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Transition Metal Doping Reveals Link between Electron T_1 Reduction and ^{13}C Dynamic Nuclear Polarization Efficiency

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Abstract

Optimal efficiency of dissolution dynamic nuclear polarization (DNP) is essential to provide the required high sensitivity enhancements for *in vitro* and *in vivo* hyperpolarized ^{13}C nuclear magnetic resonance (NMR) spectroscopy and imaging (MRI). At the nexus of the DNP process are the free electrons which provide the high spin alignment that are transferred to the nuclear spins. Without changing DNP instrumental conditions, one way to improve ^{13}C DNP efficiency is by adding trace amounts of paramagnetic additives such as lanthanide (e.g. Gd^{3+} , Ho^{3+} , Dy^{3+} , Tb^{3+}) complexes to the DNP sample which has been observed to increase solid state ^{13}C DNP signals by 100–250%. Herein, we have investigated the effects of paramagnetic transition metal complex R-NOTA ($\text{R}=\text{Mn}^{2+}$, Cu^{2+} , Co^{2+}) doping on the efficiency of ^{13}C DNP using trityl OX063 as the polarizing agent. Our DNP results at 3.35 T and 1.2 K show that doping the ^{13}C sample with 3 mM Mn^{2+} -NOTA led to a substantial improvement of the solid-state ^{13}C DNP signal by a factor of nearly 3-fold. However, the other transition metal complexes Cu^{2+} -NOTA and Co^{2+} -NOTA complexes, despite their paramagnetic nature, had essentially no impact on solid-state ^{13}C DNP enhancement. W-band electron paramagnetic resonance (EPR) measurements reveal that the trityl OX063 electron T_1 was significantly reduced in Mn^{2+} -doped samples but not in Cu^{2+} - and Co^{2+} -doped DNP samples. This work demonstrates, for the first time, that not all paramagnetic additives are beneficial to DNP. In particular, our work provides a direct evidence that electron T_1 reduction of the polarizing agent by a paramagnetic additive is an essential requirement for the improvement seen in solid-state ^{13}C DNP signal.

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ASSOCIATED CONTENT

Supporting Information

Complete microwave frequency sweep and polarization buildup data, sample numerical calculation of theoretical DNP spectra, and sample fittings for EPR saturation recovery data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

The authors declare no competing financial interest.

Keywords

DNP; NMR; EPR; Hyperpolarization

1. INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool for probing molecular structure and chemical dynamics. However, NMR is relatively insensitive, particularly for a low-gyromagnetic ratio (γ) and low natural abundance nuclei such as ^{13}C .¹ Historically, there have been two primary methods used to combat this insensitivity challenge. The first is the “brute force” method in which signal is improved by using large sample size, prolonged scans with signal averaging, higher magnetic field, or reduced temperatures.² Alternatively, highly polarized electrons may be used to improve the nuclear spin populations by way of dynamic nuclear polarization (DNP).^{2–4} While first used to create polarized targets for particle physics experiments, DNP is now primarily used for polarizing nuclear spins for NMR studies. For a time, this was limited to polarizing and monitoring nuclear spins in the solid state magic angle spinning (MAS) DNP⁵ while liquid studies were confined to the Overhauser Effect.^{6,7} The field was revolutionized, however, with the invention of dissolution DNP in 2003.⁸ In this technique, nuclear spins are polarized in the solid state and then rapidly dissolved using a superheated solvent to yield a “hyperpolarized” solution whose NMR signal is enhanced several thousand-fold over thermal equilibrium.^{9,10} This highly increased signal has been and continues to be harnessed for many applications, particularly ^{13}C metabolic imaging both in vitro and in vivo.^{11–17} However, despite its heavy usage in both preclinical and clinical studies, many questions with remain regarding optimization of the polarization procedure and the underlying physics of DNP under the conditions common to dissolution DNP.

One of the most important considerations when preparing a DNP sample is the choice of polarizing agent, typically provided by stable organic free radicals, as the source of free electrons from which high polarization is transferred.¹⁸ Among the organic free radicals tested for DNP,^{18–21} the most commonly used radicals for dissolution DNP are the trityls and nitroxides.^{10,22,23} Nitroxides, with their broad electron paramagnetic resonance (EPR) spectra, have been used to directly polarize ^{13}C with some success,^{24,25} but have been found to be more efficient for polarizing ^1H followed by cross polarization of ^1H spins to ^{13}C .^{26–31} Trityl radicals, on the other hand, have narrow EPR spectra so are ideal for direct polarization of ^{13}C .^{22,32} Furthermore, trityl has the added advantage that ^{13}C polarization can be additionally enhanced by the addition of paramagnetic compounds such as gadolinium based contrast agents (GBCA) or other lanthanide complexes.^{33–39} This paramagnetic modulating effect is widely known and implemented but less well understood.^{40–42} The common perception is that the reduction of electron T_1 of the polarizing agent brought about by the addition of paramagnetic agent is responsible for the increase in polarization.^{33,41} This is corroborated by measurements of the electron T_1 for samples doped with the various paramagnetic agents that have been shown to enhance ^{13}C DNP.^{32,35,36,38} In each case, the electron T_1 was shortened by about one order of magnitude. Furthermore, changes in electron spectra have not been observed with the addition of

paramagnetic agents, suggesting that the T_1 effect and the interaction between paramagnetic agent and nuclei are the sole determining factors behind the polarization increase.

In this work, we have tested, for the first time, the effect of paramagnetic transition metal complexes R -NOTA ($R=\text{Mn}^{2+}$, Cu^{2+} , Co^{2+}) on ^{13}C DNP of $[1-^{13}\text{C}]$ acetate at 3.35 T and 1.2 K. There is a wealth of research exploring the utility of manganese and copper-based contrast agents, suggesting that they may be beneficial agents in DNP.^{43–49} Cobalt, too, has been used for similar purposes, though not to the same degree as the other two ions.⁵⁰ Furthermore, Mn^{2+} has been used as a polarizing agent for MAS DNP studies.⁵¹ These prior studies suggested a potential role for these metal ions as additives to DNP using trityl. Chelated ions were chosen for this study because the ultimate goal of many DNP studies is a biomedical application which requires chemical inertness. Additionally, a ligand could improve the likelihood of benefit to DNP as the chelation of the ion could lead to slower tumbling of the metal ion, and hence greater effect on the nuclear and electron spins in the sample.⁵² NOTA (Figure 1) was chosen as the ligand for these studies because it is known to form stable complexes with first row divalent transition metal ions and those ions remain in the divalent oxidation state. The main goal of this study was to investigate the impact of paramagnetic transition metal complexes on the ^{13}C DNP efficiency in pursuit of expanding the list of paramagnetic additives in dissolution DNP.

2. EXPERIMENTAL SECTION

2.1 Sample Preparation

All reagents and solvents were purchased from commercial sources and used as received unless otherwise noted.

The ligand 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) was purchased from Macrocyclics™ (Plano, TX). Complexes used in experiments were prepared by mixing a ligand with 5% excess stoichiometric amounts of Mn^{2+} chloride stock solutions. The reaction mixtures were adjusted and maintained to a pH 6.5–6.8 by adding NaOH. The presence of free metal checked by using xylenol orange as the endpoint indicator in acetate buffer (pH = 5.8).⁵³ Purity of the complexes were checked using an analytical HPLC eluting with 10 mM ammonium acetate and gradient of 95 % MeCN/5 % 10 mM ammonium acetate for 30 minutes.⁴⁵ The metal concentrations were determined via Inductively Coupled Plasma (ICP) from UTD. Chemical structures and IUPAC names were obtained using Chemaxon MarvinSketch 6.2.0.⁵⁴

All samples were prepared in 100 μL volumes containing 3 M $[1-^{13}\text{C}]$ sodium acetate (24.9 mg) and 15 mM trityl OX063 (2.14 mg). Samples were additionally doped with trace concentrations (0.5 – 20 mM) of Co^{2+} -NOTA, Cu^{2+} -NOTA, or Mn^{2+} -NOTA. Transition metal solutions in 100 μL 1:1 v/v glycerol:water were diluted from aqueous stock solutions containing 75.3 mM Co-NOTA, 57.0 mM Cu-NOTA, or 61.7 mM Mn-NOTA. These stock solutions were diluted to between 0.5 and 20 mM metal ion complex and used to prepare the final DNP samples containing acetate and trityl at the appropriate concentration. A control sample containing no transition metal was also studied. Samples were prepared immediately prior to DNP experiments.

2.2 Dynamic Nuclear Polarization

All DNP experiments were performed in a HyperSense polarizer (Oxford, UK) with rootsblower attachment operating at 3.35 T and 1.2 K. Abbreviated microwave frequency sweeps were performed for a selection of the samples, allowing for a good estimate of the location of optimum irradiation frequency.³⁹ Samples were irradiated for 120 s per frequency in steps of 5 MHz over a range from 94.05 to 94.25 GHz. For a select few samples, only a small number of frequencies were measured around the expected positive polarization peak P(+). Based on these sweeps, P(+) was estimated and samples were irradiated at P(+) for 1–2 hours, monitoring the ¹³C NMR signal every 180 s using a small tip angle RF pulse. Data were then fit using single exponential function and extrapolated to 10,000 s. Be-cause of the comparative difficulty in measuring a thermal equilibrium ¹³C NMR signal, relative polarization levels with respect to the control sample are reported, which has previous precedent in the literature.^{32–34}

The optimum concentration for each metal was deter-mined as the lowest concentration that yields at least 90% of the maximum polarization achieved. Once these were determined for Mn (3 mM), Co (1 mM), and Cu (4 mM), samples containing the optimum concentration were polarized for 2 hours and rapidly dissolved using 4 mL deionized water as the dissolution solvent. Samples were shuttled to a 9.4 T high resolution Varian NMR magnet (Agilent Technologies, CA) where the ¹³C NMR signal was monitored every 2 s using a 2° RF pulse. Final concentration of metal ion complexes in solution were 73 μM (Mn), 24 μM (Co), and 97 μM (Cu).

2.3 Electron Paramagnetic Resonance

Samples were prepared containing 3 M [1-¹³C] sodium acetate, 15 mM trityl OX063, and the optimum concentration of one of the three transition metals in 50 μL 1:1 v/v glycerol:water. Additionally, a control sample was prepared with no transition metal. These samples were studied using electron paramagnetic resonance (EPR) in the W-band (94 GHz). Measurements were performed at the National High Magnetic Field Laboratory (NHMFL) in Tallahassee, FL on a Bruker E680 EPR spectrometer (Bruker Biospin, Billerica, MA) using a Bruker TE011 cylindrical cavity. Sample temperature was regulated between 5 and 200 K using a CF1200 helium flow cryostat (Oxford Instruments, UK). Before insertion into the cavity, samples were loaded into 0.15 mm ID thin quartz capillary tubes. The electron T_1 was measured using saturation recovery at a series of temperatures between 5 and 200 K, and EPR spectra were measured at each temperature point using the field-swept electron spin-echo method. These EPR spectra of the transition metal complexes were measured using a quasi-optical high-frequency EPR (HiPER) spectrometer at the NHMFL with an induction-mode cylindrical sample holder. The sample temperature was regulated using a CF1200 helium flow cryostat (Oxford Instruments, UK).

2.4 Data Analysis

All data were analysed using Igor Pro version 6.2 (Wave-metrics, Lake Oswego, OR). Post-dissolution ¹³C NMR data were processed using ACD labs version 12.0 (Advanced Chemistry Development, Toronto, Canada).

3. RESULTS AND DISCUSSION

Paramagnetic additives are known to cause a narrowing of the DNP spectrum which is a plot of the nuclear polarization as a function of microwave frequency.^{35–39,55} The microwave frequency sweep plots of relative ^{13}C polarization shown in Figure 2 are crucial in the DNP optimization steps using transition metal doping since these determine the locations of the optimum microwave irradiation frequencies $P(+)$ and $P(-)$ for DNP. As shown in Figure 2a, Mn^{2+} doping resulted in a significant narrowing effect on the DNP spectrum, with polarization peak separation $|P(+)-P(-)|$ decreased from ~ 100 MHz (control) to ~ 65 MHz (2 mM Mn^{2+}). This behavior is similar to that observed in samples doped with gadolinium or other lanthanide ions.^{35–39} The shift of the polarization peaks is apparent even at the lowest concentration (1 mM) studied for Mn^{2+} -NOTA in which $|P(+)-P(-)| \sim 75$ MHz as displayed in Figure 2a. On the other hand, this narrowing effect of the DNP spectra is less prominent or very minimal for ^{13}C samples doped with Cu^{2+} -NOTA and Co^{2+} -NOTA at similar low concentrations as shown in Figure 2b and 2c (see also additional ^{13}C DNP spectra in Figure S1, ESI). In particular, the polarization peak separation for Cu^{2+} - and Co^{2+} -doped samples only decreased by 5–10 MHz. At higher concentrations of these metal complexes, the ^{13}C DNP spectrum narrowing can become more prominent. For instance, Co^{2+} -NOTA doping at a relatively high concentration of 20 mM, as shown in Figure 2b, resulted in polarization peak shift comparable to those obtained with Mn^{2+} -doped samples.

After locating the optimum microwave irradiation frequencies and their corresponding shifts with transition metal doping, the relative ^{13}C polarization buildup curves and maximum ^{13}C DNP intensities taken at $P(+)$ irradiation frequencies were measured according as shown in Figure 3 (see also Figure S2, ESI for more detailed measurements of ^{13}C polarization buildup curves). It is apparent in Figure 3 that the addition of Mn^{2+} -NOTA was highly beneficial, while copper and cobalt led to minimal enhancements of the DNP signal over reference. It was found that as the concentration of manganese was increased, the ^{13}C polarization enhancement was increased, up to about 3 mM Mn^{2+} -NOTA. For a small concentration range, there was a rough plateau in polarization efficiency, above which the polarization enhancement began to retreat toward that of the reference sample. A somewhat similar trend on a weaker scale was observed with Cu^{2+} -NOTA and Co^{2+} -NOTA. There was a range of concentrations that enhanced the polarization to the greatest degree, albeit only to about 1.1 times that of the reference polarization, as opposed to the 2.5-fold increase achieved with manganese. For high concentrations of cobalt, which produced a narrowing of the DNP spectrum on par with manganese, the polarization was reduced below that achieved with a reference sample. This shows that, although a narrowing of the DNP spectrum seems to be associated with polarization increase,^{35–39,55} it does not guarantee a beneficial effect.

These results may be understood in light of the thermal mixing model of DNP using the W-band EPR results. While there are other possible mechanisms that could be responsible for polarization, there is a wealth of evidence that suggests thermal mixing is dominant under the conditions used herein.^{9,22,41,56,57} In the most current models of thermal mixing, the shape of the EPR spectrum as well as the electron T_1 of the radical used largely determine both the shape of the DNP spectrum and the maximum achievable polarization.^{56,58,59} Though there is an only minimal change in trityl's EPR spectra with the addition of

transition metal (Figure 4a), the electron T_1 measurement is more demonstrative. Electron T_1 for the sample optimally doped with manganese is drastically shortened with respect to the control sample, while the samples doped with copper and cobalt have T_1 values nearly identical to the control as shown in Figure 4b.

A numerical calculation using the most recent model of thermal mixing⁵⁹ shows that the shortened trityl OX063 electron T_1 in the Mn^{2+} -doped sample is adequate to cause a narrowed DNP spectrum on par with what we found experimentally (Figure S3, ESI). The minimal change in DNP spectra for copper and cobalt-doped samples matches well with the observed lack of change in the trityl electronic T_1 . Other, similar models have also shown that change in electron T_1 is adequate to cause the observed narrowing of the DNP spectrum.
40–42

It should be noted that other mechanisms could equally well describe the changes observed in DNP spectra. Most prominent is the work put forth by the Vega group in which polarization is described as combined solid and cross effects.^{60–62} As the concentration of manganese is increased, there is an increase in the cross effect contribution, which causes a narrowing of the DNP spectrum. Because there is little effect on trityl electron properties and the DNP spectra with copper and cobalt-doping, there is minimal change between the contributions of the cross and solid effects.

As with the microwave frequency sweep results, the polarization buildup in the solid state may be understood using EPR results and the thermal mixing model. The differences in polarization among optimally doped samples are particularly straightforward. As discussed above, polarization efficiency is determined in part by the electron T_1 of the free radical.
40–42,56,58,59 In the thermal mixing model of DNP, this is seen quite clearly as the minimum achievable spin temperature and hence maximum polarization is given by^{56,58}

$$T_{\min} = \left(\frac{2DT_L \sqrt{\eta(1+f)}}{\omega_e} \right)$$

In this equation, D is the EPR linewidth, T_L is the lattice temperature, f is the nuclear relaxation leakage factor, ω_e is the electron resonance frequency, and η is the ratio T_{1e}/T_{1D} of electron spin lattice relaxation time to the electron dipolar relaxation time. As T_{1e} is shortened, the lowest achievable spin temperature is reduced, which corresponds to higher polarization levels. Newer models predict the same sort of behavior but do not have a simple analytical solution, reducing their use for qualitative discussion.^{40–42,59}

Manganese causes a drastic shortening of T_1 , and thus increased polarization, while copper and cobalt have a minimal effect on T_1 , leading to barely enhanced DNP. In addition to electron T_1 effects, we expect that the presence of metal ions within the solution will also result in increased nuclear relaxation both by shortening nuclear T_1 and by increasing the rate of nuclear spin diffusion, which increases the leakage factor f , reducing the achievable polarization. At the optimal concentration of metal ions, a balance is reached between the enhancement gained through increased electron relaxation and the detrimental effects caused by nuclear relaxation. As the concentration of metal ions is increased further, the nuclear

relaxation effects dominate, while electron relaxation effects must reach a saturation point, given that there must be some lower limit on electron T_1 .

To elaborate, the dipolar interactions between nuclei and transition metal ions have a $1/r^6$ dependence. There will be some region around the paramagnetic ion in which nuclei are fast relaxing as a result of this dipolar coupling. These nuclei will then “talk” with slower relaxing nuclei at greater distances via spin diffusion. At low concentrations of transition metal, this is expected to be a comparatively small effect. As the metal concentration is increased, the greater number of fast-relaxing nuclei begins to be in greater competition with the polarization transfer from electrons. At some threshold concentration (~5 mM for Mn, ~6 mM for Co and Cu), the relaxing effect begins to overpower the polarization improvement achieved by DNP, and the enhancement begins to be reduced from the optimum value.

It should be noted that the nuclear relaxation effects would minimally affect the shape of the microwave DNP spectrum as they are independent of irradiation frequency. For the case of cobalt, as the concentration of metal ions is increased to the point that T_1 is reduced adequately to cause spectrum narrowing, the concentration is also high enough that the metal ions are increasing nuclear relaxation effects. This leads to poor polarization despite the observed narrowing of DNP spectrum.

The enhancement of the ^{13}C liquid-state NMR signal after dissolution was performed on optimally doped samples agreed well with the solid-state results. Samples doped with manganese had comparatively high enhancement that were slightly lower than values reported in the literature for gadolinium doping.^{22,33,34,36} Gadolinium has been shown to improve liquid state enhancement by as much as 3-fold over an undoped reference sample, while manganese is shown here to improve enhancement by 2-fold. Copper and cobalt, on the other hand, had very similar enhancement to the control sample containing no transition metal ions as displayed in Figure 5. These results are largely expected based on solid state polarization results, though there are a number of subtleties worth mentioning.

Though cobalt-doping has approximately the same enhancement effect in the solid state as copper-doping, the liquid state enhancement is marginally less than for copper. This is a result of the slight reduction of electron T_1 caused by cobalt contrary to copper, which causes no change. During the 8 s shuttling time from polarizer to magnet, the ^{13}C in the cobalt-doped sample relaxes more than in the copper-doped sample. Manganese causes the same effect to a greater degree. Though the ^{13}C polarization of the optimally doped manganese sample is increased about 2.5-fold over the reference sample in the solid state, the liquid state enhancement is only about 2-fold greater than the reference. As is seen in Figure 5, ^{13}C T_1 is shortened by about 10 s as compared to the reference sample, which accounts for the disparity between solid and liquid-state polarization results.

Finally, while the EPR results are primarily presented for the purposes of explaining DNP results, there are several features of these results that merit discussion. The field-swept EPR spectra of samples (Figure 4) are minimally affected by the addition of transition metal. Fitting spectra with a Voigt profile reveals that the approximate broadening of the control

and doped samples are very similar, with Mn^{2+} -NOTA and control samples having similar broadening (~25 MHz). The trityl spectra for copper and cobalt samples are slightly broader (28 MHz and 34 MHz, respectively). On the other hand, T_1 measurements showed significant differences between samples. T_1 was determined from saturation recovery data by fitting with a double exponential function as has been reported previously.^{32,35,36,38,55} Sample fittings are shown in Figure S4, Supporting Information. Of the two relaxation time constants obtained by this fitting, the longer was recorded as electron T_1 while the shorter was assumed to describe electron-electron interactions.^{63,64} Once T_1 was determined for each temperature point, the relaxation rate ($1/T_1$) was plotted vs temperature on a log log plot. Then, regions of similar slope (on the log-log plot) were fit using a power law equation, $1/T_1 = bT^\alpha$. It was found that the T_1 of the samples doped with copper and cobalt were nearly identical to the control sample. The T_1 of trityl in the manganese doped sample was drastically reduced.

In addition to the differences between trityl T_1 observed among the transition metal dopants, the values provided by the power law fit give insight into the electron relaxation processes that dominate in the temperature and field regimes.⁶⁵ At high temperatures, all samples had $\alpha > 3$ in Figure 4b, which indicates a dominance of multiple phonon Orbach processes and a possible combination with the Raman process. For the manganese-doped sample, a transition occurs at a relatively high temperature (100 K), reducing the power law to $\alpha \sim 1.25$ as shown in Figure 4b, suggesting that in this temperature range, electron relaxation is largely dominated by the direct process. The copper sample, too, has only a single transition occurring at 60 K, below which $\alpha \sim 1.75$, suggesting a combination of Raman and direct process dominate relaxation. The control and cobalt samples had nearly identical results in which the log-log plot slope changes at about 40 K and the second at about 15 K. In the middle temperature range, $\alpha \sim 2$, indicating a dominance of Raman process. Below 15 K, direct processes seem to dominate, with $\alpha \sim 0.5$.

The different responses of trityl OX063 EPR relaxation in the presence of different transition metal dopants shown in Figure 4b can potentially be understood in light of the EPR spectral results shown in Figure 6 in which we invoke the model proposed by Rakowsky et al.⁶⁶ This model adapts the BPP type of relaxation to the case of two electrons, one fast-relaxing and one slow-relaxing.^{67,68} In the current work, we take trityl OX063 to be the slow-relaxing spin and the transition metal ion to be fast-relaxing. This model takes the form:

$$\frac{1}{T_{1s}} = \frac{1}{T_{1s}^0} + S(S+1)[D+C+E]$$

$$D = \frac{d^2 T_{2f}}{1 + (\omega_f - \omega_s)^2 T_{2f}^2 + d^2 T_{1f} T_{2f}}$$

$$C = \frac{c^2 T_{2f}}{1 + \omega_s^2 T_{1f}^2}$$

$$E = \frac{e^2 T_{2f}}{1 + (\omega_f + \omega_s)^2 T_{2f}^2}$$

where T_{1S}^0 is the spin lattice relaxation time of trityl with no metal dopant, d^2 , c^2 , and e^2 are terms depending on inter-spin distance and orientation, s and f denote quantities describing slow and fast-relaxing electrons, and ω is the resonant frequency of the specified spin. It is expected that the C term will be approximately equal for each transition metal ion and that the E term is approximately negligible for each of the ions, given the large frequencies in use. The D term, however, is expected to change between transition metal ions based on the EPR spectra of the transition metal ions. For manganese, the spectrum is comparatively narrow and centered at approximately the same frequency as trityl, suggesting that the average $(\omega_f - \omega_s)^2$ will be comparatively small and thus D relatively large. Copper's EPR spectrum has a significant offset and is somewhat broader than manganese, while cobalt's EPR spectrum is extremely wide, both of which suggest large average $(\omega_f - \omega_s)^2$ and hence small D . Furthermore, the multiplicative factor $S(S + 1)$ is largest for Mn^{2+} ($S = 5/2$) and smaller for Co^{2+} ($S = 3/2$) and Cu^{2+} ($S = 1/2$), all of which gives reason for the significant electron T_1 shortening induced by Mn^{2+} -NOTA and the minimal effect observed with copper and cobalt doping.

As shown in this work, the different relaxation results of trityl in the presence of transition metal dopants have resulted in different ^{13}C DNP responses. It should be emphasized that the function of these paramagnetic additives here is not the role of a polarizing agent, instead these dopants are meant to perturb the electronic properties of the free radical polarizing agent. Mn^{2+} was the only transition metal dopant in this study that improved ^{13}C DNP because of its electron T_1 shortening effect on trityl OX063 free radical. This can be explained in the context of thermal mixing model in which reduced electron T_1 of the polarizing agent results in lower spin temperature of the electron dipolar system (EDS).^{56,58} Due to the thermal contact of the EDS with the nuclear Zeeman system, the nuclear spins eventually acquire the same lower spin temperature as the EDS, translating to improvement in the observed DNP-enhanced polarization. For this reason, the absence of DNP improvement for Cu^{2+} - and Co^{2+} -doped samples can be ascribed to their negligible influence on trityl electron relaxation. In the broader context, the results here imply that a perturbation of the electronic properties of the free radical polarizing agent, being at the nexus of the hyperpolarization process, can have significant impact on ^{13}C DNP efficiency.

4. CONCLUSION

In conclusion, we have investigated the effects of transition metal complex R -NOTA ($R=Mn^{2+}$, Cu^{2+} , Co^{2+}) doping on ^{13}C DNP efficiency. The improvement in ^{13}C DNP signal by Mn^{2+} doping was in line our initial expectation given the current trend in previous DNP

works using DNP-enhancing paramagnetic additives such as the lanthanide complexes. However, the absence of ^{13}C DNP improvement in samples containing the other transition metal dopants Cu^{2+} and Co^{2+} was somewhat contrary to our initial hypothesis given the paramagnetic nature of these additives. While the shortening of trityl OX063 electron T_1 associated with DNP-enhancing Mn^{2+} dopant was also observed in samples with lanthanide DNP enhancers, our work demonstrates for the time that the absence of DNP enhancement in Cu^{2+} - and Co^{2+} -doped samples is accompanied by the null effect of these additives in trityl OX063 relaxation. These results provide a strong evidence that not all paramagnetic additives can be beneficial to DNP and that electron T_1 reduction of the polarizing agent by a paramagnetic additive is a key requirement for the observed improvement in ^{13}C DNP efficiency.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

The structures of the chemicals used in this work: (a) Trityl OX063, (b) The NOTA complex in which Co^{2+} , Cu^{2+} , or Mn^{2+} are chelated, and (c) $[1-^{13}\text{C}]$ sodium acetate, the substrate being polarized.

Figure 2.

^{13}C microwave frequency sweeps for DNP samples doped with (a) Mn^{2+} -NOTA, (b) Co^{2+} -NOTA, and (c) Cu^{2+} -NOTA. The DNP spectrum of a control sample is shown in each plot for comparison. The up and down arrows represent the positive P(+) and negative P(-) polarization peaks, respectively.

Figure 3.

(a) Representative ^{13}C polarization buildup curves for a control sample and samples doped with optimum concentrations of Mn^{2+} -NOTA (3 mM), Cu^{2+} -NOTA (4 mM), and Co^{2+} -NOTA (1 mM). (b) Relative maximum ^{13}C DNP signals for different concentrations of transition metal complexes.

Figure 4.

(a) W-band EPR spectra for control, 1 mM Co^{2+} -NOTA, 4 mM Cu^{2+} -NOTA, and Mn^{2+} -NOTA. Spectra shown correspond to the free electron of trityl OX063 and are fit using a Voigt profile (shown in black). (b) Temperature dependence of electron T_1 for samples studied in (a). Dashed lines shown are power law fittings of regions of similar trend.

Figure 5.

(a) Representative thermal and hyperpolarized signal for the sample optimally doped with manganese. (b) Comparison of liquid-state ^{13}C NMR enhancements at 9.4 T and 295 K 8 s following dissolution for control and transition metal-doped samples. (c) Relaxation of the ^{13}C NMR signal following dissolution.

Figure 6.

W-Band EPR spectra of transition metal complexes and trityl OX063 free radical measured using the HiPER EPR spectrometer at the National High Magnetic Field Laboratory: (a) Co^{2+} -NOTA (inset: expanded view of trityl EPR spectrum), (b) Cu^{2+} -NOTA and Mn^{2+} -NOTA. Note that the Co^{2+} -NOTA EPR spectrum was separated from Cu^{2+} - and Mn^{2+} -NOTA spectra for clarity, since the former has a much wider spectral window of 6 T.