

Flow ^1H Dynamic Nuclear Polarization Studies in Normal Liquids and Supercritical Fluid Carbon Dioxide

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(Abstract)

Flow ^1H dynamic nuclear polarization (DNP) experiments of small probe molecules (i.e. chloroform and benzene) were conducted in normal liquids (i.e. neat benzene, deuterated benzene, chloroform) and supercritical fluid CO_2 . Initial data collected on the ^{14}N NMR line width of acetonitrile in normal liquids and SF CO_2 resulted in a 2-6 factor increase in the molecular motion of the molecule in the SF--the result of which corresponds to the expected factor increase in DNP signal enhancements in the low viscosity fluid. Observed DNP signal enhancements were 2-5 times greater in the supercritical fluid versus the normal liquids in both the solid-liquid intermolecular transfer (SLIT) and liquid-liquid intermolecular transfer (LLIT) experiments. Significant changes in the electron spin-spin (T_{1s}) and spin-lattice (T_{2s}) relaxation times of the nitroxide radical TEMPO in neat benzene, deuterated benzene, and SF CO_2 were noted; the $T_{1s}T_{2s}$ product (calculated from DNP saturation plots) of the LLIT DNP data were compared.

Due to the high pressures and elevated temperatures necessary for optimum flow DNP with SF CO_2 (e.g. $P = 2310$ psi, $T = 313$ K), high pressure flow cells were developed (and, also, adapted to a commercially available probe in the NMR regime) using PEEK (polyetheretherketone) material.

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Chapter 1: Introduction

1.1 General Remarks

The technique of dynamic nuclear polarization (DNP) had its origins with the theoretical prediction by Overhauser¹ in 1953, that if the conduction electron spins in a solid metal were saturated, the electron spin polarization could be transferred to the nuclear spins, resulting in as much as a 1000-fold increase in nuclear spin polarization. In the nuclear magnetic resonance (NMR) regime, the observable result of this phenomenon is enhanced signal intensity. DNP enhancements, therefore, are obtained via the Overhauser polarization phenomenon. By 1962, DNP was utilized for liquids² and since 1965, up until the present, the results of static liquid and flow DNP experiments, including various reviews of the subject, have been reported in the literature.³⁻⁵

Over the past fifty years, DNP has been used to study NMR sensitivity enhancements, probe molecular motion in solutions, and aid in the measurement of electron spin-lattice relaxation times (T_1). In our labs, flow DNP has been developed and used primarily for the measurement of NMR signal enhancements⁶⁻⁸--which necessarily includes information on the motional properties of tested sample solutions. In the present work, the results of DNP used in the determination of electron T_1 relaxation times will be newly presented. Beginning in 1988, our lab was the first to report a flow ^1H DNP transfer experiment⁶ and, subsequently, report on the signal enhancements obtained for simple probe molecules such as benzene and chloroform, analyzed in the neat liquid.^{7,8} In the same year, supercritical fluid (SF) chromatography coupled to ^1H NMR detection was also performed in our laboratory.⁹ In the latter experiment, the use of SF CO_2 as the flowing solvent, proved to be advantageous in eluting a hydrocarbon sample mixture in a shorter amount of time and with greater resolution, versus a "normal", neat liquid solvent. In both the case of flow DNP and SF CO_2 - ^1H NMR, the above-mentioned research has demonstrated that signal enhancements and resolution enhancements of small probe molecules can be obtained, through the use of these techniques. However, as of this time, the coupling of supercritical fluids with flow ^1H DNP detection has not been fully investigated, nor reported in the literature.

1.2 Objective of Present Research

In the present research, therefore, the major goal was to investigate the use of SF CO_2 with ^1H DNP detection. This goal was accomplished, in part, by conducting fundamental studies of small probe molecules (i.e. acetonitrile and benzene) in order to : 1) obtain the optimum field strength at which SF CO_2 -DNP can be performed (Chapter 3); 2) obtain signal enhancements for the probe molecule in the liquid-liquid and solid-liquid intermolecular flow transfer DNP experiments in SF CO_2 (Chapters 4 and 5); and 3) use the flow ^1H DNP technique as an aid in the measure of electron T_1 relaxation times for a simple nitroxide (Chapter 6). In the process of fulfilling these research goals, the development of a high-pressure probe cell made out of PEEK material became necessary and so Appendix I will address this topic.

Chapter 2: Background For the Use of Supercritical Fluid (SF) CO₂ in Flow Transfer DNP

2.1 Introduction

Supercritical fluids (SF's), in general, exhibit unique properties of low viscosity, moderate density, and high diffusivity, as compared to normal gases and liquids. Table 2.1 lists a comparison between the properties of gases, liquids, and supercritical fluids.¹¹ SF's are commonly used in extractions, processing, and chromatographic separations and have become an important class of solvents, especially for continuous flow processes. The solvating ability of SF's can be readily varied by changes in temperature and pressure, which affect the SF density. One can increase the pressure or decrease the temperature of a system, in order to increase the density and therefore the solvating power of the SF. Therefore, density is the major state effect in solvation for SF's. In addition to density effects, chemical effects including polarity, hydrogen bonding, and acid-base interactions are important in the dissolution process, as well.¹¹ In regards to SF viscosity, it is interesting to note, that it has been experimentally determined¹², for a SF at constant density and at temperatures above the critical point, viscosity is approximately constant and independent of temperature. This flexibility of SF's can offer an advantage over normal liquids and gases used as solvents in liquid chromatography, for example. Beginning in the 1980's, SF properties were utilized in detection techniques, i.e. high-pressure NMR experiments, where researchers such as Evilia and Jonas demonstrated resolution enhancements of quadrupolar nuclei (i.e. ¹⁴N, ¹⁷O, ³³S) in SF ethylene.¹⁰ The researchers, also, investigated transport and relaxation mechanisms of nuclei in these high pressure solvents.^{11,13}

In order to operate in the supercritical region, temperatures and pressures at or above the critical point (T_c and P_c) must be used. SF CO₂ is commonly used as a solvent of choice in SF applications because of its benevolent chemical properties. In addition, it has the advantage of relatively low critical point parameters, $T_c = 31^\circ\text{C}$ and $P_c = 74\text{ atm (1072 psi)}$, with a corresponding critical density of $\rho_c = 0.468\text{ g/mL}$ and $\eta_c = 0.03\text{ cP}$.

Dynamic nuclear polarization (DNP) is a double resonance spectroscopic technique that has been proven to be very powerful, compared to conventional NMR, in the detection of chemical compounds. As was mentioned in the general remarks section of Chapter 1, flow DNP has been conducted in normal liquid systems, where a free radical is either dissolved directly in the sample solution (this is referred to as liquid-liquid intermolecular transfer or abbreviated as LLIT) or where a free radical is immobilized onto silica gel (this is called solid-liquid intermolecular transfer or SLIT). Both methods yield enhanced signals, but as is shown in reported SLIT DNP enhancements from our lab¹⁴ for example, overall enhancements of sample nuclei (in normal liquids) can be reduced as a result of the decreased molecular motion of the immobilized free radical.

Property		Gas	Liquid	Supercritical Fluid
Density	ρ [g/ cm ³]	10^{-3}	1	0.3
Diffusivity	D [cm ² /s]	10^{-2}	5×10^{-4}	10^{-3}
Viscosity	η [g/cm • s]	10^{-4}	10^{-2}	10^{-4}

Table 2.1. Physical properties of gases, liquids, and supercritical fluids.

There are other chemical and physical factors that can decrease the overall DNP enhancement in the flow transfer experiment e.g. poor saturation of electron transitions, poor transfer efficiency of the polarization detected at the high field probe, etc.--and these will be addressed in Chapters 4 and 5. However, the mobility of the free radical and the compound being analyzed is important because of the requirement in DNP that the extreme narrowing condition, or $(\omega_0\tau_c)^2 \ll 1$, be satisfied--in order to achieve the highest enhancements. In DNP, the electron resonant frequency (where $\omega_0 = \omega_e$), is governed by the magnitude of the magnetic field and the molecular correlation time, τ_c , of the sample molecule. In the extreme narrowing condition, then, if the field strength is increased or the τ_c is shortened, DNP enhancements will be at an optimum. Since SF's have molecular correlation times on the order of gases (10^{-12} - 10^{-13} s), compared to longer times for normal liquids ($\tau_c \approx 10^{-9}$ - 10^{-11} s), large, enhanced NMR signals should be expected, if the field strength remains (ω_e) constant.

2.2 DNP Theory

Dynamic nuclear polarization occurs when any system of interacting spins I and S are coupled by cross-relaxation processes. In liquid DNP, polarization transfer occurs when a free radical solute representing the electron spin (S_z) and a diamagnetic solvent carrying the nuclear spin (I_z) interact in a random, continuous manner. Figure 2.1 shows the energy level diagram for the electron-nuclear, two-spin system with the accompanying transition probabilities. The radiationless zero quantum (W_0) and the double quantum (W_2) transitions, in particular, contribute to the DNP phenomenon because they involve the simultaneous flip of the nuclear and electron spins. Under thermal equilibrium, the nuclear spins are distributed among the energy levels according to a Boltzmann distribution. When perturbed from its equilibrium state, the spin system returns to thermal equilibrium with the surrounding "lattice" by a first-order relaxation process characterized by a time T_1 , the spin-lattice relaxation time. The intensity of the NMR signal (I_z) is proportional to the spin state populations ($N_{\alpha\alpha} + N_{\beta\alpha}$) - ($N_{\alpha\beta} + N_{\beta\beta}$) and under thermal equilibrium is denoted (I_0). When the electron spin transition (W_1^S) is saturated, $N_{\alpha\beta} \cong N_{\beta\beta}$ and $N_{\alpha\alpha} \cong N_{\beta\alpha}$ with relaxation to thermal equilibrium involving interactions through W_1 , W_2 , and W_0 . Consideration of these transition probabilities leads to the observed NMR enhancement, which can be described as follows:

$$A = (I_z - I_0) / I_0 = -\rho f s |\gamma_s| / \gamma_I. \quad [1]$$

The coupling factor (ρ) reflects the type of nuclear-electron interaction being involved and can be defined in terms of the transition probabilities,

$$\rho = (W_2^D - W_0^D - W_0^{Sc}) / (W_2^D + W_0^D + W_0^{Sc} + 2W_1^D) \quad [2]$$

for the dipolar and scalar mechanism of interaction. (Dipolar dominated enhancements generally result in negative NMR signals where radiation is emitted, and scalar dominated enhancements result in positive NMR signals, arising from absorption of

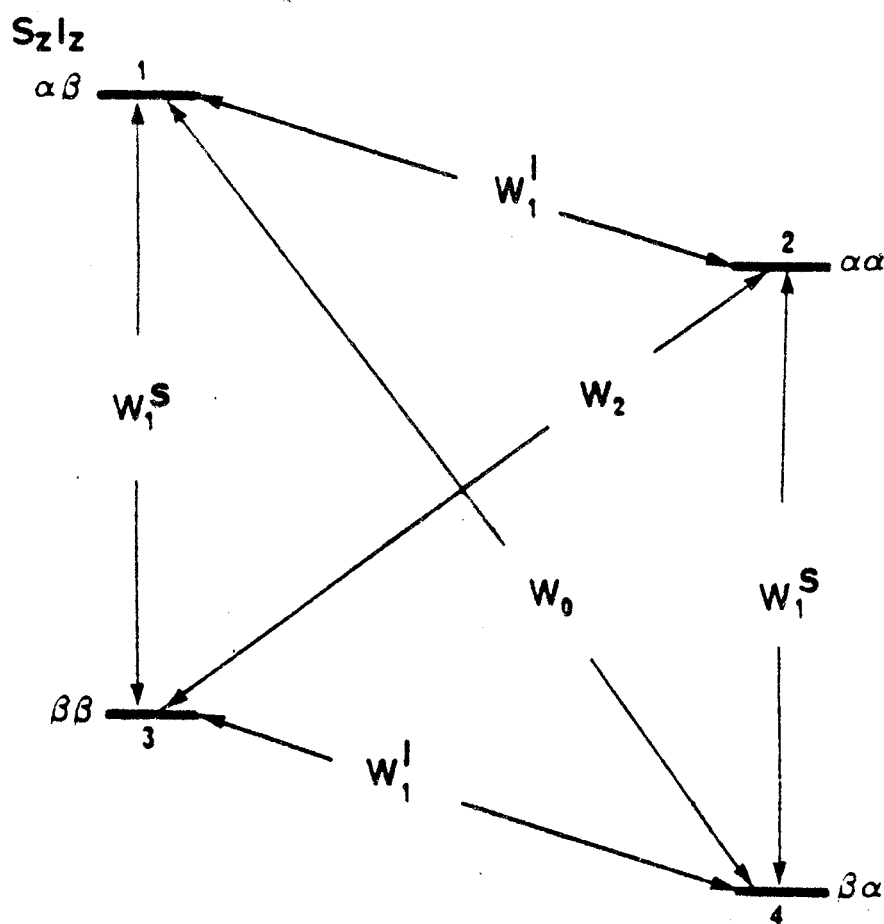


Figure 2.1. Energy level diagram for a coupled electron-nuclear spin system ($S = \frac{1}{2}$ and $I = \frac{1}{2}$).

radiation.) For the ^1H DNP studies presented in this work, dipolar interactions are thought to dominate therefore Equation [2] reduces to

$$\rho = (W_2^D + W_0^D) / (W_2^D + W_0^D + 2W_1^D) \quad [3]$$

The coupling factor can have any value ranging from -1.0 for a purely scalar interaction ($W_2^D = W_0^D = W_1^D$), to the case of a dominant dipolar interaction ($W_0^{\text{Sc}} = 0$) which has a limiting value of $+0.5$ under the extreme narrowing condition.

The leakage factor (f) measures the fraction of the total nuclear relaxation rate that is a result of nuclear-electron interactions. It can be expressed in terms of transition probabilities:

$$f = (W_0^D + W_0^{\text{Sc}} + 2W_1^D + W_2^D) / (W_0^D + W_0^{\text{Sc}} + 2W_1^D + W_{10}) \quad [4]$$

Since the nuclear spin-lattice relaxation rates in the presence and absence of a free radical are given by $(T_1)^{-1} = W_0^D + W_0^{\text{Sc}} + 2W_1^D + W_2^D + W_0^{\text{Sc}} + 2W_{10}$ and $(T_{10})^{-1} = 2W_{10}$ respectively, the leakage factor can be readily determined by the relationship

$$f = 1 - (T_1 / T_{10}) \quad [5]$$

The electron saturation factor (s) is a measure of the degree to which the electron transitions are saturated. When the electron transitions are completely saturated, $s = 1$. It can be expressed in terms of the following relationships:

$$S = (S_0 - S_Z) / S_0 \quad [6]$$

$$S_Z = S_0 / (1 + \gamma_s^2 B_{1s}^2 T_{1s} T_{2s}) \quad [7]$$

T_{1s} and T_{2s} are the electron spin-lattice and spin-spin relaxation times, respectively. The microwave magnetic field is given by B_{1s} . Substituting equation [7] into [6], we obtain

Since B_{1s}^2 is proportional to P , S can be obtained by a plot of the inverse enhancement

$$S = \frac{\gamma_s^2 B_{1s}^2 T_{1s} T_{2s}}{1 + \gamma_s^2 B_{1s}^2 T_{1s} T_{2s}} \quad [8]$$

A^{-1} versus the inverse microwave power P^{-1} . After substituting equation [8] into [1], this plot should yield a straight line, having the form

$$A^{-1} = \left(\rho f \frac{\gamma_s}{\gamma_l} \right)^{-1} \left(1 + \frac{1}{\gamma_s^2 B_{1s}^2 T_{1s} T_{2s}} \right) \quad [9]$$

The ultimate enhancement A_∞ is the extrapolated enhancement for complete saturation ($s = 1$) and complete dominance of nuclear relaxation by the nuclear-electron interaction ($f = 1$),

$$A_\infty = \rho \gamma_S / \gamma_I \quad . \quad [10]$$

From the above equation, the important coupling factor can be obtained,

$$\rho = A_\infty \gamma_S / \gamma_I \quad . \quad [11]$$

For dipolar dominated interactions $\rho = 0.5$ yielding $A_\infty = 0.5 (-\gamma_S / \gamma_I) = -330$, for ^1H observation. For scalar interactions $\rho = -1$ yielding $A_\infty = -1 (-\gamma_S / \gamma_I) = 660$ for ^1H observation.

Chapter 3: Fundamental Study of Molecular Correlation Times (τ_c) in Normal Liquids and Supercritical Fluids

3.1 Introduction

The extreme narrowing condition, $(\omega_e \tau_c)^2 \ll 1$, presented in Chapter 2, can be defined in more rigorous terms as the maximum in spectral density for the range of molecular motion possible. The spectral density function $J(\omega)$ is a Lorentzian function and is given as:

$$J(\omega) = 2\tau_c / (1 + \omega^2 \tau_c^2) \quad [12]$$

The spectral density can be thought of as the intensity of field fluctuations at a particular frequency, necessary for the efficient (or inefficient) relaxation of nuclei. Figure 3.1 shows the spectral density as a function of motional frequency for three different systems, where $\omega = \tau_c^{-1}$. Regions A, B, and C describe the degrees of available motion in solids, viscous liquids, and normal liquids, respectively. For the case of normal liquids, the extreme narrowing condition is represented by the large plateau, which extends over a large range of resonant frequencies, as can be seen on the plot for region C. This signifies that the spectral density is at a maximum for a large range of resonant frequencies (where $\omega = \omega_0$), resulting in efficient relaxation of nuclei over those frequencies. The transition probabilities for the interaction of the unpaired electron and nuclei fluctuating in step with the random molecular motion in SF's is best represented by the spectral density function in the region of fast molecular motion.

For observation of the ^1H at 4.7 T in liquids, the resonance frequency (ν) is 200 MHz, corresponding to $\omega_0 = 1.26 \times 10^9$ radians/sec. If a τ_c value of 5×10^{-12} sec is assumed for liquids, the extreme narrowing condition is approximately equal to 4.0×10^{-5} --which fulfills the relationship $(\omega_0 \tau_c)^2 \ll 1$. Electron resonant frequencies are on the order of Gigahertz ($\nu_e = 9.3$ GHz) at X-band, at a field strength of 3.3 kG. In normal liquids using $\tau_c = 5 \times 10^{-11}$ sec, $(\omega_e \tau_c)^2 \approx 8.5 \times 10^{-3}$, which is a value less than one. For optimum ^1H DNP, if the magnetic field is increased to e.g. 14 kG or 60 MHz ^1H , the resulting electron frequency equals $\nu_e \approx 40$ GHz, resulting in a value of $(\omega_e \tau_c)^2 \approx 1.2$, not satisfying the extreme narrowing requirement. If, however, the molecular motion of the sample nuclei being monitored was increased, e.g. through the use of supercritical fluids, the extreme narrowing condition could be extended to higher effective fields. Using SF CO_2 , molecular correlation times are on the order of 5×10^{-12} sec, giving $(\omega_e \tau_c)^2 \approx 1.5 \times 10^{-2}$ and resulting in maximum DNP signals.

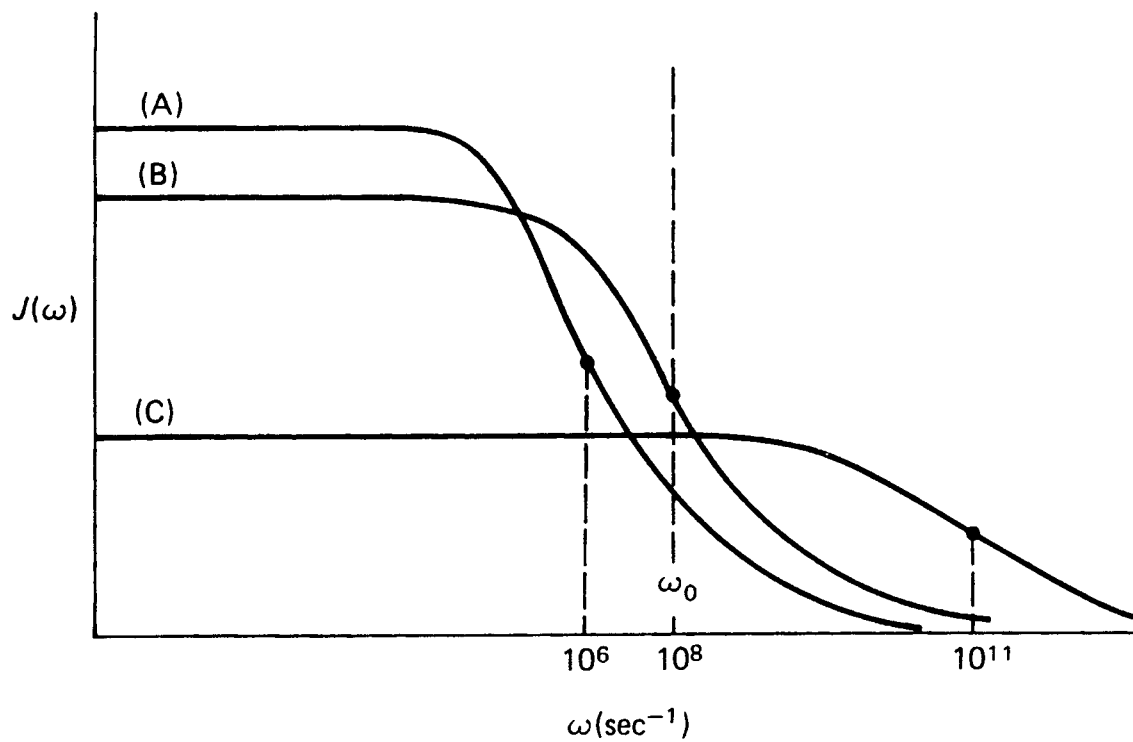


Figure 3.1. A plot of the spectral density as a function of molecular motion for solids (A), viscous liquids (B), and normal liquids (C).¹⁵

Studies of molecular correlation times for small probe molecules in SF's and gases, have been conducted by the pioneering researchers Robert and Evilia. They observed that for quadrupolar nuclei such as ^{14}N ($I = 1$) and ^{17}O ($I = 5/2$) in NO and CO_2 , under supercritical conditions, line narrowing factors of 2-6 resulted.¹⁶ However, this is in contrast to that predicted (up to 200) from known supercritical fluid viscosities. A researcher from our lab, Lee Allen in 1988, demonstrated the line narrowing of ^{14}N for the small probe molecule CH_3CN , when SF CO_2 was used as the solvent.¹⁷ For nuclei having spins $I \geq 1$ under the extreme narrowing condition, quadrupolar interactions dominate nuclear relaxation. Whereas in normal liquids, broad lines are observed due to the higher viscosity, in SF's, narrowed lines observed for quadrupolar nuclei indicate that other relaxation mechanisms are competing with quadrupolar relaxation under conditions of high molecular motion. The efficiency of quadrupolar relaxation, in the extreme narrowing limit, is directly proportional to the correlation time, τ_c , which is, in turn, directly proportional to the solution viscosity. This relationship is obtained via the Debye equation, which also relates the correlation time inversely with solution temperature.¹⁸ Therefore, through the measurement of NMR signals of quadrupolar nuclei in sample molecules, the molecular correlation time can be directly calculated. The following equation¹⁷ can be used to calculate τ_c for quadrupolar nuclei:

where: $T_{2q} \equiv$ quadrupolar spin-spin relaxation

$$\pi\Delta\nu_{1/2} = 1/T_{2q} = \frac{3\pi^2 (2I + 3) \chi^2 \tau_c}{10 I^2 (2I - 1)} \quad [13]$$

$I \equiv$ the nuclear spin

$\chi \equiv$ the quadrupolar coupling constant (for ^{14}N , $\chi = 3.74$ Hz)

The range of τ_c times obtained in normal liquid systems and SF's for a sample molecule can be compared and ultimately used to determine the optimum field strength at which to conduct flow SF CO_2 ^1H DNP.

3.2 Experimental

A Bruker WP-270 nuclear magnetic resonance spectrometer was used to obtain ^{14}N spectra at 20 MHz. Typical acquisition times of 30 to 45 minutes were used in acquiring ^{14}N spectra of 7% v/v acetonitrile. All samples were N_2 degassed and the spectra were collected at room temperature. Acetonitrile and other various solvents were used as received from Aldrich.

3.3 Discussion of Results and Conclusions

The ^{14}N line width values for 7 % CH_3CN in normal liquid systems of varying viscosity is shown in Table 3.1. The particular solvent systems were chosen mainly based on the range of macroscopic viscosities, η , where glycerol is almost 2 orders of magnitude more viscous than e.g. CCl_4 . The percentages of higher viscosity solvents i.e. glycerol, cyclohexanol, and ethylene glycol, correspond to an increase in the overall viscosity of the solvents, resulting in the observed trend of broadened NMR signals. In the case of the chloroform/cyclohexanol and carbon tetrachloride/glycerol systems, ^{14}N line widths leveled off or actually decreased, indicating the insolubility of the 7 % acetonitrile sample in the more viscous solutions. From the overall data, it can be seen that the water/glycerol system gave the greatest increase in the NMR signal. By substituting the measured line width values into equation [12], τ_c times for 7% CH_3CN in water/glycerol can be calculated and are shown in Table 3.2. Also represented in the table are the calculated τ_c times in less viscous solvent systems $\text{CCl}_4/\text{CH}_3\text{CN}$ and neat CH_3CN . As is expected, since τ_c is directly proportional to $\Delta V_{1/2}$, an increase in the τ_c times are observed as the viscosity of the solutions increase.

The magnitude of rotational motion of the small probe molecule CH_3CN , in highly viscous and normal liquids, were compared to that of τ_c times measured in SF_6/CO_2 . Table 3.2 includes τ_c times obtained for 7% acetonitrile in SF_6/CO_2 , where the temperature has been varied, at constant pressure.¹⁷ As mentioned previously, τ_c is inversely related to temperature and it can be observed from the SF_6 data that as the temperature is increased, the correlation time is shortened. A plot of the calculated molecular correlation times for acetonitrile in normal liquids and SF_6/CO_2 is shown in Figure 3.2. A visual analysis of the data from Table 3.2 reveals that there is a factor decrease of 2-6 in molecular correlation times for a simple molecule, when SF_6/CO_2 is used as the solvent. The plot in Figure 3.2 suggests that there is on average a factor of four increase in the rotational motion of a small molecule when dissolved in SF_6/CO_2 . This finding is in agreement with the results of the researchers Allen, Evilia, and Jonas, as previously mentioned, with the explanation given by Jonas¹³ that at optimum (critical point) conditions, limited solute solubility is obtained at the expense of lower solvent viscosities. Nevertheless, for the purposes of conducting flow transfer ^1H DNP using SF_6/CO_2 , ω_e can be increased by this same factor, while satisfying the relationship $(\omega_e\tau_c)^2 \ll 1$. As mentioned earlier in section 3.1, if DNP is presently conducted at 0.33 T ($\omega_e = 9.3$

Table 3.1. ^{14}N line width $\Delta\nu_{1/2}$ values for 7% (v/v) CH_3CN in normal liquid systems.

<i>*Percentage of higher viscosity solvent</i>		0%	4%	8%	16%	32%	40%
$\Delta\nu_{1/2}[\text{Hz}]$:	$\text{H}_2\text{O/glycerol}$	163	170	278	--	344	603
	$\text{CHCl}_3/\text{cyclohexanol}$	175	180	200	199	161	--
	Acetone/cyclohexanol	88	92	97	107	140	--
	$\text{CCl}_4/\text{cyclohexanol}$	156	180	229	256	346	--
	$\text{CCl}_4/\text{glycerol}$	153	151	155	155	154	--
	$\text{CHCl}_3/\text{ethylene glycol}$	178	172	180	317	476	--

-Line width values have a RSD of 5%.

Table 3.2. ^{14}N molecular correlation times for 7% CH_3CN .

<i>*Percentage of higher viscosity solvent</i>		0%	4%	8%	32%	40%
τ_c [psec]:	$\text{H}_2\text{O/glycerol}$	2.04 ± 0.37	2.19 ± 0.38	3.48 ± 0.38	4.30 ± 0.45	7.54 ± 0.59
	$\text{CCl}_4/\text{CH}_3\text{CN}$	1.91 ± 0.40				
	Neat CH_3CN	1.06 ± 0.30				
T [K]=		361	327	307	295	
$^a\text{SF CO}_2$		0.21	0.33	0.43	0.62	

a—Supercritical fluid data collected by Lee Allen, 1988 Ph.D. thesis. RSD values ≤ 0.04 .

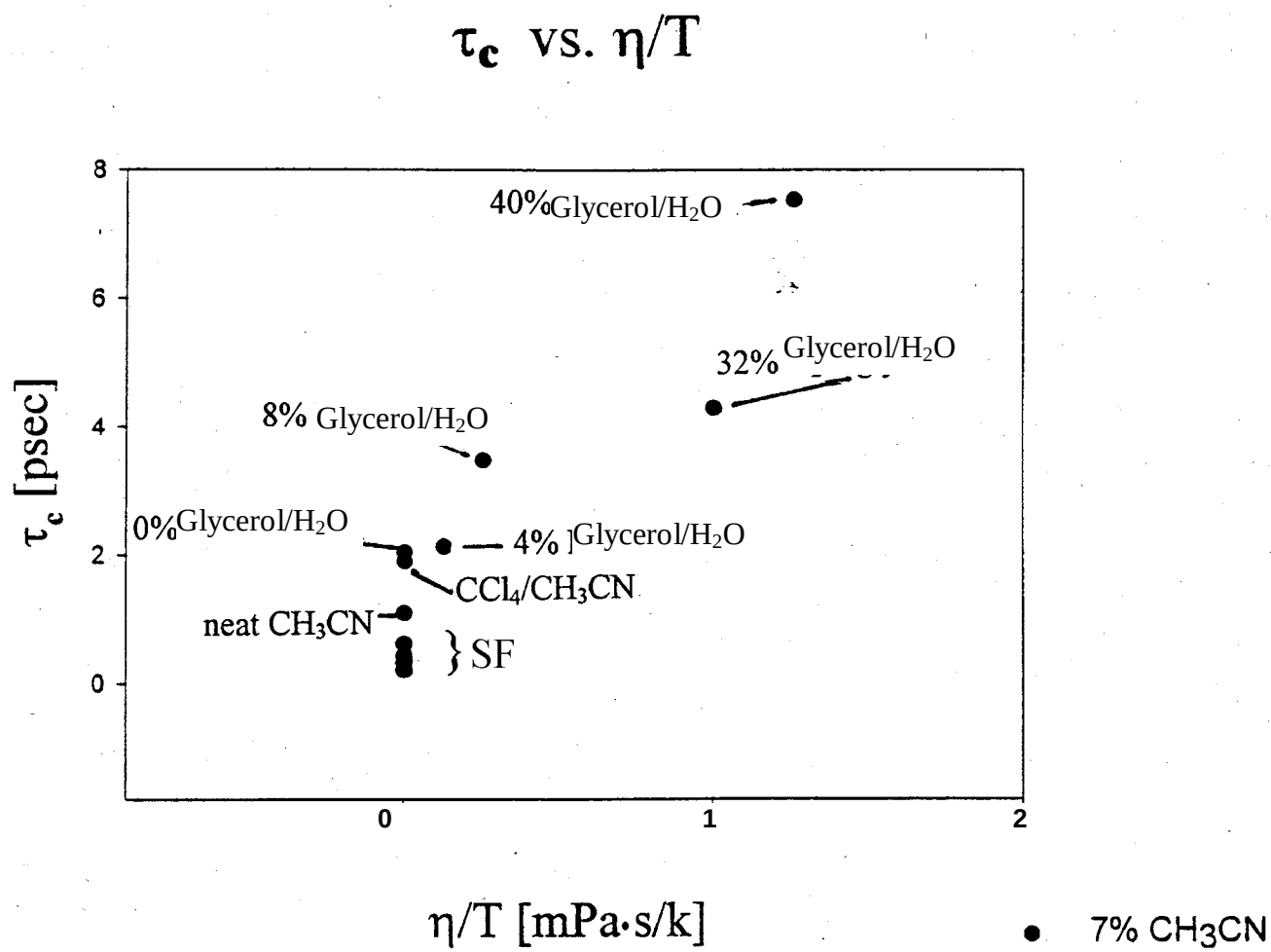


Figure 3.2. Plot of τ_c vs. η/T for 7% CH_3CN

GHz), then $4 \times \omega_e$ would be equivalent to saturating electron spins at 1.4 T. An optimum field strength of 1.4 T, then, would be ideal for achieving maximum DNP enhancements. This result, also, suggests that DNP can be conducted where sample spins are both polarized and monitored at the same magnet--at 1.4 T and 60 MHz ^1H DNP detection. This would, perhaps, facilitate and simplify how flow transfer DNP is currently performed, where electron spins are saturated and nuclear spins are detected at two different magnets. (The experimental setup will be presented in greater detail, in the next chapter.)

Chapter 4: Fundamental Study With Benzene, Using the Coupled Technique of LLIT ^1H DNP /SF CO_2

4.1 Introduction

The hydrogen nucleus (^1H) is one of the most well studied in liquid DNP experiments (and conventional NMR) because of its high sensitivity and easy detection even at low magnetic fields (< 7.5 mT), compared to other nuclei.⁵ Almost all previous ^1H DNP studies indicate dipolar dominated ^1H -electron interactions (i.e. the enhanced ^1H signals are negative or inverted). Enhancements extrapolated to the dipolar limit of -330 for ^1H DNP conducted at low magnetic fields are obtainable, regardless of the type of free radical or liquid solvent employed. Because the ^1H enhancement is less sensitive to change of the chemical system, ^1H DNP is generally considered uninteresting from a chemical viewpoint. However, ^1H DNP studies are important in variable field and molecular motion studies, where experiments have been conducted in magnetic fields ranging from 1.5 mT to 1.25 T.

The variation of interaction of the unpaired electron and nuclei in DNP with the magnetic field is often referred to as “modulation” of the coupling. In ^1H DNP, magnetic dipoles of the electron and nucleus interact, and at the resonance frequency, the coupled spin system undergoes relaxation. More specifically, in the case of dipole-dipole relaxation, the local, oscillating magnetic field generated by the rotating electron spin density of the paramagnetic species can induce a local magnetic field (of oscillating nuclear spin density) at the nucleus of the diamagnetic species being monitored. The results of ^1H DNP, then, can be described in terms of a dipolar interaction modulated by rotational diffusion, although contributions from translational diffusion (i.e. isotropic motion along the same axis, through 3D space) cannot be discounted.¹⁹

Rotational diffusion is a modulation mechanism for dipolar interactions in DNP where relaxation occurs via a time-dependent, rotational tumbling of (radical) solute-solvent complexes. This type of modulation mechanism is characterized by the reorientational or rotational correlation time, τ_r , and treats the tumbling complex as a sphere. If the molecular motion between the interacting nuclear and electron spins are at the resonance frequency, relaxation transitions will be induced. For rotational modulation of the dipolar coupling²⁰, the radical induced dipolar transition probabilities are given by the following expressions:

$$2W^D_1 = 3W^D_0 = W^D_2/2 = (3/10) \gamma_s^2 \gamma_i^2 \hbar^2 \tau_r (n_p N_e / N_p) d_r^{-6} J_r(\omega_i) \quad [14]$$

where

$$\tau_r = (4\pi b^3 \eta / 3kT), \quad J(\omega_i) = 1 / (1 + \omega_i^2 \tau_r^2) . \quad [15]$$

In the above equations, n_p is the number of receptor nuclei bound near each electron, N_e is the unpaired electron concentration, N_p is the total nuclear concentration, d_r is the average pair radius, and b is the effective tumbling radius of the molecular complex.

The rotational spectral density function, $J_r(\omega_i)$ is expressed in terms of τ_r . It is sufficient to note that from equations [14] and [15], the relaxation transition probabilities contain

important parameters of molecular collision in liquids: e.g. the correlation time, τ , and radical-solvent distance of closest approach, d . Since DNP is closely related to these transition probabilities, DNP measurements can provide a way to obtain detailed information about molecular motion and collision. For exclusive rotational dipolar diffusion, equation [16] can in addition be written, relating the important DNP coupling factor, ρ , to the spectral density function (where the definition of $J(\omega)$ given in equation [15] has been substituted):

$$\rho = \frac{1}{1.4 + 0.6(1 + \omega_e^2 \tau_r^2)} \quad [16]$$

If the equation is expressed in terms of τ_r , ρ is then related to the molecular motion of the molecule. DNP theory relates that the coupling factor will reach a maximum value of 0.5 (for dipolar dominated polarization transfer) in the extreme narrowing limit of fast molecular motion, when $\omega_e^2 \tau_r^2 \ll 1$. It can be seen, therefore, by equations [16] and [10] (refer to Ch. 2) that as the molecular correlation time becomes shorter, DNP ultimate enhancements can reach a maximum value. DNP has, thus, been applied to study fast molecular motion (with correlation times $< 10^{-8}$ sec), weak molecular complexation, and stereo specific collisions in liquids. The reviews of Richards et. al. and Potenza discuss the specific methods for obtaining the abovementioned physical parameters (i.e. τ_r , ρ , etc.) from DNP measurements.^{21,22}

When the DNP experiment is conducted where the free radical is dissolved directly in the sample solution, this is known as the liquid-liquid intermolecular transfer experiment or LLIT. The time-dependent interaction of an electron spin (2,2,6,6-tetramethylpiperidine-1-oxyl or TEMPO) with a nuclear spin (^1H nucleus of a benzene molecule) is shown in Figure 4.1. This model depicts the interaction of a typical solvent system used in the LLIT experiment. As mentioned in Chapter 1, flow transfer ^1H DNP (or LLIT) has been used in our labs to demonstrate the increased sensitivity of the DNP technique over conventional flow NMR, in normal liquid systems.²³ In general, the following factors affect the DNP signal enhancement: 1) the external field strength; 2) the ratio of scalar to dipolar coupling; 3) the temperature of the system; 4) the viscosity of the solution; 5) the free radical; 6) the chemical environment of the nuclei; and, 7) the type of nuclei being monitored. In the case of flow transfer ^1H DNP, where SF CO_2 is used as the flowing solvent, it is evident that based on the unique chemical and physical properties of the solvent, the aforementioned factors will differ from that of normal liquid systems, thus affecting the overall DNP enhancements. Larger ultimate DNP enhancements are expected, therefore, for this supercritical fluid, flow system.

4.2 Experimental

The instrumental apparatus used in the flow transfer DNP experiment for normal liquids and SF CO_2 is shown in Figure 4.2. In the flow transfer experiment, the nuclear

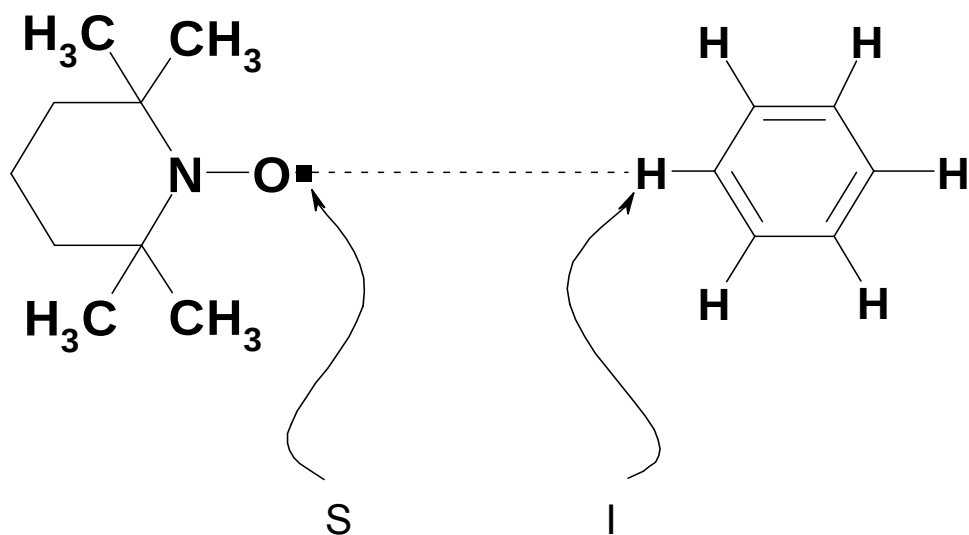


Figure 4.1. Time-dependent Hamiltonian interaction of an electron spin S with a nuclear spin I .

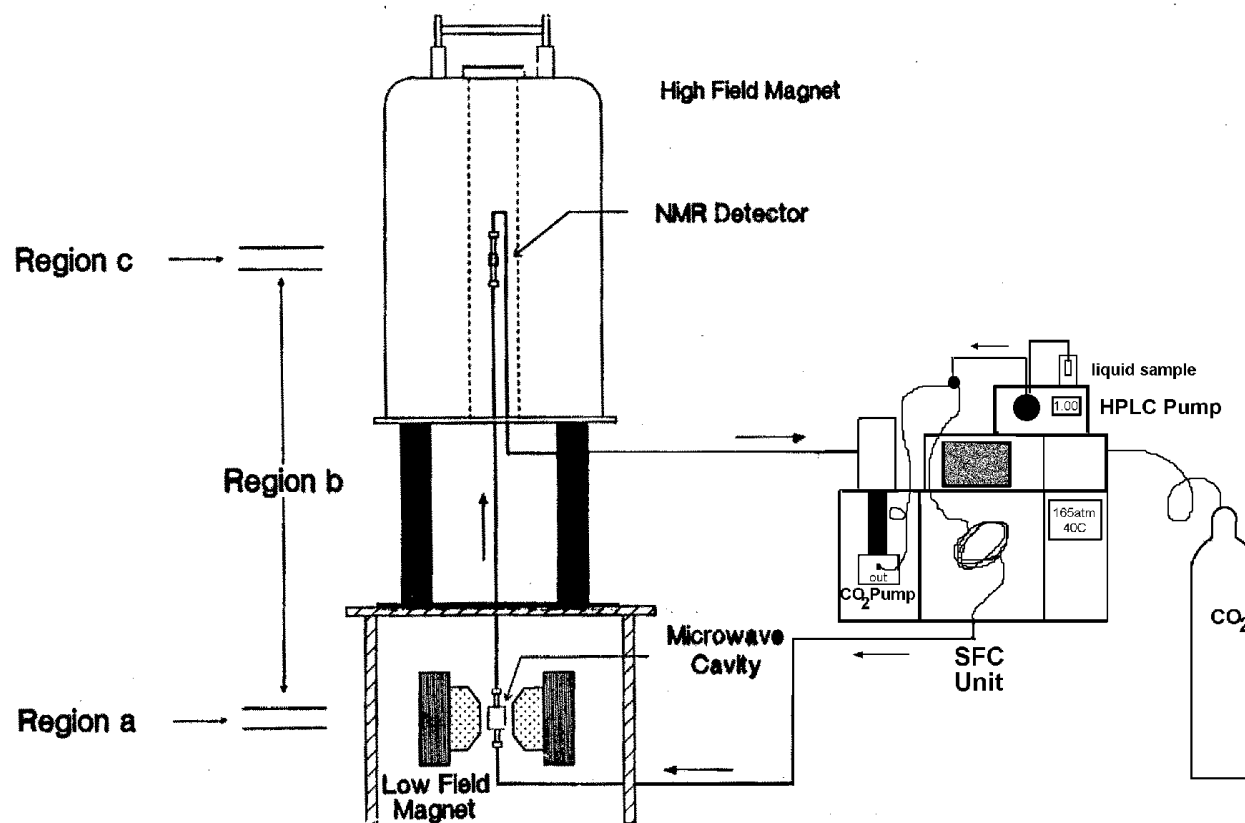


Figure 4.2. The low-to-high magnetic field transfer DNP experimental apparatus.

spins are polarized in a low magnetic field, designated as region a, and rapidly transferred via region b to a high magnetic field c where the polarization is monitored. A variable electromagnet (0-0.6 T) is positioned directly under a 4.7 T (200 MHz) superconducting magnet, at a distance of 1.2 m apart. The microwave frequency (9.3 GHz) is generated in a HP 8684B Signal Generator, klystron source and is amplified by a Varian "k" series TWT amplifier. The microwave power, at the X-band TE₁₀₂ microwave cavity, can be adjusted by an attenuator in the range of 1-20 Watts. In this way, the sample is polarized at the low magnetic field (0.33 T) and then transferred to a high magnetic field detector, where a Helmholtz detection coil is employed in a commercial probe.

In the present DNP apparatus, it is important to note, that the electron spin system of the nitroxide free radical can only be saturated at one, specific wavelength. The EPR spectrum of any nitroxide radical is usually a triplet, due to the hyperfine coupling of the free electron (which is localized on the N-O functional group²⁴) with the ¹⁴N nucleus (spin quantum number I= 1). And, it is usually the central line, or the line of highest intensity, that is irradiated. Figure 4.3 shows the EPR spectrum of a TEMPO nitroxide radical at low concentrations. The theoretical result is that at very low concentrations (< 0.005 M), ¹⁴N labeled nitroxide radicals will have ultimate enhancements reduced by a factor of three, $A_{\infty}/3$ (e.g. -330/3 for H¹ DNP enhancements)--since all electron spin transitions cannot be saturated. However, since the other two sets of transitions (or EPR lines) of the triplet are indirectly and partially saturated via radical induced collisions of anti-parallel spin states, ultimate enhancement factors are not significantly reduced by the 1/3 factor. In the case of the exchange narrowing limit (where there exists fast electron-electron exchange for the three separate transitions) observed at high radical concentration, the EPR spectrum is a singlet. In theory, if all electron transitions can be saturated simultaneously at one wavelength, then the extrapolated enhancements will approach the appropriate dipolar limit (-330 for protons).²² Consequently, the use of high radical concentrations in the DNP experiment results in a broadened nitroxide singlet and broadened EPR lines, in general (in addition to broadened NMR signals)--making it experimentally more difficult to saturate the electron spins at a specific wavelength (or EPR field strength). This effect decreases the degree of electron spin saturation and, similarly, reduces DNP enhancements.

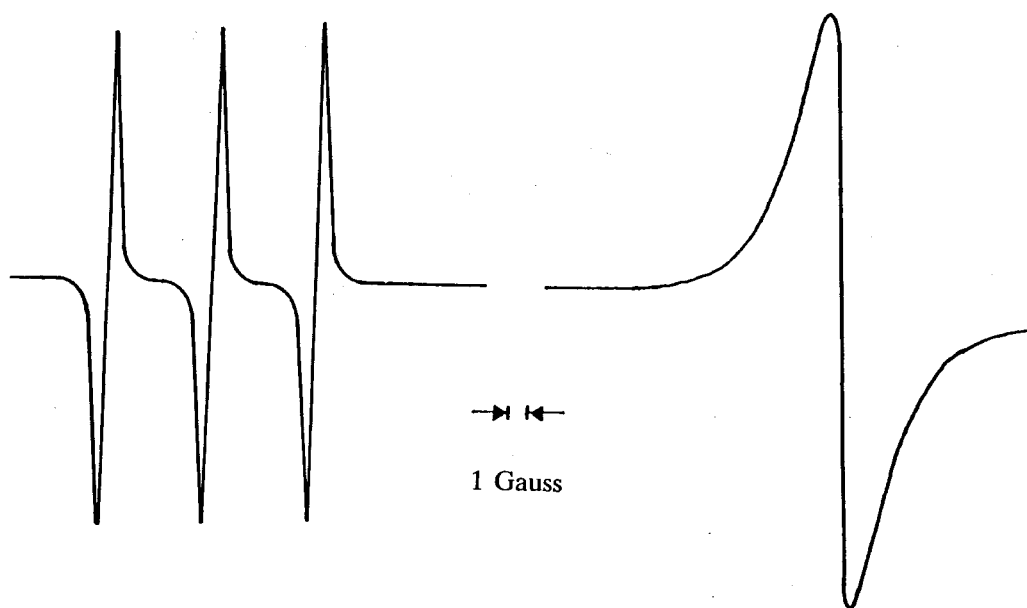


Figure 4.3 EPR spectra of 1×10^{-3} M (seen on the left) and 1×10^{-1} M TEMPO (the right) in neat benzene.

The SF CO₂ and normal liquid LLIT experiments were performed in the following manner. For flow ¹H DNP performed under supercritical fluid conditions, N₂ degassed neat liquid benzene (Aldrich) containing free radical TEMPO (0.1 M-0.01M) originates at the HPLC pump and flows at a rate of 0.1 mL/min. PEEK (polyetheretherketone) tubing (Upchurch), capable of withstanding pressures and temperatures of up to 3000 psi and 100°C, was used as the solvent transfer lines in the DNP apparatus. Typical volume amounts in regions a, b, and c are 160, 80, and 60 µL, respectively. Analytical grade CO₂ (not containing a Helium pressurized head), provided by Air Products, is pumped from a Suprex SFC/200A Multipurpose Unit, syringe pump and is equilibrated to a flow rate of 1.01 mL/min and 165 atm (2310 psi). The liquid sample is introduced into the CO₂ flow through a stainless steel mixing tee and at a total flow rate of 1.11 mL/min the sample solution enters the SFC oven via stainless steel tubing where it is heated to 40°C. At this total flow rate, the liquid sample concentration is maintained at 9% v/v, ensuring the solubility of the liquid solutes (benzene and TEMPO) in SF CO₂ solvent (ρ_{CO2} = 0.805 g/mL). Additionally, this rate was found to be optimum based on transfer and residence times for the flow ¹H DNP apparatus shown in Figure 4.2.

For normal liquid flow (e.g. neat benzene and 9% benzene/ deuterated benzene), the sample originates at the HPLC pump. The SF CO₂ flow is closed off and the sample passes through the SFC unit oven where it is heated to 40 °C. The sample passes through the remaining portions of the flow DNP apparatus via PEEK tubing in the directions of the arrows, depicted in Figure 4.2. Typical liquid flows for flow transfer ¹H DNP using benzene solvent range from 5-8 mL/min., with backpressure values ranging from 200-1000 psi.

In the low and high field regions, the development of high pressure PEEK flow cells was necessary, in order to contain the flowing sample in the low field cavity and the high field probe. The PEEK sample cells are depicted and discussed in more detail in Appendix I.

4.3 Discussion of Results and Conclusions

The LLIT ¹H DNP data for neat liquid benzene and benzene dissolved in deuterated (D6) benzene and SF CO₂ are presented in Table 4.1. DNP data was collected for the benzene samples at two different TEMPO concentrations. The spin-lattice relaxation times, T₁ and T₁₀, for benzene in the presence and absence of TEMPO, respectively, are taken from data collected by Kun Hsiang Tsai, 1990, for samples containing the same concentration of TEMPO (0.01 and 0.001 M), as described in Table 4.1.²⁵ T₁ values for 9% benzene in D6 benzene were approximated to be the same as that of the neat liquid since the only physical change in the solvent sample is a decrease in the concentration of hydrogen nuclei. Values of the spin-lattice

Table 4.1. LLIT ^1H DNP Data for Benzene.

Sample	$(A_{\text{obs}})_{\text{max}}$	T_{10} (sec)	T_1 (sec)	f	s	$A_{\infty}^{\text{d,e}}$
Neat Benzene/ 0.001M TEMPO	-2.9	18.5 ^a	5.3 ^a	0.72	0.62	-116 $\pm$ 14
9% Benzene/ D6 Benzene/ 0.001M TEMPO	-2.4	"	"	"	0.68	-120 $\pm$ 18
9 % Benzene/ SF CO ₂ / 0.001M TEMPO	-5.4	4.4 ^b	1.9 ^c	0.57	0.28	-341 $\pm$ 36
Neat Benzene/ 0.01M TEMPO	-3.1	18.5	1.3	0.93	0.57	-141 $\pm$ 21
9% Benzene/ D6 Benzene/ 0.01M TEMPO	-3.2	"	"	"	0.49	-146 $\pm$ 20
9 % Benzene/ SF CO ₂ / 0.01M TEMPO	-5.2	4.4 ^b	1.6 ^c	0.64	0.21	-293 $\pm$ 44

Relative standard deviations, RSD, are given following $\pm$ symbol.

a) Spin-lattice relaxation times at 4.7 T for neat benzene and 9% benzene in SF CO₂ collected by Kun-Hsiang Tsai, 1990 and Ziqi Sun, 1992, respectively.^{25,26}

b) Spin-lattice relaxation time obtained at 40 °C, ~ 165 atm from Lee Allen's Ph.D. thesis.¹⁷

c) RSD is ~ 6-20 %.

d) Ultimate DNP enhancements corrected for transfer efficiency, Q, according to data collected by Tsai 1990.²⁵

e) Estimated RSD values are from regression analysis: high-field spin-lattice relaxation time (T_{10} and T_1), ~ 5-10 %; high-field leakage factor (f), ~ 5-9%; saturation factor (s), ~ 5%; ultimate enhancement (A_{∞}), 10-18%.

relaxation times for 9% benzene in SF CO₂ solvent were collected by Ziqi Sun, 1992.²⁶ This collection of T₁ and T₁₀ values obtained at 4.7 T were used to calculate the leakage factor, f, according to equation [5], at the high field magnet (region c). It can be noticed that the f factor is larger for samples containing a higher concentration of the free radical, which would indicate a greater coupling of the nuclear-electron interaction. In SF CO₂, the lower values of the leakage factor (where f is less than unity) indicate that there is a decrease in the effectiveness of the electron spin to relax the nuclear spin, compared to the neat liquid case. This may be due in part to the larger error associated with the T₁ determination in SF CO₂, especially for 9% benzene in 0.001 M TEMPO. However, a basic trend shows an increase in the leakage factor at higher TEMPO concentrations.

Based on data presented in Table 4.1, saturation values, s, for LLIT DNP collected in SF CO₂ are smaller values by comparison to the normal liquids'. These results are evidence, in part, of a broadened EPR signal, as can be seen by a field plot in Figure 4.4, showing the width of the EPR line of 0.001 M TEMPO in SF CO₂. This plot, in fact, shows a width for the TEMPO singlet to be approximately 9 Gauss, which is consistent with the EPR broadened line width of 10⁻² M TEMPO in SF n-hexane--which is reported to be on the order of 10 Gauss.²⁷ (One can observe and compare the exchange narrowed single peak width of the 10⁻³ M TEMPO, EPR signal dissolved in SF CO₂ with the width of one EPR line of the 10⁻³ M TEMPO triplet, dissolved in normal liquid benzene, shown in Fig. 4.3.) As already suggested, broadened EPR lines make it more difficult to saturate the electron transitions by the microwave power and can affect optimal DNP enhancements. Additional experimental evidence collected by the researcher Batchelor²⁷ demonstrates that at a TEMPO concentration of 10⁻² M, the EPR line width in SF n-hexane, ethane, and ethanol increases dramatically (by a factor of 8-10) near or at T_c and P_c. As temperatures and pressures increase above T_c and P_c for the SF solvents, the line width increases to a much lesser extent. Likewise, in the same study, a large increase in the electron-electron exchange rate is observed at T_c and P_c for the same concentration of TEMPO in ethanol and n-hexane--the value of which quickly decreases and levels off at T's > T_c and P's > P_c. Batchelor, as well as Randolph and Carlier²⁸, suggest that the increased line widths of nitroxides and increased Heisenberg exchange in SF's at the critical parameters may be due to critical clustering between solute-solvent molecules. This clustering creates a local density augmentation compared to the bulk solvent and increases the proximity and probability of collisions between spins²⁸, thus increasing the spin exchange rate. At the same time, in his research, Batchelor found that under conditions of constant temperature (T_c) and pressures greater than P_c for the above mentioned SF's and at the specified TEMPO concentration, the electron-electron exchange rate is independent of pressure and remains constant. However, the overall effects of line broadening, and thus the saturation of the electron spin-system, does not inhibit the resulting increased signal enhancements in SF CO₂, as will be presented in the next paragraph.

EPR Field Plot of 9% benzene/0.001 M TEMPO in SF CO₂

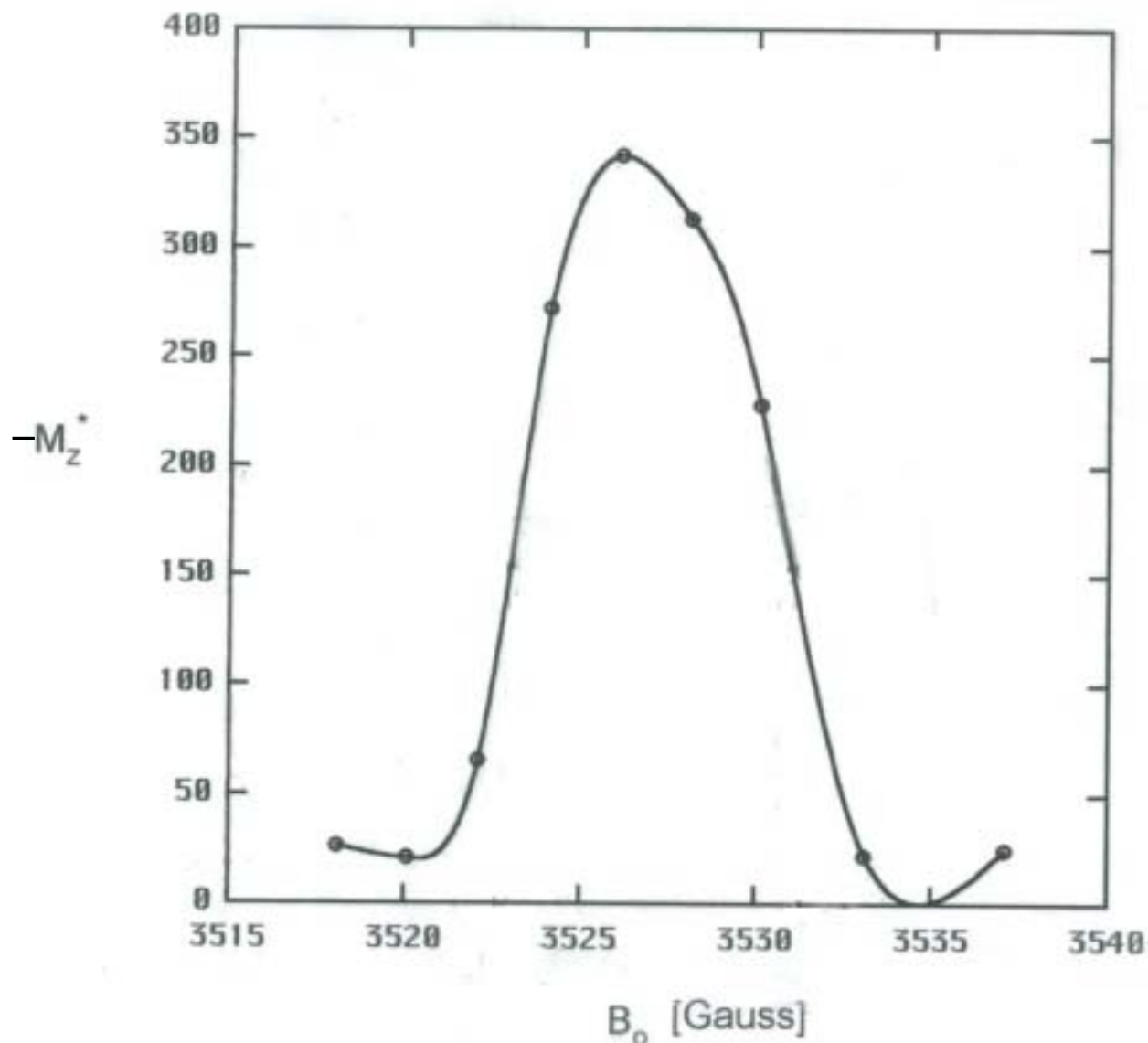


Figure 4.4. The nuclear magnetization versus field strength at the EPR cavity for 9% benzene/ 0.001 M TEMPO/ SF CO₂. The magnetization data (¹H DNP) was collected at 9.49 GHz and 5 Watts of power.

The static, flow, and stopped-flow ^1H spectra for 9% benzene/0.001 M TEMPO dissolved in SF CO_2 is illustrated in Figure 4.5. This series of ^1H spectra, in fact, is representative of all of the benzene samples (i.e. neat benzene, benzene in D6 benzene, and benzene in SF CO_2) tested in the present research using the LLIT and SLIT ^1H DNP experiment. A negatively enhanced signal suggests that the nuclear-electron time-dependent interaction for benzene/TEMPO is exclusively dominated by the dipolar relaxation mechanism. Based on the maximum observed DNP enhancements given in Table 4.1 for the sample spectra presented in Figure 4.5, an approximate factor increase of 5.0 in the DNP signal ($(A_{\text{obs}})_{\text{max}} = -5.4$) versus the flow NMR signal can be observed (albeit in the negative direction). Spectrum c shows that at a TEMPO concentration of 0.001 M, the theoretical maximum ^1H DNP enhancement for benzene dissolved in supercritical fluid is achieved. When s , f , and the transfer efficiency, Q , are corrected, a calculated value of $A_{\infty} = -341 \pm 36$ is obtained. When the experimental error is taken into account, a similar result is obtained for the 0.01 M TEMPO/ benzene sample. The higher ultimate enhancement values obtained in SF CO_2 can be compared to lower values obtained in the neat liquids. A comparison of the A_{∞} values for 9% benzene in D6 benzene and SF CO_2 with the same TEMPO concentration shows there to be a significant increase in the DNP signal; there is no comparable difference in the DNP signals for benzene in the neat and deuterated solvents. The decrease in A_{∞} in normal liquids can be attributed to a decreased mobility of benzene in the neat solvent versus supercritical fluid carbon dioxide solvent. Subsequently, there is a factor increase in A_{∞} by approximately 3. This result is in agreement with the findings of the molecular motion study for small molecules presented in Chapter 3. If the molecular correlation time for a small molecule (i.e. benzene in this case) is shortened in supercritical fluids by a factor of 2-6, then at a constant frequency, ω , an increase in the NMR signal should result by the same factor.

Table 4.2 shows estimated rotational correlation times for the benzene/TEMPO interaction (refer to Figure 4.1). The correlation times were calculated based on equations [14] and [15], and the expression derived for a rotationally modulated mechanism, exclusively dominating the dipolar interaction, given by Dorn et. al., 1993.¹⁹ The data shows an increase in molecular motion (or shorter τ_r 's) for benzene/TEMPO by a factor of 3 to 4 in SF CO_2 , at the two different TEMPO concentrations. In a study of the molecular motion of benzene in 2.5×10^{-2} M galvinoxyl, the researchers Kramer et. al.²⁹ used the technique of liquid DNP to report a value of the correlation time—for the electron-nuclear interaction. The reported value was 34 psec at 23°C and this value agrees well with the values reported for benzene at the two different TEMPO concentrations. These results are, again, in similar agreement with the factor range of molecular correlation times calculated for acetonitrile when the motion of the small probe molecule in liquids is compared to its motion in SF's. In general, magnetic resonance theory relates the molecular correlation time as being inversely proportional to the spin-spin and spin-lattice relaxation times for the sample nucleus. Indeed, Figure 4.6, which relates the spin-spin and spin-lattice relaxation times of small molecules to

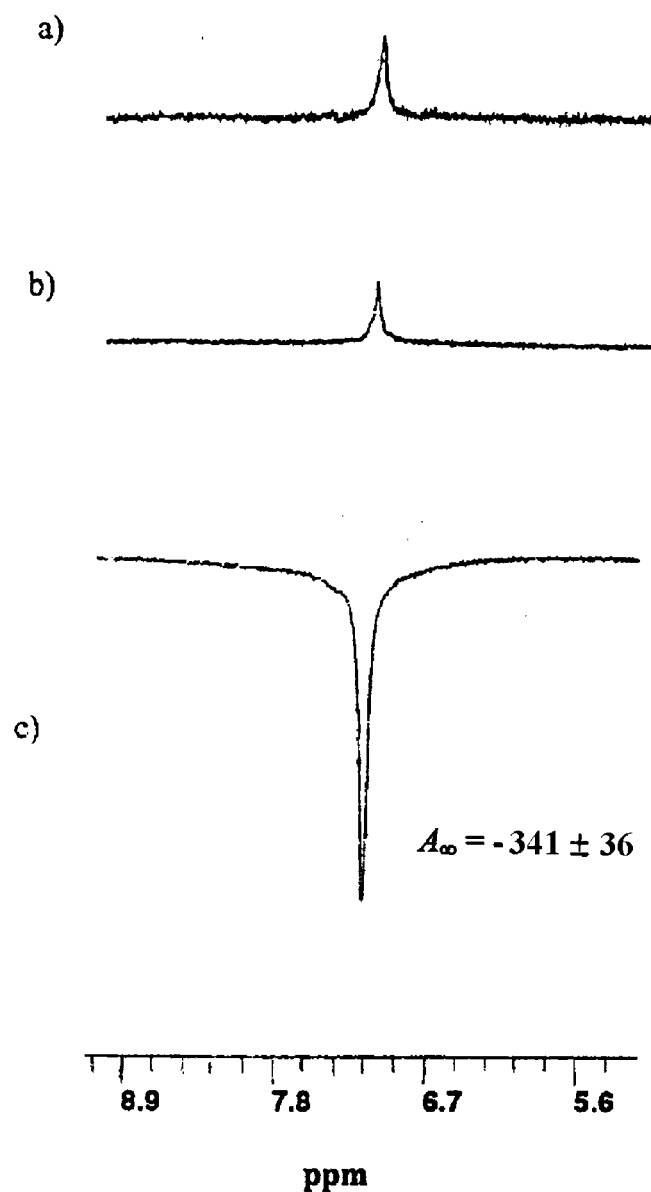


Figure 4.5. SF CO₂ ¹H DNP spectra of 9% benzene in 0.001 M TEMPO. (a) Static ¹H NMR (S/N = 20), (b) flow ¹H NMR (S/N = 17) and (c) flow ¹H DNP (S/N = 124) were collected at 32 scans, 4096 points; the total flow for spectra b and c was 1.1 mL/min.. SF CO₂ conditions under which spectra were collected are as follows: d_{CO2} = 0.805 g/mL, SFC oven temperature 40°C, P_{CO2} = 2398 psi.

molecular motion, shows this increase in T_1 and T_2 , as τ_c times become shorter, especially in the region of motional narrowing (where $T_1=T_2$). Ultimately, an increase in the DNP enhancement in supercritical fluids versus normal liquids is the result of an improved spectral density, J , at the frequency observed, due to motional narrowing. That is, in supercritical fluids, there is an increase in the probability that the frequency of random molecular motion of the molecule (depending on the solvent environment) is on the order of the Larmor frequency, ω_e , of the electron when the condition of $\omega_e\tau_r \ll 1$ is satisfied. Once again, since A_∞ is directly proportional to ρ , when $\omega_e\tau_r \ll 1$, then ρ can reach a maximum value and, the ultimate enhancement will, correspondingly, approach the dipolar limiting value of -330--leading to the highest possible signal in the H^1 DNP experiment.

Table 4.2. Molecular Rotational Correlation Times for Benzene/TEMPO system in Normal Liquids and SF CO₂.

Molecular Correlation Time , τ_r , [psec]			
LLIT Sample	Neat Benzene	9% Benzene/ D6 Benzene	9 % Benzene/ SF CO ₂
0.001M TEMPO	39	42	10
0.01M TEMPO	35	36	12

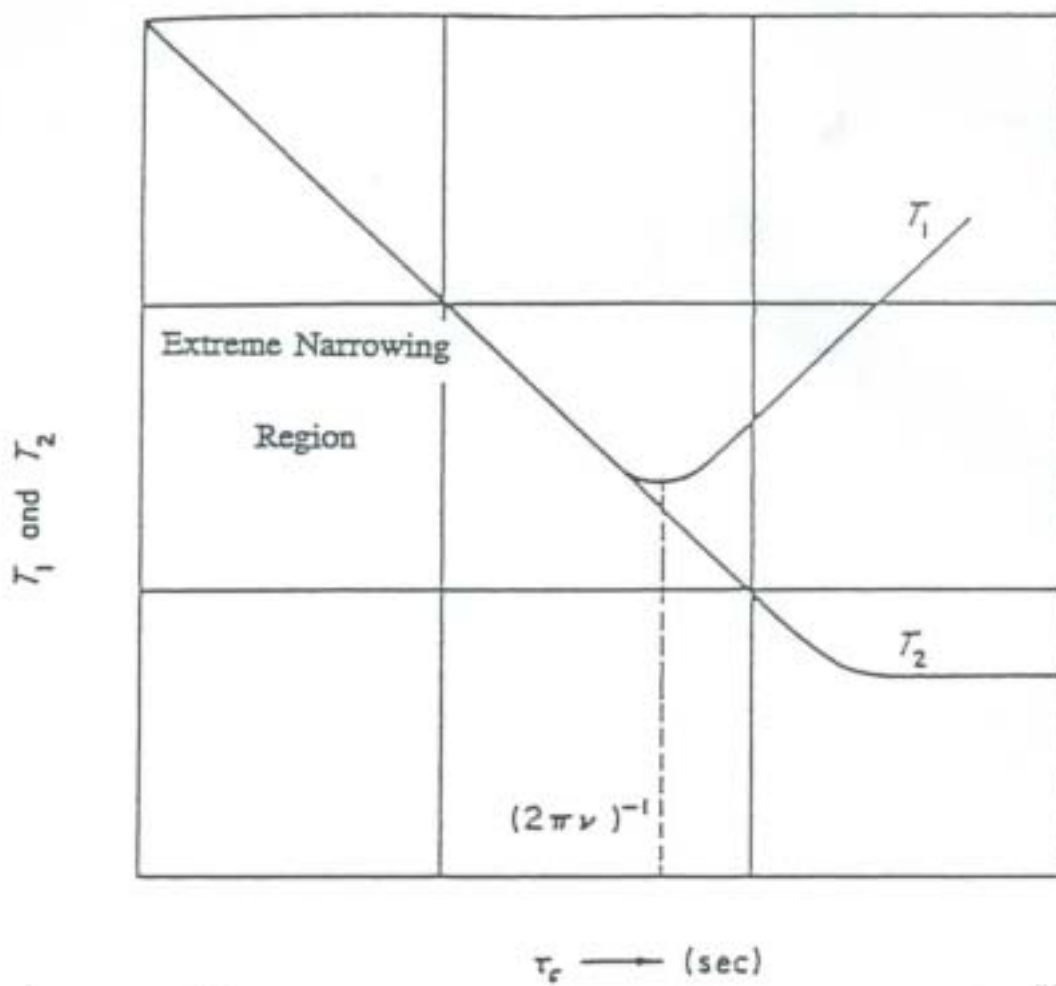


Figure 4.6. A log-log plot of T_1 and T_2 versus the correlation time τ_c ³⁰

Chapter 5: Fundamental Study With Benzene and Chloroform, Using the Coupled Technique of SLIT ^1H DNP /SF CO_2

5.1 Introduction

The solid-liquid intermolecular transfer, or SLIT DNP, experiment is particularly attractive because in addition to the high sensitivity and improved spectral resolution, compared to normal liquid LLIT DNP, it provides high efficiency in the low to high magnetic field transfer experiment.⁷ This result is due, in part, to the absence of the free radical in the flowing bolus stream (after polarization transfer)--avoiding a potential problem of paramagnetic line broadening of the NMR signals. The practical advantages of SLIT DNP over L²IT DNP has been demonstrated in our laboratories by its application in recycled-flow and liquid chromatography techniques.^{9,23} For example, in the aforementioned coupled techniques, the recovery of the sample (or the recycling of the sample) through the system is possible since the radical is not present in the solution. In addition, the possibility of extending the detection technique of SLIT DNP to supercritical fluid chromatography systems seems feasible for the reasons just mentioned. Figure 5.1 illustrates the general location of the SLIT material or immobilized nitroxide radical in the solid-liquid intermolecular transfer or SLIT ^1H DNP experiment. The material is contained in a flow cell, positioned in the low field cavity (refer back to Figure 4.2), where polarization of the electron spins occurs. The, more specific, depiction of the time-dependent interaction of an immobilized free radical, interacting with a solvent molecule, is shown in Figure 5.2. Immobilized free radicals have been used in flow NMR experiments since the 1980's, by researchers such as Bruck et. al.³¹ and more recently by Albert et. al.³², for the efficient pre-magnetization of samples in the main magnetic field for flow NMR experiments. The general approach used in this work was to make use of the efficient electron-nuclear relaxation processes in the presence of free radicals to quickly bring the nuclear spins in a flowing liquid to equilibrium in the magnetic field. Gilpin and co-workers³³ have conducted EPR studies investigating the effects of various polar and nonpolar solvents on chromatographic grade silica modified with immobilized TEMPOL molecules and reported changes in the hyperfine coupling constant with solvent polarity. Large differences in the hyperfine line shapes between bound TEMPOL and that free in solution was observed for the nonpolar solvents. In the case of polar solvents, the line shapes were significantly more narrow, indicative of increased group mobility on the surface. The results were rationalized in terms of a major conformational change in the immobilized nitroxide probe as a result of the solvent's ability to strongly interact with unreacted silanol groups on the surface. It is with this motivation that, Rossi Gitti, synthesized in our labs (late 1980's) a variety of immobilize radicals for the specific application in the newly, developing technique of flow DNP.³⁴ Gitti and co-workers^{7,34} found that SLIT DNP enhancements in normal liquid systems were influenced by motional characteristics of the immobilized nitroxides, the concentration or number of spins of the material, and by

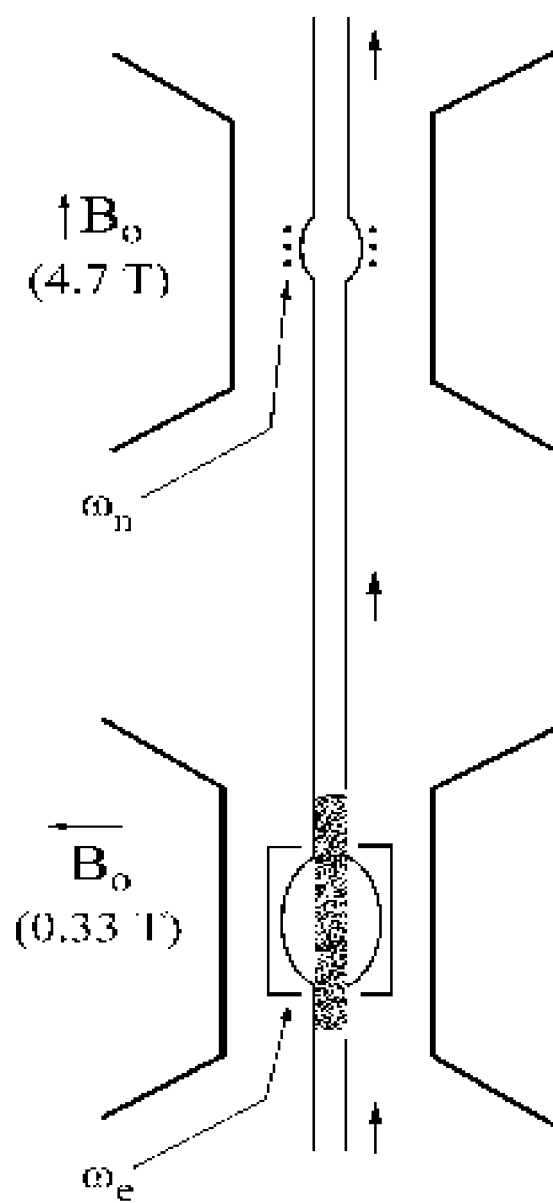


Figure 5.1. Depiction of the position of the immobilized nitroxide or SLIT material (shaded grey), in the low-to-high field DNP apparatus.

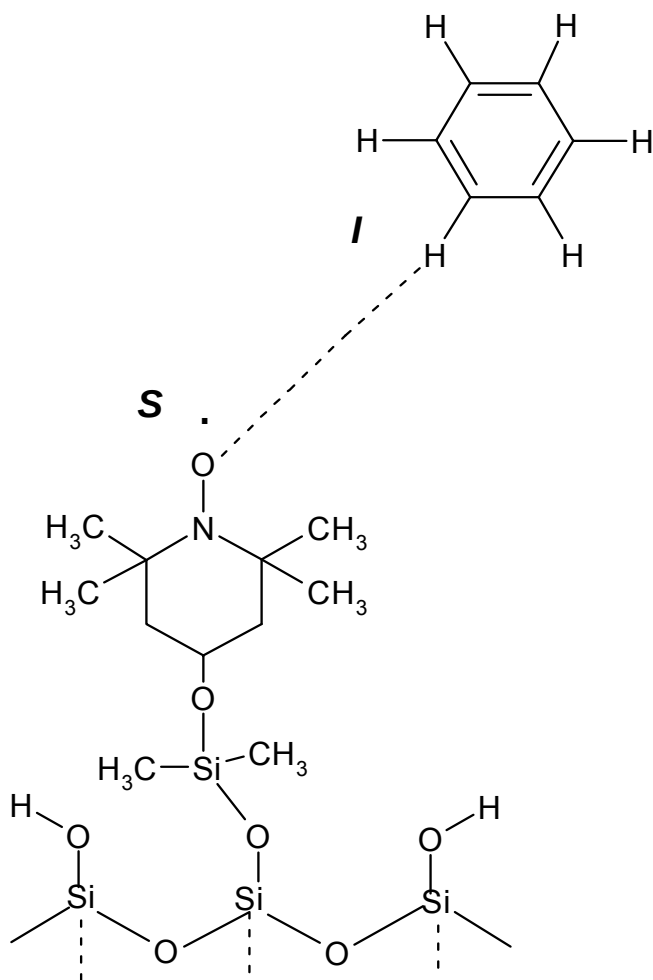


Figure 5.2. Time-dependent Hamiltonian interaction of an immobilized nitroxide electron spin S and a nuclear spin I on the benzene molecule in normal liquid flow ^1H DNP.

surface/solvent interactions relating back to the findings of the previously mentioned Gilpin study. More recently, in 1998, the researcher Batchelor conducted studies on the EPR line width of free-radical TEMPO in normal liquid and supercritical fluid ethane, n-hexane, and ethanol. EPR line minimizing and broadening effects were observed at sub-critical and supercritical point parameters (ρ , T, and P), respectively, in the study. Line broadening of the EPR signal for free radical TEMPO in supercritical fluid CO₂ has been observed by Dukes et. al.³⁵ These studies were performed using non-immobilized radical. Nevertheless, they suggest that the motional characteristics of the nitroxides' change in the normal liquid versus the supercritical fluid: e.g. the correlation times are different in normal liquids versus supercritical fluids. Therefore, flow ¹H DNP was conducted using SLIT material synthesized in our labs, under normal liquid and supercritical fluid conditions, and the resulting DNP signal enhancements were compared.

5.2 Experimental

The experimental setup of Figure 4.2 was used in conducting SLIT ¹H DNP for normal liquid samples and those dissolved in SF CO₂. The experimental procedure for conducting SLIT ¹H DNP is essentially the same as that for LLIT ¹H DNP, described in the experimental section of Ch.4. However, the immobilized nitroxide material was packed in a PEEK rod (of the same dimensions used in the LLIT experiments) and positioned in the cell of the low field cavity. In the case of normal liquid, SLIT DNP, the sample was introduced at the liquid pump, at flow rates ranging from 4-6 mL/min. When, SF CO₂ was used as the flowing solvent, the liquid pump flow was adjusted to obtain sample concentrations of the normal liquids at 5, 9, and 33% v/v in SF CO₂.

The SLIT materials used in the present study are shown in Figure 5.3. All SLIT materials were synthesized in our labs, according to procedures developed by Rossi Gitti, detailed in the doctoral dissertation of her research.³⁴ The specific, immobilized nitroxides used in the present research were chosen based on their availability and their successful application in previous DNP work from our labs. SLIT materials 2 and 4 were synthesized in the summer of 1999 by Holger Fischer. SLIT materials 1 and 3 were used as is, synthesized by Gitti. It is important to note that SLIT material 1 consists of ¹⁵N labeled nitrogen. The EPR spectrum for a ¹⁵N nitroxide radical is a doublet due to the electron hyperfine interaction with this nucleus (¹⁵N, I= 1/2). Figure 5.4 shows the EPR spectrum for SLIT material 1 in neat benzene. The saturation of the electron spin at a field strength corresponding to the highest intensity or sharpest EPR line, in this case, should result in a reduction in the DNP ultimate enhancements by a factor of two ($A_{\infty}/2$ or for ¹H DNP, $-330/2$). This is in comparison to the factor of three reduction of the ultimate enhancements for ¹H DNP (e.g. $-330/3$) when ¹⁴N is the isotope in greatest abundance (¹⁴N, I= 1). Therefore, the use of SLIT material 1 implies higher observable DNP enhancements, under analogous conditions.

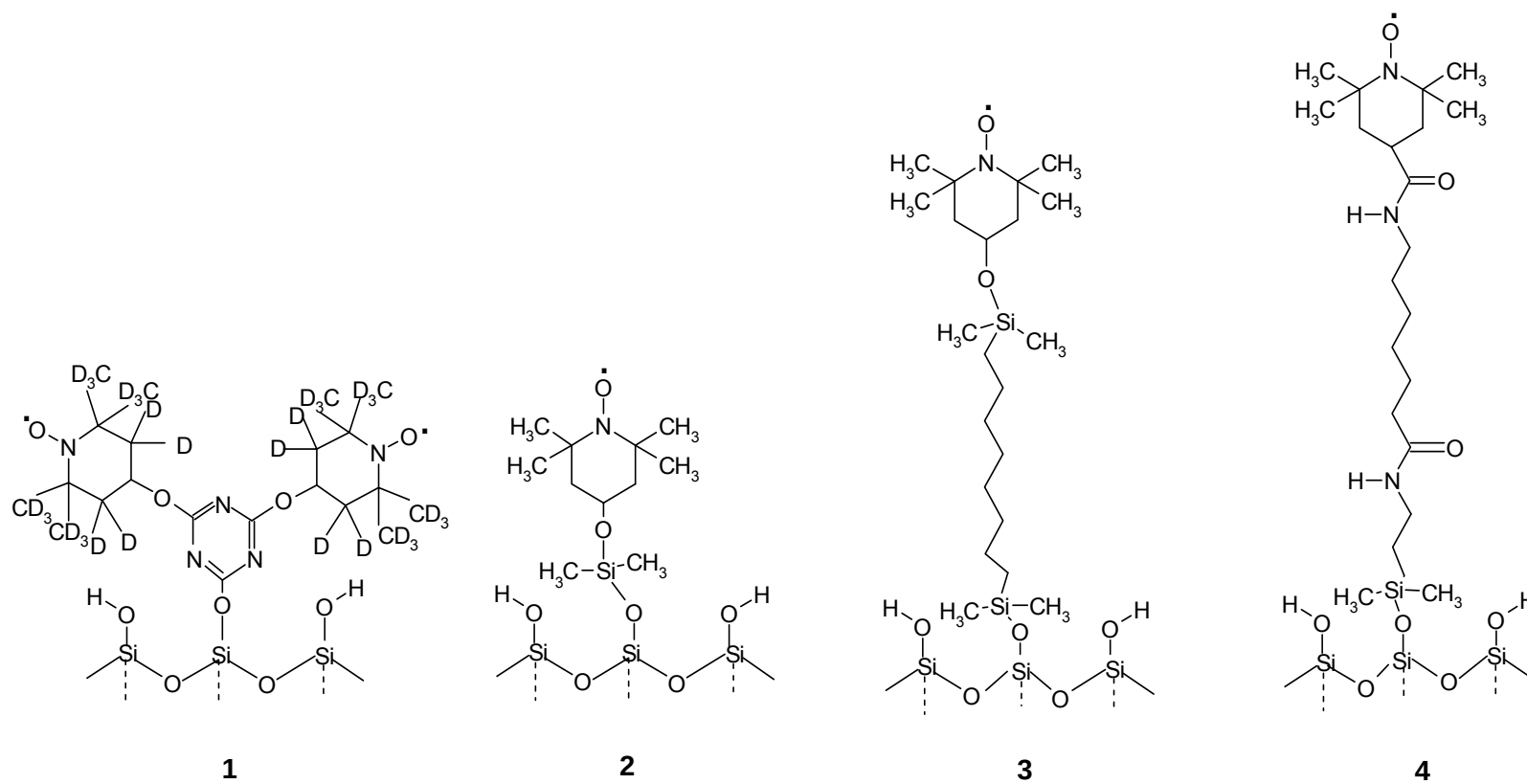


Figure 5.3. Immobilized nitroxide material used in SLIT ^1H DNP in normal liquids and SF_6/CO_2 . SLIT material 1 contains ^{15}N labeled nitrogen.

5.3 Discussion of Results and Conclusions

The SLIT ^1H DNP data for neat liquid benzene and benzene dissolved in deuterated (D6) benzene and SF CO_2 for materials 2 and 3, are presented in Table 5.1. SLIT ^1H DNP data for material 4 was obtained in the neat liquid benzene solely and is also given in the table. Table 5.2 presents SLIT ^1H DNP data for material 1 in neat chloroform and chloroform dissolved in SF CO_2 , at various concentrations. The leakage factor at the high field magnet for SLIT materials 2, 3, and 4 was obtained from previous research conducted in our labs, using the same materials under normal liquid flow conditions.^{8,14} (The determination of, f , for SLIT 4 was not possible for reasons discussed later in this section.) Additionally, the values for the degree of nuclear- electron coupling in the case of SF CO_2 flow were approximated for SLIT 2 and 3 materials, based on experimental flow data collected for SLIT 3. In general, the degree of electron to nuclear coupling in the SLIT DNP experiment correlates well with a high leakage factor for the experiment, compared to LLIT DNP. Transfer efficiencies (that is the efficiency with which the polarized sample bolus is transferred from the low to high field magnet and subsequently detected) have experimentally been found to be in the range of 60-80%¹⁴; the SLIT data reported presently reflect this finding.

Figures 5.5 and 5.6 show the static, flow, and stopped-flow ^1H spectra for 9% benzene in SF CO_2 , when DNP is conducted using SLIT materials 2 and 3, respectively. Based on the maximum observed enhancement values, $(A_{\text{obs}})_{\text{max}}$, given in Table 5.1 for these respective samples, the approximate factor 10 increase in the DNP signal (in the negative direction) versus the normal flow NMR signal can be observed. This factor increase in signal, for the same sample, is twice as large in the flow SF CO_2 SLIT DNP experiment versus the flow SF CO_2 LLIT experiment reported in Table 4.1. This observed signal enhancement could be attributed, initially, to the expected gain in polarization transfer efficiency in the DNP experiment when the free radical TEMPO is immobilized (SLIT experiment), rather than dissolved directly, in the sample flow (LLIT experiment). When the $(A_{\text{obs}})_{\text{max}}$ values for SLIT 2 and 3 in SF CO_2 , are compared to the neat liquid benzene values, a factor increase of approximately 3 and 6 are observed, respectively. Figures 5.7 and 5.8 show the DNP spectra collected for benzene under similar conditions, when SLIT 2 and 3 are immobilized in the low field cavity. The observed DNP enhancements can be seen in the figures by a comparison of the signal intensities. Again, these increases are in agreement with the predicted increase of DNP signal enhancements in SF's, similarly found for the LLIT data, reported in Chapter 4. The fact that the DNP signal of neat benzene using SLIT 3 is twice as large as that for SLIT 2, is indicative of the differences in the increased chain length of the two SLIT materials (refer to the structures given in Figure 5.3). The subsequent A_{∞} values, calculated for the DNP signals (spectra c) in the respective sample Figures 5.5 and 5.6, are -205 ± 24 and -246 ± 36 . These values are the

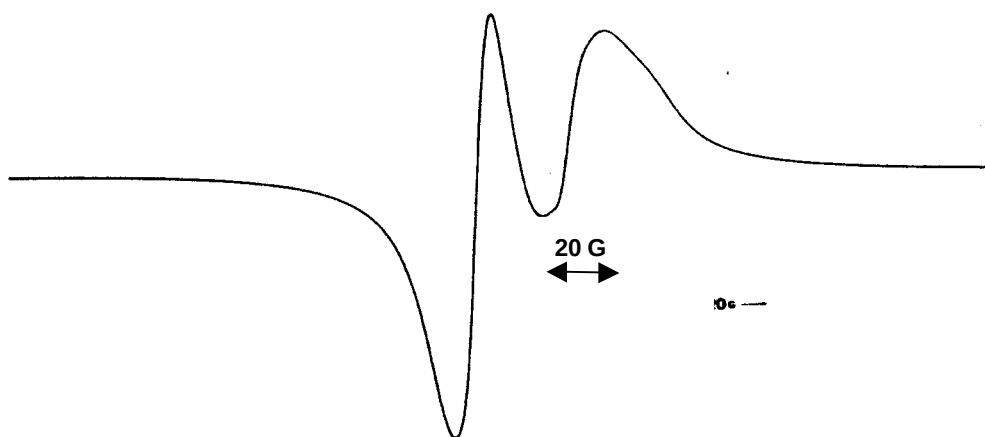


Figure 5.4. ^{15}N labeled SLIT 1 material in neat benzene.

Table 5.1. SLIT ^1H DNP Data for Benzene.

Immobilized Nitroxide Sample	$(A_{\text{obs}})_{\text{max}}$	f_{c}	s	A_{∞}
SLIT 2 Neat Benzene	-1.7	0.73 ^a	0.17	- ^b 43 $\pm$ 21
SLIT 2 9% Benzene/ d6 Benzene	-2.7	“	0.15	- ^b 67 $\pm$ 15
SLIT 2 9% Benzene/ SF CO ₂	-9.1	0.73	0.33	-205 $\pm$ 24
SLIT 3 Neat Benzene	-3.2	0.72 ^a	0.33	-73 $\pm$ 14 ^a (-56 $\pm$ 7)
SLIT 3 9% Benzene/ d6 Benzene	-2.7	“	0.26	-61 $\pm$ 17
SLIT 3 9% Benzene/ SF CO ₂	-10	0.73	0.26	- ^b 246 $\pm$ 36
SLIT 4 Neat Benzene	-0.2	--	0.15	--

a)Data obtained from K. Tsai, 1990.

b)Ultimate enhancement values corrected for an approximated transfer efficiency of Q= 0.80.

Table 5.2. SLIT ^1H DNP Data for Chloroform

SLIT 1 Immobilized Nitroxide Sample	$(A_{\text{obs}})_{\text{max}}$	s	$^a f$	$^b A_{\infty}$
Neat Chloroform	-1.8	0.87	0.80	-41 ± 12
33% Chloroform/ SF CO ₂	-5.8	0.74	0.80	-131 ± 20
9% Chloroform/ SF CO ₂	-6.0	0.83	0.80	-135 ± 22
5% Chloroform/ SF CO ₂	-4.8	0.58	0.80	-108 ± 18

a) Leakage factor approximated based on findings reported by Tsai et. al 1991.

b) Ultimate enhancement values corrected for transfer efficiency based on a value of, $Q = 0.80$.

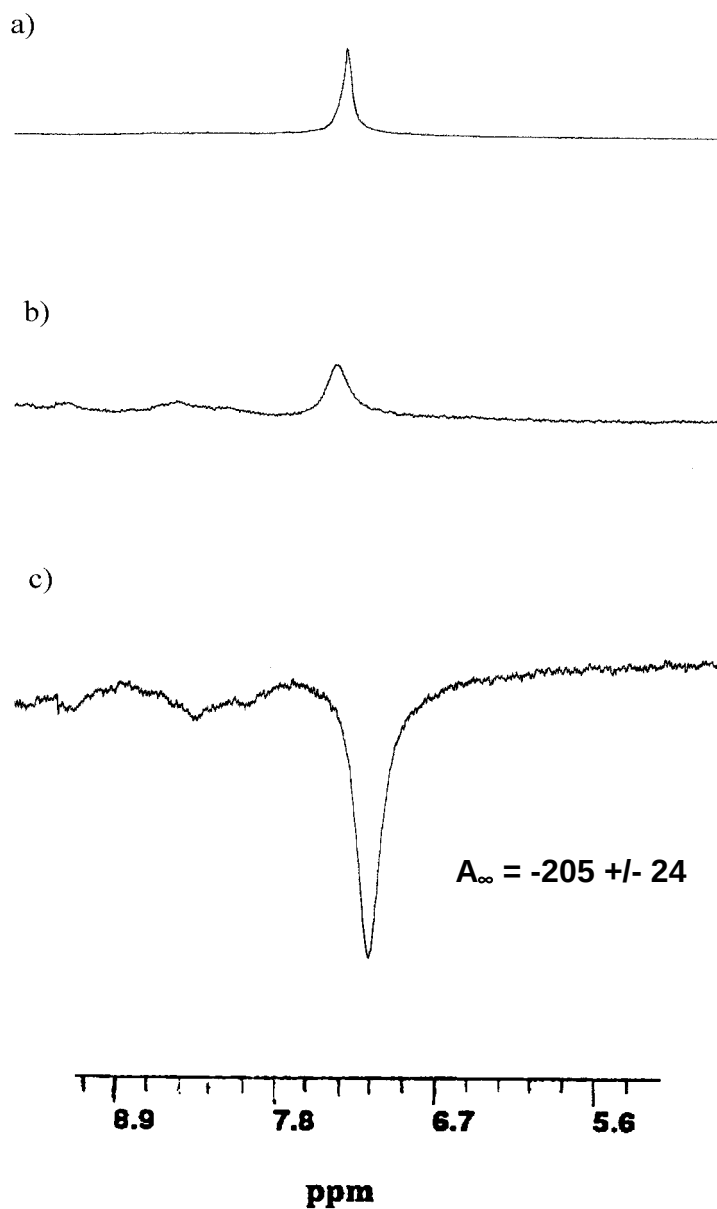


Figure 5.5 . SF CO₂ ¹H DNP spectra of 9% benzene using **SLIT 2**. (a) Static ¹H NMR (S/N = 25) and (b) flow ¹H NMR (S/N = 17) were collected at 32 scans, 4096 points; the total flow for spectra b and c was 1.1 mL/min. and (c) flow ¹H DNP (S/N = 104) was collected at 16 scans. SF CO₂ conditions under which spectra were collected are as follows: d_{CO2} = 0.805 g/mL, SFC oven temperature 40°C, P_{CO2} = 2398 psi.

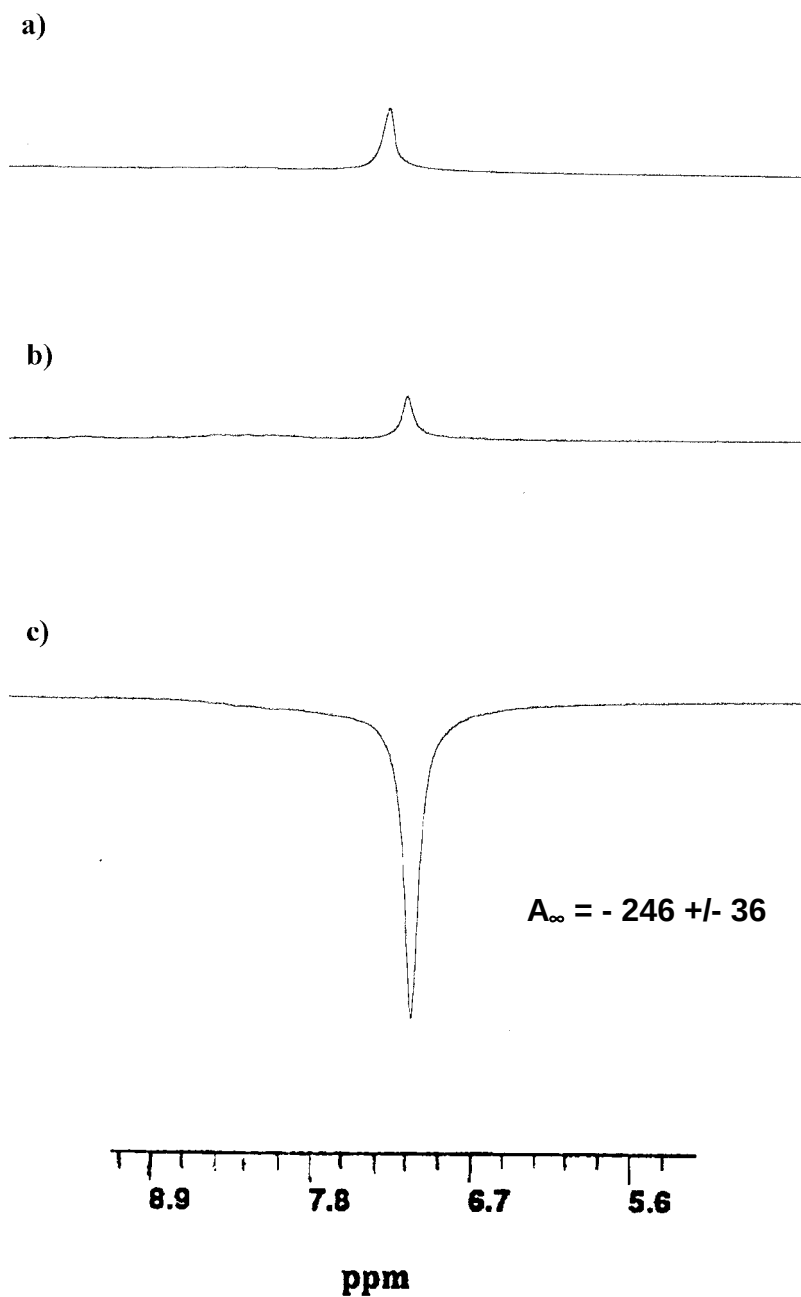
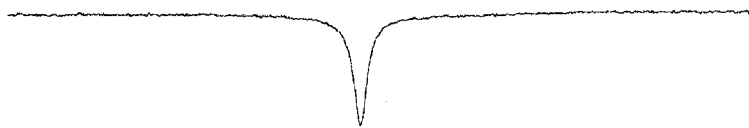


Figure 5.6. SF CO₂ ¹H DNP spectra of 9% benzene using **SLIT 3**. (a) Static ¹H NMR (S/N = 25), (b) flow ¹H NMR (S/N = 17) and (c) flow ¹H DNP (S/N = 134) were collected at 32 scans, 4096 points; the total flow for spectra b and c was 1.1 mL/min.. SF CO₂ conditions were approximately the same, as given in Fig. 5.5.

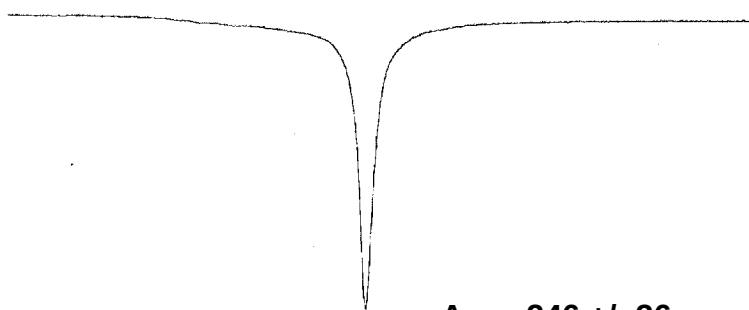
a)



b)



c)



$$A_{\infty} = -246 \pm 36$$

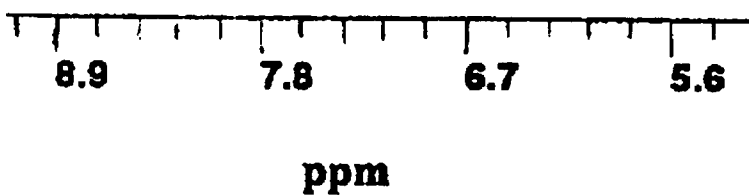


Figure 5.7. SLIT ^1H DNP spectra of benzene using **SLIT 3** in neat benzene (a), d_6 benzene (b), and 9% benzene in SF_6 CO_2 (c). The conditions under which spectra (a) and (b) were collected are as follows: flow rate 5.0 mL/min, 32 scans, 4096 points, back pressure 800 psi. SF_6 CO_2 conditions were approximately the same except for a flow rate of 1.11 mL/min.

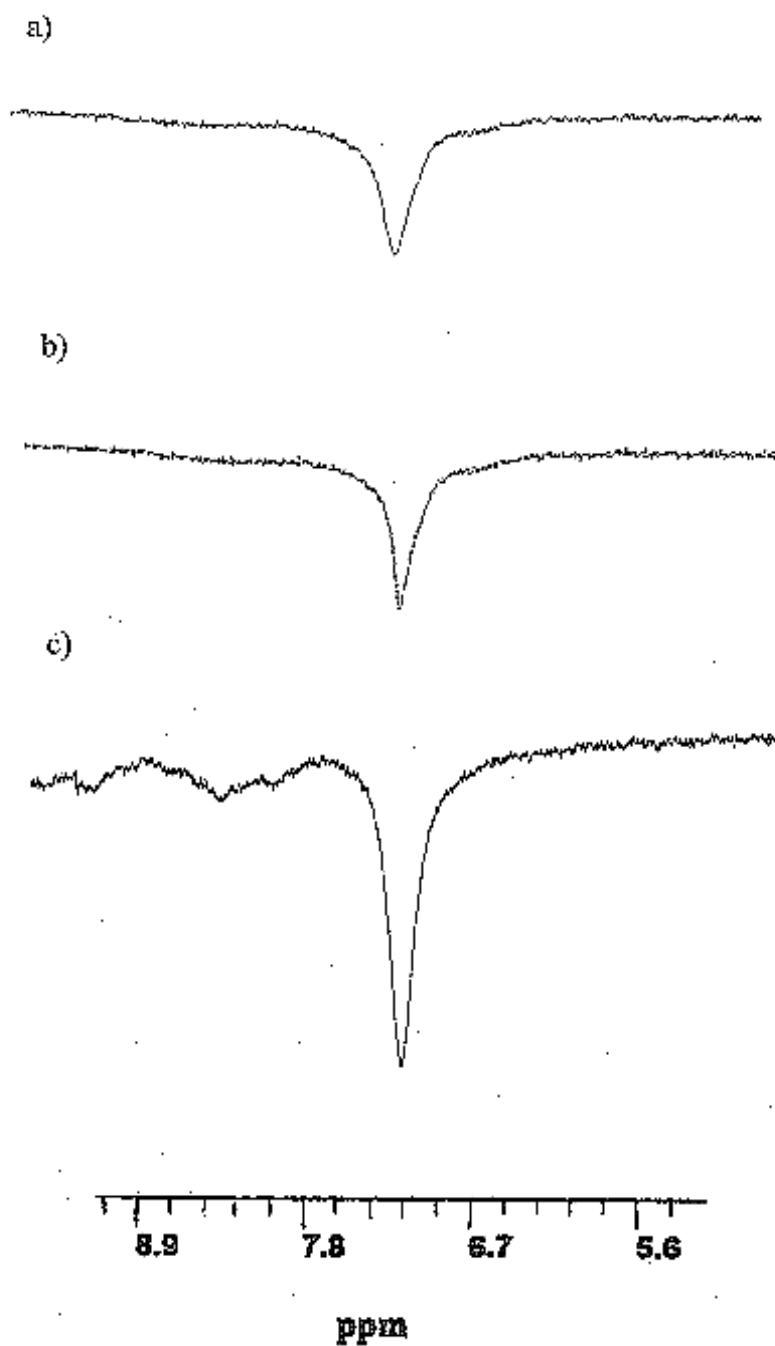


Figure 5.8. SLIT ^1H DNP spectra of benzene using **SLIT 2** in neat benzene (a), d₆ benzene (b), and 9% benzene in SF CO₂ (c). The conditions under which spectra (a) and (b) were collected are as follows: flow rate 4.5 mL/min, 32 scans, 4096 points, back pressure 750 psi. SF CO₂ conditions were approximately the same except for a flow rate of 1.11 mL/min..

largest ultimate enhancement values obtained in our laboratories for SLIT ^1H DNP of benzene and approach the theoretical value of -330. The A_∞ value for ^1H DNP of neat benzene using SLIT material 2 was reported by Tsai in 1990 and is given in Table 5.1. The value is comparable to the value reported in the present work and is approximately one-third the value of that obtained in SF CO_2 . As in the case of the LLIT experiment, D6 benzene was used as the solvent for the determination of the signal enhancement for 9% benzene, using the various SLIT materials. The use of deuterated benzene in DNP resulted in little change in the calculated ultimate enhancement values as compared to neat benzene. Therefore, it can be assumed that the motional properties i.e. rotational correlation times of the SLIT materials in the normal liquids are similar. In addition, then, the time-dependent interaction of the sample and the immobilized nitroxide must also be similar in benzene and D6 benzene and it is for this reason that the A_{obs} and A_∞ values are comparable.

Table 5.3 shows estimated rotational correlation times, τ_c , for the benzene/TEMPO (immobilized nitroxide) interaction in the normal liquids (chloroform, benzene, and D6 benzene) and SF CO_2 for SLIT materials 1-3. The values were obtained in the same manner as the τ_c 's presented in Chapter 4 for the LLIT ^1H DNP of benzene. The data in the present table shows that in SF CO_2 the molecular motion of the coupled sample system increases and, therefore, the time of the interaction becomes shorter. As the maximum ultimate enhancement value is approached for a sample system, a limiting value for molecular motion is also reached, based on the calculations. Again, a 3-4 factor increase in molecular motion is observed in going from the use of normal liquids in flow DNP versus SF 's. The time for the motion of the interaction in benzene and D6 benzene is approximately the same and this corresponds directly with the enhancement data, as already mentioned.

Normal liquid flow and SF CO_2 ^1H DNP was also conducted for chloroform, using SLIT material 1. The data for the experiment is listed in Table 5.3. Figure 5.9 shows a comparison of the SLIT ^1H DNP spectra, under the same experimental conditions, for neat liquid chloroform and 5, 9, and 33% v/v chloroform in SF CO_2 . It has been shown experimentally that the ultimate enhancement values for SLIT ^1H DNP in neat chloroform are approximately twice as large as those obtained for neat benzene¹⁴, under analogous conditions of flow, SLIT material, etc.. As noted earlier, ^{15}N labeled SLIT 1 was used based on its availability in the lab, but also for its potential to result in theoretical enhancement values one-third greater than ^{14}N containing nitroxides. The data listed in Table 5.3, then, were analyzed in light of the predicted increase in signal enhancements for ^{15}N labeled immobilized nitroxides and previous data suggesting larger enhancements for chloroform versus benzene. As can be seen, there is an approximate factor 3 increase in signal enhancements when chloroform is dissolved in SF CO_2 , as compared to the neat liquid. The highest DNP signal enhancement is obtained at a sample concentration of 9% chloroform in SF CO_2 . At 5% chloroform, it

Table 5.3. Molecular Rotational Correlation Times for Benzene/Immobilized Nitroxide and Chloroform/Immobilized Nitroxide Systems in Normal Liquids and SF CO₂

Rotational Correlation Time, τ_r [psec]				
SAMPLE	Neat Benzene	9% Benzene/ D6 Benzene	9 % Benzene/ SF CO ₂	
SLIT 2	67	69	25	
SLIT 3	77	62	19 ^a	
	Neat Chloroform	33% Chloroform/ SF CO ₂	9% Chloroform/ SF CO ₂	5% Chloroform/ SF CO ₂
SLIT 1	79	38	38	44

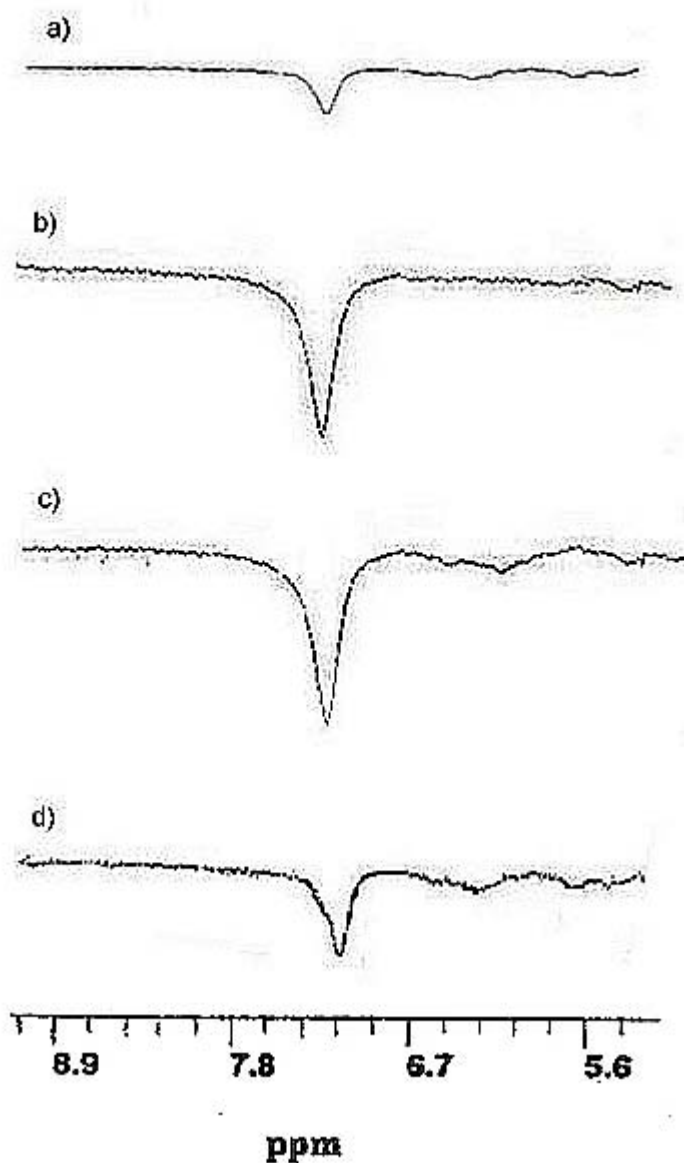


Figure 5.9. SLIT ^1H DNP spectra of a) neat chloroform, b) 33% chloroform in SF_6/CO_2 , c) 9% chloroform in SF_6/CO_2 , and d) 5% chloroform in SF_6/CO_2 . The conditions of spectra b)-d) were as follows: 32 scans, 4096 points; total flow for spectra was 1.1 mL/min.. SF_6/CO_2 conditions under which spectra were collected are as follows: $d_{\text{CO}_2} = 0.805 \text{ g/mL}$, SFC oven temperature 40°C , $P_{\text{CO}_2} = 2398 \text{ psi}$. Spectrum a) was collected at a flow rate of 5.2 mL/min.

would be expected that the sample liquid assumes the properties of the bulk solvent (e.g. SF CO₂), the result of which should yield the highest A_{∞} values. However, the observed maximum enhancement and, therefore, the ultimate enhancement decreases at this concentration. This result might be attributed to an inaccuracy in the measure of the concentration, due to limitations in the HPLC pump's ability to deliver accurate sample volumes (concentrations) at flow rates lower than 0.1 mL/min. In this case, the concentration of chloroform might have been less than 5%, resulting in the insufficient saturation of electron spins (as evidenced by a poor s value, given in Table 5.2) and lower DNP signals.

The rotational correlation times listed in Table 5.3 for the DNP interaction of chloroform with the immobilized nitroxide SLIT 1 material, additionally, support an increase in the mobility of the system, in SF CO₂. The general trend is observed that as the concentration of the neat liquid sample is decreased in the bulk solvent, SF CO₂, the time of the electron-nuclear DNP interaction becomes shorter. This results directly in an increase in ultimate signal enhancements for SLIT ¹H DNP of chloroform.

As one compares the SLIT structures in Figure 5.3, it is clear to observe that the largest DNP ultimate enhancements were obtained for SLIT 3. The longer chain length of the immobilized nitroxide increases its mobility. (Previously reported rotational correlation times for SLIT materials 1-3 in neat benzene demonstrate a shortening of τ_r times as the chain length increases.¹³) In SF CO₂, the mobility of the SLIT material is enhanced. Based on the longer chain length of SLIT material 4, one would expect comparable or even higher enhancements with the other, shorter chain counterparts. However, as the data presented in this chapter demonstrates, it became experimentally difficult to saturate the electron spins due to a broad EPR signal; e.g. s values measured for SLIT 4 were low and corresponding A_{obs} values were essentially zero. This could be due to the decreased mobility of the nitroxide due to hydrogen bonding interactions taking place at the amide groups with exposed hydroxyl groups on the silica gel surface. This type of interaction would inhibit the mobility of the nitroxide chain, even in the presence of SF CO₂. Hydrogen bonding interactions are also possible with neighboring nitroxides bound to the silica gel backbone. In a similar way, then, nitroxide mobility would be inhibited and the SLIT TEMPO/¹H interaction would be longer, resulting in lower enhanced signals.

Chapter 6: Study of the Indirect Measure of the Electron Spin-Spin and Spin-Lattice Relaxation Times (T_{2s} , T_{1s}) Using the Detection Techniques of EPR and ^1H DNP

6.1 Introduction

In Chapters 4 and 5, the increase in DNP signal enhancement values were reported in SF CO_2 for benzene and chloroform model systems. The increased signal enhancements that were obtained in the SF, were shown (via calculated τ_r 's) to be directly related to the increased mobility of the coupled nitroxide/sample nuclei interaction in the solvent. As already demonstrated in Chapter 3 of this thesis, the molecular motion of a sample in a particular solvent environment (i.e. the molecular correlation time) can be measured using conventional NMR and EPR techniques. In general, magnetic resonance theory relates the molecular correlation time as being inversely proportional to the spin-spin and spin-lattice relaxation times for the sample nucleus that is being monitored in a given chemical species. It can be expressed that $\tau_r \propto 1/T_1$, $1/T_2$ and, according to the Stokes-Einstein relation, $\tau_r \propto \eta/T$, for small spherical molecules. However, in the case of nitroxide spin-labels, the electron spin-spin and spin-lattice relaxation times have been shown, experimentally, to have opposite dependencies on viscosity, than versus the nuclear T_1 's.^{27,36-39,49} In one researcher's findings, for a change of two orders of magnitude in η/T , the T_{1s} increases by one order of magnitude.³⁶ At constant nitroxide concentration, then, the relaxation times have a simple direct proportionality to the molecular rotational correlation time. Since, then, for the electron, $\tau_r \propto T_{1s}$, T_{2s} , the electron spin-spin, T_{2s} , and electron spin-lattice, T_{1s} , relaxation times—and the product of these two relaxation times—should decrease as motion increases (and τ_c times become shorter), namely in SF's. The ultimate enhancement, defined by Equation [10] in Chapter 2, shows the NMR enhancement to be directly proportional to the coupling factor, ρ , (which, as a review, is a parameter of the DNP experiment representing the degree to which the nucleus of a diamagnetic species and the electron of the paramagnetic species interact, thus enhancing the magnitude of the NMR transition). For the spin systems studied in this thesis, it is assumed that dipole-dipole interaction of the proton nucleus and electron is the dominant form of relaxation responsible for DNP enhancements. The coupling factor, ρ , has previously been related to the molecular correlation time in Chapter 4, Equation [16]. From this equation, it can be seen that as τ_r decreases, and as $\omega_e \tau_r \ll 1$, then ρ can reach a maximum value of 0.5. The result of this expression would show that at shorter correlation times, higher values of A would be obtained, as previously mentioned and related in Equation [10], Ch.2.

The electron spin-lattice and spin-spin relaxation times, then, will be affected by a change in τ_r , which in turn implies a change in the viscosity of the solution environment. In the case of electron spin-lattice relaxation, it is assumed that an electron-nuclear dipolar interaction is the relaxation mechanism that governs the T_{1s} times presented in this chapter. A spin-rotational and/or spin-diffusion mechanism has been presented in the literature^{37,40}, that may, in addition, contribute to the overall value of the electron

spin-lattice relaxation rate for nitroxides. It will be sufficient to note that all of these mechanisms of relaxation are related to the τ_r of the nitroxide (through proposed, mathematical theories) and thus DNP experiments conducted in different media, where τ_r can change significantly, can influence the degree to which the mechanisms contribute to the overall T_1 relaxation rate. (However, it is not in the scope of this research to identify and quantitate the individual contributions of the above mechanisms, which contribute to the total T_1 relaxation rate.) For spin-spin relaxation, this time is influenced by the rate at which unpaired electron spins collide with one another and change their spin states; T_{2s} is inversely related to this rate. The rate of spin-exchange has already been shown in this thesis to be dependent on nitroxide concentration and on changes in temperature and pressure, especially where SF's are concerned. As related from liquid DNP theory presented in Ch. 4, when the electron exchange rate increases to a maximum value (and the EPR line for TEMPO is exchange narrowed into a singlet), then it is possible that all electron spins can be saturated in the DNP experiment and the maximum signal enhancement, A_∞ , for DNP achieved.

Equation [9] was presented as the theoretical expression for the equation of the line obtained when the saturation parameter, s , and the value of A_∞ for a given sample system is to be ascertained, as explained in the DNP theory section of Chapter 2. This expression is obtained when the inverse of the experimentally observed enhancement values is plotted versus the inverse of the EPR field strength—the graph of which is referred to as a saturation plot. (An example of a saturation plot is shown in Figure 6.1.) The slope, m , of this line can be expressed as follows

$$m = C \cdot 1/\gamma_s^2 T_{1s} T_{2s} \quad [17]$$

where C is a constant for the DNP experiments conducted according to the instrument setup shown in Figure 4.3 and is proportional to $(\rho f (\gamma_s/\gamma_I))^{-1}$. The magnitude of the slope varies inversely with the product $T_{1s} T_{2s}$ and can provide both qualitative and quantitative information about the relaxation times of free radicals (and thus the mobility of free radicals) in the technique of DNP.

Quantitative information about T_{1s} can be obtained indirectly from the slope relationship shown in Equation [17] via the DNP technique. In the early 1960's, researchers such as Muller-Warmuth et. al.^{41,42} and Richards et. al.⁴³ used DNP to measure electron T_{1s} 's at EPR field strengths of 15 Gauss and of free radicals at 3300 Gauss. The researchers utilized information from saturation plot data and line width data to measure relative T_{1s} 's. McCain and Myers⁴⁴ in 1967 used DNP to measure the relative T_{1s} of the eight hyperfine lines of VO^{2+} . When compared to the T_{1s} calculated from the nonsecular terms (terms contributing to both T_1 and T_2) of the theory derived by Wilson and Kivelson⁴⁵ based on line width analyses, the measured values agreed well with the theory. In addition, William Glad, in his 1982 doctoral thesis, utilized DNP

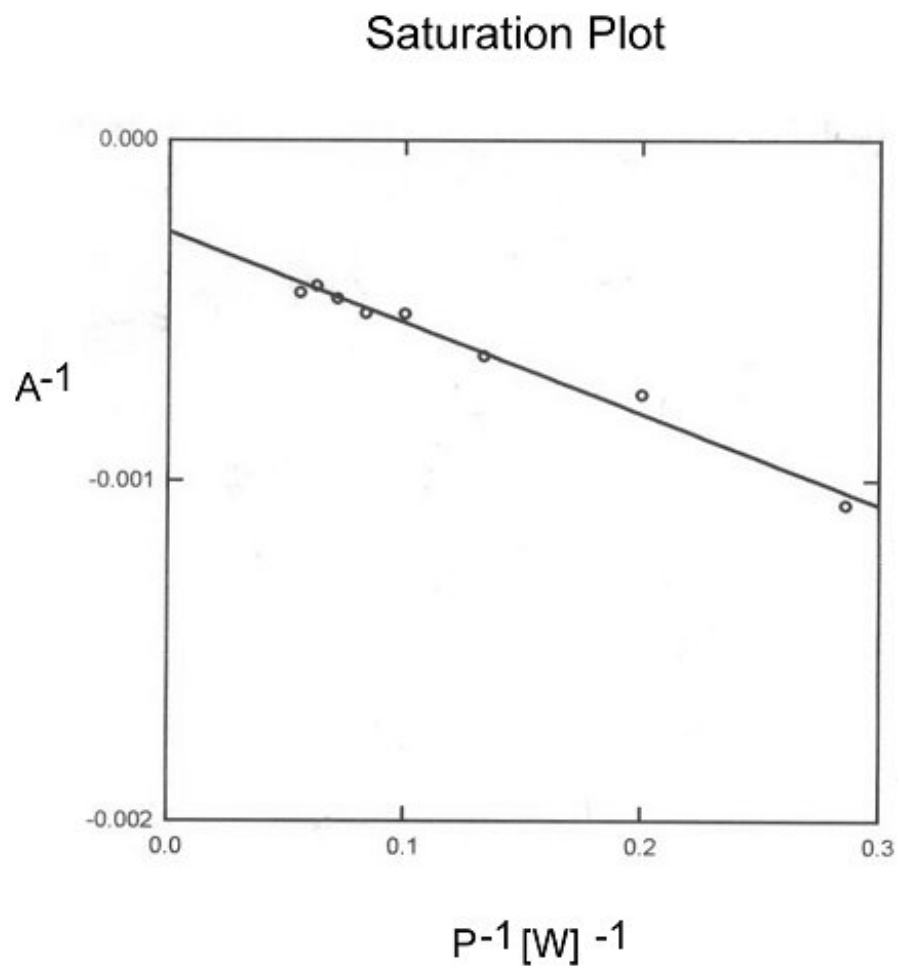


Figure 6.1. LLIT ^1H DNP saturation plot of neat sec-butyl benzene. Microwave power was varied from 3.5 to 20 W. The value of the slope is $-2.7 \times 10^{-3} \text{ G}^2$.

to indirectly measure electron T_{1S} 's in paramagnetic metal ions and compared these results to literature values, according to the methods used by the aforementioned researchers.³⁹ Of course, electron spin-lattice relaxation times can be quantitatively measured, directly by spectroscopic techniques such as pulsed saturation recovery and electron-electron double resonance EPR.⁴⁰

Qualitative information about the electron spin-spin and spin-lattice product makes possible the determination of trends in relaxation times, for some of the LLIT liquid systems tested in this thesis. In fact, in the DNP work just mentioned, conducted by Glad³⁹, even though his work was a quantitative determination of T_{1S} for metal ions in solution, he did make note of the qualitative relationship that the DNP enhancement had, specifically, with the $T_{1S}T_{2S}$ product and did use this information in support of and in the analysis of his results. It was noticed in the present research (as will be presented in the results section of this chapter) that there was a large difference in slope values between the saturation plots of the LLIT DNP data of 9% benzene in normal liquid benzene, deuterated benzene, and SF CO₂. When compared, it was found that there were, on average, 2 orders of magnitude difference between the values. This trend in large differences in saturation plot slopes, in fact, was observed for all SLIT and LLIT data, as DNP was conducted for the same sample in normal liquids (i.e. benzene and deuterated benzene) and in SF CO₂. The observation of less positive slope values in going from normal liquid solvents to SF's had never been noted in our labs before, due to the fact that most DNP experiments to date, have been conducted in normal, non-deuterated liquid solvents. The relationship of the slope to the electron relaxation times of the free radical dissolved or immersed in the solvent flow (as is the case of the immobilized nitroxide in SLIT DNP), suggested that a major change was occurring in the spin-spin and/or spin-lattice relaxation time--since e.g. the concentration of TEMPO and other experimental/instrumental conditions remained constant, specifically in the normal liquid systems. On further consideration, specific questions were raised as to how and why T_{1S} and T_{2S} (or the product of these two quantities) were changing in such a large manner. The observation, for example, of a changing T_{2S} for the same concentration of TEMPO dissolved in neat benzene and deuterated benzene would suggest that Heisenberg exchange between nitroxide radicals was different in the two solvents. This observation is in contrast to the fact that deuterated benzene and neat benzene have similar viscosities. And, it would not be expected, nor has it been presented in the literature, that the T_{2S} for the free radical should be influenced by this fact alone. In addition, a changing T_{1S} for TEMPO in these same solvents may imply that a specific type of spin-lattice relaxation mechanism is dominant in the solvent. The decreased concentration of ¹H nuclei in the deuterated benzene systems (and SF CO₂ systems) versus the increased concentration and presence of hydrogen nuclei in the bulk solvent of the neat benzene systems, has been suggested by some researchers^{37,39} (in 1994), to influence electron T_{1S} 's. The spin diffusion theory for the spin-lattice relaxation mechanism of free radicals states that for a coupled electron-nuclear system, the electron magnetization can obviously relax the magnetization of protons in the bulk solvent.⁴⁶ Robinson et. al.⁴⁰ proposed that just as in spin diffusion theory, the converse is also true: the proton spin system in the solvent can relax the electron spins. According to predicted results of Robinson et. al.⁴⁰ and

Mailer et. al.³⁷, T_{1S} of the nitroxide spin-label should decrease by an order of magnitude in bulk solvents where nuclear spins (i.e. ^1H nuclei) are present—and where the electron and nuclear spins are coupled. However, the researchers Koptug et. al.⁴⁷ in 1996, more recently, challenged Robinson's claim by experimentally showing that for TEMPONE dissolved in benzene and CS_2 , there was no experimentally observable change in the T_{1S} --indicating that there is no apparent involvement of nuclear spins in the solvent with nitroxide spins in solution. However, the researchers admit that further experimentation on the matter of this theory should be examined: considering that the full range of temperatures--in which Robinson et. al.⁴⁰ used in their study to collect T_{1S} data--was not used by Koptug. Additionally, the researcher--and other researchers cited in his study--admit that the "question about the mechanism of spin-lattice relaxation in nitroxides...remains to be a controversial issue in the literature."^{37,47} It remains at the present time that the confirmation of the proposed theory or the refutation of it, has not been substantiated in the literature--nor further experimental work presented thereof.

In the present research, therefore, it is of interest to use saturation plot, slope data from DNP measurements already presented in Chapter 4 of this thesis, for purposes of gathering qualitative information about the $T_{1S}T_{2S}$ product. A full treatment of the DNP data involving the analysis of DNP line widths and obtaining qualitative data for T_{1S} , in particular --according to the methods of Muller-Warmuth, McCain, Glad and others--will not be made. The information gathered from the proposed analysis of electron relaxation times in the normal liquid and SF DNP systems can be used to gauge the influence of, e.g. deuterated solvents and/or SF CO_2 on electron relaxation times. And, this information can be used to consider current theories of relaxation for electron spin-lattice and spin-spin relaxation and compare expected results of these theories to trends observed in our data. It is important to emphasize that this analysis will be carried out, in light of the fact that even after all the many years of research performed on nitroxide radicals, one researcher concludes at the end of his comprehensive study of T_{1S} times (research conducted in 1996) that, ultimately, "the main relaxation mechanism...[for spin labels]...remains unknown"⁴⁷. Since T_{2S} has been measured for the nitroxide radical via EPR, in some instances, attempts were made to solve for a quantitative value of T_{1S} . However, any reported electron spin-lattice relaxation time values reported in this chapter, would not necessarily be comparable to reported literature values.

6.2 Experimental

In order for the $T_{1S}T_{2S}$ product to be evaluated, it is necessary to determine the value of the constant C, shown in the relationship of Equation [17], under the same conditions (i.e. the physical dimensions of the DNP instrumental setup: using the same flow cells and magnet field strengths, in addition to sample flow rate, viscosity, and temperature, etc.) in which the DNP data referred to in the introduction of this chapter, was collected. The value of C can be found using DNP, if T_{1S} and T_{2S} are accurately measured using a known, standard sample. Once the value of C is found for the standard, this same value can be used to solve for the $T_{1S}T_{2S}$ product--in a variety of sample systems tested in the same DNP instrument setup--using Equation [17]. In the

work of Percival and Hyde³⁶, EPR saturation-recovery was used to measure the electron spin-lattice relaxation times of simple nitroxides, in which temperature was varied (from +31 to -81°C) and viscosity was varied, over two orders of magnitude. The value of T_{1S} for the free radical TEMPOL was reported in the study, at a similar concentration and temperature as was TEMPO used in normal liquid benzene, LLIT DNP experiments in our labs (with concentrations for the free radical on the order of 0.001 and 0.01 M and temperatures at or around 25°C). With a known value of T_{1S} , for a nitroxide spin-label similar in structure to that of TEMPO, and obtained in conditions of temperature and solution viscosity similar to those in which the liquid benzene/TEMPO systems were tested in the LLIT DNP experiment, it was possible to conduct LLIT DNP on the sec-butyl benzene/TEMPOL system (used in the research cited above) in order to ascertain a value of C , “sensitive” to the instrumental DNP setup that is used in our labs. Again, the sec-butyl benzene/TEMPOL system gave a reliable value of T_{1S} , such that it was possible to assume that the value of the constant C (the value of which is governed largely by the magnetogyric ratios of the electron to the hydrogen nucleus, which is a constant) would be representative of the physical parameters of the DNP setup in our labs--and that this value of the constant C would be a reproducible value, for use in solving for the $T_{1S}T_{2S}$ product in other liquid systems. It has been shown previously by the researchers Kramer et. al.²⁹, that in an investigation of the molecular motion and spin-lattice relaxation of benzene, toluene, and some ethers in free-radical solutions using DNP, “if no considerable differences in the size and in the shape of the radicals occur,” different types of radicals do not change the (measured T_1 and τ_c) results. That is, nitroxides of similar structure (and at similar concentrations) do not influence the physical properties of the solvent (in which information such as nuclear spin-lattice and molecular correlation times are being obtained) and indeed can be used as interchangeable spin probes, for the abovementioned types of measurements. This fact would, additionally, allow for the use of the experimentally determined constant, C , in evaluating the $T_{1S}T_{2S}$ product in the normal liquid systems tested in this research, as well as in the case of SF CO₂ LLIT DNP. Therefore, LLIT ¹H DNP of sec-butyl benzene was conducted at approximately 25°C and a TEMPOL concentration of 7.8×10^{-4} M. (The TEMPOL molecule differs from TEMPO only by the substitution of a hydroxyl group in the C-4 position of the six-member ring.) The same instrumental setup shown in Figure 4.2 (used in the collection of all previous DNP data), including the same flow cells--positioned in the low field and high field regions of the DNP setup--were used for the experiment. The enhancement data was collected over a range of microwave powers and a saturation plot resulted in the experimentally determined slope for the equation of the line, given in Equation [9]. Additionally, the EPR spectrum of the sample was obtained under the same conditions of viscosity and temperature and a calculated value of the T_{2S} from line width data was found. The resulting known values of T_{2S} and T_{1S} and the experimentally determined value of the slope, m , were substituted into Equation [17] and the value of C was obtained.

6.3 Discussion of Results and Conclusions

The EPR spectrum of 7.8×10^{-4} M TEMPOL in sec-butyl benzene is shown in Figure 6.2. From a line width analysis based on Kivelson theory⁴⁸, the T_{2S} for TEMPOL

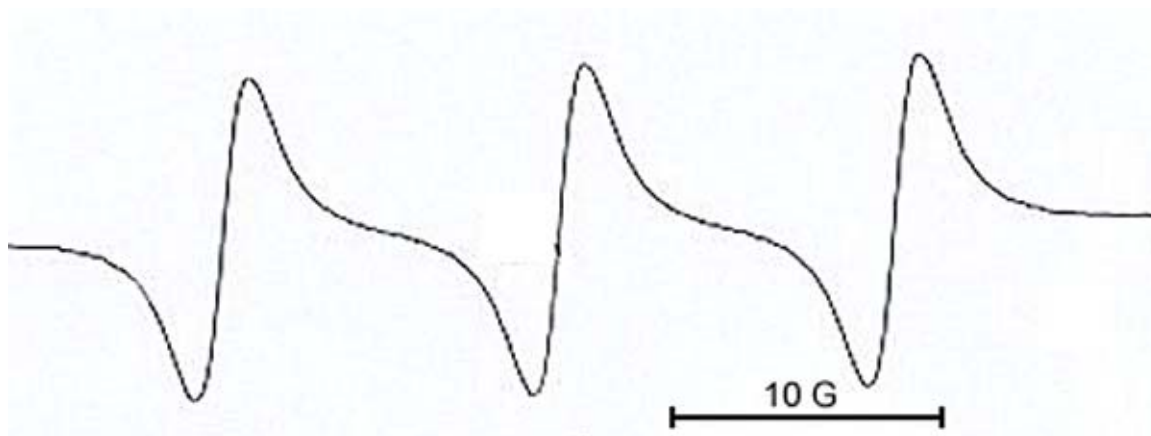


Figure 6.2. EPR spectrum of 7.8×10^{-4} M TEMPOL in neat sec-butyl benzene.

was found to be 2.9×10^{-8} s. Figure 6.1 shows the DNP saturation plot, the slope of which is used for the determination of the value of C. Using the value of the slope, $-2.7 \times 10^{-3} \text{ G}^2$, the value of $T_{1S} = 1.7 \times 10^{-7}$ s, as reported by Percival and Hyde³⁶ and the value of $\gamma_S^2 = (-1.76 \times 10^7 \text{ rad./s} \cdot \text{G})^2$, was substituted into Equation [17], yielding $C = -4.2 \times 10^{-3}$. This resulting value was, subsequently, substituted back into Equation [17] and used to solve for the $T_{1S}T_{2S}$ product of other LLIT ^1H DNP systems.

Figures 6.3 and 6.4 show the saturation plots for LLIT DNP of 9 % benzene/0.001 M and 0.01 M TEMPO respectively, dissolved in neat benzene, deuterated benzene, and SF CO_2 . As can be seen from the figures, the slopes of the lines become more negative (and decrease in value), as LLIT DNP is conducted in normal liquids, deuterated benzene, and SF CO_2 , respectively. The values of the slopes in a given solvent are reported in each figure and also in tabulated form, in Table 6.1. The trend in values shows that the slopes differ by two orders of magnitude in going from normal liquid DNP to SF CO_2 DNP. Considering the inverse relationship of the slope, m , to the $T_{1S}T_{2S}$ product, one would expect the magnitude of the product to, necessarily, change by the same factor, only, in the opposite direction. The negative value of the slopes, however, reverses this apparent trend and what is observed for the slope data is also observed in the analysis of the product of the relaxation times data.

The calculated $T_{1S}T_{2S}$ values for each solution are given in Table 6.2. Although these values cannot be quantitatively compared to any existing literature values, the qualitative trends in the values are made more apparent when comparing the different DNP solvent systems. In general, the data suggests that the product of the electron relaxation times is becoming shorter, by approximately two orders of magnitude, in going from the normal liquids to SF CO_2 , at both concentrations of TEMPO. Based on the direct relationship that T_{1S} and T_{2S} for the nitroxide are shortened as the viscosity (and density) of the bulk solvent decreases, then the calculated values support this trend. In the case where the $T_{1S}T_{2S}$ product decreases from deuterated benzene to neat benzene by an approximate order of magnitude at the two TEMPO concentrations specified, if it is assumed that the value of the electron spin-spin relaxation time does not change (based on constant viscosity and constant TEMPO concentrations between the two solvents), then it is the electron spin-lattice relaxation time that must be changing. It is possible that the increased presence of protons in the neat benzene, and thus the proton magnetization (coupled to electron spins), is acting to relax the magnetization of the electrons, via the mechanism proposed by Robinson et. al.⁴⁰ and Mailer et. al.³⁷ based on spin diffusion theory. According to the data in Table 6.2, the T_{1S} does indeed decrease by an order of magnitude, as the researchers propose.

Saturation Plot 0.001 M TEMPO

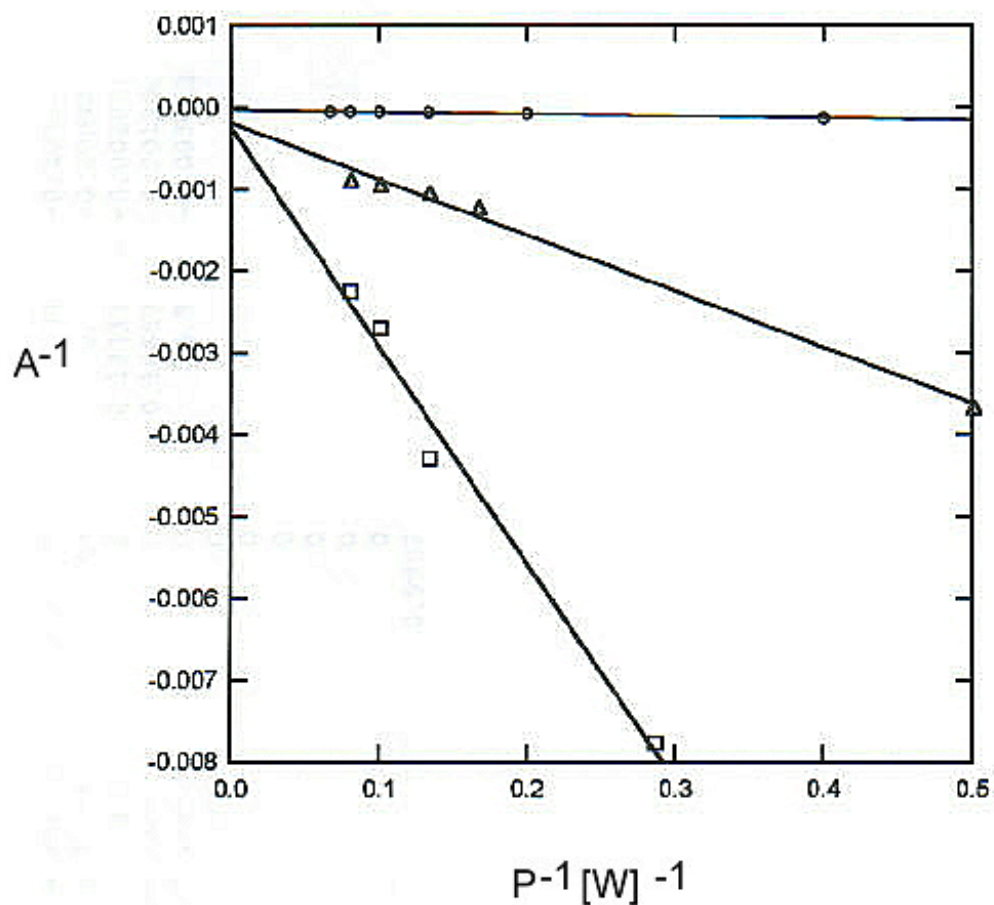


Figure 6.3. LLIT 1H DNP saturation plot showing the inverse enhancement versus the inverse microwave power for 9% benzene in deuterated benzene ($\circ$), neat benzene (Δ), and 9% benzene in SF_6 ($\square$) in 0.001 M TEMPO. Microwave power was varied from 2 to 15 W. The value of the slope for each sample is given as follows: ($\circ$)- $2.5 \times 10^{-4} G^2$, (Δ)- $5.9 \times 10^{-3} G^2$, and ($\square$)- $2.7 \times 10^{-2} G^2$.

Saturation Plot 0.01 M TEMPO

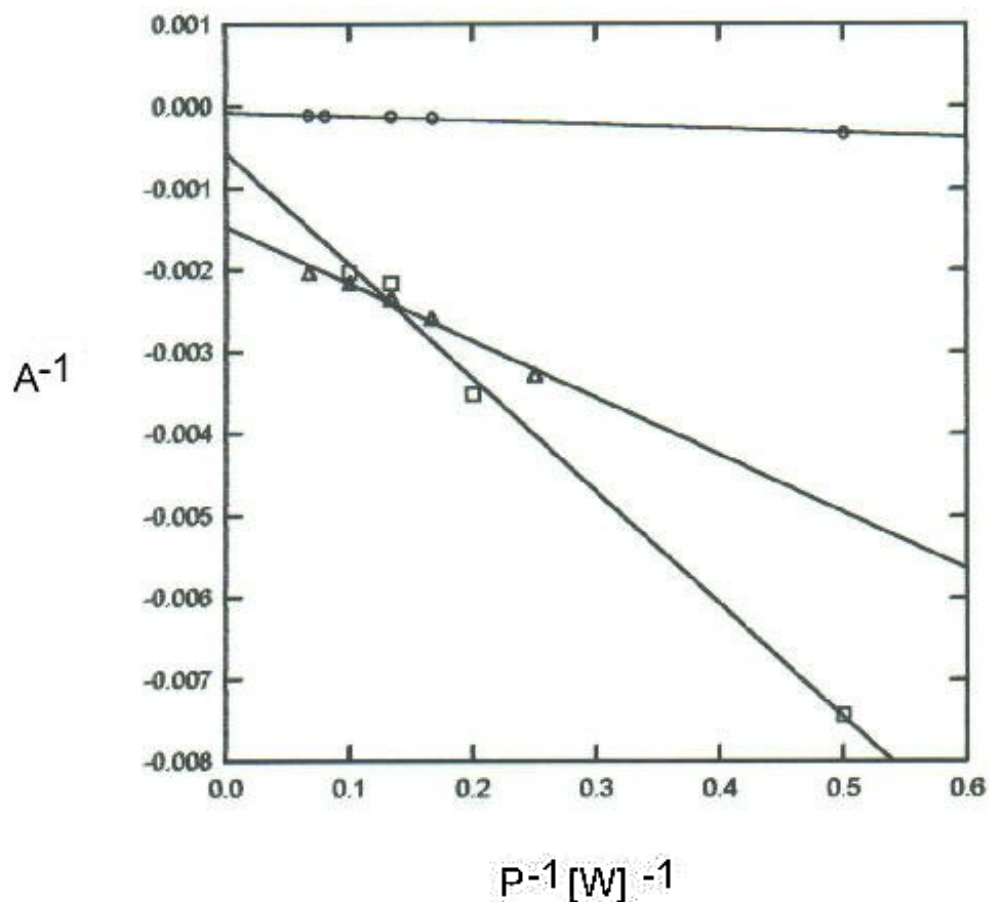


Figure 6.4. LLIT ^1H DNP saturation plot showing the inverse enhancement versus the inverse microwave power for 9% benzene in deuterated benzene ($\circ$), neat benzene (Δ), and 9% benzene in SF_6 ($\square$) in 0.01 M TEMPO. Microwave power was varied from 2 to 15 W. The value of the slope for each sample is given as follows: ($\circ$)- $5.0 \times 10^{-4} \text{ G}^2$, (Δ)- $7.0 \times 10^{-3} \text{ G}^2$, and ($\square$)- $1.4 \times 10^{-2} \text{ G}^2$.

Table 6.1. Slope values of saturation plots for DNP of deuterated benzene, benzene, and SF CO₂ using 0.001 M and 0.01 M TEMPO.

LLIT DNP Sample	Slope, [Gauss] ²		
	D6 benzene	benzene	SF CO ₂
0.01 M TEMPO	-5.0 x 10 ⁻⁴	-7.0 x 10 ⁻³	-1.4 x 10 ⁻²
0.001 M TEMPO	-2.5 x 10 ⁻⁴	-5.9 x 10 ⁻³	-2.7 x 10 ⁻²

--R² values for best-fit lines ≥ 0.95, for all data plots.

Table 6.2. Calculated $T_{1S}T_{2S}$ products for 0.001 M and 0.01 M TEMPO in deuterated benzene, benzene, and SF CO₂.

LLIT DNP Sample	$T_{1S}T_{2S}$, [s ²]		
	D6 benzene	benzene	SF CO ₂
0.01 M TEMPO	2.7×10^{-14}	1.9×10^{-15}	9.7×10^{-16}
0.001 M TEMPO	5.4×10^{-14}	2.3×10^{-15}	5.0×10^{-16}

However, as discussed earlier, their theory has not been substantiated, sufficiently. Nor can the current data presented be a confirmation of the theory, since it is more qualitative than quantitative, in nature. Otherwise, T_{1s} values in this case should not change, since all other mechanisms of electron relaxation should be the same for the two solvents.⁴⁰

If the change in T_{2s} alone is considered, this value would be expected to decrease, based on EPR line width measurements made in normal liquid benzene and SF CO₂. Magnetic resonance theory states that the EPR line width measured at half-height is inversely related to the spin-spin relaxation time. Researchers have shown, in general, the increased line widths obtained in SF's in e.g. EPR experiments where solvents are utilized under normal conditions of temperature and pressure and at their critical point parameters.²⁸ The researcher Dukes et. al. show a broadened EPR spectrum, specifically, for TEMPO dissolved in SF CO₂ versus a sharper-lined spectrum for the same concentration of the nitroxide dissolved in normal liquid benzene, when demonstrating the use of a high-pressure EPR probe.³⁵ Fig. 4.4 is a field plot showing evidence for a broadened EPR signal for TEMPO at a smaller concentration. Overall, decreasing spin-spin electron relaxation times and an increased electron-electron spin exchange rate between nitroxides, would be indicative of the increased mobility of the nitroxide in SF's.

In reference to Table 6.2, the $T_{1s}T_{2s}$ product compared at the two different TEMPO concentrations for the normal liquids shows an increase in overall value on going from the more concentrated to the less concentrated TEMPO sample systems. An opposite trend is observed for SF CO₂, where the product decreases. A comparison of the normal liquid data (the neat benzene and deuterated benzene) at the two concentrations of TEMPO in Table 6.1 show that the (negative) slope values increase and become more positive as the concentration of TEMPO decreases. A larger value of the slope supports, on the one hand, an increase in T_{2s} , or longer times, at lower nitroxide concentrations. Since, the Heisenberg spin-exchange rate increases with increasing concentration of radical, this result in the data would be supportive of a decreased electron-electron exchange rate between nitroxide radicals, at lower concentrations. Decreased spin exchange, also, would result in a decreased EPR line width, based on theory. Figure 4.2 shows, in fact, the characteristic narrowed EPR line of TEMPO in neat benzene, at decreasing TEMPO concentrations—and when T_{2s} times are longer. (Comparable EPR spectra could be shown for decreasing concentrations of TEMPO in deuterated benzene solvent, as well as for TEMPO dissolved in SFs.²⁸)

If T_{1s} alone is considered, it could be said that an increase in this value also contributes to the overall increase in the $T_{1s}T_{2s}$ product in the normal liquid systems (in going from a higher to lower concentration of the free radical). However, the literature shows that the experimental findings of researchers both support and refute the abovementioned trend for (nitroxide) free radicals. For example, fundamental research using EPR techniques to measure electron T_1 's has shown that at radical concentrations of 10^{-3} M and greater, electron T_1 's decrease with increasing concentration, reflecting faster intermolecular dipole-dipole relaxation.^{29,36,47,49} In some

of the same research, spin-lattice relaxation times have, in fact, been shown to be concentration independent at low nitroxide concentrations of $<10^{-3}$ M.⁴⁷ Other fundamental research, dealing specifically with the use of the DNP technique in the estimation of molecular correlation times and electron spin-lattice relaxation times for benzene and other organic molecules dissolved in free radical solutions, experimentally contradict the above research.²⁹ In the unique case where the Overhauser phenomenon is in effect in solutions containing free radicals (as is the case with the DNP data being currently presented), measured T_{1s} 's are shown to be directly proportional to the free radical concentration, at concentrations of $\geq 10^{-3}$ M, where dipolar electronic relaxation predominates.²⁹ In addition, the researchers Schwartz et. al.³⁸ reported "an absence of any noticeable dependence of T_{1s} on TEMPONE nitroxide concentration in the range 10^{-4} to 3.5×10^{-3} M". Undoubtedly, variations in the experimental findings of all researchers can be attributed, in part, to the different experimental techniques used by each and by the different theory on which calculations for the data were based. It is the case that in comparing the DNP (normal) liquid systems at the two different concentrations of TEMPO, there should not be a significant change in the viscosity of the solutions. (In fact, the work of Percival and Hyde³⁶ cited above found that a change of 2 orders of magnitude in the viscosity of a solution was needed in order to effect a change of 1 order of magnitude in the T_{1s} times.) Literature findings, thereby, support that there are only minor differences in T_{1s} times measured in different solvents of comparable viscosity.⁴⁷ So, it is plausible that T_{1s} should not really change at all—especially in the range of concentrations used in the present study.

Unreported, calculated T_{2s} 's obtained for the DNP liquid systems tested in our labs (calculated in order to obtain a value for the $T_{1s}T_{2s}$ product) reveal that in going from the lower to higher concentration of TEMPO, the value of T_{2s} changed by a factor of approximately 2 in deuterated benzene and by a lesser factor in the neat benzene solvent. (Actual values were 20 and 44 ns for 0.01 M and 0.001 M TEMPO in d6-benzene, respectively, and 15 and 26 ns for 0.01 M and 0.001 M TEMPO in neat benzene, respectively). As can be seen from the change in the $T_{1s}T_{2s}$ product in Table 6.2, this value also changes by the same approximate factors, based on the above calculated values. Therefore, it can be said that it is really the change in T_{2s} alone that is governing the change in the product of the relaxation times. The trend, then, in increasing relaxation times of the normal liquid systems, cannot necessarily be supported nor refuted by the literature. And, it is possible that minor variations in T_{1s} cannot be detected, nor are not significant enough, to produce a noticeable change in the present analysis of relaxation time products, for the free radical.

In the case of SF CO_2 , as already indicated, the opposite trend is observed where the slope values become less positive and decrease in a negative direction, as the concentration of the nitroxide is decreased. A comparison, then, of the $T_{1s}T_{2s}$ product in SF CO_2 at decreasing nitroxide concentration, shows an overall shortening or decrease in value of electron spin-spin and spin-lattice relaxation times—which would imply increased mobility of the nitroxide. Based on the above reasoning used for the interpretation of the normal liquid slope trends, the decrease in slope value by a factor of approximately 2 would indicate, specifically, shorter electron spin-spin relaxation

times (and, subsequently, an increased EPR line width). It, similarly, suggests greater electron-electron exchange rates at decreased radical concentrations in SF CO₂. Apparently, one would expect the Heisenberg spin-exchange rate to increase with increasing concentration of free radical, as is the case in normal (viscosity) liquids. However, in the case of SF's, the research of Randolph and Carlier²⁸ (presented briefly in Ch. 4) shows that the experimentally determined exchange rate is independent of radical concentration, as was determined for the free radical di-tert-butyl nitroxide (DTBN) dissolved in SF ethane, over the range 0.5 to 2 mM. For a constant DTBN concentration in the SF, the exchange rate was affected, however, by changes in pressure and temperature, especially in the vicinity of T_c and P_c. In the present research, no experimental evidence was gathered e.g. EPR line width data, to corroborate a change (or lack thereof) in the EPR line width (and hence a change in the spin-exchange rate, which is inversely proportional to T_{2s}) at the two different concentrations of the nitroxide, under SF conditions. However, if the rate of spin exchange does not change significantly for the SF solvent systems based on the research cited, then the change in the T_{1s}T_{2s} product as seen in Table 6.2 can be attributed to a change, mainly, in T_{1s}.

Overall, the change in the electron spin-spin and spin-lattice relaxation time (which is shown to be dependent on the viscosity and temperature of the solution) can be attributed to the "sensitivity" of SF's in which slight changes in temperature and pressure, especially at the critical point, can dramatically alter their physical properties i.e. viscosity, density, etc.. Of course, as has been the subject of this thesis, the molecular motion in molecules dissolved in SF's is also greatly affected, based on these effects. For instance, the literature shows that at the critical point, local density augmentations and an increase in the intrinsic viscosity of solvents, versus the bulk solvent, occur due to solvent-solute and solute-solute clustering.^{27,28,50} The result of this clustering has been shown to increase molecular correlation times at the critical point for naphthalene dissolved in SF CO₂, as the many solvent molecules—treated as one large molecular entity—act to slow the motion of the naphthalene solute at the critical point conditions.^{12,50} And, solvent clustering about dilute solute molecules is proposed as being responsible for an increase in the electron-electron spin exchange rate of DTBN dissolved in SF ethane, by a factor of 3, compared to spin exchange in normal liquids.²⁸ Much evidence for SF clustering exists in the literature, however, it is still purportedly an issue of debate.⁵⁰⁻⁵³ Nevertheless, it is possible, then, that variations in temperature, namely sub-critical T's, could have influenced EPR line width's and spin-lattice relaxation times, even. It was shown in the work of Batchelor²⁷ (presented briefly in Ch. 4) that at constant pressure (slightly above the P_c) and at a TEMPO concentration of 10⁻² M, as the temperature was varied in SF ethanol, ethane, and n-hexane, a small decrease in the EPR line width was observed at sub-critical temperatures, before a huge increase in the line width (by a factor of @ 4) was observed at the critical point temperature and corresponding temperatures above T_c. Essentially, the EPR line width, ΔV_{1/2}, was found to be directly proportional to T when operating at a constant pressure and slightly above P_c (that is, working within 1 to 2 pressure units above P_c for a particular substance). Under conditions of constant pressure well above the P_c, when T is increased, ΔV_{1/2} was found to be approximately

constant, with only a small increase (by a factor of approximately 0.5) in the EPR line. In another experiment, where the pressure was varied at constant temperature (above the T_c), a similar trend in line width results was observed for a constant concentration of TEMPO dissolved in the SF's. At subcritical pressures, there was a slight narrowing in the EPR line width, with a large increase as the pressure was decreased to P_c and values below. In this case, it was demonstrated that $\Delta v_{1/2} \propto 1/P$ when operating at a constant temperature, slightly above T_c ; at P 's well above P_c , $\Delta v_{1/2}$ was shown to remain relatively constant with only a slight decrease. The pronounced increase in $\Delta v_{1/2}$ at T_c and P_c is undoubtedly explained by the occurrence of solvent-solute critical clustering (which is proposed to enhance spin exchange, as well). This is especially supported by researchers already mentioned above, since, once the experiment is no longer being conducted under critical (point) conditions for a particular solvent, the phenomenon of marked differences in the data results (as compared to normal liquids) usually disappears—that is, the clusters disappear at P 's and T 's, outside of the critical point region. With these experimental results, it is possible that in the present research, subcritical temperatures (T 's below 33°C for SF CO₂) at the EPR cell (refer to Fig. 4.1), could have resulted in longer than expected spin-spin relaxation times for the case of 0.01 M TEMPO/SF CO₂ system (since it was more difficult to maintain a constant temperature of the DNP solvent system throughout the PEEK cells and tubing, than it was to maintain a constant pressure). Thereby, the value of the $T_{1s}T_{2s}$ product was inflated, compared to the value reported for 0.001 M TEMPO. However, since it was the case that in the present research, SF CO₂ DNP was conducted at P 's well above the P_c for CO₂ (for purposes, initially, of ensuring the solubility of the benzene and TEMPO solutes) and at elevated T 's above the T_c , the results of the above research again suggest that it is more probable that T_{2s} should not have changed significantly, then, between the two solvent systems, based on TEMPO concentration alone.

Since T_{2s} and T_{1s} for nitroxides are directly proportional to the molecular correlation time, then a significant change in τ_r would affect these two values. The results of the above research imply that if the molecular motion of the nitroxide was, for example, decreased due to critical clustering effects (especially in the vicinity of P_c and T_c), this would have a great effect on both T_{1s} and T_{2s} values. Therefore, it can only be suggested at the present time, that between the two SF CO₂ solvent systems, there might have been a difference in P and T operating conditions when the experiments were being conducted, that would account for the factor of 2 difference in the value of the $T_{1s}T_{2s}$ product. If τ_r decreases due to clustering effects, then the $T_{1s}T_{2s}$ product should become smaller in value, also. This value is smaller in the case of the SF system that has the smaller concentration of TEMPO: and so perhaps it was this system in which the operating parameters of T and P were not optimal for the DNP experiment, and, therefore, critical clusters were present in some degree. Also, it can be observed that for the 0.001 M TEMPO/SF CO₂ system, since the value of the $T_{1s}T_{2s}$ product is smaller, this suggests that the motion of TEMPO is faster in this system, and, therefore, ultimate DNP enhancements should be larger. And, in fact, A_∞ values are larger based on the results presented in Ch. 4. Unfortunately, there is no experimental data in the literature that, specifically, provides T_{1s} data for TEMPO dissolved in SF's at more than

one concentration (nor under the same conditions of temperature and pressure of the present research), that allows for the direct comparison of the two solvent systems: in order to verify the above claims and to offer another explanation for the observed trend in the data, based on a changing T_{1s} alone. It is difficult, therefore, to verify if the data presented in Table 6.2 for the SF's, shows the proper trends. To be sure, if there were any slight variations in conditions of T and P, especially in the vicinity of the critical point between the two SF CO₂ systems, then this fact alone could greatly alter the results of one system, versus the other, and yield uncharacteristic or inaccurate results.

Overall Summary and Conclusions

LLIT and SLIT dynamic nuclear polarization experiments of simple probe molecules (i.e. benzene and chloroform), where the sample nuclei to be detected were dissolved in SF CO₂, were shown to be advantageous over the use of normal liquids. The increased molecular motion (of the sample molecules) in the supercritical fluid versus neat liquids resulted in observed signal enhancements 2-6 times greater than the normal NMR signals. This result is significant in that it is the largest observed enhancement obtained for benzene and chloroform, using the flow DNP technique, to date. (And, as of this time, similar results have not been reported in the literature.) Ultimately, these results demonstrate the high sensitivity of the flow DNP technique for detecting sample nuclei when SF CO₂ is used as the solvent--resulting in the detection of samples in the range of 1 µg to 100 ng (or molecules in the 100-1000 Dalton range).

On commencing this research, initial data, showing a decreased ¹⁴N NMR line width in low viscosity liquids predicted the 2-6 factor increase in the motion of molecules dissolved in SFs--and, thus, the enhanced NMR signals that would result via the DNP technique, when using supercritical fluids as the flowing solvent. Subsequent experiments where the spin-spin and spin-lattice electronic relaxation times of the nitroxide radical (utilized in the DNP experiment) were analyzed based on DNP results, also, demonstrated how shorter T_{1s} T_{2s} product times were indicative of increased motion of free radical molecules in SFs versus normal liquids. Faster motion of the nitroxide, once again, would suggest the increased mobility of the paramagnetic species--to interact and transfer polarization to the diamagnetic chemical species to be detected at the NMR--thus enhancing NMR signals.

In general, signal enhancements obtained through SF CO₂ LLIT and SLIT DNP offer improved sensitivity over conventional NMR and/or normal liquid DNP detection techniques. The flow technique, also, affords the possibility of being directly coupled to the analytical techniques of SF chromatography or extraction. (The advantage of using SF CO₂ as a mobile phase versus normal liquids, to obtain increased resolution of chromatographic solutes, has already been noted in the introduction to this thesis.) The coupling of these techniques would be useful in the monitoring of environmental samples containing small organic molecules, similar to the organic molecules analyzed in this research.

Future efforts in supercritical flow DNP-NMR will investigate the use of SF CO₂ in extending the extreme narrowing condition from 3.3 kG, to higher fields. However, in considering the findings of the present research, by decreasing τ_r with the use of SFs, the operating field strength at which electron spins are currently polarized was effectively increased from 0.33 Tesla to 1.4 Tesla (or 3300 Gauss to 14000 Gauss). At this field strength, the predicted NMR sensitivity is equivalent to a 1000-1500 MHz ¹H NMR instrument. Furthermore, this sensitivity factor (gained via the DNP technique) is 1.3-2 times greater than what is feasible today with a conventional 750 MHz ¹H NMR instrument (17.6 Tesla).

References

1. Overhauser, A.W. *Phys. Rev.*, **1953**, 92, 411.
2. Dwek, R.A.; Richards, R.E.; and Taylor, D. *Annu. Rev.*, **1969**, 2, 293.
3. Bates, R.D. *J. Magn. Reson.*, **1982**, 48, 111.
4. Hausser, K.H. and Stehlik, *Adv. Mag. Reson.*, **1968**, 3, 79.
5. Muller-Warmuth, W. and Meise-Gresch, K., *Adv. Mag. Reson.*, **1983**, 11, 1.
6. Dorn, H.C.; Wang, J.; Allen, L.; Sweeney, D. and Glass, T. *J. Magn. Reson.*, **1988**, 79, 404.
7. Gitti, R.; Wild, C.; Tsiao, C.; Zimmer, K.; Glass, T.E. and Dorn, H.C. *J. Am. Chem. Soc.*, **1988**, 110, 2294.
8. Tsai, K.H. and Dorn, H.C. *Appl. Magn. Reson.*, **1990**, 1, 231.
9. Allen, L.A.; Glass, T.E.; and Dorn, H.C. *Anal. Chem.*, **1988**, 60, 390.
10. Baker, J.M.; Brown, D.R.; and Jonas, J. *J. Am. Chem. Soc.* **1984**, 88, 5425.
11. Gouw, T.H.; Jentoft, R.E., *J. Chrom.* **1972**, 68, 303-323.
12. Divina T. Miranda, Ph.D. Thesis, **1992**, University of New Orleans.
13. Lamb, W.J.; Hoffman, G.A.; and Jonas, J. *J. Chem. Phys.* **1981**, 74, 6875.
14. Dorn, H.C.; Glass, T.E.; Gitti, R. and Tsai, K.H. *Appl. Magn. Reson.*, **1991**, 2, 9.
15. Drago, R. S. "Physical Methods for Chemists", 2nd ed. Surfside Scientific Publishers: Florida **1992**, 326.
16. Robert, Jan and Evilia, Robert. *J. Am. Chem. Soc.*, **1985**, 107, 3733.
17. Lee Allen, Ph.D. Thesis, **1988**, Virginia Polytechnic & State University.
18. Parkhurst, H.J.; Lu, Y.; and Jonas, J. *J. Chem. Phys.*, **1971**, 55, 1368.
19. Dorn, H.C.; Gu J.; Bethune, D.S.; Johnson, R.D.; and Yannoni, C.S. *Chem. Phys. Lett.*, **1993**, 203, 549.
20. Poindexter, E.H.; Stewart, J.R.; and Caplan, P.J. *J. Chem. Phys.*, **1967**, 47, 2862.
21. Richards, R.E.; Dwek, R.A.; and Taylor, D. *Annu. Rev. NMR Spectrosc.*, **1969**, 2, 293.
22. Potenza, J.A. *Adv. Mol. Relaxation Processes*, **1972**, 4, 229.
23. Stevenson, S.; Glass, T.; and Dorn, H. C. *Anal. Chem.*, **1998**, 13, 2623-8.23.
24. Stone et. al. *Proc. N. A. S.: Chemistry*, **1965**, 54, 1010.
25. Kun Hsiang Tsai Ph.D. Thesis, **1990**, Virginia Polytechnic & State University.
26. Unpublished data collected by Ziqi Sun, **1992**, Virginia Polytechnic & State University.
27. Batchelor, S.N. *J. Phys. Chem. B*, **1998**, 102, 615-9.
28. Randolph, T. and Carlier, C. *J. Phys. Chem.* **1992**, 96, 5146-5151.
29. Kramer, K. D.; Muller-Warmuth, W.; and Schindler, J. *J. Chem. Phys.* **1965**, 43, 31-43.
30. "High Resolution NMR Theory and Chemical Applications", Becker, E., Ed., Academic Press: New York, 1980.
31. Bruck, D.; Dudly, C.S.; Fyfe; and Delden, J.V. *J. Magn. Reson.*, **1981**, 51, 51.
32. Albert, K. *Analytiker-Taschenbuch*, 20, **1998**, 107.
33. Gilpin, R.K.; Kasrur, A.; and Gelerinter, E. *Anal. Chem.*, **1987**, 59, 1177.
34. Rossi Gitti, Ph.D. Thesis, **1989**, Virginia Polytechnic Institute & State University.
35. Dukes, K.; Harbron, E.; Forbes, M.; and DeSimone, J. *Rev. Sci. Instrum.*, **1997**, 68, 2505-10.

36. Percival, P. W. and Hyde, J. S. *J. Magn. Reson.* **1976**, 23, 49-57.
37. Mailer, C.; Robinson, B. H.; and Haas, D. A. *Bull. Mag. Res.* **1992**, 14, 30-34.
38. Schwartz, R. N.; Jones, L.L.; and Bowman, M. J. *Am. Chem. Soc.* **1979**, 83, 3429.
39. Glad, W.E., Ph. D. Thesis, **1982**, University of California, Berkeley.
40. Robinson, B. H.; Haas, D. A.; Mailer, C. *Science*, **1994**, 263, 490-3.
41. Muller-Warmuth, W. Z. *Naturforschg.*, **1963**, 18a, 1001-7.
42. Haupt, J. and Muller-Warmuth, W., *Z. Naturforschg.*, **1962**, 17a, 1011-22.
43. Richards, E. and White, J. W., *Proc. Roy. Soc. (London)*, **1964**, A283, 459.
44. McCain, D. C. and Myers, J. *Phys. Chem.*, **1967**, 71, 192.
45. Wilson, R. and Kivelson, D. *J. Chem. Phys.*, **1966a**, 44, 154.
46. Blumberg, W. E. *Phys. Rev.*, **1960**, 119, 79.
47. Koptug, I. V.; Bossmann, S. H.; and Turro, N. J. *J. Am. Chem. Soc.* **1996**, 118, 1435-45.
48. Atkins, P.W. and Kivelson, D., *J. Chem. Phys.* **1966**, 44, 169-74.
49. "Spin Labeling II Theory and Applications," Ed. Lawrence Berliner, p191, Academic Press: New York, 1979.
50. Jan Marie Robert, Ph.D. Thesis, **1987**, University of New Orleans.
51. Economou, I. G. and Donahue, M. D. *AIChE J.* **1990**, 36(12), 1920.
52. Brennecke, J. F.; Debenedetti, P. G.; Eckert, C. A.; and Johnston, K. P. *AIChE J.* **1990**, 36(12), 1927.
53. Petsche, I. and Debenedetti, P. *J. Phys. Chem.* **1991**, 95, 386.

Appendix I: Development of High Pressure Sample Cells for the EPR Cavity and the NMR Probe

Introduction

Before the present research endeavor, of conducting DNP with SF CO₂, the sample cell used in our labs for flow DNP in the low field cavity was constructed out of ceramic (Omegatite 450 from Omega Engineering Inc.), while the high field flow cell in the NMR probe was constructed out of glass. The ceramic cell had a low dielectric constant, meaning that it could be easily tuned in the microwave field and a high mechanical strength, for which it could withstand high back pressures of 1000 psi and greater. The high field probe was sufficient enough to withstand normal liquid flow backpressures and did not produce a background signal that would interfere with NMR signal detection. For SF CO₂ flow DNP--requiring much higher pressures, up to 165 atm, to achieve optimum enhancement conditions--it was concluded, and evidenced, that the glass high-field probe would not be able to withstand breakage and prevent the leakage of the gas-like sample solvent. In addition, after initial experimentation, it was observed that the brass Swagelock fittings used to connect PEEK tubing to the ends of the rod-like cells, in the low and high field magnet cavities, continually leaked CO₂. Due to this leaking, the DNP setup was continuously disturbed and had to be dismantled at various places to tighten fittings: this, consequently, affected DNP experimental conditions such as tuning of the EPR cavity and tuning of the NMR probe, etc.. A change in the "plumbing" of the present setup, therefore, was needed to correct this problem.

As previously mentioned in the experimental section of Ch. 4, PEEK has a high pressure and temperature rating (the material is able to withstand pressures up to 3000 psi and temperatures of 150°C). The material is chemically inert, flexible as tubing, and yet, rigid enough so that it can be machined. A consideration was given to using quartz glass: a stronger material than amorphous glass that has been used widely in high-pressure NMR and liquid DNP studies.^{29,50} Quartz, can withstand the high pressures (up to 6000 psi²⁹) of supercritical fluid conditions and has a lower dielectric constant than glass, making it more resistant to heating, especially in the EPR cavity where tuning can be affected. Similar properties can be attributed to sapphire, and this material was considered as well. However, quartz, and namely sapphire, are much more expensive than PEEK and were not readily available for experimentation in our labs. PEEK rods were available in our labs and seemed the more sensible choice to use for testing in the DNP flow setup. Based on the abovementioned qualities of PEEK, it was determined that solid PEEK rods (from Global Plastics) could be machined, according to the same dimensions of the cells used in normal liquid flow DNP, for use with SF CO₂. Rods, according to the dimensions specified in Figure I.1, were made by Fred Blair (Physics Department, Va. Tech.) and commercially available PEEK end fittings and ferrules (Upchurch Scientific) were used in place of brass fittings. Figure I.1 is a structure diagram showing the position and dimensions of the cells, and of the PEEK fittings and tubing, used for the research presented in this thesis. Initial tests

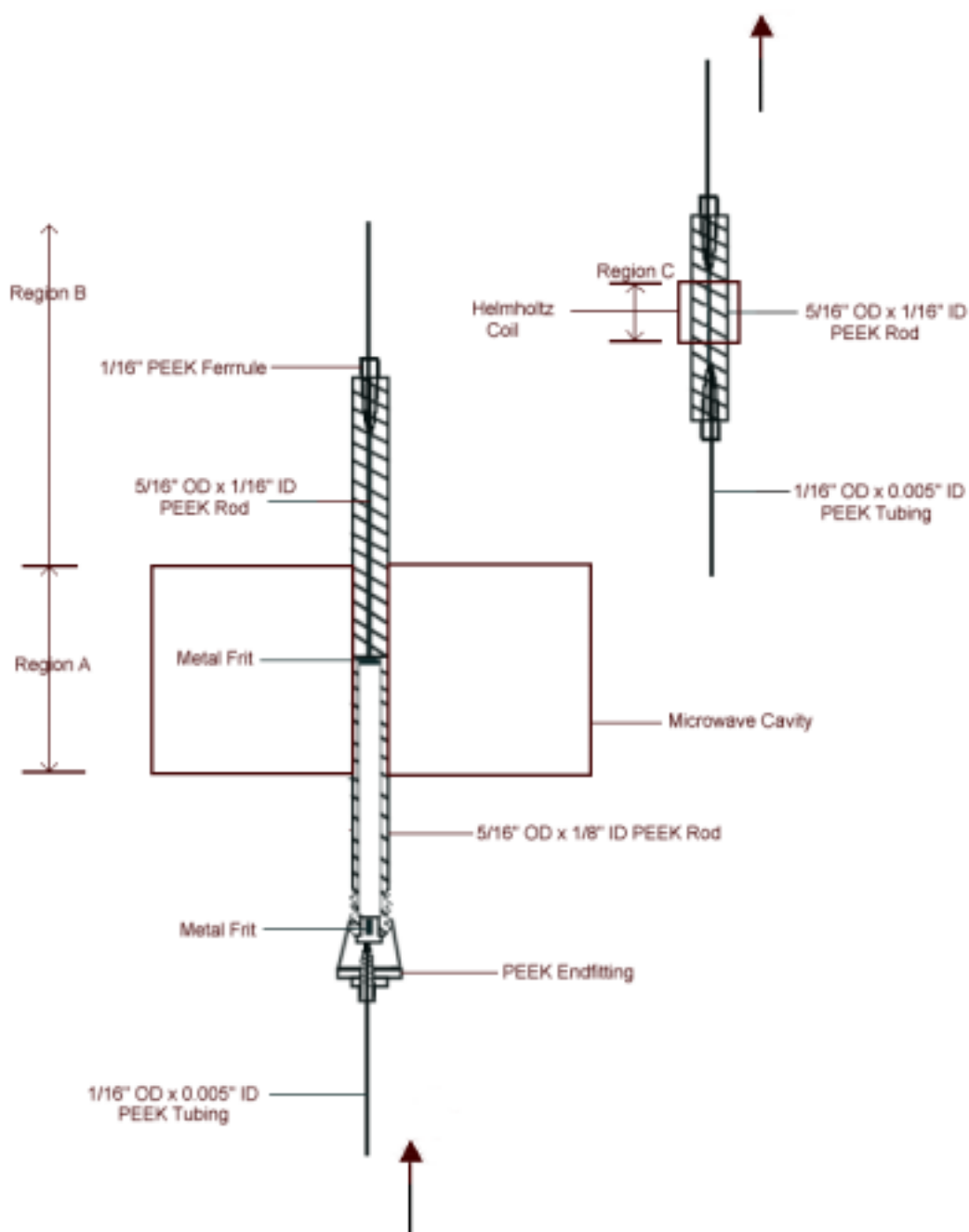


Figure I.1. Structure diagram of the low and high field PEEK flow cells and PEEK tubing used in the DNP experiment. The direction of flow and the regions in which polarization (region A) and transfer of the polarized sample (region B) occurs, and its detection by NMR (region C), are indicated. (These regions correspond to the same regions for the DNP setup depicted in Fig. 4.2.)

using flowing SF CO₂ revealed that the PEEK was able to contain the high-pressure fluid and leaking (evidenced by large drops in the backpressure) was minimal.

The PEEK rods also had to be tested for how well the material, containing flowing solvent, would tune in the EPR cavity and if the material would have a background signal at the NMR probe. It was found that the PEEK rod tuned as sharply and as effectively as the ceramic cell formerly did, only at a slightly different frequency. This is important in effectively saturating electron spins in the DNP experiment. And, after solid-state NMR tests were conducted by Tom Glass (Analytical Services, Chemistry Department, Va. Tech.) with a small portion of PEEK rod, there was no evidence of a ¹H background signal that would interfere in NMR detection of the sample being analyzed in the DNP experiment. Subsequently, DNP parameters such as saturation and observed signal enhancements, especially in the case of normal liquid flow DNP, were comparable with results obtained formerly in our labs. With these favorable test results, it was possible to commence DNP research using the new PEEK cells.

VITA

Sandra Irene Salido was born on December 28, 1973 in Lynchburg, VA. She is the youngest of five children (four older brothers); her mother was a nurse and her father was a mechanical engineer. She graduated with a BS degree in chemistry from Davidson College in 1996. In the fall of 1996, she began her PhD graduate studies at Virginia Polytechnic and State Institute under the direction of Dr. Harry Dorn. Her PhD research focused on the use of a non-conventional, flow nuclear magnetic resonance technique called dynamic nuclear polarization, for the detection of small, probe organic molecules. The use of supercritical fluid carbon dioxide as a flowing solvent was, also, implemented in this work. Upon nearing the completion of her studies at VA Tech, she taught one year (2000-2001) as a Visiting Assistant Professor at Roanoke College in Salem, VA. She returned to VA Tech and finished her PhD in the fall 2002.