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Electron Decoupling with Cross Polarization and Dynamic Nuclear Polarization Below 6 K

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Abstract

Dynamic nuclear polarization (DNP) can improve nuclear magnetic resonance (NMR) sensitivity by orders of magnitude. Polarizing agents containing unpaired electrons required for DNP can broaden nuclear resonances in the presence of appreciable hyperfine couplings. Here we present the first cross polarization experiments implemented with electron decoupling, which attenuates detrimental hyperfine couplings. We also demonstrate magic angle spinning (MAS) DNP experiments below 6 K, producing unprecedented nuclear spin polarization in rotating solids. ^{13}C correlation spectra were collected with MAS DNP below 6 K for the first time. Longitudinal magnetization recovery times with MAS DNP ($T_{1\text{DNP}, 1\text{H}}$) of urea in a frozen glassy matrix below 6 K were measured for both the solid effect and the cross effect. Trityl radicals exhibit a $T_{1\text{DNP}}(^1\text{H})$ of 18.7 s and the $T_{1\text{DNP}}(^1\text{H})$ of samples doped with 20 mM AMUPol is only 1.3 s. MAS below 6 K with DNP and electron decoupling is an effective strategy to increase NMR signal-to-noise ratios per transient while retaining short recovery periods.

Graphical Abstract

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Keywords

Dynamic nuclear polarization; Cryogenic magic angle spinning; Electron decoupling; 2-D NMR

1. Introduction

Solid-state nuclear magnetic resonance (NMR) spectroscopy is a powerful tool for studying a wide variety of molecular architectures.[1–3] When employed in conjunction with magic angle spinning (MAS), long spin coherence lifetimes deliver spectral resolution sufficient to interrogate structure at atomic resolution. Internal magnetic interactions, such as isotropic scalar couplings and anisotropic dipolar couplings, allow the determination of molecular connectivities and three-dimensional structures. MAS NMR also enables molecular structural characterization within endogenous and heterogeneous environments relevant to biomedical and materials science applications. However, MAS NMR typically suffers from low signal-to-noise ratios.

Poor signal-to-noise in NMR primarily arises from the small polarization of nuclear Zeeman states at thermal equilibrium. Dynamic nuclear polarization (DNP) is one technique for biasing the polarization of spins of interest well beyond what is achievable thermally, theoretically leading to sensitivities orders of magnitudes higher.[4,5] When performing DNP, a millimolar concentration of a stable exogenous radical is typically doped into the sample. Upon irradiation of the sample with microwaves matching DNP transition

frequencies, the large Boltzmann polarization of the radical electrons can be transferred to the nuclei, with a theoretical maximum gain in polarization proportional to the ratio of magnetic moments in the high temperature limit $\left(\frac{P_e}{P_{^1H}} \approx \left|\frac{\gamma_e}{\gamma_{^1H}}\right| = 658 \text{ for } h\nu_e \ll k_B T\right)$. [6–8]

Reducing the thermal energy of the sample is another method of increasing spin polarization. MAS DNP is commonly performed at 100 K, but experiments at 25 K, at which temperature spin polarizations are $(298 \text{ K})/(25 \text{ K}) = 12$ -times larger in magnitude than at room temperature, have recently been demonstrated. [9–12] Spin polarization is further increased by cooling the sample to below 6 K. The associated NMR sensitivity gains achievable with cryogenic DNP below 6 K result in much shorter signal averaging times.

The hyperfine couplings mediating DNP transfers can, however, dramatically shorten nuclear spin relaxation and impair spectral resolution. [9,11,13–18] We recently demonstrated that hyperfine interactions can be attenuated with a train of chirped microwave pulses applied during the NMR free induction decay (FID). [19] These initial electron decoupling experiments were performed by directly transferring the polarization of the electrons to ^{13}C nuclei. The $T_{1\text{DNP}}$ of the carbon nuclei leads to long magnetization recovery periods to maximize the signal-to-noise ratio per unit time. Signal averaging can be accelerated by first polarizing protons through DNP and then relying on proton spin diffusion to distribute the enhanced polarization throughout the sample.

DNP to protons followed by cross polarization to carbon nuclei therefore dramatically improves signal-to-noise per unit time. Additionally, proton-proton spin diffusion allows for hyperpolarization of carbons distant from the broadening hyperfine interactions of the electrons, leading to further improvements in spectral resolution. [20]

Here we demonstrate for the first time electron decoupling in rotating solids in conjunction with cross polarization. We record electron decoupling with cross polarization below 6 K and at 90 K. Further, we observe short polarization build-up times with cryogenic MAS DNP below 6 K.

2. Experimental Methods

A custom-built, 4-channel, 3.2 mm MAS, transmissionline NMR probe with a Redstone spectrometer (Tecmag, Houston, TX) was used to record all data. Larmor frequencies for ^1H and ^{13}C were 300.184 MHz and 75.495 MHz, respectively, at $B_0 = 7.05 \text{ T}$. All spectra were recorded as rotor-synchronized, echo-detected, cross-polarization MAS (CPMAS) experiments. The Hartmann-Hahn condition was $\nu_{^1\text{H}} = 50 \text{ kHz}$ and $\nu_{^{13}\text{C}} = 52 \text{ kHz}$ with a contact time of 1 ms. For $\pi/2$ pulses and two-pulse phase-modulated (TPPM) decoupling on ^1H , a nutation of $\nu_{^1\text{H}} = 90 \text{ kHz}$ was used. The ^{13}C refocusing pulse had a nutation of $\nu_{^{13}\text{C}} = 100 \text{ kHz}$. To destroy any residual polarization, saturation trains (SAT) on both ^1H and ^{13}C were employed before a DNP polarization time (τ_{pol}). Polarization build-up times ($T_{1\text{DNP}}$) were recorded using a saturation recovery sequence. Typical spinning frequencies were $5000 \pm 20 \text{ Hz}$ but varied in each experiment (see Supporting Information for details).

Experimental parameters corresponding to each experiment are shown in Table 1 of the Supporting Information.

All spectra were referenced to adamantane at 193 K, and that chemical shift reference was used to assign chemical shift values at 6 K. Enhancements were found by dividing the area of the spectra taken with microwaves on by the area of the spectra obtained with microwaves off. The areas were determined by fitting the peaks in DMfit.[21] Microwave irradiation was produced by a custom-built frequency-agile gyrotron with an output power of 15 W,[19,22] corresponding to approximately 5 W ($\nu_{1s} = 380$ kHz) at the sample due to loss within the waveguide. The microwave power was attenuated with a 3% transmittance attenuator (Tydex LLC, St. Petersburg, Russia) placed in the waveguide. For low power experiments, 0.70 W was measured out of the attenuator with an estimated 0.23 W ($\nu_{1s} = 81$ kHz) at the sample. Microwave powers were measured using a calorimeter. The microwave irradiation frequency was 197.674 GHz for AMUPol and 197.950 GHz for trityl. For experiments utilizing electron decoupling (eDEC), the irradiation during τ_{pol} was at the zero-quantum solid effect condition for 1H (197.950 GHz); during the acquisition period, chirped pulses were applied around the EPR resonance condition (197.640 GHz) as shown in Figure 1a. The chirped pulses were 13.75 μs in length and 87 MHz wide in frequency.[19] Microwave chirps were produced using the spectrometer's arbitrary waveform generator and were amplified by a linear amplifier (Trek Inc., Lockport, NY) attached to the gyrotron.[22]

Two-dimensional spectra were obtained on L-proline using the proton-driven spin diffusion (PDS) NMR sequence[23] and the STATES method.[24] All microwave and RF frequencies were the same as for CPMAS experiments. For the PDS taken below 6 K, a 30 μs dwell time was used in both the indirect and direct dimensions. 64 points were collected in the indirect dimension (t_1) and 256 points in the direct dimension. Four transients were recorded with a $\tau_{pol} = 1$ s. For the PDS at 90 K, 32 transients were acquired in each slice with a polarization time of 3 s. 256 points were collected in the direct dimension and 128 in the indirect dimension. For all PDS experiments, the dwell used in both dimensions was 30 μs , and both dimensions were zero filled to 1024 points. The mixing time was 5 ms, and the spinning frequency was 5500 ± 100 Hz. The PDS pulse sequence is shown in Figure 1b. The microwave frequency was shifted off of the DNP condition to the nominal output frequency of the gyrotron at 197.839 GHz following the z-filter.

Sample temperatures below 6 K were achieved with liquid helium as variable-temperature (VT) fluid directed at the center of the spinning rotor. Ultra-high-purity helium gas at 80 K was used for MAS bearing and drive gases. The sample temperature was monitored at the interface of the VT outlet and NMR stator with a Cernox temperature sensor (Lake Shore Cryotronics Inc., Westerville, OH). The temperature at this point was regarded as the sample temperature as demonstrated previously.[25] A Lakeshore temperature controller was used to monitor the temperature of the sample, the incoming transfer lines, and the exhaust line.

Three standard samples were prepared for this set of experiments. The first was a 4 M [$U-^{13}C, ^{15}N$] urea sample (Cambridge Isotopes, Tewksbury, MA). Urea was dissolved in a solution of 60% d8-glycerol, 30% D_2O , and 10% H_2O . AMUPol (CortecNet Corp., Brooklyn, NY) was added to bring its concentration to 20 mM. The second sample was

made by preparing a 4 M [U-¹³C, ¹⁵N] urea sample in 60% d8-glycerol, 30% D₂O, and 10% H₂O. Trityl (Finland radical; Oxford Instruments, Abingdon. UK) was added to give a 40 mM radical concentration. The final sample consisted of 4 M [U-¹³C, ¹⁵N] L-proline (Cambridge Isotopes) dissolved in 60% d8-glycerol, 30% D₂O, and 10% H₂O. This sample contained trityl at 40 mM. High concentrations (40 mM) of trityl were employed to establish the effect of electron decoupling. Approximately 36 μL of each sample was added to separate 3.2 mm zirconia rotors.

3. High-resolution DNP-NMR below 6 K in model systems

To validate DNP-enhanced NMR spectroscopy below 6 K with a frequency-agile microwave source (gyrotron), a series of experiments were performed using nitroxide biradicals or tertiary carbon monoradicals as polarizing agents on [U-¹³C, ¹⁵N] urea and [U-¹³C, ¹⁵N] L-proline within a frozen glassy matrix. Biradicals such as AMUPol, using the cross-effect mechanism, yielded the highest signal enhancements. A 282-fold signal enhancement of urea with 20 mM AMUPol was recorded under MAS with a sample temperature below 6 K (Figure 2a). It should be noted that this is not a true enhancement value, as samples with AMUPol are susceptible to depolarization.[9] Due to combined DNP enhancement and improved thermal Boltzmann polarization, only a single transient was required to achieve an excellent signal-to-noise ratio. Remarkably, the polarization build-up time ($T_{1\text{DNP}}$, ¹H) was only 1.3 s (Figure 2c), making the optimal recycle delay to maximize signal-to-noise per unit time only 1.6 s ($1.26 \cdot T_1$). [26] Polarizing agents also yield short polarization build-up times at 90 K ($T_{1\text{DNP}} = 1.3$ s), [13,27,28] but here we demonstrate similar build-up times below 6 K. While $T_{1\text{DNP}}$ time constants in static samples at 1.2 K can be several minutes to hours, [29,30] these short recovery time constants are comparable to those observed at room temperature. [31] Therefore, MAS DNP below 6 K delivers unprecedented NMR sensitivity per unit time.

The enhancement in Figure 2b demonstrates the solid effect using the tertiary monoradical, trityl, below 6 K. In contrast to the cross effect, the solid effect enhancement is polarization time dependent due to the effect of microwave intensity on magnetization buildup during DNP. [16,17,32,33] Accordingly, we observed a solid effect DNP enhancement of 51-fold with a polarization time (τ_{pol}) of 3 s (Figure 2b) and 32-fold with a τ_{pol} of 30 s. The $T_{1\text{DNP}}$ (¹H) for urea with trityl was 18.7 s below 6 K (Figure 2d). Shorter longitudinal recovery times (in this case, τ_{pol}) with solid effect DNP will be accessible with higher microwave intensity. DNP provided a signal increase of 51-fold with a further improvement of 41-fold due to Boltzmann spin polarization at 6 K compared to 298 K. At these temperatures, the polarization of the electrons at ~198 GHz has moved out of the high temperature approximation commonly employed in magnetic resonance calculations and the full

Boltzmann expression, $P_e = \tanh\left(\frac{h\nu_e}{2k_B T}\right)$, must be employed (see Supporting Information).

Therefore, MAS DNP below 6 K can enhance NMR signals by over 2,000-fold over room temperature experiments.

Significantly higher DNP enhancements were obtained with an electron nutation frequency (ν_{1s}) of 380 kHz (5.0 W incident power) [34] compared to $\nu_{1s} \approx 83$ kHz (0.23 W incident

power). Microwave power was dependent on DNP enhancement with trityl monoradicals using the solid effect and also with nitroxide biradicals polarizing through the cross effect (Figure 2e, f). Two-fold and 26-fold gains in DNP signal enhancement were obtained for AMUPol and trityl, respectively, using 5.0 W versus 0.23 W.

Therefore, high-power microwave sources such as the gyrotron oscillator employed here improve DNP enhancements not only at higher temperatures (>80 K)[35] but also in MAS DNP below 6 K. Higher microwave powers also efficiently polarize nuclear spins with direct hyperfine couplings, resulting in a concomitant larger observed hyperfine shift (Figure 2e). [25,36,37]

4. Electron decoupling with cross polarization

We previously introduced electron decoupling (eDEC), employing direct electron-to-carbon DNP transfers on samples of urea with trityl at 90 K.[19] Note, moderate spinning frequencies of 4 kHz result in a significant effects of electron decoupling compared to higher spinning frequencies (see Supporting Information). Here we show electron decoupling of DNP-enhanced NMR spectra recorded with MAS below 6 K, also employing cross polarization (CP). Figures 3a and 3b illustrate the first CPMAS electron decoupling experiments with urea and L-proline, respectively. Polarization transfer from electron to proton spins, followed by ^1H - ^1H spin diffusion, results in much faster longitudinal magnetization recovery over direct ^{13}C polarization. Following a subsequent polarization transfer from protons to carbons via CP, the ^{13}C free induction decay (FID) is recorded under both proton and electron decoupling (Figure 1a). Electron decoupling on urea and L-proline improved signal intensity ($5 \pm 2\%$ area increase) and resolution (11 ± 2 Hz narrowing) on average (Figure 3a and 3b), with an additional attenuation of the hyperfine shift resulting from the DNP polarizing agent. This is the first demonstration of electron decoupling attenuating an observable hyperfine shift in rotating solids.

Detrimental hyperfine interactions are reduced using proton spin diffusion to relay enhanced polarization far from the radicals using CP. At 90 K, CP with electron decoupling on a sample of urea narrowed the ^{13}C resonance by 10 Hz. The overall linewidth of the resonance utilizing CP with electron decoupling approached the linewidth observed with no radical present (Table 1). ^{13}C direct DNP followed by electron decoupling narrowed the resonance to 306 Hz, while CP with electron decoupling gave a linewidth of 250 Hz. When electron decoupling was performed below 6 K, a similar trend was observed (Table 1). Note that, overall, there was more broadening from the radicals below 6 K compared to 90 K, suggesting that further DNP-NMR spectra improvements can be realized with improved electron decoupling.

5. Proton-driven spin diffusion below 6 K

Carbon-carbon correlation spectra were recorded on $[\text{U-}^{13}\text{C}, ^{15}\text{N}]$ L-proline with trityl below 6 K to demonstrate the time savings afforded by DNP at this temperature in multidimensional experiments. The proton-driven spin diffusion (PDS) pulse sequence is shown in Figure 1b. The total experimental time of the PDS experiment performed below 6

K was 24 minutes (Figure 4a). For comparison, a PDSD at 90 K is shown containing a similar signal-to-noise ratio (Figure 4b); however, this experiment took 16 hours.

6. Conclusions

The radicals introduced in DNP can lead to broadening due to hyperfine interactions. Electron decoupling provides a means to mitigate these unwanted interactions. Performing electron decoupling with cross polarization has the dual advantages of faster recovery times and narrower resonances compared to direct polarization. One-dimensional NMR experiments employing CPMAS and electron decoupling on biomolecules frozen in a glassy matrix resulted in 11 Hz narrowing and a 5% increase in signal area.

The already sizeable enhancements afforded by DNP can be further improved by cooling the sample to below 6 K. DNP below 6 K leads to a 51-fold solid effect enhancement and a 282-fold cross-effect enhancement. The longitudinal magnetization recovery times remain short at these temperatures, allowing for large enhancements and quick recovery times. MAS below 6 K combined with high-power frequency-agile microwave sources also provides a promising avenue for the implementation of pulsed DNP.[38–41]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- DNP MAS NMR below 6 K.
- 2-D DNP MAS NMR below 6 K.
- Electron decoupling applied to CPMAS pulse sequences.
- Electron decoupling of the hyperfine shift with MAS.

Figure 1.

(a) Cross-polarization magic angle spinning (CPMAS) with electron decoupling. (b) Proton-driven spin diffusion (PDSD) CPMAS.

Figure 2. DNP CPMAS NMR spectra below 6 K on model systems. **(a), (b)** Enhancement spectra of urea with AMUPol and trityl, respectively. Spectra in black represent no microwave irradiation while spectra in red are with microwave irradiation. $T_{1\text{DNP}}$ of ^1H for urea with AMUPol **(c)** and trityl **(d)**. **(e), (f)** Microwave power dependence on urea with AMUPol and trityl, respectively. 5.0 W of microwave power incident to the sample is shown in red and 0.23 W of microwave power is shown in black. * denote spinning side bands.

Figure 3.

Electron decoupling with cross polarization. (a) CPDNP spectra of urea with trityl. (b)

Carbonyl region from the ^{13}C spectrum of L-proline with trityl. Black spectra represent no electron decoupling, while red spectra were recorded with electron decoupling.

Figure 4.

PDSD taken on $[\text{U-}^{13}\text{C}, ^{15}\text{N}]$ L-proline with trityl below 6 K (a) and at 90 K (b). The 1D spectrum at the top of each figure is the slice of the 2D spectrum indicated by the dashed line. The total experiment time was 16 hours for the spectrum recorded at 90 K, while the experiment at 6 K required only 24 minutes.

Table 1.

Effect of radicals and electron decoupling on linewidths of [U- ^{13}C , ^{15}N] urea with and without trityl. Electron decoupling (eDEC) coupled with cross polarization (CP) results in ^{13}C linewidths approaching those without any radical present.

Experiment	90 K linewidth (Hz)	Below 6 K linewidth (Hz)
^{13}C direct - no eDEC	339 ± 2	349 ± 2
^{13}C direct - eDEC	306 ± 2	328 ± 6
CP – no eDEC	259 ± 1	322 ± 6
CP – eDEC	250 ± 2	310 ± 6
CP – no radical present	228	225