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Enhanced Efficiency of ^{13}C Dynamic Nuclear Polarization by Superparamagnetic Iron Oxide Nanoparticle Doping

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Abstract

Attainment of high NMR signal enhancements is crucial to the success of *in vitro* or *in vivo* hyperpolarized NMR or imaging (MRI) experiments. In this work, we report on the use of a superparamagnetic iron oxide nanoparticle (SPION) MRI contrast agent Feraheme (ferumoxytol) as a beneficial additive in ^{13}C samples for dissolution dynamic nuclear polarization (DNP). Our DNP data at 3.35 T and 1.2 K reveal that addition of 11 mM elemental iron concentration of Feraheme in trityl OX063-doped 3 M $[1-^{13}\text{C}]$ acetate samples resulted in a substantial improvement of ^{13}C DNP signal by a factor of almost 3-fold. Concomitant with the large DNP signal increase is the narrowing of the ^{13}C microwave DNP spectra for samples doped with SPION. W-band electron paramagnetic resonance (EPR) spectroscopy data suggest that these two prominent effects of SPION doping on ^{13}C DNP can be ascribed to the shortening of trityl OX063 electron T_1 as explained within the thermal mixing DNP model. Liquid-state ^{13}C NMR signal enhancements as high as 20,000-fold for SPION-doped samples were recorded after dissolution at 9.4 T and 297 K, which is about 3 times the liquid-state NMR signal enhancement of the control sample. While the presence of SPION in hyperpolarized solution drastically reduces ^{13}C T_1 , this can be mitigated by polarizing smaller aliquots of DNP samples. Moreover, we have shown that Feraheme nanoparticles (~30 nm in size) can be easily and effectively removed from the hyperpolarized liquid by simple mechanical filtration, thus one can potentially incorporate an in-

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ASSOCIATED CONTENT

Supporting Information

^{13}C DNP spectra and polarization buildup curves of 3 M $[1-^{13}\text{C}]$ sodium acetate in 1:1 v/v glycerol:water with 15 mM trityl OX063 doped with different concentrations of SPION, representative electron T_1 magnetization recovery curves and corresponding exponential fittings, modified Borghini modelling of ^{13}C microwave DNP spectra, and NMR and UV-Vis spectroscopic data on Feraheme filtration.

Notes

The authors declare no competing financial interest.

line filtration for these SPIONS along the dissolution pathway of the hyperpolarizer—a significant advantage over other DNP enhancers such as the lanthanide complexes. The overall results suggest that the commercially-available and FDA-approved Feraheme is a highly efficient DNP enhancer that could be readily translated for use in clinical applications of dissolution DNP.

1. INTRODUCTION

Dynamic nuclear polarization (DNP), originally used to create solid polarized targets for particle physics experiments, has seen more use recently as a method of creating large non-equilibrium polarization in samples of nuclear spins for study by NMR.^{1–6} However, until the invention of the dissolution method in 2003, the technique was largely limited to the solid state.^{7,8} In dissolution (*ex situ*) DNP are polarized at cryogenic temperature and intermediate magnetic fields prior to a rapid dissolution using a superheated solvent. This results in the production of a liquid-state sample of “hyperpolarized” nuclear spins at a physiologically compatible temperature whose NMR signal is enhanced greater than 10,000-fold over thermal equilibrium. This method has been particularly advantageous for low-gyromagnetic ratio nuclei, allowing highly sensitive NMR signal detection of low-concentration solutions that would otherwise be very challenging to measure.^{9–11} Unfortunately, the hyperpolarized signal is relatively short-lived, decaying according to the longitudinal relaxation time (T_1) of the nuclei being imaged, typically on the order of 30 to 60 s for ^{13}C -labeled compounds.^{12,13} Despite the transient nature of the signal enhancement, it still may be used for a plethora of applications, most notably *in vivo* metabolic tracing.^{5,12,14–22} As dissolution DNP moves rapidly into the realm of clinical research thanks to the commercial SPINlab polarizer (GE Healthcare, UK), there are elements of the technology left to explore.^{23,24} Though standard methods of sample preparation and polarization can yield liquid-state samples polarized to greater than 10,000-fold over thermal equilibrium, further chemical additives or instrumentation methods could improve the technology further.^{25–31} However, as the primary applications of the dissolution method revolve around biomedical studies, safe, non-toxic methods of improving signal strength are of great importance.

The DNP procedure requires a source of free electrons within the sample from which polarization is transferred to nuclear spins.¹ Of the radicals that have been used in DNP, the most commonly used belong to the classes of the TEMPOs or the trityls.^{32–37} TEMPO, having a wide electronic spectrum, is more suitable for polarizing large-gyromagnetic ratio (γ) like ^1H , though it has been used with limited success to polarize ^{13}C as well.^{25,38,39} Trityl free radicals, on the other hand, have narrow electronic spectra, making them ideal for direct polarization of low- γ nuclei such as ^{13}C .^{32,36,40} Using these radicals, ^{13}C polarization levels of 10–30% at 3.35 T and 50% at 5 T may be routinely reached.^{7,8,41,42} Additionally, when using trityl, the signal enhancement may be further increased through the addition of trace amounts of paramagnetic agents such as gadolinium, an effect that is very pronounced at low field, and somewhat subdued as the field strength is increased.^{43–47} While the signal enhancement achieved by gadolinium doping is highly desirable, the growing concerns about toxicity of these agents will likely limit their use in clinical studies.⁴⁸ The free radical is relatively easy to remove from solution via mechanical filtration of a low pH solution, but

the removal of paramagnetic dopants within the time window afforded by T_1 relaxation is more difficult.^{23,26} For this reason, additives such as gadolinium are not typically used in clinical samples. In order to avoid this sacrifice of additional signal enhancement, paramagnetic agents that have low toxicity and are easily removed from solution are needed.

One possible candidate is the superparamagnetic iron oxide nanoparticle (SPION) ferumoxytol, often known as the drug Feraheme (see structure in Figure 1), which is used to treat patients with iron deficiency anemia. In addition to this primary use, Feraheme has proven useful as a magnetic resonance imaging (MRI) contrast agent due to its strong paramagnetism.^{49,50} This property stems from the structure of ferumoxytol, which consists of an iron oxide core of about 10 nm in diameter surrounded by polyglucose sorbitol carboxymethylether (PSC) as displayed in Figure 1.^{51,52} Additionally, the SPION is water-soluble, which allows it to easily be introduced in DNP samples. With this in mind, we have performed a comprehensive study of DNP with Feraheme as an additive in order to explore its possible benefit to the process.

2. EXPERIMENTAL SECTION

2.1 Sample Preparation

All chemicals were obtained and used without further purification. The samples used for comparison of solid state ^{13}C polarization were made to contain 3 M (24.8 mg) $[1-^{13}\text{C}]$ sodium acetate (Cambridge Isotope Lab, Tewksbury, MA) and 15 mM (2.14 mg) trityl OX063 (GE Healthcare, UK) in 100 μL 1:1 v/v glycerol:water. These samples were further doped with Feraheme in varying concentrations. Pharmaceutical Feraheme (AMAG Pharmaceuticals, Inc, Waltham, MA, Lot AC6335) contains 30 mg/mL (537 mM) elemental iron, or approximately 122 mg/mL, which far surpasses the concentration required to have a marked effect on both T_1 and T_2 in imaging studies, suggesting that dilution is necessary. Based on previous studies using gadolinium, it is expected that only a trace amount of paramagnetic agent will be necessary to achieve significant DNP improvement, and the stock solution was diluted accordingly. Specifically, a secondary stock solution containing 100 mM elemental iron in 2000 μL 1:1 v/v glycerol:water was made by mixing 372 μL Feraheme, 628 μL deionized water, and 1000 μL glycerol. This stock solution was then diluted to concentrations of 0.2, 2, 3.5, 5, 6.5, 8, 9.5, 11, 12.5, 14, 15.5, 20, 24.5, 29, 34.5, 40, 60, 80, and 100 mM elemental iron for study in DNP. This corresponds to a range between 0.5 mg/mL to 22.7 mg/mL SPION. A control sample using pure glycerol:water was also prepared. While this work is primarily concerned with the magnetic properties of Feraheme, and thus the concentration of magnetic centers (i.e. nanoparticles), iron concentrations are more readily and accurately calculated due to nanoparticle polydispersity.^{51–53} As such, iron concentration will be used to describe samples for the duration of this article. For W-band EPR, 50 μL samples were prepared in the same manner for control and 11 mM iron (Feraheme) samples.

2.2 Dynamic Nuclear Polarization

For samples containing 2, 3.5, 8, 11, 14, 20, 29, and 60 mM iron, microwave frequency sweeps were run by irradiating samples for 120 s in steps of 5 MHz between 94.05 and

94.25 GHz. This was performed using a pre-programmed sequence in the HyperSense polarizer (Oxford, UK) at 3.35 T and 1.2 K. Samples with concentrations between those listed above are assumed to have similar positive polarization peak (P(+)) when the DNP spectra for the concentrations above and below were approximately identical. For concentrations above 60 mM, short sweeps around the expected P(+) (94.08 to 94.12 GHz) were measured to verify the approximate location of P(+). It should be noted that though the microwave frequency step time is comparatively short, previous work has shown that longer irradiation time results in a similar P(+) location to the short irradiation case used herein.³⁵

Once the positive polarization peak was determined, samples were removed from the polarizer and brought to room temperature for 5 minutes to ensure the relaxation of any residual hyperpolarization. Samples were then returned to the polarizer and polarized at the microwave frequency corresponding to P(+). Based on the solid-state polarization results, the optimal concentration was selected as the lowest concentration that yields the highest polarization achieved. Dissolutions were performed for different volumes (10 μ L, 50 μ L, 100 μ L) of sample containing the optimal concentration of Feraheme (11 mM elemental iron). A 100 μ L control sample containing no Feraheme was also studied using the dissolution technique. Samples were dissolved using 4 mL deionized water and shuttled to a 9.4 T high resolution NMR magnet (Agilent Technologies, CA), where the ^{13}C NMR signal was monitored every 2 s using a 2° RF pulse. The shuttling time from polarizer to NMR magnet was approximately 8 s. Final Feraheme concentrations in solution were 0 μ M (control), 27.5 μ M (10 μ L), 136 μ M (50 μ L), and 268 μ M (100 μ L).

2.3 W-Band Electron Paramagnetic Resonance (EPR)

W-band EPR measurements were performed at the National High Magnetic Field Laboratory (NHMFL) in Tallahassee, FL on a (Bruker Biospin, Billerica, MA) using a Bruker TE₀₁₁ cylindrical cavity. The sample temperature was regulated using a CF1200 helium flow cryostat (Oxford Instruments, UK). Samples were loaded into 0.15 mm ID thin quartz capillary tubes prior to insertion in the cryostat. Field swept EPR spectra and electronic T_1 were measured at a series of temperatures between the low and high limits of the system (5 and 200 K). T_1 was measured using saturation recovery.

Electron magnetization recovery curves were fitted using a double-exponential fitting equation:

$$M(t) = M_a - M_b e^{-t/T_{1a}} - M_c e^{-t/T_{1b}} \quad (1)$$

This fitting provided two different time constants, the larger of which was attributed to electron T_1 while the smaller was attributed to electron-electron cross relaxation effects.^{54,55} The electron relaxation rate (T_1^{-1}) was plotted vs. temperature on a log-log scale, and regions of similar slopes were fitted using a power law equation. Field-swept EPR spectra were fitted with a Gaussian function.

2.4 Filtration of Feraheme

In order to determine whether Feraheme could be easily removed from solution, a simple filtration was attempted. Feraheme doped solutions were forced through a Whatman Anotop 25 syringe filter with 0.02 μm pores (GE Healthcare, UK). This was performed both on a mock dissolution sample of water with Feraheme and on a sample that had been polarized and dissolved. The mock sample contained 0.268 mM elemental iron in deionized water to mimic the concentration of Feraheme in a post-dissolution DNP sample. This sample was evaluated by measuring UV-Vis absorbance and proton T_1 before and after filtration. The ^{13}C T_1 of the dissolution sample was measured pre-filtration by the decay of the hyperpolarized signal and measured post-filtration by inversion recovery in the same magnet.

2.5 Data Analysis

Liquid state NMR data were analyzed using VNMRJ software (Agilent Technologies, CA) and ACD/Laboratories version 12.0 (Advanced Chemistry Development). All other data analysis was performed using Igor Pro version 6.37 (Wavemetrics, Lake Oswego, OR). Modeling of the theoretical ^{13}C DNP spectra was performed using MATLAB (Mathworks, Nattick, MA).

3. RESULTS AND DISCUSSION

Representative ^{13}C microwave DNP spectra, which are frequency sweep plots of relative ^{13}C polarization levels near the expected EPR frequency of the free radical, are shown in Figure 2 in the absence and presence of Feraheme doping at DNP conditions of 3.35 T and 1.2 K. Inspection of Figure 2 reveals that Feraheme or SPION doping on the samples has significant effect on the shape of the ^{13}C DNP spectra. Similar to previous studies using gadolinium and other lanthanide ions, the DNP spectra of samples doped with Feraheme were narrowed by up to 50 MHz with positive and negative polarization peaks shifted by as much as 25 MHz as shown in Figure 2.^{35,44–47} Since the locations of the optimum microwave irradiation frequencies P(+) and P(–) are shifting with SPION doping, it is imperative to track microwave ^{13}C DNP spectra for samples with different concentrations of SPION (see Figure S1, Supporting Information).

Concurrent with this shift in P(+) and P(–) of the microwave DNP spectra, another striking effect of SPION doping is the substantial improvement of the ^{13}C DNP signal by a factor of 3. As can be seen from Figure 3, the relative solid-state ^{13}C polarization is maximum for SPION concentrations between 11 mM and 40 mM (see also Figure S2, Supporting Information for detailed polarization buildup curves). In this case, we have chosen 11 mM SPION as the optimum concentration for ^{13}C DNP because it is the least amount of nanoparticle doping that yielded close to the maximum ^{13}C polarization. It should be noted that 40 mM SPION yielded slightly higher solid-state ^{13}C polarization, however this slight advantage can be negated in the liquid-state after dissolution because of greater ^{13}C T_1 reducing effects. Above iron concentrations of 40 mM, the polarization begins to be reduced, though even at 100 mM, the ^{13}C polarization is 1.5-fold higher than the polarization

achieved by the reference sample. These results may be understood by considering W-band EPR results and the thermal mixing model of DNP which will be discussed subsequently.

At the conditions under which polarization took place, namely $B_0=3.35$ T, $T=1.2$ K, and use of trityl OX063 free radical as polarizing agent, the DNP mechanism has often been attributed to thermal mixing—a spin temperature-based description of polarization.^{8,32,56,57} Thermal mixing, which occurs when the EPR linewidth of the polarizing agent is greater than or comparable to the nuclear Larmor frequency, involves a microwave-driven dynamic cooling of the electron spin-spin interaction (ESSI) reservoir. A thermal link is established between the nuclear Zeeman system and ESSI due to their comparable energies, thus in the end, the nuclear spins acquire the same lower spin temperature as the ESSI reservoir, translating to higher nuclear polarization.^{8,32,56,57} In the thermal mixing model of DNP, the maximum achievable polarization for spin-1/2 nuclei is given by:^{58–60}

$$P_{max} = \tanh\left(\beta_L \frac{\omega_e \omega_I}{4D} \frac{1}{\sqrt{\eta(1+f)}}\right) \quad (2)$$

In the above equation, β_L refers to the inverse lattice temperature, ω to the electron or nuclear Larmor frequency, D to the dipolar width of the EPR spectrum, η to the ratio of electronic and dipolar relaxation times (T_{1e}/T_D), and f to a nuclear relaxation leakage factor. Because the field and temperature are held constant between samples, the parameters that determine differences between the polarization of samples are D , η , and f .

To shed light on the DNP behavior with SPION doping, we have monitored the effect of Feraheme on the EPR spectral and relaxation properties of the trityl OX063 polarizing agent. The electron T_1 values of trityl OX063 were extracted from electron magnetization recovery curves fitted with a double-exponential function (see Figure S3 of the Supporting Information), where the longer T_1 component is the actual electron T_1 relaxation and the shorter T_1 is attributed to electron cross relaxation effects. As is shown in Figure 4a, there is negligible difference between the EPR spectra of control and Feraheme doped samples, suggesting that D remains relatively constant. However, addition of Feraheme causes a reduction in electron T_1 (increase in electron relaxation rate) as seen in Figure 4b to which an increase in polarization may be attributed based on a reduction of the parameter η . A more recent model of thermal mixing suggests the same increase in polarization with electron T_1 reduction, though there is no simple analytical solution in this model, making it of less use for qualitative discussion.⁶¹ Similarly, the narrowing of DNP spectra may also be attributed to the reduction of electron T_1 . When electron T_1 is shortened, the model presented by Wenckebach⁶¹ predicts a narrowing of the DNP spectrum (Figure S4, Supporting Information). After a certain concentration of Feraheme is added, it seems that the reduction of electron T_1 is saturated, resulting in similar shaped spectra for all concentrations studied above 11 mM. As the polarization begins to decrease at concentrations greater than 40 mM, it is expected that the Feraheme begins to have a greater depolarizing effect due to a combination of longitudinal nuclear relaxation and spin diffusion. This, in turn, increases the nuclear relaxation leakage factor, f , and hence decreases the achievable polarization. Thus, both the narrowing of the ^{13}C DNP spectra and

the substantial increase in ^{13}C DNP signals with SPION doping can be explained in the context of thermal mixing model of DNP.

When samples are dissolved and studied in the liquid state, the presence of Feraheme has a significant effect on ^{13}C relaxation, reducing T_1 by as much as 70% compared to a reference sample (~15 s vs ~50 s). This shortened T_1 combined with the 8 s shuttling time from polarizer to NMR magnet results in a liquid state signal enhancement less than 10% above the reference for a standard 100 μL sample doped with Feraheme (11 mM Iron, final concentration in solution 268 μM). This effect can be alleviated through the use of smaller aliquots of DNP samples as shown in Figure 5. Polarizing a 10 μL sample for use in dissolution DNP reduces the final concentration of Feraheme in solution 10-fold (27.5 μM), and the ^{13}C relaxation time for such a sample is only marginally shortened relative to a control sample. As a result, the enhancement of the ^{13}C NMR signal over thermal equilibrium is nearly 4 times greater than that achieved using a control sample. However, while small samples may be useful for *in vitro*, perfusion, or small animal studies, they would be ill suited for most clinical applications.

For studies in which large volumes of ^{13}C DNP sample are necessary, the addition of Feraheme would be counterproductive unless it could be removed from solution at the time of dissolution. Ferumoxytol is a somewhat large nanoparticle (~30 nm) with a highly negatively charged core, which suggests that it could be filtered from solution.^{51,52} This was initially tested on a test dissolution sample. Prior to filtration, the test sample had an absorbance at 302 nm of about 0.37 which was reduced to approximately 0.007 after filtration, a reduction of about 98% (Figure S5 Supporting Information). Proton relaxation tells a similar story, with pre-filtration $T_1 = 0.15$ s, increasing to $T_1 = 2.82$ s after filtration, which nearly returns to the length of the relaxation time of the control ($T_1 = 3.13$ s) (Figure S5, Supporting Information). Based on these results, as much as 98% of the Feraheme is removed through filtration. For the 100 μL sample doped with 11 mM elemental iron (Feraheme), the ^{13}C liquid state T_1 was approximately 15 s, down from the control sample's 46 s. After filtration, ^{13}C T_1 was increased to 30 s (Figure S6, Supporting Information). Though there is room for improvement, this shows that ^{13}C T_1 can be significantly improved by sample filtration. In modern clinical polarizers, this type filtration could be included relatively simply in the sterile fluid path, allowing for greatly enhanced ^{13}C polarization over a standard DNP sample with a lesser reduction in T_1 . Previous results⁴⁵ have suggested that the benefit of adding paramagnetic additives such as lanthanides in terms of DNP signal improvement is diminished at higher magnetic field such as 5 T when compared to the DNP results at 3.35 T. It remains to be seen whether this is the case for the DNP performance of Feraheme at 5 T which is the operating field of the commercial SPINLab polarizer. This will be an interesting subject of future study especially when considering the fact that Feraheme is already FDA-approved and unlike Gd-DOTA, can be easily filtered by simple mechanical filtration after dissolution.

4. CONCLUSION

We have shown that the commercially-available and SPION-based MRI contrast agent Feraheme or Ferumoxytol can substantially increase the DNP-enhanced solid-state ^{13}C

polarization by a factor of almost 3-fold. EPR results suggest that the two prominent effects of SPION doping in DNP, namely the narrowing of the ^{13}C DNP spectra and significant improvement in ^{13}C DNP signals, could both be ascribed to the electron T_1 reduction of trityl OX063 polarizing agent as explained within the thermal mixing DNP mechanism. The strong paramagnetism of the nanoparticle that is beneficial to solid-state polarization becomes a liability upon dissolution, causing a significant reduction in ^{13}C T_1 . Using a small initial sample volume can mitigate this, which was shown by the measurement of liquid-state ^{13}C NMR signals enhanced as high as 20,000-fold which is about 3 times better than the control sample. Furthermore, as a relatively large nanoparticle, Feraheme can be easily removed from hyperpolarized solution by simple filtration methods and thus one can devise a simple in-line filter along the dissolution pathway of the polarizer. This attribute of Feraheme is a main advantage over the other paramagnetic DNP enhancers such as the lanthanide complexes. Thus, the overall results suggest that the FDA-approved Feraheme is a highly beneficial DNP enhancer that could potentially be ready for translation into clinical use in hyperpolarization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(a) Simplified schematic of the functionalized nanoparticle Feraheme or ferumoxytol, which consists of an iron oxide core surrounded by carboxymethylated dextran. (b) Structure of trityl OX063, the free radical used in this work.

Figure 2. Normalized microwave frequency sweeps for select concentrations of Feraheme in DNP samples. The sweep profiles for samples with concentrations above 11 mM are essentially identical to the data shown for 11 mM. The up and down arrows indicate the positive P(+) and negative P(-) polarization peaks, respectively.

Figure 3.

Relative solid-state ^{13}C polarization data at 3.35 T and 1.2 K: (a) ^{13}C polarization build up curves shown for select samples. Data are fit with single exponential equations and scaled such that the reference sample builds up to unity. (b) Relative maximum ^{13}C DNP signals of samples doped with varying concentrations of Feraheme.

Figure 4.

(a) W-band (3.35 T) frequency-swept EPR spectra of trityl OX063 at 5 K for control and Feraheme-doped (11 mM elemental iron) samples. Spectra are overlaid with a Gaussian fit. (b) Temperature dependence of trityl OX063 electron T_1 for control and Feraheme-doped samples. The dashed lines are power law fits.

Figure 5.

Liquid-state ^{13}C NMR results at 9.4 T and 297 K: (a) Representative hyperpolarized and thermal ^{13}C NMR signals for the dissolution of a 10 μL DNP sample. (b) Hyperpolarized signal decay monitored using 2-degree RF pulses every 2 s for dissolution of control and DNP samples with different SPION concentrations in the liquid-state. (c) Liquid-state ^{13}C NMR signal enhancements relative to thermal equilibrium NMR signals measured after 8 s after dissolution with different concentrations of Feraheme.