeast. Fleishmann's active dry yeast was cultured in the same manner as previously described and used for hyperpolarized studies in the manner described in Appendix E.

Unfortunately, glucose and fructose metabolism was not visible when administered to yeast. In each case, the parent compound was visible at least for a short time, but no metabolic products were seen. One possible explanation is that uptake or metabolism of glucose and fructose by bakers' yeast is too slow to be imaged in the short window afforded by quickly relaxing substrates. It is also possible that a more optimized protocol for performing these metabolic studies would lead to visible metabolism.

Pyruvate was relatively successful at monitoring yeast metabolism. For $[1\text{-}^{13}\text{C}]$ pyruvate, metabolism to carbon dioxide and further conversion to bicarbonate was visible in real time (Figure 5.5). This is as expected based on Figure 1.9, which shows that the C1 location of pyruvate is converted into CO_2 through anaerobic fermentation. The C2 location could provide more information, as it would be converted to ethanol. Unfortunately, the experimental

results show that $[2-^{13}\text{C}]$ pyruvate is ineffective for monitoring this metabolic pathway (Figure 5.6). Specifically, the spectrum of pyruvate is clearly visible, but no visible metabolic products appear in real time. When the spectra are summed together, several products are visible. The problem lies in the structure of the products. Though the C2 location of pyruvate is isolated from proton spins which grants it long relaxation time, as it is metabolized into ethanol, a proton is introduced only a single bond-length away. This causes very fast relaxation, making it difficult to view accumulation of metabolic product.

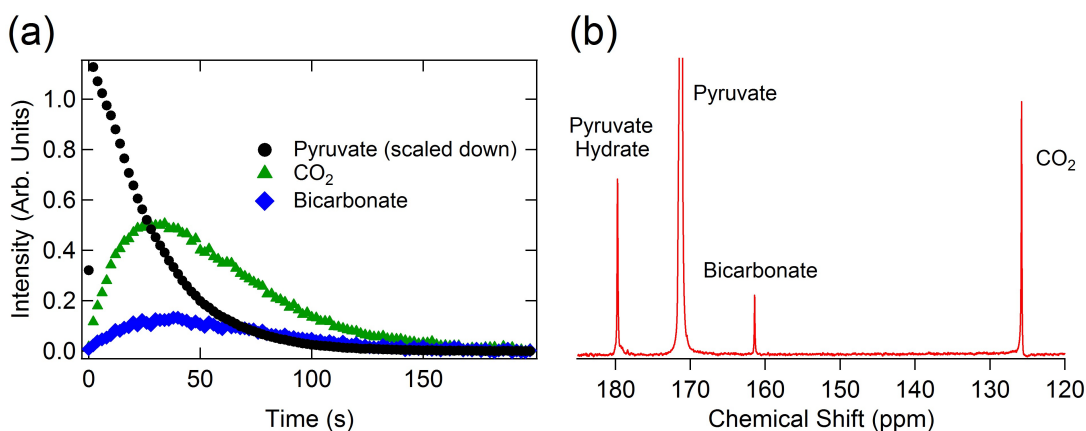


Figure 5.5. (a) Real time tracking of the metabolism of $[1-^{13}\text{C}]$ pyruvate and its conversion to carbon dioxide and bicarbonate. The pyruvate data is scaled down so that it is more easily compared to the products. (b) Sum of the first 50 spectra measuring metabolism.

Overall, it was found that $[1-^{13}\text{C}]$ sodium pyruvate was the only substrate tested that provided the opportunity to monitor yeast metabolism in real time. However, it has limited utility as the metabolic product of this substrate is of lesser importance.

5.3 Prostate Cancer Metabolism

Prostate cancer cells (PC-3) were obtained and cultured using the methods described in Appendix D. These cells were used for a number of experiments and were studied using both conventional NMR and hyperpolarized ^{13}C and ^{15}N magnetic resonance.

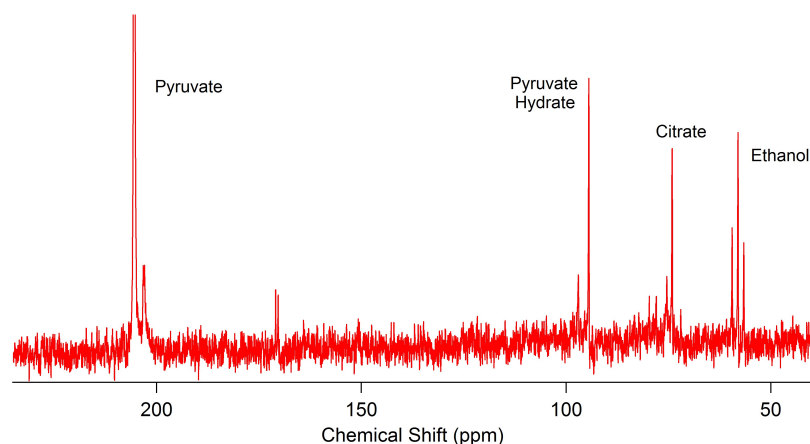


Figure 5.6. Sum of the first 50 spectra measuring yeast metabolism of $[2\text{-}^{13}\text{C}]$ pyruvate.

5.3.1 Conventional NMR using PCA Extracts

In order to have a sense of the metabolism of $[1\text{-}^{13}\text{C}]$ choline, it was studied using the PCA extracts method described in Appendix D. The times at which extracts were taken were determined based on the expected rate of metabolism of the substrate in question. Choline was expected to metabolize relatively slowly, so it was studied using long time delays between “snapshots”.

^{13}C -labeled choline was added to standard base medium (DMEM) to a concentration of 0.1 mM - roughly 1.2 times biological concentration - and added to cultured PC-3 cells. Extracts were taken at 0, 1, 4, 19, and 24 hours. Extracts were lyophilized, the powder redissolved in D_2O , and the samples studied using ^1H and ^{13}C NMR. Because the samples were extremely dilute, particularly the labeled choline, long scan times were necessary to see the peaks of interest. Despite low SNR, evidence of choline being metabolized to phosphocholine was visible in the 24 hour extract sample (Figure 5.7). The long time scales necessary for this metabolic pathway seem to suggest choline is not a good candidate for hyperpolarized studies, given that the T_1 decay of polarization only gives a few minutes of usable scan time. However, it is possible that the large signal enhancement will show small amounts of phos-

phocholine production that would be invisible to conventional NMR without prohibitively long scan times.

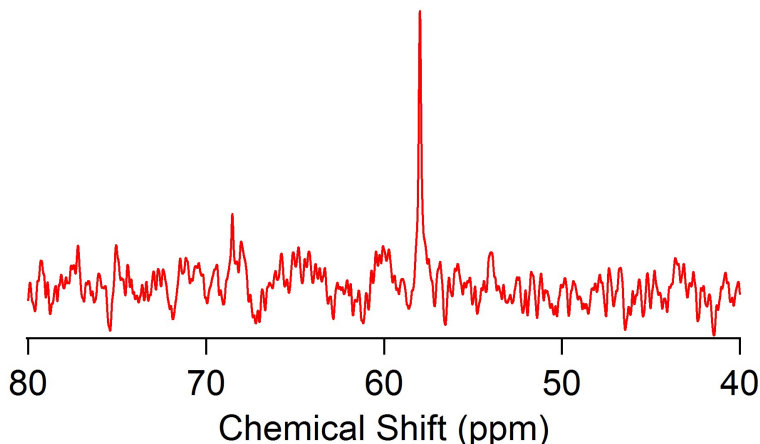


Figure 5.7. NMR spectrum of the extract of PC-3 cells that had been cultured in $[1-^{13}\text{C}]$ choline for 24 hours. The single peak corresponds to phosphocholine, indicating that choline has been metabolized by the PC-3 cells.

5.3.2 Hyperpolarized ^{13}C and ^{15}N Magnetic Resonance

While the substrates of primary interest in this work are glucose, fructose, and choline, pyruvate was also studied since $[1-^{13}\text{C}]$ pyruvate has long been the standard for *in vitro* and *in vivo* metabolic studies using ^{13}C magnetic resonance spectroscopy [43, 54, 195].

The HyperSense was used to polarize all samples. Each sample studied contained the maximum concentration of ^{13}C or ^{15}N -labeled substrate (Table 5.2) with 15 mM trityl and 2 mM Ho^{3+} -DOTA. Samples were polarized for a minimum of 2 hours until the polarization build-up curve approached a maximum. Samples were dissolved in 4 mL phosphate buffered saline with 1 mM EDTA. 250 μL of hyperpolarized liquid was mixed with approximately 80 million cells suspended in 1.5 mL glucose-free DMEM (Sigma, St. Louis, MO) and placed in either a 400 MHz Varian NMR spectrometer or the 1 T Magritek SpinSolve spectrometer.

Table 5.2. The approximate maximum soluble concentration in 1:1 v/v glycerol:water of the substrates studied

Substrate	Concentration (M)
Glucose	5.0
Fructose	5.0
Choline	3.0
Pyruvate	1.4

Glucose and Fructose As mentioned previously, hyperpolarized glucose and fructose have successfully been used in a number of metabolic studies. However, when these were tested in PC-3 prostate cancer cells *in vitro*, metabolism was not visible though the parent compounds did show up in the NMR spectrum. Most likely, the decay of the hyperpolarized signal occurred too rapidly for metabolism to be imaged.

Choline ^{13}C -labeled choline is known to have short T_1 [34, 193], so it was not tested using dissolution DNP. Rather, ^{15}N -labeled choline was used as a substrate given its long hyperpolarization lifetime (Figure 5.3). However, when this experiment was performed, no metabolism was seen (Figure 5.8). There was an interesting feature in the decay of the integrated intensity of the hyperpolarized signal in that, after 100 s, the intensity began to rise before decaying again. However, the sum of the first 50 spectra showed no secondary spectra or change in choline’s spectrum. It could be that the choline was metabolized into phosphocholine, but the chemical shift was too small to discern. Alternatively, it could just be an artifact. In either case, it does not appear that ^{15}N -labeled choline is effective for monitoring PC-3 metabolism.

Pyruvate In spite of the failures of the emphasized substrates, the use of hyperpolarized magnetic resonance for studying yeast and PC-3 metabolism was still desired. Because of this, the field standard pyruvate was used to investigate these cells’ metabolism. Because of the wealth of research using pyruvate in cancer metabolism, it was known to be a promising substrate for *in vitro* studies of PC-3.

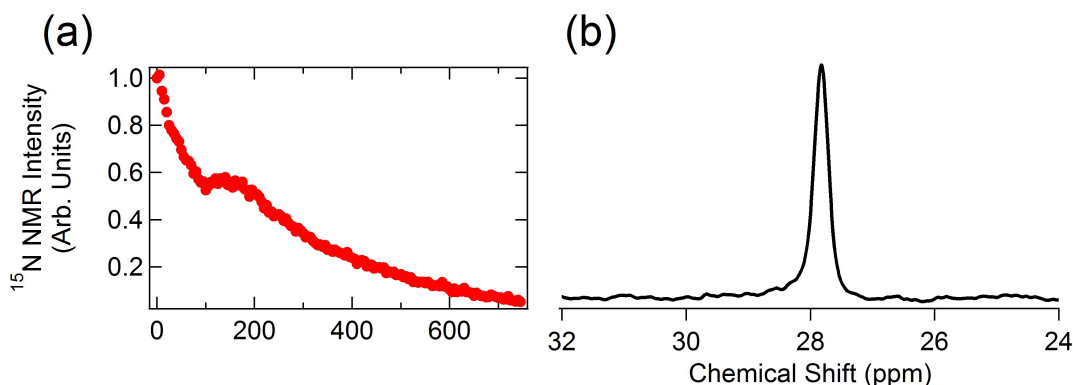


Figure 5.8. (a) Decay of the ^{15}N hyperpolarized signal after administering hyperpolarized ^{15}N -labeled choline to a PC-3 cancer cell suspension. (b) Sum of the first 50 spectra measured after administering choline to the cells.

Commonly, *in vitro* studies are performed at high magnetic fields [71, 196, 198, 199]. However, animal scanners and clinical MRI's tend to be at lower magnetic fields (1 - 3 T). As such, it may be useful to use a lower field strength for *in vitro* studies in order to more closely match the conditions commonly used for *in vivo* studies. Additionally, at low field, relaxation rates tend to be slower which lengthens the usable imaging time. The availability of spectrometers at 1 and 9.4 T allows for a comparative study of PC-3 metabolism at these two fields.

The SpinSolve is somewhat limited in its available parameters, so the 400 MHz spectrometer was adjusted to match the SpinSolve. To that end, an array of 100 scans were taken with a 4 s delay between each 10-degree RF pulse.

The production of lactate was visible almost immediately, already appearing with significant SNR on the second scan (Figures 5.9 and 5.10). The lactate signal increased rapidly for a short time until the effect from T_1 relaxation began to dominate the signal. This was apparent at both 9.4 T and 1 T, though it was more prominent at higher field.

These results show that smaller, benchtop NMR spectrometers are adequate for monitoring metabolism in real time when using hyperpolarized ^{13}C . This has been verified by other groups [200], and given the low cost, improved technology, and portability of permanent

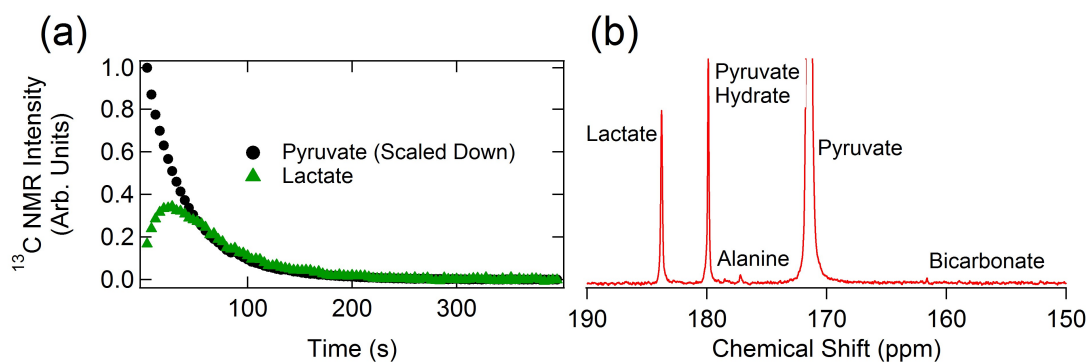


Figure 5.9. (a) Real time metabolism of PC-3 cells in suspension after administering hyperpolarized ^{13}C -labeled pyruvate. Scans were measured at 9.4 T (b) Sum of the first 50 spectra measured after administering pyruvate to the cells.

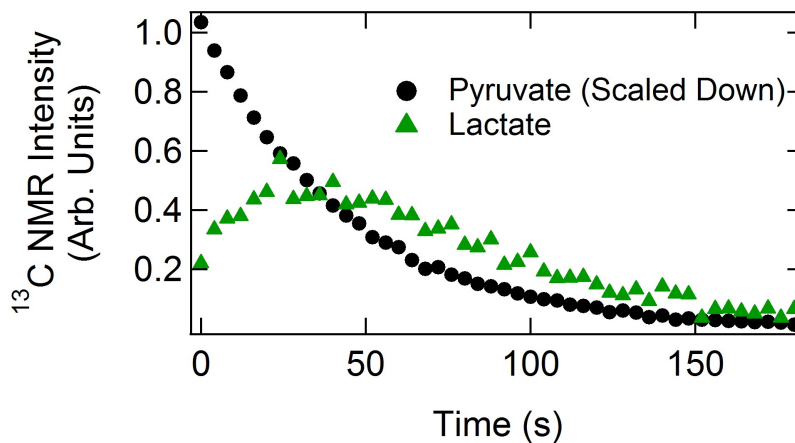


Figure 5.10. (a) Real time metabolism of PC-3 cells in suspension after administering hyperpolarized ^{13}C -labeled pyruvate. Scans were measured at 1 T

magnet high resolution NMR, they will likely see increased use for hyperpolarized magnetic resonance studies.

CHAPTER 6

LOW MAGNETIC FIELD NMR

Because of NMR's insensitivity, much work has been done to increase the magnetic fields in which NMR experiments are performed. For this reason, most magnetic resonance measurements are taken using superconducting magnets. While these magnets have many advantages, including the ability to reach very high fields and high field homogeneity, they can be expensive to procure and maintain. As such, low field methods of conducting NMR are desirable as they tend to be compact, relatively portable, and comparatively inexpensive. In particular, earth's magnetic field NMR has recently garnered a great deal of attention.

6.1 Earth's Field Nuclear Magnetic Resonance (EFNMR)

Earth's magnetic field, though very weak (≈ 0.5 Gauss), has a number of properties that make it useful as B_0 for NMR experiments. On very large scales, earth's magnetic field has large fluctuations in magnitude, but on small scales, it is very homogeneous, a property essential to NMR [112, 201]. Additionally, it is ubiquitous, allowing for NMR measurements to be taken nearly anywhere. Finally, it may be done very cheaply, expanding the circle of those who have access to NMR far more than could ever be hoped with conventional superconducting magnets. [141].

Two different EFNMR systems have been used to take measurements. The commercially available Terranova MRI (Magritek, New Zealand) is a fully functioning earth field NMR system with gradient coils allowing for shimming of the magnetic field and imaging. A second spectrometer system has been built based on the design presented in reference [141] as described in Chapter 3.

These systems are very similar in bore size and in the size and shape of the transmit/receive coil. However, the shielding and polarizing coil for the two differ dramatically.

The Terranova has no external shielding, while the homebuilt system has a thick aluminum tube that encompasses the transmit/receive and polarizing coils. Additionally, the Terranova uses thicker wire, larger diameter, and higher current in the polarizing coil, allowing for a greater magnetic field for the polarizing pulse.

6.1.1 Terranova EFMRI

The Terranova EFMRI system has been used primarily for introducing students to the principles of NMR and MRI (Figure 6.1), studying relaxation properties of water at Earth's field, and testing the feasibility of low-field contrast-enhanced MRI.

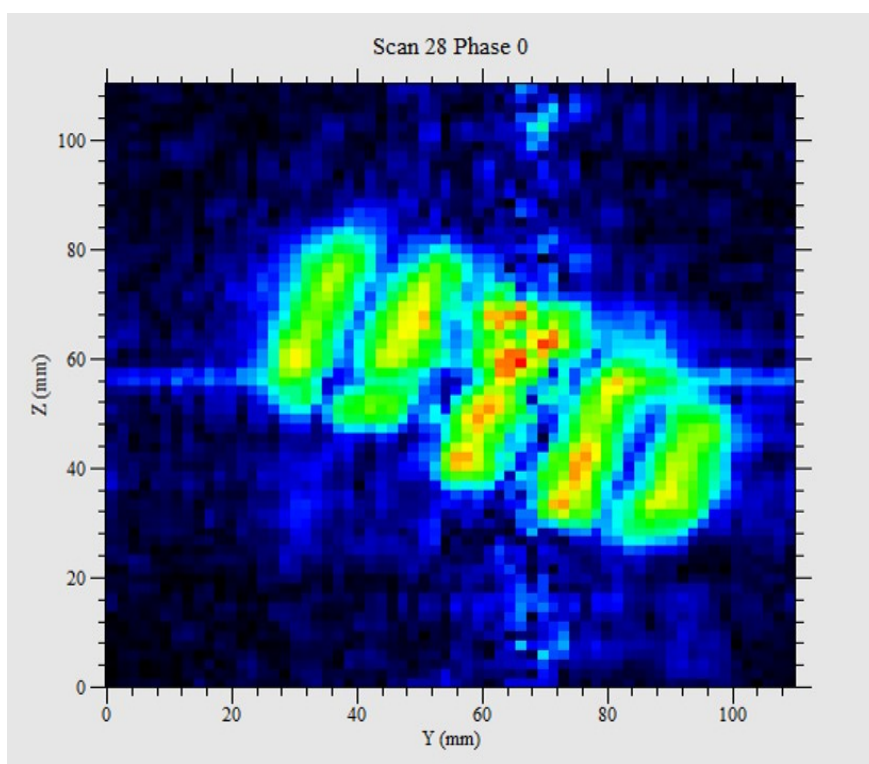


Figure 6.1. An MRI Image obtained of a custom 3-D printed phantom designed and printed by the author. The phantom was filled with water and measured using a gradient echo pulse sequence (See Appendix A) in the Terranova MRI.

While the relaxation properties of nuclei in solution are well studied and understood, there is a dearth of information on relaxation at low magnetic fields. This is likely because

of the relatively low sensitivity achievable at earth's magnetic field. However, the Terranova is sensitive enough to gather a great deal of data.

In particular, the temperature dependence of the water proton T_1 was measured between about 5 °C and 100 °C. For this, a 750 mL wine bottle was thoroughly rinsed and used to hold the sample. The top of the bottle was sealed using a cork. Temperature was monitored manually using a DigiSense temperature sensor whose metal probe was stuck through the cork at the top of the bottle. Because the probe was metal, it had a significant effect on the homogeneity of the magnetic field. To alleviate this effect, the wine bottle was positioned so that the entirety of the temperature probe was outside of the probe. Furthermore, the system was set up and shimmed with the temperature sensor in place which led to signal intensity and resolution approximately the same as what was achievable without the temperature probe.

The sample was then heated or cooled to the desired temperature by immersion in either a boiling water or ice water bath. It was quickly inserted into the Terranova probe and an initial NMR signal taken. Then, using the T_1 measurement pulse sequence supplied with the Terranova software, the proton T_1 was measured. During the measurement time, the temperature was recorded every 10 s, and at the end of the measurement, the nominal temperature was recorded by averaging each temperature point.

In this manner, proton T_1 was measured between 5°C and 100° for samples of pure water and water doped with varying concentrations of copper chloride (CuCl_2), namely 0.125 mM, 0.25 mM, 0.5 mM, 1 mM, and 2.5 mM.

Copper has long been used as a contrast agent due to its paramagnetic properties [177, 178, 179, 202]. By studying its effect on proton T_1 , insight may be gained into the relaxation mechanisms at earth's magnetic field.

It was found that for all samples, T_1 varied approximately linearly with temperature (Figure 6.2). For each sample, the slope of this line was positive, but grew progressively more shallow with the addition of CuCl_2 .

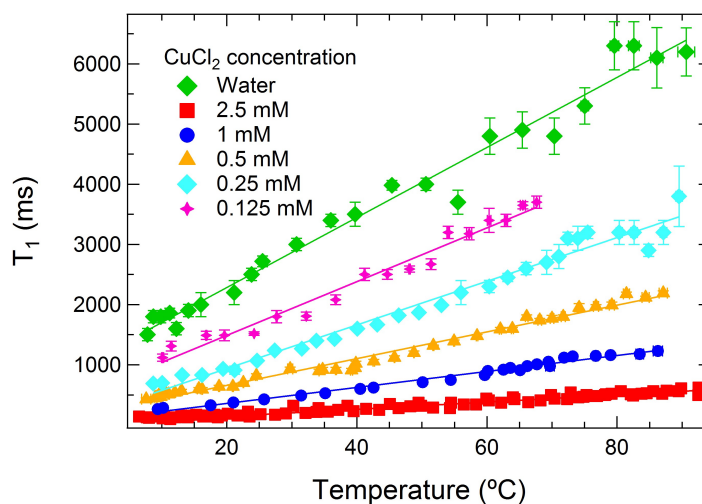


Figure 6.2. Temperature dependence of proton relaxation times for water doped with varying concentrations of copper chloride measured at Earth’s magnetic field.

These results show that the relaxation is dominated by temperature dependent interactions between water protons when there is little paramagnetic additive. As CuCl_2 is added, relaxation due to the paramagnetic additive gradually begins to have a dominant impact on relaxation. The relaxation due to CuCl_2 has a weaker temperature dependence which results in the flattening of the slope of the fit line [185, 190]. Furthermore, the significant impact of CuCl_2 on proton relaxation in earth’s magnetic field indicates that copper could be a useful low-field contrast agent for Earth’s field MRI.

The Terranova MRI offers the opportunity to investigate low-magnetic-field NMR phenomenon in a very accessible way. The results described above are only a fraction of what this instrument is capable of. There is a great deal left to be done to explore more paramagnetic agents, other proton-heavy liquids, and contrast-enhanced imaging.

CONCLUSION

The field of dissolution dynamic nuclear polarization, as a relatively young technique, has a huge number of unanswered questions even as applications in biomedical research are abundant. Herein, several important advances have been presented. Included in this are the details of the construction and testing of two different polarizers, both of which are among the highest magnetic field dissolution DNP polarizers in the world. Furthermore, one of the two polarizers is completely cryogen-free, the first of its kind and likely a foreshadowing of future DNP technology.

In addition to the instrumentation presented, experiments detailing optimization procedure for dissolution DNP were discussed. Studies using derivatives of the TEMPO radical investigated the effect of wide-linewidth radicals on ^{13}C DNP and helped to confirm widely held views regarding polarization mechanisms and the benefit of solvent deuteration. Additionally, many different paramagnetic additives were tested in conjunction with DNP using the trityl free radical. These results not only expand the pool of additives known to be effective beyond gadolinium, but also provide insight into the physics of DNP by confirming the link between electronic T_1 and DNP efficiency.

These optimization results were put to immediate use by applying the learned techniques to *in vitro* yeast and cancer cell metabolism. This was done using both conventional methods and hyperpolarization at low and high magnetic field. In particular, the use of a 1 T benchtop NMR system to monitor hyperpolarized ^{13}C metabolism was proven to be effective.

Furthermore, several low magnetic field alternatives to conventional high magnetic field NMR were discussed, and a permanent magnet NMR system as well as an Earth's magnetic field NMR system were constructed. The use of these instruments was somewhat limited, but they have been shown to be fully functioning and ready for further experiment.

As dissolution DNP continues to grow as a field of study, the instruments and optimization methods discussed herein provide both knowledge of the underlying physics and

advances in current DNP technology. While there is much left to be accomplished, this work represents an important step forward in the understanding of dissolution dynamic nuclear polarization.

APPENDIX A

NMR PULSE SEQUENCES

The most basic pulse sequence in NMR is a single 90° pulse after which the signal is monitored (Figure A.1). The signal measured is the precession of the bulk magnetization inducing a current in the NMR receive coil and is known as a free induction decay (FID). This is commonly used for conventional NMR. For hyperpolarization studies, a 90° pulse is not typically used. Rather, the signal is so greatly enhanced that very small tip angles (2° - 10°) are sufficient to monitor the signal. Further, this allows many scans to be measured with only a single hyperpolarization, whereas a 90° pulse would use all of the polarization in a single scan.

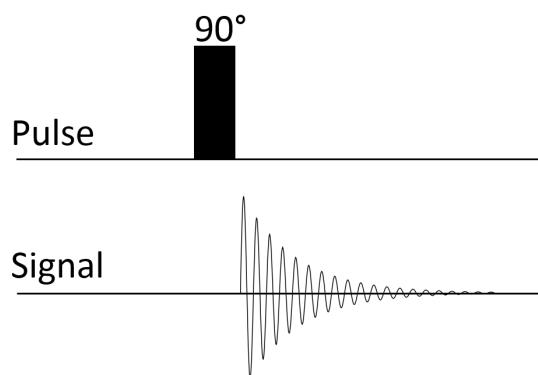


Figure A.1. The single pulse NMR experiment, which is the most basic NMR pulse sequence.

Only slightly more complex is the Spin Echo pulse sequence (Figure A.2). In this sequence, a 90° pulse is applied to the sample followed by a 180° pulse. This pulse serves to rephase the spins after the signal has decayed leading to an “echo” that is monitored in the same manner as a standard FID. This particular sequence is often used as a part of greater MRI pulse sequences or in cases of poor magnetic field homogeneity.

In order to measure T_1 relaxation time, a pulse sequence called inversion recovery is often used (Figure A.3). In this sequence, a 180° pulse is applied to invert the signal and then a

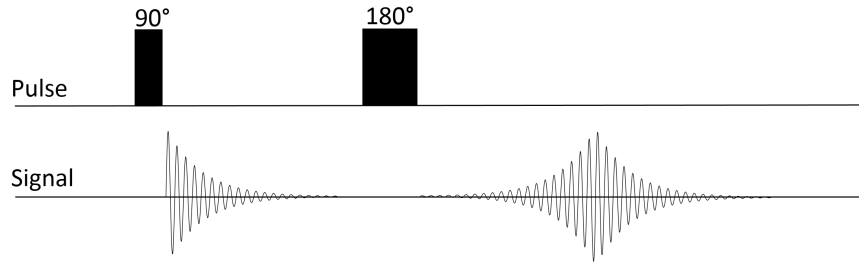


Figure A.2. The Spin Echo Pulse Sequence

90° pulse is used to monitor the signal after a time delay known as the inversion time. This is repeated multiple times with different inversion times and a long time interval between scans (4-5 times the estimated T_1). By fitting an exponential curve to the data, the T_1 may be gathered from the data.

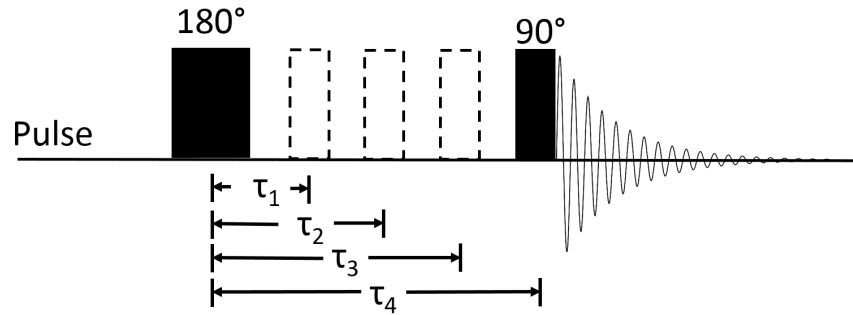


Figure A.3. The inversion recovery pulse sequence used to measure T_1 of nuclei.

One of the most basic imaging sequences used in MRI is the gradient echo pulse sequence (Figure A.4). In this sequence, a slice select gradient is applied during the 90° and 180° pulses of a spin echo pattern. Immediately following the pulses, a phase encoding gradient is applied to induce a phase shift. Then, the signal is monitored in the presence of a frequency encoding gradient.

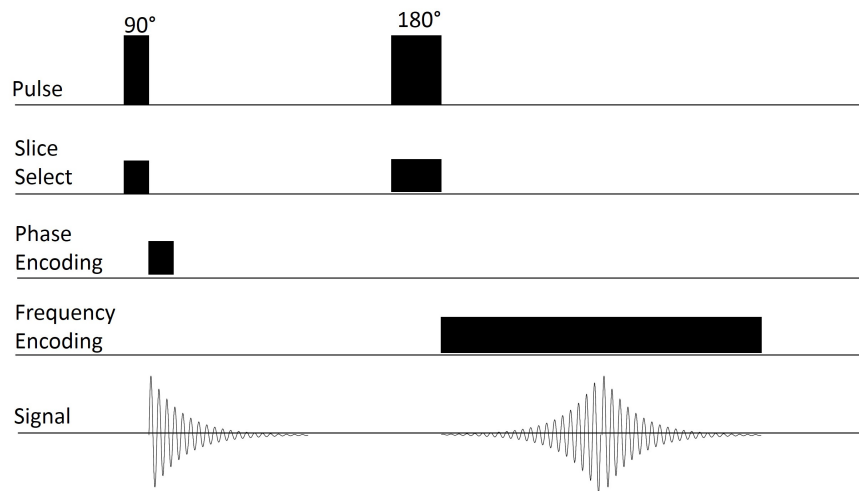


Figure A.4. A simple MRI pulse sequence - Gradient Echo.

APPENDIX B

THERMAL MIXING NUMERICAL SIMULATIONS

For a time, one of the major downsides to using the Thermal Mixing mechanism to describe polarization results was the poor agreement between theoretical calculations and experimental results. Borghini's original model was able to qualitatively describe polarization, but failed to match experimental results quantitatively [132, 133, 134]. This was due to the assumptions made during the derivations, which required strong field and high temperature approximations [132]. These models produce a set of rate equations, known as Provotorov equations [203] that describe the evolution of the spin temperature of the spin systems. In the model proposed by Wenckebach [135], these approximations are removed, giving the model rigor not found elsewhere. However, this model has the downside that there is no analytical solution to the equations which are presented, which make it difficult to apply to qualitative discussion. It has proven adept at predicting experimental polarization behavior and has provided solid quantitative agreement with experiment. The model hinges on the derivation of generalized Provotorov equations that can be used to calculate theoretical polarization values based on the experimental conditions and electronic properties of the free radical being used.

$$0 = -2W(\omega_m) \tanh \frac{1}{2} (\omega_0 \alpha + (\omega_m - \omega_0) \beta_{NZ}) - \frac{1}{2} \left[\int_{-\infty}^{\infty} d\omega g(\omega) \tanh \frac{1}{2} (\omega_0 \alpha + (\omega - \omega_0) \beta_{NZ}) - P_L \right] \quad (\text{A.1})$$

$$0 = -2W(\omega_m)(\omega_m - \omega_0) \tanh \frac{1}{2} (\omega_0 \alpha + (\omega_m - \omega_0) \beta_{NZ}) - \frac{1}{2} \int_{-\infty}^{\infty} d\omega g(\omega)(\omega - \omega_0) \tanh \frac{1}{2} (\omega_0 \alpha + (\omega - \omega_0) \beta_{NZ}) \quad (\text{A.2})$$

In these equations, $g(\omega)$ is the EPR spectrum function of the free radical, $W(\omega) = 1/2\pi\omega_0 1S^2 g(\omega)$ with $\omega_0 1S = \gamma_s \mathbf{B}_1$ where γ_s is the electron gyromagnetic ratio and $\mathbf{B}_1$ is

the alternating magnetic field of the microwave, ω_0 is the center of the EPR spectrum, ω_m is the frequency of microwave irradiation, α is the inverse lattice temperature and β_{NZ} is the inverse nuclear Zeeman temperature. The estimated polarization can then be calculated using

$$P_I = \tanh \frac{1}{2} \omega_{0I} \beta_{NZ} \quad (\text{A.3})$$

where ω_{0I} is the nuclear Larmor frequency.

By approximating the EPR spectrum of trityl using a Gaussian function, the estimated shape of the DNP spectrum can be calculated (Figure B.1). The change in spectrum with the addition of paramagnetic agent can also be simulated by adjusting the electron relaxation rate while keeping all other parameters constant. This was done numerically using a script written in MATLAB. Though the simulation is not perfect, it clearly indicates that a narrowing of spectrum and increase in nuclear polarization is to be expected with a shortened electronic T_1 .

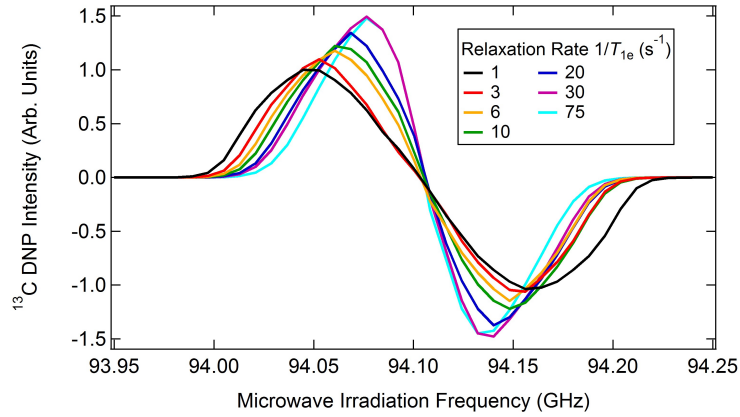


Figure B.1. Numerical simulation of DNP spectra for ^{13}C polarization using a narrow line-width radical such as trityl. The electron relaxation rate was adjusted to simulate the addition of paramagnetic agent. Simulations are scaled such that $1/T_{1e} = 1$ has a maximum intensity of 1.

APPENDIX C

OPERATION OF HOMEBUILT DNP POLARIZERS AT UTD

There are two polarizers that have been built at The University of Texas at Dallas, and both have specific procedures that must be followed in order to avoid damaging the instruments and to ensure safety and optimal data taking.

6.4 T Hyperpolarizer

As described in Chapter 3, this polarizer uses a superconducting magnet and a liquid helium cryostat, and, as such, requires great care in order to operate. This appendix provides the basics necessary to operate the system. Prior to operation, the user should consult manuals for each of the components of the polarizer in order to ensure safety and proper operation.

Initializing the System/Filling the Cryostat

To begin the operation of the polarizer, it is important to turn on the instruments that will monitor the conditions. The LakeShore temperature controller should be turned on and should display temperatures from the Cernox temperature sensors in the bottom of the cryostat and in the DNP probe. Additionally, the NMR cable should be plugged into the KEA NMR spectrometer (Magritek), with the appropriate duplexer (50-100 MHz) in place. The network analyzer should be close enough to the spectrometer that the matching and tuning of the coil may be checked easily. Connect the microwave source to the waveguide at the top of the DNP probe.

The first step to filling the cryostat is to purge the system.

- Close the ball valve attached to the roots blower. Turn on the roots blower and slowly open the ball valve to evacuate the cryostat.
- Connect a helium gas line to the toggle valve (Figure C.1) on the KF-50 cross.

- Open the two stage regulator on the helium gas tank such that there is positive pressure
- Close the ball and butterfly valves, and using the toggle valve, pressurize the cryostat to positive pressure.
- Slowly open the butterfly valve, and then the ball valve. Particularly with the ball valve, open very slowly and listen for a change in the sound of the pump to indicate when gas is being pumped from the cryostat.
- Repeat this purging process three times.

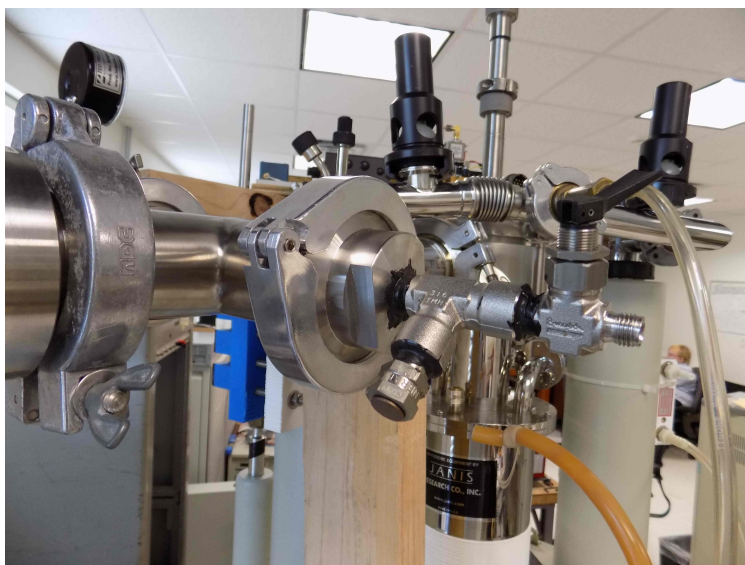


Figure C.1. The toggle valve used in purging the system.

After the cryostat has been purged, with the ball and butterfly valves open, it may be filled with liquid helium. Prior to filling the cryostat, the amount of liquid helium available must be measured. For one polarization run, 15 L is the minimum amount used. It is also important to note that the transfer line does not reach to the bottom of a standard 100 L helium dewar and will not be able to access approximately the final 10 L in the tank. If there is adequate helium, prepare the transfer line and the helium dewar by bringing them near

the magnet at a point where the ceiling is tall enough to accommodate the transfer line. If a dewar smaller than 100 L is used, a hose clamp should be placed on the transfer line so that the transfer line does not reach all the way to the bottom of the dewar. This is to avoid damaging the dewar or picking up contaminants that may have settled at the bottom.

- On the transfer line, turn the knob flow valve regulator clockwise (when viewed from the top) to open the needle valve at the bottom of the transfer line on the storage dewar leg.
- Insert the long leg of the transfer line into the storage dewar very slowly until it has been completely inserted. Tighten the brass O-ring seal nut to form a tight seal. There should be no gas coming out of the other end of the transfer line. As soon as the transfer line is below ceiling level, the dewar may be moved into position to fill the cryostat.
- Insert the other side of the transfer line (“male” bayonet) into the cryostat. Push firmly into the “female” bayonet and tighten the O-ring compression nut. Once this has cooled for a short time, push the tip again and re-tighten the nut in order to ensure the brass tip at the end of the transfer line is fully into the cryostat.
- Close the needle valve valve on the transfer line, and open the flow valve on the cryostat, opening and closing several times to ensure that the valve does not freeze shut.
- Using helium gas, pressurize the dewar to between 0.5 and 1 PSIG.
- Monitor the temperature displayed on the Lakeshore temperature controller. Once both the DNP probe and cryostat read 4 K, the helium sensor may be turned on and should be set to update every 10 s. This will enable the monitoring of the level of liquid helium in the cryostat.

- Once the cryostat is full (30" LHe), stop the transfer by closing the flow valve on the cryostat and the needle valve on the transfer line. It is also essential to depressurize the dewar by closing off the helium gas line and opening the pressure release valve on the top of the dewar.

As the cryostat is filling, the sample should be prepared in a standard sample cup. The sample cup should be deposited slowly into a liquid nitrogen bath in order to completely freeze the sample. Once the sample is frozen and the helium sensor reads about 5 inches of helium, the flow valve on the cryostat should be closed and the dewar depressurized. Then, connecting a helium line to the toggle valve once again and closing the ball and butterfly valves, the cryostat should be pressurized to positive pressure. The stopper placed in the quick coupler at the top of the DNP probe may be removed and the sample dropped into the probe. Replace the stopper and slowly open the butterfly and ball valves. Open the flow valve on the cryostat and pressurize the helium dewar in order to continue filling the cryostat. This all must be done quickly in order to avoid excessive loss of helium. The cryostat should be filled up to 30 inches of helium.

Polarization

Once the sample has been inserted into the probe and as the cryostat continues to fill, connect the NMR cable from the DNP probe to the network analyzer and tune the coil to the appropriate frequency (68.768 MHz for ^{13}C). Once there, connect the cable to the KEA NMR spectrometer and run a scan to verify that the sample is in the proper position. If no signal is seen, the elevator may need to be removed from the probe and the sample re-positioned.

It should also be noted that polarization experiments should only be run while the temperature is relatively stable and the liquid helium level is above about 1 inch. Polarization data could be inconsistent if this is not observed.

Microwave Frequency Sweep

In order to know the optimal irradiation frequency, a microwave sweep must be run. Unfortunately, there is currently no simple program to initiate this. Rather, it all must be undergone manually. To begin, open Prospa and open two different windows of the 1Pulse macro under the KEA-NMR menu. In one window, set the parameters to those desired for signal acquisition. This will be referred to as the “acquisition program”. Typical parameters are shown in Figure C.2. It is also important to change the file settings such that it will save the experiments incrementally within a larger directory. In the other window, set it such that many high power pulses are applied to the sample with short repetition time in order to kill the signal. This will be referred to as the “kill program”. Typical parameters are also shown in Figure C.2.

Then, after determining the range of frequencies over which a sweep is desired, turn on the microwave source and open the LabView executable which controls it (“Set VDIE Synthesizer”). Set the frequency to the first point in the sweep, and run the kill program. As soon as the kill program has finished running, start a stopwatch to monitor the time. After the amount of time designated per point (usually 5 minutes), run the acquisition program. It can be a good idea to immediately integrate the displayed signal using the integrate1D macro in the 1D menu in Prospa. Change the microwave frequency to the next point and run the kill program, starting the stopwatch when it is finished. Repeat these steps over the sweep range desired.

Polarization Build-up Monitoring

Once the optimum irradiation frequency has been determined (180.02 GHz for trityl OX063, 180.22 GHz for TEMPONE), polarization buildup curves may be obtained. For this, a pulse program has been written in Prospa in order to automatically monitor DNP buildup. Open the DNPBuildupMonitor macro under the KEA-NMR menu. Typical parameters are shown

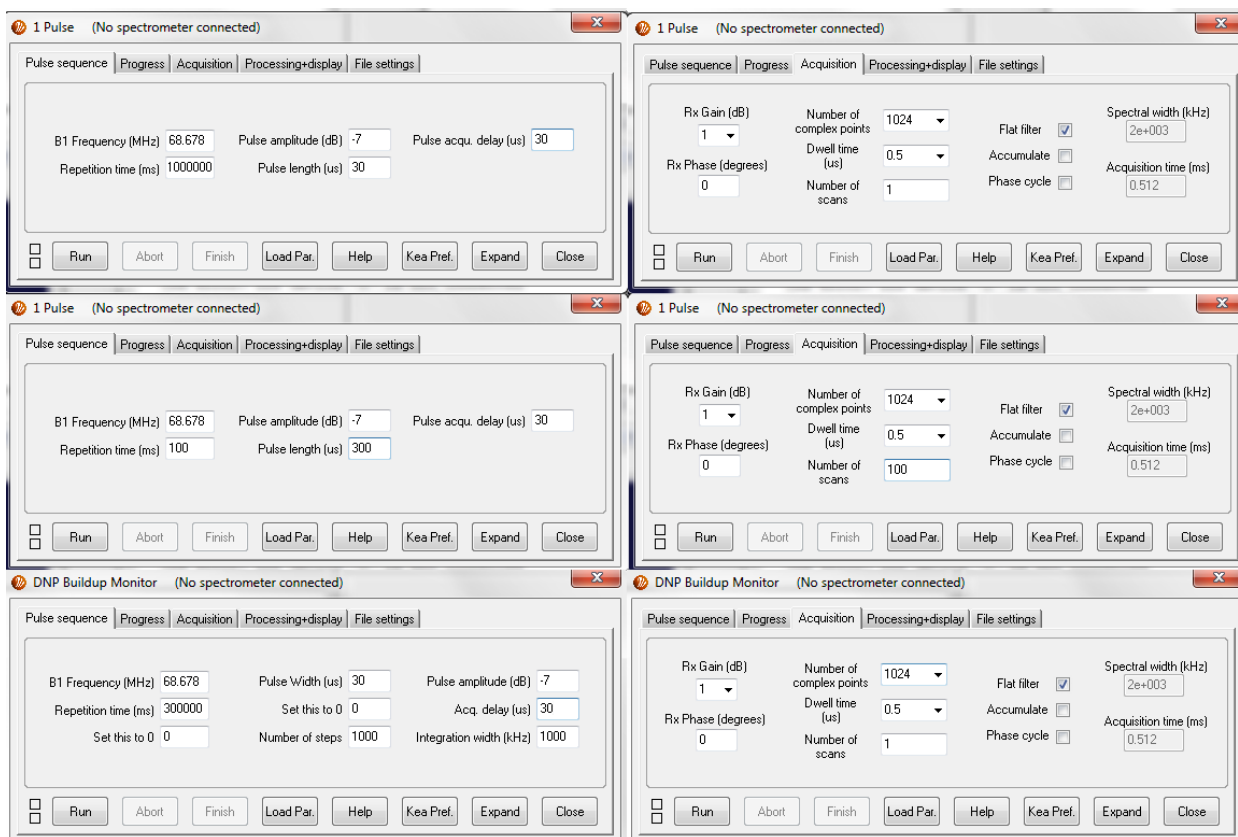


Figure C.2. Standard parameters for acquiring microwave frequency sweep data (top row), killing the built-up polarization (middle row), and monitoring the DNP build-up curve (bottom row).

in Figure C.2. It is important to note that, unless you know the precise number of points desired in the build-up curve, the number of steps should be set at some high number, and the experiment ended when desired by pressing the “Finish” button (If “Abort” is pressed, the data will be lost). To begin a build-up, set the microwave to the desired frequency and run a kill program. Immediately after, begin the DNP Buildup Monitor program.

Dissolution

Dissolution may be undergone when the liquid helium level has dropped below about 2 inches. Typically, this will take three people in order to perform the dissolution safely and efficiently. First, make sure all of the Swagelok quarter-turn valves on the dissolution wand

are closed. Additionally, make sure the tubing which delivers the hyperpolarized liquid is prepared and leads to a beaker for liquid collection. Then, open the topmost valve on the dissolution wand, insert approximately 4 mL of solvent into the dissolution wand, and close the valve again. Connect a helium line to the dissolution wand, and using the quarter turn valve, pressurize to 40 PSI. Then, plug in the heater and set the temperature to 400° F. Once the temperature has reached 400 and the pressure has reached 100 PSI, the ball valve to the roots blower should be closed and the cryostat pressurized to positive pressure. The stopper at the top of the DNP probe is then removed and the dissolution wand inserted all the way to the bottom of the probe, at which point the bottommost valve and the helium line valve should be opened rapidly.

This will send the hyperpolarized liquid to the prepared beaker, and it may then be used for hyperpolarized ^{13}C NMR experiments.

Shutdown

Once all experiments have been performed, the system must be shut down. Begin by removing the transfer line male bayonet from the cryostat female bayonet, then removing the transfer line from the helium dewar. Make sure that the helium dewar pressure release valve is open. Failure to do so will lead to pressure build-up in the dewar which can cause rapid boil-off of helium in the best case scenario. In the worst case, the dewar could rupture. Turn off all instruments, including helium sensor, microwave source, KEA spectrometer, network analyzer, and LakeShore temperature controller. Flush the dissolution wand three times by inserting 4 mL water and using helium gas to blow it out into a waste beaker until the transfer tube is dry.

Cryogen-free DNP System

This system uses a Cryomagnetics Incorporated cryogen-free sweepable superconducting magnet made available through collaboration with Dr. Mark Lee. The operation of the magnet system is explained in great detail both in the documentation for the magnet and in a document prepared by Dr. Lee's lab. As such, this discussion will simply cover various tricks and lessons learned and should not be considered a comprehensive document.

System Preparation

The system in Dr. Lee's lab consists of a MOKON chiller, a helium compressor, a helium circulation system, and the magnet. Before beginning to power the system, the chiller should be examined. Check the water level and ensure that the water level is no less than six inches from the top of the tank. To do this, the grate on the top of the chiller must be removed.

If the probe is configured to use a saddle coil, it may be inserted at this time. If it is configured with a solenoid, it should remain outside of the magnet until the magnet base temperature is reached.

Next, the system must be charged with ultra-pure helium gas. The process by which this is done is found in the system documentation and will not be covered here. After this process, the helium exchange system should be pressurized to 2 psi and the sample space to -10 psi with ultra-pure helium gas.

Once the system is charged, the process pump on the chiller may be turned on. It should be allowed to run for about 5 min, monitoring that the flow is between 2.5 and 3.5 GPM (Figure C.3). If the flow is not in an acceptable range, the bypass valves connected to the chiller may need to be adjusted. After several minutes of appropriate flow, the chiller compressor may be turned on. Once the outgoing water temperature has reached 50 °F, the helium compressor can be turned on. Note that there is both a main power switch to turn on the machine, and a power button to start the compression.



Figure C.3. Left: The control panel for the MOKON chiller used to cool the helium compressor. Process pump and compressor controls are labeled. Right: The flow meter attached to the chiller. The flow indicator is labeled and shows an appropriate value for the running of the system.

The temperature displayed on the chiller will begin to go up, but should stabilize around 75 °F after about an hour. The ingoing and outgoing water temperatures for the helium compressor should be monitored periodically as well, particularly in the short time after turning on the system. This is done by pressing the SERVICE/RTN button on the compressor unit. After this is pressed, the display will read “MONITOR SENSORS”, at which point the sensors may be cycled through by pressing the “SELECT” button (Figure C.4). Additionally, the Model TM-600 temperature monitor should be turned on to view the magnet temperature. T1 is the shield temperature and T2 is the magnet temperature. Common values for the sensor readings at different times in the system’s operation are shown in Table C.1.

It will take approximately 24 hrs for the magnet to reach its base temperature of about 3 K. Once this is reached, the sample space should be at approximately 100 K. At this point, the solenoid-configured probe should be inserted. To do this, connect the helium

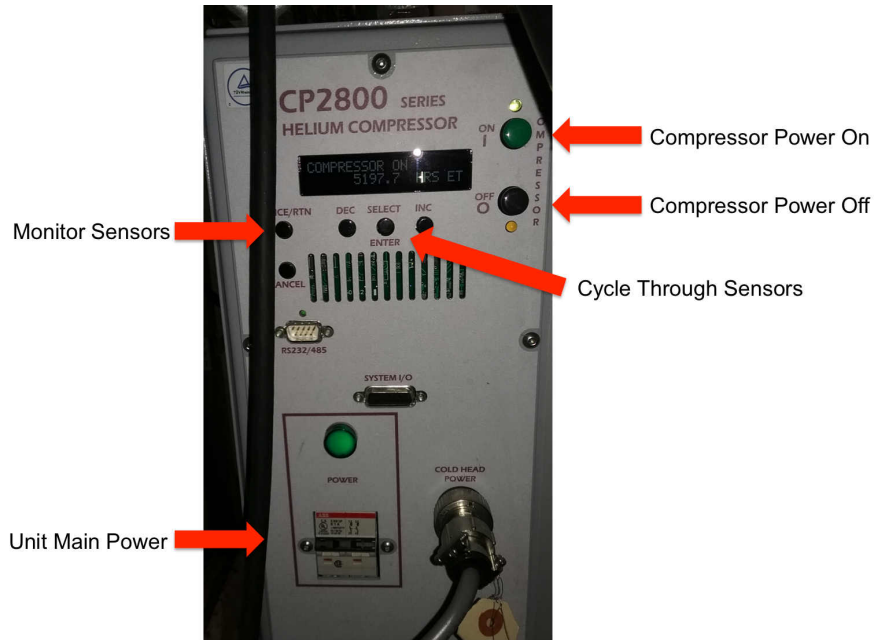


Figure C.4. The front panel of the helium compressor used in the cryogen-free magnet system. Important buttons and switches are indicated. Not pictured is the main breaker of the unit, which is a large switch under the main power. If the system trips, the breaker will need to be flipped.

Table C.1. Typical values for the sensor readings on the CP2800 Series Helium Compressor

Parameter	0 hrs	1 hr	6 hrs	24 hrs
Chiller Temperature (°F)	52	70	71	63
Compressor Water In (°F)	53	71	71	64
Compressor Water Out (°F)	53	99	100	89
Compressor Oil Temperature (°F)	74	110	110	97
Compressor Helium Temperature (°F)	74	142	143	136
Compressor δP (psi)	247	245	231	179
Magnet Temperature (K)	293	285	150	3

gas line to the sample space through the valve system used in charging the magnet system. Open the helium valve to over-pressurize the sample space and remove the sample space cap. Then, carefully slide the probe into the sample space, making sure that the probe is oriented correctly (waveguide will connect to microwave source). Once the probe is in, close the helium valve and tighten the collar on the top of the magnet. Connect the waveguide to the microwave source, and plug the temperature controller and NMR cable to the appropriate connections on the top of the probe.

After the probe is in place, the helium circulation should be started by turning on the scroll pump next to the compressor (Figure C.5). Then, the needle valve on the magnet should be adjusted until the flow-meter on top of the magnet reads approximately 4 SCFH. After a short time, the sample space should begin to cool down. This may be monitored using the LakeShore 336 temperature controller. There are two different LabView programs (“Big_Temperature_Display.vi” and “NML Data Acquisition.vi”) that can be used to track the temperature.



Figure C.5. The circulation pump used to bring the sample space temperature down to a base of 1.7 K.

With no further adjustment, the sample space should be able to reach below 10 K. Then, a roughing pump and helium gas line should be attached to the sample space using the same process as for charging the magnet. Vacuum out the sample space. Then, fold the helium

gas line about 4 inches from the valve, open the valve, then close it again while holding the line closed. This will allow a very small amount of exchange gas into the sample space which is necessary to reach operating temperature.

At this point, the sample temperature is likely between 5 and 10 K. To bring this down to the operating temperature of 2 K, the needle valve must be adjusted. However, this is a delicate process and must be done slowly to prevent the valve from being clogged and the temperature going up to 100 K. Close the needle valve (clockwise when looking down at the magnet) by 1 tick mark at a time, monitoring the temperature for 10-20 minutes after moving the valve. Once below 4 K, only turn the needle valve half a tick mark at a time. Immediately after turning the valve, the temperature should go up slightly then down, where it will eventually stabilize. The base temperature is about 1.7 K. Once this is reached, DNP experiments may be performed.

Magnet Operation and Polarization

The first step in conducting a DNP experiment is to bring the magnet to field. First, turn on the Model 4G Superconducting Magnet Power Supply (Figure C.6). Then, set the HI LIMIT or LO LIMIT to the desired value by pressing SETUP → LIMITS. Navigate to the limit that will be set using the up or down arrows and then type in the value using the key pad. Press SAVE, then EXIT and SAVE again. For polarization using the 180 GHz microwave source, the magnetic field should be set at 6.42393 T which corresponds to 33.3132 A. For other magnetic field strengths, the current required can be calculated based on the fact that the field strength varies linearly with current. The multiplicative factor to use is 5.1858 A/T. It is important to note that this value was determined experimentally and differs from the value of 5.2301 A/T reported in the system documentation.

Next, ensure that the charging rate is safe for the magnet by pressing SETUP → RATES. For 0 to 47.05 (maximum current for the magnet), the maximum safe charge rate is 0.0399



Figure C.6. The magnet power source used to energize the cryogen-free system. The series of images shows the process by which the limits are set. (a) Power source home page that is automatically displayed when the power is turned on. The SETUP menu is indicated (b) Power source SETUP menu with the LIMITS menu indicated. (c) Power source LIMITS menu with navigation arrows indicated. (d) LIMITS menu after a value has been changed. Select SAVE and EXIT to retain the input values and return to the main menu.

A/s. Typically, a smaller value (0.02 A/s) is used to ensure that the charging is safe. If needed, the rate can be changed in the same way as the limits.

Finally, the magnet may be brought to field by pressing HI LIMIT or LO LIMIT on the home page. The greatest danger of quench is at the beginning, so the magnet temperature should be monitored closely until the current has reached about 10 A. If the temperature gets above 4.4 K, press PAUSE and wait for the temperature to reduce to a safer value.

Once the magnet stabilizes at the appropriate field, the microwave source should be turned on. This source is the same as used for the conventional DNP system described above and is operated in the same manner. The source is relatively high power (100 mW) which

causes significant heating to the sample. Because of that, the source should be attenuated to about 30 mW by applying a DC bias of 3.66 V to the BNC connection marked “UCA” on the microwave source.

From this point, polarization proceeds exactly as described for the dissolution DNP system described above. The only difference is that instead of monitoring the liquid helium level, the temperature should be closely monitored to make sure that the magnet is not in danger of quenching and that the sample is stable at 1.7-2.2 K. If set up properly, this system has very good temperature stability and can be run for long stretches of time. However, the system should not be left alone if the magnet is energized.

Magnet Shutdown

To power down the magnet, simply press ZERO on the magnet power supply. The magnet will then discharge according to the charging rate that is set. As always when the current in the magnet is changing, monitor the magnet temperature closely. If the temperature gets above 4.4 K, press PAUSE and wait for the magnet to cool before continuing. Once stable at 0 A, the supply can be turned off. The chiller and compressor can remain on as long as experiments are planned for the near future, but the microwave source, DC Bias, NMR spectrometer, and RF power amplifier should be powered off before leaving.

Shutting down the magnet system entails a series of steps that must be done in the proper order. First, the helium should be “throttled back” so that helium gas is not wasted. This is done by closing the injection valve (green Swagelok valve on top of the magnet) while the circulation pump is still on. Wait until the pressure on the gas handling system in the back room is back to where it was initially (2 psi). Once it reaches this pressure, the black valve underneath the valve to the sample space should be closed and the circulation pump turned off. Next power down the helium compressor, first by stopping compression with the OFF button, then powering down the unit by flipping the main power switch. Finally, power

down the chiller by powering off the compressor, waiting about 5 minutes, and powering off the process pump.

Changing Samples

The method by which samples are changed depends on the configuration of the probe. If configured for a solenoid, the entire probe must be removed to change the sample. To do this, the circulation pump must be turned off and the sample temperature brought to 100 K. Over-pressurize the sample space with helium gas as described previously, then loosen the collar and remove the probe, replacing it with the sample space cap.

The probe must sit for a time to defrost. Then, carefully remove the Teflon tape holding the sample and Cernox temperature sensor. Use tweezers wrapped in Teflon tape to ensure that insulation is not scratched off of the cables.

Gently pull the copper microwave chamber off of the bottom of the probe to expose the solenoid. Carefully remove the coil by heating the solder connecting it to the NMR cable. Finally, unwrap the Teflon tape around the solenoid, exposing the sample tube.

Using a toothpick, remove the vacuum grease sealing the tube and collect the sample using a pipette. Carefully wash the tube. Then, the tube may be filled with 100 μL of the new sample and sealed with a small amount of vacuum grease. Replace the tube in the solenoid and wrap with Teflon tape. Connect the coil back to the probe by soldering the leads back to the NMR cable. Fix it in the correct orientation for polarization (open end of the solenoid toward waveguide) and secure it using Teflon Tape. Replace the microwave cavity and use Teflon tape to secure all of the probe components so that there is no vibration.

Finally, the probe may be returned to the magnet and brought to the base temperature as outlined above.

The sample exchange is much simpler for the saddle coil configuration. The temperature may remain at the base. Connect the helium gas line and over-pressurize the sample space.

Remove the cap from the quick connect coupler and stick the sample grabber down into the magnet to retrieve the sample cup. Place the cap back on. Then, remove the old sample from the sample grabber and replace it with the new sample cup. Note that the new sample should be frozen within the sample cup. Again, over-pressurize the sample space, remove the cap, and use the stick to place the sample in position. Replace the cap, vacuum out the sample space, and replace the exchange gas as described above.

APPENDIX D

CELL CULTURE PROTOCOL

Basic Precautions

In performing cell culture, care must be taken in order to ensure personal safety and to preserve the integrity of the cell line being studied. To that end, the following precautions must be observed.

- Always perform cell culture in a fully functioning laminar flow hood. Ensure the blower is on when the hood is raised. Turn on UV light when not in use to disinfect surfaces. Spray with 70% ethanol and wipe down inside of hood both before and after using.
- Always wear Nitrile lab gloves when working. Spray hands with 70% ethanol prior to working with cells.
- Prior to beginning work, spray surfaces of hood with 70% ethanol and wipe down with a paper towel.
- Never introduce any item into the hood unless it is in sterile packaging or has been cleaned with 70% ethanol.
- Do not open cell dishes outside of the hood.
- Warm all cell culture liquids (PBS, medium, trypsin) in a warm water (37° C) bath prior to using. Store liquids in a refrigerator when not in use.
- Do not touch pipette tips or vacuum tip to anything other than the substances on which they are being used. If a tip accidentally comes into contact with anything, discard and use a new tip.

- When working with different cell lines, never use the same pipette tips for the different lines. Discard pipette tips after each use. Failure to do so could result in contamination of one or more cell lines.
- If working with different cell lines, use a Bunsen burner to sterilize vacuum tip or use a new tip before using aspirator.

All equipment used in preparing and culturing cells must be sterile in order to prevent contamination or damage to cells. The following equipment is necessary for a fully stocked cell culture lab.

- Laminar flow hood (Figure D.1)
- Carbon dioxide incubator (Figure D.2)
- Aspirator (outside hood with line leading into hood)
- Portable pipette aid for serological pipettes with recharger (in hood)
- Sterile, individually packaged serological pipette tips, particularly 5, 10, and 25 mL sizes (in hood)
- Small pipettes, (0.5-10 μ L, 20-200 μ L, and 200-1000 μ L) (in hood)
- Sterile pipette tips for the above pipette sizes (in hood)
- Cell culture dishes, particularly 10 and 15 cm (may be stored in sterile packaging outside hood)
- Conical centrifuge tubes, 15 and 50 mL (may be stored outside hood in sterile packaging)
- Sterile cell culture medium filters (may be stored outside hood in sterile packaging)

- Hemacytometer for counting cells (outside hood)
- Cell counter (outside hood)
- Inverted Microscope (outside hood)
- Centrifuge (outside hood)
- Mr. Frosty (ThermoFisher Scientific) for freezing cells



Figure D.1. The laminar flow hood used for cell culture.

Preparing Cell Culture Medium

Typically, cell culture medium is not purchased fully prepared. Rather, the base medium and several additives are procured separately and must be mixed prior to use in cell culture experiments. The components are:

- Base Medium: Common Example: Dulbecco's Modified Eagle Medium (DMEM)
- Fetal Bovine Serum (FBS)



Figure D.2. The incubator used for cell culture. The incubator should be kept at 37°C and 5% CO₂. Additionally, the water tray within the incubator needs to be kept filled with 1.5 L of sterilized distilled water.

- Glutamine solution
- Penicillin/Streptomycin Solution

Other necessary solutions for cell culture are

- Phosphate buffered saline (PBS)
- Trypsin, either .05% or .25%
- Trypan blue dye (if measuring cell viability)

Before first use, the base medium should be kept in a refrigerator, and FBS, glutamine solution, and penicillin/streptomycin should be frozen, the FBS in 50 mL sterile centrifuge tubes, the glutamine solution and penicillin/streptomycin in 15 mL sterile centrifuge tubes. Standard labeling for each is S for FBS, Q for Glutamine, and P/S for penicillin/streptomycin. Ultimately, the medium will contain 10% FBS, 4 mM of glutamine, and 10 mL penicillin/streptomycin per 1 L of medium. To begin the process, all ingredients should be

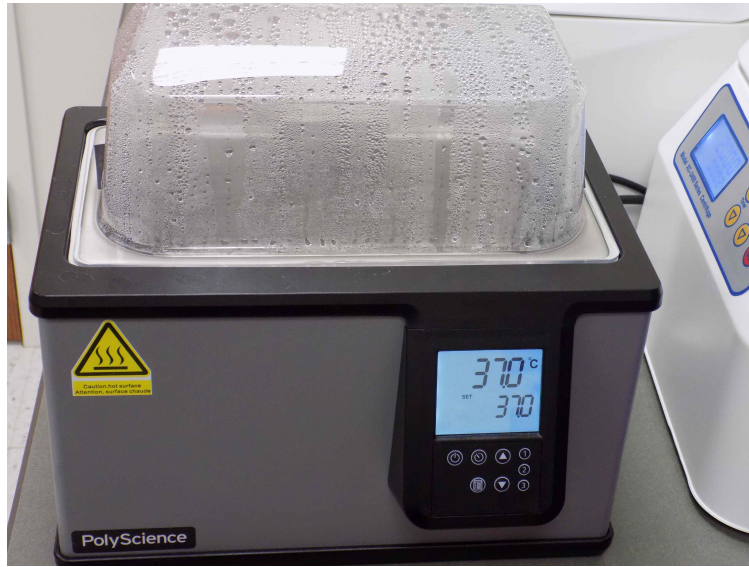


Figure D.3. The warmer that is used to thaw and warm media components to 37°C.

placed in a warm water bath until thawed (Figure D.3). Subsequently, they should be sprayed with 70% ethanol, wiped clean, and placed in the clean hood. Pipette FBS, glutamine, and penicillin into base medium taking care to avoid touching the pipette tip to any surfaces to reduce the chance of contamination. Gently swish the bottle in a circular motion to mix medium. Avoid heavy splashing of the liquid, and try to keep the medium from reaching the top of the bottle. If contamination is thought to have occurred, the medium must be filtered through a sterile cell culture medium filter. Label bottle with the date of mixing. Media may be stored in a standard refrigerator along with trypsin and PBS. Each time these are used, they should be warmed in a water bath and cleaned with 70% ethanol before being introduced into the hood.

Initializing a Cell Line

When cell lines are not being actively propagated, they must be stored in liquid nitrogen. If cells are stored at some higher temperature for an extended period, they may lose their viability. Cell lines are stored in a freezing medium. In order to initialize a cell line, they

must be thawed, removed from the freezing medium, and introduced into cell culture dishes. Cells should be thawed rapidly by immersing the cell vial in a warm water bath. Then, the vial of cells should be moved to the hood. Remove the cell suspension from the vial and move it to a 50 mL centrifuge tube using a 1 mL pipette. Add about 20 mL of medium to the tube and centrifuge at 300 g's for 5 minutes. Make sure that the centrifuge is balanced. Using the vacuum, remove the medium down to the cell pellet, being careful not to vacuum up the cells. Add to the tube 10 mL of medium, pipetting up and down many times in order to evenly suspend all of the cells. Using the pipette, transfer cells to a 10 cm cell culture dish. Once in the dish, cells must be evenly distributed on the bottom of the dish by gently swishing the dish in the following pattern:

- forward and back (ten times)
- side to side (ten times)
- forward and back (ten times)
- side to side (ten times)
- forward and back (five times)

The dish may then be stored in a 37 °C, 5%CO₂ incubator. In larger labs, cells will often need first to be stored in a “pending” incubator until it can be verified that cells are free from contamination, at which point they may be stored in a “clean” incubator. Make sure to verify with the lab manager to check if this is necessary.

Transferring Cells

In order to keep cells growing and healthy, the medium must be changed every 2-3 days. Additionally, cells should only be allowed to reach 60-80% confluency (dish coverage). If too

great confluency is reached, mutations could result. Therefore, it is good practice to transfer cells to a new dish every 2-3 days. However, in order to determine if transferring cells is necessary, it is a good idea to look at your cells using a bottom lens microscope to estimate confluency (Figure D.4).

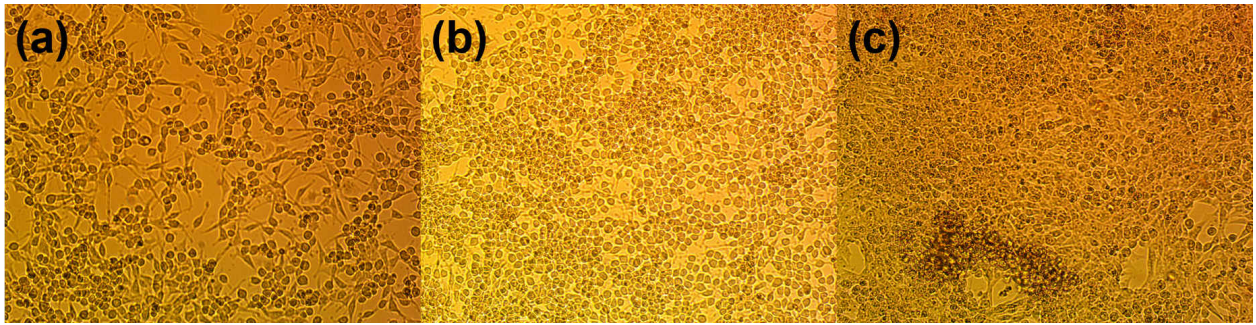


Figure D.4. The image through an inverted microscope of cell dishes with (a) low confluency, (b) appropriate confluency for transferring cells, and (c) over-confluency at which point cells should be discarded.

To split cells, introduce PBS, trypsin, medium, and the cells to be transferred into the hood using proper clean procedure. Begin by aspirating out the old medium from the dish. Tilt the dish so that media may be aspirated without damaging cells. Next, pipette PBS into the dish to wash the cells, administering to the dish wall without touching the wall by tilting the dish at about 30° . By administering to the wall, it is ensured that cells are not detached from the bottom of the dish prematurely and that the pipette is not contaminated. Swish the dish gently to cover the entire bottom with PBS, and then, tilting the dish slightly, aspirate out the PBS. Next, administer trypsin to the dish. Tilt the dish to cover the bottom and let sit for several minutes. If cells are known to be hard to detach, move dish to the incubator to aid in detaching cells. The dish may also be gently tapped on the side to aid in detaching cells. Cells sloughing off the bottom of the dish should be visible when the cells are adequately detached.

At this point, medium should be added to inhibit trypsin activity. If cells will not be counted, add 2-3 times the amount of trypsin added. If cells are to be counted, add more

medium, 5-6 times as much as trypsin. Use the pipette to spray medium along the bottom of the dish, holding it at a tilt so that all of the cells will pool in one place. Then, if counting cells, transfer the suspension to a centrifuge tube. If not counting cells, add an appropriate number of cells to the new dish as well as enough fresh medium to bring the amount to that shown in Table D.1. Distribute cells evenly using the above swishing pattern. The dish may then be placed back into the incubator.

Table D.1. Useful cell numbers and medium quantities for cell culture (Values are approximate)

Tissue Culture Dish	Amount of PBS	Amount of Trypsin	Amount of Medium	Number of Cells to Seed	Number of Cells at Confluency
60 mm	1 mL	0.5 mL	3 mL	0.8×10^6	3.2×10^6
10 cm	2-3 mL	1 mL	10 mL	2.2×10^6	8.8×10^6
15 mm	3-5 mL	2 mL	25 mL	5×10^6	25×10^6
6-Well Plate	1 mL	0.5 mL	3 mL	0.3×10^6	1.2×10^6
96-Well Plate	—	—	100 μ L	5000	—

Counting Cells

Once the cell suspension has been placed into a 15 mL centrifuge tube, cover the tube while the hemacytometer is prepared. Using 70% ethanol, clean both the platform and the glass cover using a Kimwipe. Place the glass slide on the plate so that it covers about half of the notch as shown in Figure D.5. Going back to the cell suspension, pipette the suspension in and out using a 5 or 10 mL pipette tip to uniformly distribute the cells. Then, pipette out 10 μ L and insert into notch of the hemacytometer. The spread of suspension under the glass slide should be visible, and should fill the whole space. Place the hemacytometer under a microscope and find the center grid. Using a counter, count all cells that are within the 4 grids diagonally adjacent to the center, as shown in Figure D.6. If the number of cells counted is N , the cell count for the cell suspension is $N \div 4 \times 10^4$ cells/mL.

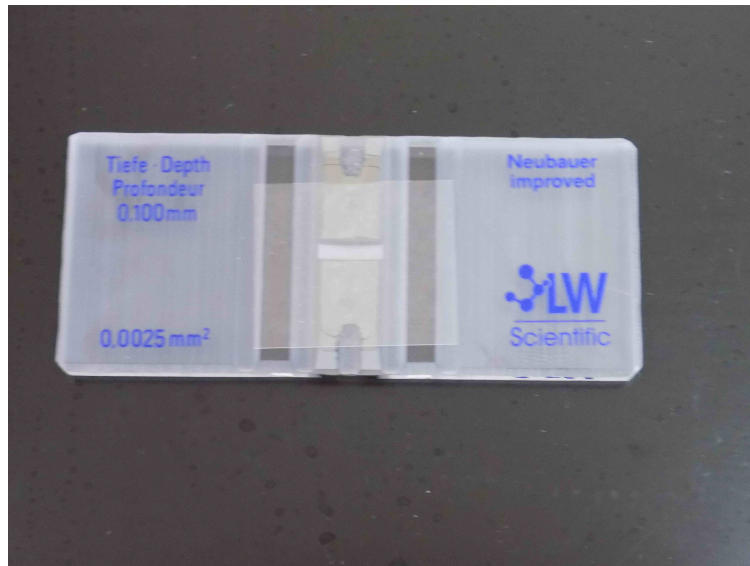


Figure D.5. A hemacytometer

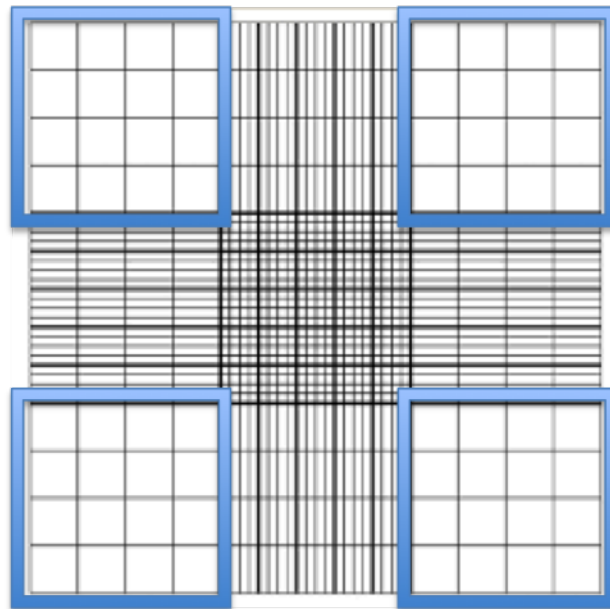


Figure D.6. View through a microscope of a hemacytometer. Count the cells in the squares indicated

Using a similar procedure, it is also possible estimate the viability of the cells. The viability is a measure of what percentage of cells in the suspension are living. From the original cell suspension, pipette out 100 μ L of cell suspension into a micro-centrifuge tube. Add 100 μ L of Trypan blue dye. Using the same procedure as previously, add 10 μ L to the hemacytometer. Living cells have intact cell membranes that are impermeable to the dye, while dead cells have compromised membranes. Therefore, living cells will look the same as previously under the microscope, while dead cells will be dark blue. Count the number of dead cells and the total number of cells. By taking the number of dead divided by the number of living, the percentage of dead cells is obtained. Subtract this percentage from 100% in order to obtain the viability.

Freezing Cells

If a cell line is no longer actively needed for experiments, it may be frozen for storage. Following the same procedure as for transferring cells, wash the cells with PBS and loosen them with trypsin. Use about 5 mL of medium to inhibit trypsin once cells are detached and pipette the suspension into a 15 mL centrifuge tube. Centrifuge at 300 g's for 5 minutes and vacuum out medium down to the cell pellet. Add in the freezing medium and pipette in and out in order to evenly suspend cells. The freezing medium is made with 90% FBS and 10% DMSO. Each vial of cells to be frozen should contain about 0.5 mL of freezing medium, so the amount of freezing medium to add depends on the number of vials of cells desired. Label each vial with the cell type and date. Finally, cells are placed in a "Mr. Frosty" container filled with isopropyl alcohol and placed in a -80 °C freezer. This prevents the cell medium from freezing too quickly and damaging the cells. Once cells are frozen, the vial should be moved to liquid nitrogen for long term storage.

Perchloric Acid (PCA) Extracts

Caution: Perchloric Acid (PCA) is a strong acid that can be very dangerous if not handled with proper caution. Make sure to consult the MSDS for PCA and to follow all proper precautions before using.

Taking extracts of cells is a common method by which to take a "snapshot" of the cells' metabolism. A labeled substrate is applied to cultured cells and at desired time intervals, the cells are destroyed and their contents are harvested, allowing for the imaging of the metabolic products present in the remains.

Cells are cultured as described above. In order to have enough cells for NMR of cell extracts, it is suggested to use at least two 15 cm cell culture dishes for each time snapshot. Cells should be allowed to grow until they are 80 - 100% confluent. Prepare the labeled cell culture medium by adding enough substrate so that it is at or slightly above biological concentration in the medium. It may be desirable to use a specialized base medium with none of the unlabeled substrate added (Ex: Use choline-free DMEM when taking choline cell extracts). Aspirate out the old medium. Cells do not need to be trypsinized but prior to the addition of the labeled medium, they should be washed multiple times with PBS. Keeping the cells in the same culture dishes, add the labeled medium, being careful to add to the side of the dish so as not to disturb the cells. Place cells into incubator.

When it is time to take the cell extracts, remove cells from incubator to a fume hood, and aspirate or pipette out all of the medium. Wash the cells with a small amount (4 mL per dish) of saline solution. Apply 4 mL of 12% PCA to each dish. Using a plastic scraper, rub the cells off the bottom of the dish. Tilt the dish, and, using the scraper, gather the PCA and cell residue into a pool at the bottom of the dish. Collect into a (50 mL) centrifuge tube. Apply 4 mL of 'utrapure' (milli-Q) water into the dish. Follow the same procedure as above to gather the water into the same centrifuge tube. Slightly different volumes of

liquid or concentrations of PCA may be used, but the final solution should have 4-6% PCA in order to ensure complete cell destruction.

Centrifuge the tube for five minutes at 2000 g's. Because the cells have been killed, centrifuge speeds can be very fast since cell death is no longer an issue. Once completed, collect the liquid, which contains all of the water soluble molecules that were contained by the cells, into another centrifuge tube. This solution contains everything necessary for NMR experiments but is very highly acidic. Therefore, it should be neutralized using 8 M KOH. For 16 mL of 6% PCA, just over 1800 μ L of KOH is needed. Test the pH using a pH strip. Add about 10 μ L at a time until the pH of the solution is between 7 and 8.

During the neutralization process, PCA will precipitate out and collect in the bottom of the tube. Centrifuge at 2000 g's for 5 minutes. Collect the remaining liquid into a tube and store in a freezer.

This solution is extremely dilute, and must be concentrated in order to perform NMR. This will be accomplished by the process of lyophilization, which removes all of the water from the sample and leaves behind a powder. This powder may then be dissolved in just enough solute (usually D₂O) to perform NMR.

MTT and XTT assays

MTT and XTT are chemicals that change color as they are metabolized by cells. This enables rapid assessment of cell viability using UV-Vis spectrophotometry, which is very useful for studying the impact of drugs on cell cultures.

Necessary materials:

- Thiazole blue tetrazolium bromide (MTT)
- DMSO or Sodium Dodecyl Sulfate (SDS) and 0.01 M HCl
- 96 well plate

- Absorbance microplate reader
- XTT
- Phenazine Methosulfate (PMS)
- 96 well plate
- Absorbance microplate reader

First, the 96-well plate must be seeded with the appropriate number of cells. From an established cell line, collect cells into a centrifuge tubes using standard methods. Count the number of cells and then dilute until the concentration is between 50,000 and 100,000 cells/mL. Seed each well of the plate with 100 μ L of this suspension. Then, incubate overnight so that the cells recover after trypsinization. The preparation of the microplate is the same for both types of assay.

In order to conduct the MTT assay, the reagents must first be mixed. Mix MTT powder with phosphate buffered saline (PBS) to form a 12 mM MTT solution. It takes about 1 mL of solution for a complete assay of a 96-well plate. With the molecular weight of MTT being 414.32, this amounts to about 5 mg MTT for every 1 mL PBS.

Aspirate out the old medium from the cell dish and apply 100 μ L of fresh medium. Then, apply 10 μ L of MTT solution to each well. After this has been accomplished, incubate the plate for 2 - 4 hours, at which point a purple precipitate should be visible at the bottom of the plate. These are formazan crystals that are the insoluble metabolic product of MTT. There are a number of methods that may be used to dissolve this precipitate. The two methods that have been used by the author are to mix sodium dodecyl sulfate (SDS) with 0.01 M HCl and add 100 μ L of this solution to each well. Then, incubate the cells for 4 hrs to overnight. The other option is to remove the medium from the wells by aspiration, taking

care not to vacuum any of the precipitate. Then, add 100 μ L DMSO to each well. The dish may then be shaken to dissolve the formazan.

After the formazan is fully dissolved, the absorbance of each well at 570 nm should be read using a microplate reader. From the absorbance, an estimate of cell viability may be made.

The XTT assay is carried out in a very similar manner. However, the XTT has the advantage that its metabolic product (resazurin) is soluble in cell culture medium, avoiding the need to add an extra solvent.

First, make a 10 mM PMS solution in PBS. Only a very small amount of PMS solution is necessary, on the order of 10 μ L. Additionally, a solution of XTT should be made using the proportion 1 mg XTT to 1 mL cell culture medium (warmed to 37°C). Immediately before applying to cells, 10 μ L of PMS solution should be added to 4 mL of XTT solution. Then, add 25 μ L of this final solution to each well. The 96-well plate should then be incubated 2-4 hours. After this time, the microplate may be shaken to ensure thorough mixing of the resazurin. Finally, using an absorbance microplate reader, measure the absorbance at 450 nm of every well.

APPENDIX E

PROTOCOL FOR *IN VITRO* HYPERPOLARIZED MRS EXPERIMENTS

When studying cells *in vitro* using hyperpolarized magnetic resonance, proper protocol must be followed in order to successfully view metabolism. Cells must be correctly suspended, a cell injection system must be prepared, and the transfer of hyperpolarized liquid and cells must be handled with speed and precision following dissolution.

Preparation of Cells

Cells should be cultured as described in Appendix D. Once an adequate number of cells (50-100 million or 2-3 15 cm dishes) have been grown, they must be carefully harvested and suspended. Cells should be washed with PBS, then trypsinized. Then, they should be transferred to a centrifuge tube and centrifuged at 300 g's for 5 minutes. Aspirate out the liquid down to the cell pellet and re-suspend the cells in PBS to wash away the old medium and trypsin. Centrifuge the suspension again at 300 g's for 5 minutes and aspirate the PBS. Finally, re-suspend the cells in 1.5 mL of the desired medium. For example, if the substrate to be studied is pyruvate, glucose, or fructose, the medium should be base DMEM with no glucose, sodium pyruvate, or L-glutamine.

Cell Injection System

Following dissolution, the hyperpolarized liquid and cell suspension must be mixed before NMR scans are taken. One way to accomplish this is to rapidly inject the cell suspension into the hyperpolarized liquid and allow the natural turbulence caused by injection to stir the mixture. For this, the materials necessary are an NMR tube cap, a syringe, and a length of microbore tubing that extends from the syringe to the NMR tube inside the NMR magnet.

Two holes should be drilled through the tube cap, and the tubing threaded through one of them to reach to the bottom of the NMR tube. Using an adaptor, connect the syringe to the tubing in order to draw the cell suspension into the tubing, usually using 1-1.5 mL of suspension.

Post Dissolution

The dissolution liquid should be collected in a small beaker. Using a pipette, transfer the amount of liquid necessary for physiological concentration of the substrate to be studied to the NMR tube with the tubing already present. Immediately lower the tube into the NMR magnet. As soon as the tube has reached the center of the magnetic field, inject the cells as rapidly as possible into the tube. Begin acquiring signal as soon as the cells have been injected.

Though there are many possible conditions that would dictate the array of scans to be taken once the cells and hyperpolarized substrate have been mixed, they may be based off of basic parameters. A fairly large pulse angle ($\approx 10^\circ$) should be used in order to see the small signal of metabolites. The repetition time will be highly dependent on the T_1 of the substrate being studied, but 2 seconds is a standard value for ^{13}C labeled compounds.

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BIOGRAPHICAL SKETCH

Peter Niedbalski was born and raised in Kearney, Nebraska. After graduating as the valedictorian from Kearney Catholic High School in 2009, he attended the University of Nebraska at Lincoln for his freshman year of college. After that year, Peter transferred to Benedictine College where he double majored in Mathematics and Physics. He graduated *summa cum laude* in 2013 and began graduate studies at The University of Texas at Dallas (UTD) that same year. He received his Masters degree in Physics in 2015 and continued his studies at UTD in the research lab of Dr. Lloyd Lumata. During his time at UTD, Peter was married to Erin and had a daughter, Bernadette. Upon completion of his doctorate, he will be joining the group of Dr. Zackary Cleveland at Cincinnati Children's Hospital Medical Center as a postdoctoral research fellow. Peter is a committed Catholic, husband, father, and scientist.

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Experimental NMR Conference Student Travel Award, 2016

Phi Kappa Phi Student Travel Scholarship, The University of Texas at Dallas Chapter, 2016

Presentation Award, Texas Section of APS Meeting, Waco, TX, 2015

Tanveer and M. Yaqub Mirza Scholarship, The University of Texas at Dallas, 2015

Excellence in Education Fellowship, The University of Texas at Dallas, 2013 - 2015

Graduated Summa Cum Laude, Benedictine College, 2013

Publications

Magnetic field dependent lifetimes of hyperpolarized carboxyl ^{13}C at cryogenic temperatures, Peter Niedbalski, Qing Wang, Christopher Parish, Fatemeh Khashami, Andhika Kiswandhi, Lloyd Lumata, Manuscript in Preparation

Transition metal doping reveals link between electron T_1 reduction and ^{13}C dynamic nuclear polarization efficiency, Peter Niedbalski, Christopher Parish, Qing Wang, Zahra Hayati, Likai Song, André F. Martins, A. Dean Sherry, Lloyd Lumata, Manuscript in Preparation

1. **Enhanced efficiency of ^{13}C dynamic nuclear polarization by superparamagnetic iron oxide nanoparticle doping**, Peter Niedbalski, Christopher Parish, Qing Wang, Zahra Hayati, Likai Song, Zackary I. Cleveland, Lloyd Lumata, Accepted for publication in Journal of Physical Chemistry C, August 17, 2017, DOI: 10.1021/acs.jpcc.7b06408
2. **^{13}C dynamic nuclear polarization using derivatives of TEMPO free radical**, Peter Niedbalski, Christopher Parish, Qing Wang, Andhika Kiswandhi, Lloyd Lumata, *Appl. Magn. Reson.* **48**, 933-942 (2017)
3. **^{13}C dynamic nuclear polarization using a trimeric Gd^{3+} complex as an additive**, Peter Niedbalski, Christopher Parish, Qing Wang, Andhika Kiswandhi, Zahra Hayati, Likai Song, Lloyd Lumata, *J. Phys. Chem. A* **121**, 5127-5135 (2017)
4. **Assembly and performance of a 6.4 T cryogen-free dynamic nuclear polarization system**, Andhika Kiswandhi*, Peter Niedbalski*, Christopher Parish, Qing Wang, Lloyd Lumata, *Magn. Reson. Chem.* **55**, 846-852 (2017)

5. **Construction and ^{13}C hyperpolarization efficiency of a 180 GHz dissolution dynamic nuclear polarization system**, Andhika Kiswandhi, Peter Niedbalski, Christopher Parish, Sarah Ferguson, David Taylor, George McDonald, Lloyd Lumata, *Magn. Reson. Chem.* **55**, 828-836 (2017)
6. **Influence of ^{13}C isotopic labeling location on dynamic nuclear polarization**, Peter Niedbalski, Christopher Parish, Andhika Kiswandhi, Zoltan Kovacs, Lloyd Lumata, *J. Phys. Chem. A* **121**, 3227-3233 (2017)
7. **Influence of Dy^{3+} and Tb^{3+} doping on ^{13}C dynamic nuclear polarization**, Peter Niedbalski, Christopher Parish, Andhika Kiswandhi, Leila Fidelino, Chalermchai Khemtong, Zahra Hayati, Likai Song, André Martins, A. Dean Sherry, Lloyd Lumata, *J. Chem. Phys.* **146**, 014303 (2017)
8. **^{13}C dynamic nuclear polarization using isotopically-enriched 4-oxo-TEMPO free radicals**, Peter Niedbalski, Christopher Parish, Andhika Kiswandhi, Lloyd Lumata, *Magn Reson Chem* **54**, 962-967 (2016)
9. **Impact of Ho^{3+} -doping on ^{13}C dynamic nuclear polarization using trityl OX063 free radical**, Andhika Kiswandhi, Peter Niedbalski, Christopher Parish, Pavanjeet Kaur, André Martins, Leila Fidelino, Chalermchai Khemtong, Likai Song, A. Dean Sherry, Lloyd Lumata, *Phys Chem Chem Phys* **18**, 21351-21359 (2016)
10. **The effect of glassing solvent deuteration and Gd^{3+} doping on ^{13}C DNP at 5 T**, Andhika Kiswandhi, Bimala Lama, Peter Niedbalski, Mudrekh Goderya, Joanna Long, Lloyd Lumata, *RSC Adv* **6**, 38855-38860 (2016)

Presentations

1. **Dynamic nuclear polarization: boosting ^{13}C MRI signals by >10,000-fold**, Peter Niedbalski, Christopher Parish, Qing Wang, Lloyd Lumata, American Chemical Society Meeting in Miniature, Texas Christian University, Fort Worth, TX (April 29, 2017)
2. **Mechanisms of ^{13}C dynamic nuclear polarization in a mixture of two free radicals**, Christopher Parish, Peter Niedbalski, Qing Wang, Andhika Kiswandhi, Likai Song, and Lloyd Lumata, American Chemical Society Meeting in Miniature, Texas Christian University, Fort Worth, TX (April, 29, 2017)
3. **Hyperpolarized ^{89}Y -EDTP and ^{89}Y -DTPP as pH sensitive MRI agents**, Qing Wang, Peter Niedbalski, Christopher Parish, Lloyd Lumata, American Chemical Society Meeting in Miniature, Texas Christian University, Fort Worth, TX (April 29, 2017)

4. **Dynamic nuclear polarization using TEMPO-based free radicals**, Peter Niedbalski, Christopher Parish, Qing Wang, Andhika Kiswandhi, Lloyd Lumata, Experimental NMR Conference, Asilomar Conference Center, Pacific Grove, CA (March 26-31, 2017)
5. **Influence of Dy³⁺ and Tb³⁺ doping on ¹³C dynamic nuclear polarization**, Peter Niedbalski, Christopher Parish, Andhika Kiswandhi, Leila Fidelino, Chalermchai Khemtong, Zahra Hayati, Likai Song, Andre Martins, Dean Sherry, Lloyd Lumata, Experimental NMR Conference, Asilomar Conference Center, Pacific Grove, CA (March 26-31, 2017)
6. **A 180 GHz dynamic nuclear polarization system using a cryogen-free and sweepable superconducting magnet**, Andhika Kiswandhi, Peter Niedbalski, Christopher Parish, Qing Wang, Lloyd Lumata, Experimental NMR Conference, Asilomar Conference Center, Pacific Grove, CA (March 26-31, 2017)
7. **Dissolution dynamic nuclear polarization enhanced by a trimeric gadolinium complex** Peter Niedbalski, Qing Wang, Christopher Parish, Zahra Hayati, Likai Song, Magnus Karlsson, Mathilde Lerche, Lloyd Lumata, Experimental NMR Conference, Asilomar Conference Center, Pacific Grove, CA (March 26-31, 2017)
8. **¹³C dynamic nuclear polarization using a mixture of trityl OX063 and 4-oxo-TEMPO at 3.35 T and 1.2 K**, Christopher Parish, Peter Niedbalski, Qing Wang, Andhika Kiswandhi, Lloyd Lumata, Experimental NMR Conference, Asilomar Conference Center, Pacific Grove, CA (March 26-31, 2017)
9. **Hyperpolarized ⁸⁹Y-EDTP and ⁸⁹Y-DTPP as pH sensitive MRI agents**, Qing Wang, James Ratnakar, Peter Niedbalski, Christopher Parish, Lloyd Lumata, Zoltan Kovacs, Experimental NMR Conference, Asilomar Conference Center, Pacific Grove, CA (March 26-31, 2017)
10. **Dynamic nuclear polarization of low-gamma nuclei**, Qing Wang, Christopher Parish, Peter Niedbalski, Andhika Kiswandhi, Zoltan Kovacs, Lloyd Lumata, Southwest Regional Meeting of the American Chemical Society (ACS), Galveston Convention Center, Galveston, Texas (November 10-13, 2016)
11. **Influence of Dy³⁺ and Tb³⁺ on ¹³C dynamic nuclear polarization**, Peter Niedbalski, Christopher Parish, Andhika Kiswandhi, Zahra Hayati, Andre Martins, Leila Fidelino, Chalermchai Khemtong, Likai Song, A. Dean Sherry, Lloyd Lumata, Southwest Regional Meeting of the American Chemical Society (ACS), Galveston Convention Center, Galveston, Texas (November 10-13, 2016)
12. **¹³C dynamic nuclear polarization using a mixture of trityl OX063 and 4-oxo-TEMPO free radicals**, Christopher Parish, Peter Niedbalski, Qing Wang, Andhika Kiswandhi, Lloyd Lumata, Southwest Regional Meeting of the American Chemical Society (ACS), Galveston Convention Center, Galveston, Texas (November 10-13, 2016)

13. **Construction and performance of a homebuilt 180 GHz dissolution dynamic nuclear polarizer**, Andhika Kiswandhi, Peter Niedbalski, Christopher Parish, Sarah Ferguson, David Taylor, George MacDonald, Lloyd Lumata, Southwest Regional Meeting of the American Chemical Society (ACS), Galveston Convention Center, Galveston, Texas (November 10-13, 2016)
14. **Influence of Ho^{3+} -doping on ^{13}C dynamic nuclear polarization**, Andhika Kiswandhi, Peter Niedbalski, Christopher Parish, Pavanjeet Kaur, Andre Martins, Leila Fidelino, Chalermchai Khemtong, Likai Song, A. Dean Sherry, Lloyd Lumata, Joint Meeting of the Texas and Four Corners Sections of the American Physical Society (APS), New Mexico State University, Las Cruces, New Mexico (October 21-22, 2016)
15. **Ho-DOTA as a polarization enhancer for dissolution dynamic nuclear polarization**, Andhika Kiswandhi, Christopher Parish, Peter Niedbalski, Pavanjeet Kaur, Zahra Hayati, Andre Martins, A. Dean Sherry, Chalermchai Khemtong, Likai Song, Lloyd Lumata, NSF Site Visit Symposium for Early Career Scientists, National High Magnetic Field Laboratory (NHMFL), Tallahassee, FL (August 29-31, 2016)
16. **Temperature dependence of proton NMR relaxation times at earth's magnetic field**, Peter Niedbalski, Christopher Parish, Andhika Kiswandhi, Sarah Ferguson, Eduardo Cervantes, Anisha Oomen, Anagha Khrishnan, Aayush Goyal, Lloyd Lumata, Experimental NMR Conference (ENC), Wyndham Grand Hotel, Pittsburgh, Pennsylvania (April 10-15, 2016)
17. **Influence of glassing matrix deuteration and Gd^{3+} doping on ^{13}C dynamic nuclear polarization at $B = 5$ tesla**, Andhika Kiswandhi, Bimala Lama, Peter Niedbalski, Mudrekh Goderya, Joanna Long, Lloyd Lumata, Experimental NMR Conference (ENC), Wyndham Grand Hotel, Pittsburgh, Pennsylvania (April 10-15, 2016)
18. **Dynamic nuclear polarization of carbonyl and methyl ^{13}C spins in acetate using trityl OX063**, Peter Niedbalski, Christopher Parish, Zoltan Kiswandhi, Lloyd Lumata, Experimental NMR Conference (ENC), Wyndham Grand Hotel, Pittsburgh, Pennsylvania (April 10-15, 2016)
19. **Development and performance of a 180 GHz dissolution DNP hyperpolarizer**, Andhika Kiswandhi, Peter Niedbalski, Christopher Parish, Sarah Ferguson, David Taylor, George MacDonald, Lloyd Lumata, Experimental NMR Conference (ENC), Wyndham Grand Hotel, Pittsburgh, Pennsylvania (April 10-15, 2016)
20. **^{13}C dynamic nuclear polarization using isotopically-enriched 4-oxo-TEMPO free radicals**, Peter Niedbalski, Christopher Parish, Andhika Kiswandhi, Lloyd Lumata, Experimental NMR Conference (ENC), Wyndham Grand Hotel, Pittsburgh, Pennsylvania (April 10-15, 2016)

21. **Influence of ^{13}C isotopic labeling location on ^{13}C DNP of acetate using TEMPO free radical**, Christopher Parish, Peter Niedbalski, Zoltan Kovacs, Lloyd Lumata, Experimental NMR Conference (ENC), Wyndham Grand Hotel, Pittsburgh, Pennsylvania (April 10-15, 2016)
22. **The effects of Ho^{3+} -DOTA doping on ^{13}C dynamic nuclear polarization: ESR and hyperpolarization studies**, Andhika Kiswandhi, Christopher Parish, Peter Niedbalski, Pavanjeet Kaur, Zahra Hayati, Andre Martins, A. Dean Sherry, Chalermchai Khemtong, Likai Song, Lloyd Lumata, Experimental NMR Conference (ENC), Wyndham Grand Hotel, Pittsburgh, Pennsylvania (April 10-15, 2016)
23. **Real-time tracking of dissociation of hyperpolarized ^{89}Y -DTPA: a model for degradation of open-chain Gd^{3+} MRI contrast agents**, Christopher Parish, Sarah Ferguson, Peter Niedbalski, Andhika Kiswandhi, Zoltan Kovacs, Lloyd Lumata, Experimental NMR Conference (ENC), Wyndham Grand Hotel, Pittsburgh, Pennsylvania (April 10-15, 2016)
24. **Hyperpolarized ^{13}C NMR lifetimes in the liquid-state: relating structures and T_1 relaxation times**, Christopher Parish, Peter Niedbalski, Zohreh Hashami, Leila Fidelino, Zoltan Kovacs, Lloyd Lumata, American Physical Society (APS) meeting, Baltimore Convention Center, Baltimore, Maryland (March 14-18, 2016)
25. **Hyperpolarized ^{89}Y NMR spectroscopic detection of Yttrium ion and DOTA macrocyclic ligand complexation: pH dependence and YDOTA intermediates**, Sarah Ferguson, Peter Niedbalski, Christopher Parish, Andhika Kiswandhi, Zoltan Kovacs, Lloyd Lumata, American Physical Society (APS) meeting, Baltimore Convention Center, Baltimore, Maryland (March 14-18, 2016)
26. **Real-time tracking of dissociation of hyperpolarized ^{89}Y -DPTA: a model for degradation of open-chain Gd^{3+} MRI contrast agents**, Sarah Ferguson, Peter Niedbalski, Christopher Parish, Andhika Kiswandhi, Zoltan Kovacs, Lloyd Lumata, American Physical Society (APS) meeting, Baltimore Convention Center, Baltimore, Maryland (March 14-18, 2016)
27. **Impact of Gd^{3+} doping and glassing solvent deuteration on ^{13}C DNP at 5 T**, Andhika Kiswandhi, Bimala Lama, Peter Niedbalski, Mudrekh Goderya, Joanna Long, Lloyd Lumata, American Physical Society (APS) meeting, Baltimore Convention Center, Baltimore, Maryland (March 14-18, 2016)
28. **Production and NMR signal optimization of hyperpolarized ^{13}C -labelled amino acids**, Christopher Parish, Peter Niedbalski, Sarah Ferguson, Andhika Kiswandhi, Lloyd Lumata, American Physical Society (APS) meeting, Baltimore Convention Center, Baltimore, Maryland (March 14-18, 2016)

29. **Temperature dependence of proton NMR relaxation times at Earth's magnetic field**, Peter Niedbalski, Christopher Parish, Andhika Kiswandhi, Sarah Ferguson, Eduardo Cervantes, Anisha Oomen, Anagha Khrishnan, Aayush Goyal, Lloyd Lumata, American Physical Society (APS) meeting, Baltimore Convention Center, Baltimore, Maryland (March 14-18, 2016)
30. **Optimization of ^{13}C dynamic nuclear polarization: isotopic labeling of free radicals**, Peter Niedbalski, Christopher Parish, Andhika Kiswandhi, Lloyd Lumata, American Physical Society (APS) meeting, Baltimore Convention Center, Baltimore, Maryland (March 14-18, 2016)
31. **Construction and ^{13}C NMR signal efficiency of a dynamic nuclear polarizer at 6.4 T and 1.4 K**, Andhika Kiswandhi, Peter Niedbalski, Christopher Parish, Sarah Ferguson, David Taylor, George MacDonald, Lloyd Lumata, American Physical Society (APS) meeting, Baltimore Convention Center, Baltimore, Maryland (March 14-18, 2016)
32. **Novel Earth's field magnetic resonance imaging of copper-doped water for inexpensive detection of disease**, Anagha Khrishnan, Andhika Kiswandhi, Peter Niedbalski, Christopher Parish, Lloyd Lumata, American Association for the Advancement of Science (AAAS) 2016 Annual Meeting, Washington, D.C. (February 11-15, 2016)
33. **Dynamic nuclear polarization of carbonyl and methyl ^{13}C spins: ^{13}C acetate samples doped with trityl OX063**, Peter Niedbalski, Christopher Parish, Lloyd Lumata, Texas Section of the American Physical Society (APS) meeting, Baylor University, Waco, TX (October 29-31, 2015)
34. **Electron relaxation of DNP free radical DPPH at W-band**, Armin Khamoshi, Pavanjeet Kaur, Peter Niedbalski, Likai Song, Lloyd Lumata, Texas Section of the American Physical Society (APS) meeting, Baylor University, Waco, TX (October 29-31, 2015)
35. **Dynamic nuclear polarization of ^{13}C -labeled amino acids**, Christopher Parish, Peter Niedbalski, Sarah Ferguson, Andhika Kiswandhi, Lloyd Lumata, Texas Section of the American Physical Society (APS) meeting, Baylor University, Waco, TX (October 29-31, 2015)
36. **The effect of isotopic labeling on 4-oxo-TEMPO free radical on ^{13}C dynamic nuclear polarization**, Peter Niedbalski, Andhika Kiswandhi, Christopher Parish, Lloyd Lumata, Texas Section of the American Physical Society (APS) meeting, Baylor University, Waco, TX (October 29-31, 2015)
37. **Optimization of ^{13}C dynamic nuclear polarization at 5 Tesla**, Andhika Kiswandhi, Bimala Lama, Peter Niedbalski, Mudrekh Goderya, Joanna Long, Lloyd Lumata, Texas Section of the American Physical Society (APS) meeting, Baylor University, Waco, TX (October 29-31, 2015)

38. **Study of the effect of glassing matrix deuteration and Gd³⁺ doping on ¹³C hyperpolarization at 5 T**, Bimala Lama, Andhika Kiswandhi, Peter Niedbalski, Mudrekh Goderya, Joanna Long, Lloyd Lumata, 44th Southeastern Magnetic Resonance Conference (SEMRC), Daytona Beach, FL (October 9-11, 2015)
39. **Novel earth's field MR imaging of copper chloride**, Anagha Krishnan, Andhika Kiswandhi, Peter Niedbalski, Christopher Parish, Lloyd Lumata, 2015 George A. Jeffrey UTD Nanoexplorers Symposium, Richardson, TX (August 12, 2015)
40. **NMR and ESR studies of the antioxidant properties of blueberry extract and L-Ascorbic Acid**, Anisha Oommen, Peter Niedbalski, Sarah Ferguson, Andhika Kiswandhi, Lloyd L. Lumata, 2015 George A. Jeffrey UTD Nanoexplorers Symposium, Richardson, TX (August 12, 2015)
41. **Influence of ¹³C isotopic labeling location of ¹³C DNP of acetate using TEMPO free radical**, Christopher Parish, Peter Niedbalski, Lloyd Lumata, American Physical Society (APS) March Meeting, San Antonio, TX (March 2-6, 2015)
42. **Dynamic nuclear polarization of carbonyl and methyl ¹³C spins in acetate using trityl OX063**, Peter Niedbalski, Christopher Parish, Lloyd Lumata, American Physical Society (APS) March Meeting, San Antonio, TX (March 2-6, 2015)

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