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# The effect of $Ho^{3+}$ doping on $^{13}C$ dynamic nuclear polarization at 5 T

Ali A. Sirusi, *[a] Eul Hyun Suh,[b] Zoltan Kovacs[b] and Matthew E. Merritt *[a]

**Dissolution dynamic nuclear polarization was introduced in 2003 as a method for producing hyperpolarized $^{13}C$ solutions suitable for metabolic imaging. The signal to noise ratio for the imaging experiment depends on the maximum polarization achieved in the solid state. Hence, optimization of the DNP conditions is essential. To acquire maximum polarization many parameters related to sample preparation can be modulated. Recently, it was demonstrated that $Ho^{3+}$, $Dy^{3+}$, $Tb^{3+}$, and $Gd^{3+}$ complexes enhance the polarization at 1.2 K and 3.35 T when using the trityl radical as the primary paramagnetic center. Here, we have investigated the influence of Ho-DOTA on $^{13}C$ solid state DNP at 1.2 K and 5 T. We have performed $^{13}C$ DNP on [1-$^{13}C$] sodium acetate in 1 : 1 (v/v) water/glycerol with 15 mM trityl OX063 radicals in the presence of a series of Ho-DOTA concentrations (0, 0.5, 1, 2, 3, 5 mM). We have found that adding a small amount of Ho-DOTA in the sample preparation not only enhances the $^{13}C$ polarization but also decreases the buildup time. The optimum Ho-DOTA concentration was 2 mM. In addition, the microwave sweep spectrum changes character in a manner that suggests both the cross effect and thermal mixing are active mechanisms for trityl radical at 5 T and 1.2 K.**

## 1 Introduction

The phenomenon of dynamic nuclear polarization (DNP) was theoretically postulated by Overhauser in conducting metals[1] and subsequently observed experimentally by Carver and Slichter.[2] Later, DNP was applied to non-conducting solids.[3,4] DNP found applications in particle physics[5] and MAS solid state NMR,[6] but it was not until 2003 that Ardenkjær-Larsen *et al.*[7] expanded DNP's applications to biology and biochemistry by dissolving the frozen sample in a solution and bringing the sample temperature to a biologically practical 298 K (dissolution DNP). Since then, dissolution DNP has been used extensively for *in vivo* and *in vitro* NMR/MRI spectroscopy, and has been the subject of multiple reviews.[8–11]

In dissolution DNP, the substrate is mixed with a glassing matrix in the presence of stable free radicals as paramagnetic centers. Both the composition of the glassing matrix and the choice of radical have substantial influence on the final polarizations. Deuteration of glassing matrix can increase the polarization by several fold for some of the radicals,[12] but can have detrimental effects with narrow linewidth radicals.[13] In addition, based on the targeted nuclei, different types of radicals with wide and narrow ESR linewidths have been used.[14–16] For low-$\gamma$ nuclei, trityl and BDPA have been used extensively[14–17] because their narrow ESR linewidths are larger than the low-$\gamma$ nuclear Larmor frequency, especially at 3.35 T, and satisfy the conditions for the highly efficient thermal mixing mechanism. Other paramagnetic centers such as $Ho^{3+}$, $Dy^{3+}$, $Gd^{3+}$, $Tb^{3+}$, and $Mn^{2+}$ have been shown to increase the polarization by reducing the electron relaxation times ($T_{1e}$) of the trityl radicals at 3.35 T.[18–23] The maximum polarization enhancement is a sign of reaching an optimum electron relaxation time $T_{1e}$ in the system.[24] Here, we have investigated the influence of $Ho^{3+}$ doping on the $^{13}C$ polarization of [1-$^{13}C$] sodium acetate at 5 T. Although most of the dissolution DNP has been done on [1-$^{13}C$] pyruvic acid,[8–11] [1-$^{13}C$] acetate can also be used as a metabolic tracer.[25–27] Furthermore, acetate was recently used as a target using the same experimental setup.[19] We have therefore chosen [1-$^{13}C$] sodium acetate for consistency of comparison to the previous work. Note that 5 T is a common field strength for DNP; the commercial DNP system for clinical applications (SPINlab™, GE Healthcare, WI) uses a 5 T magnet for polarization.

Our results show that Ho-DOTA doping not only increases the polarization but also reduces the buildup time when operating at 5 T (140 GHz ESR frequency). In addition, the spacing between maximum polarization peaks in the $^{13}C$ DNP spectra come closer upon increasing the Ho-DOTA concentration, indicating the cross effect might become a more dominant DNP mechanism at this field strength with this radical mixture.

[a] *Department of Biochemistry and Molecular Biology, University of Florida College of Medicine, Gainesville, USA. E-mail: alisirusi@ufl.edu, matthewmerritt@ufl.edu; Tel: +1 352 2948398*

[b] *Advanced Imaging Research Center, UT Southwestern Medical Center, Dallas, TX, USA*

 

# 2 Experimental methods

## 2.1 Materials

All chemicals used in this report were purchased and used without further purification. Tris[8-carboxyl-2,2,6,6-benzo(1,2-*d*:5-*d*)-bis(1,3)dithiole-4-yl]methyl sodium salt (trityl OX063) was obtained from Oxford Instruments Molecular Biotools. [1-$^{13}$C] sodium acetate was purchased from Cambridge Isotope Lab, MA. The ligand DOTA was synthesized as previously reported.[28] The holmium complex was prepared by reacting DOTA with freshly prepared holmium hydroxide (20% excess) for 5 days while maintaining the pH of the reaction mixture between 5 and 6. The pH was then raised to around 7.5 with NaOH solution and the reaction mixture was filtered to remove uncomplexed holmium hydroxide. The mixture was treated with Chelex 100 Resin, filtered and approximately 1% excess ligand was added to ensure full complex formation. The solution was freeze-dried to give the desired product. The holmium content of the final complex was 24.6% (by ICP-MS).

## 2.2 Sample preparation

All samples were prepared fresh before each DNP experiment. 24.9 mg of [1-$^{13}$C] sodium acetate (~3 M) was added to 100 μl of 1 : 1 v/v water/glycerol solution and the final products were mixed with the 2.1 mg of tris[8-carboxyl-2,2,6,6-benzo(1,2-*d*:5*d*)-bis(1,3)dithiole-4-yl]methyl sodium salt (trityl OX063) (~15 mM). Ho-DOTA doped samples were prepared by making a 50 mM stock solution of Ho-DOTA in 1 : 1 v/v water/glycerol solution and kept at −20 °C. From the stock solution 0.5, 1, 2, 3, and 5 mM Ho-DOTA samples were made. Samples were homogenized carefully using an ultrasonic bath (~10 min) and a vortexer (~1–2 min) in each step.

## 2.3 Solid state polarization measurement

All DNP experiments were performed at the University of Florida at 1.2 K in a 5 T (homebuilt) system as described in ref. 12. The NMR signals were collected using a commercial APOLLO (Tecmag Inc., Houston, TX) spectrometer. To monitor the buildup polarizations, a low flip-angle pulse (~5°) was implemented every 3 minutes. The frequency sweeps were done with 4 minutes buildup time and a flip-angle pulse ~3°. The microwave frequency was incremented manually from low to high frequency with a ~5 MHz interval and the microwave power was set to ~50 mW. The polarization was destroyed with a series of hard pulses (100 000) prior to each frequency step. Before placing the samples inside the cryostat we prepared small beads using liquid nitrogen,[29] although the same polarization levels were obtained without making beads in our system, within the experimental errors. Due to the long nuclear relaxation time at 1.4 K we report only the relative DNP polarizations. To illustrate the reproducibility, each sample was prepared three or four times.

# 3 Results and discussion

As previously observed,[18,19,30,31] the presence of $Ho^{3+}$, $Dy^{3+}$, $Tb^{3+}$, $Gd^{3+}$ complexes not only affects the polarization levels and the buildup times but also changes the peak positions

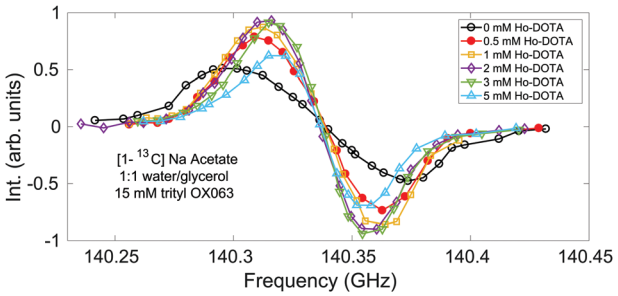


Fig. 1 $^{13}$C DNP frequency sweeps of [1-$^{13}$C] sodium acetate in 1 : 1 (v/v) water/glycerol solution in the presence of trityl OX063 radical for various concentrations of Ho-DOTA (0, 0.5, 1, 2, 3, 5 mM).

(P(+) and P(−)) of the $^{13}$C microwave DNP spectra. Therefore, it is most appropriate to find the maximum peak positions as a function of frequency prior to collecting the buildup of magnetization as a function of time. Fig. 1 shows the $^{13}$C DNP frequency sweeps for a series of Ho-DOTA concentrations (0, 0.5, 1, 2, 3, 5 mM) in 1 : 1 (v/v) water/glycerol with 15 mM trityl OX063 radical. P(+) and P(−) peaks are shifted toward each other with increasing Ho-DOTA concentration. The spacing between peak positions ($\Delta_{DNP}$) of the $^{13}$C DNP spectra are given in Table 1. Note that addition of the lowest concentration of Ho-DOTA causes a reduction of $\Delta_{DNP}$ to a magnitude similar to the $^{13}$C Larmor frequency (53.6 MHz), while concentrations of 2 mM or higher produce further, significant narrowing of the microwave DNP spectra.

After finding the maximum peak positions (P(+)) and corresponding microwave frequencies, we collected buildup curves (magnetization as a function of time). Even the minimal amount of added Ho-DOTA improves the polarization levels (Fig. 2). The optimum concentration for the highest polarization at 5 T is 2 mM Ho-DOTA. Moreover, adding Ho-DOTA reduces the buildup time at low concentrations (Table 1) while above 2 mM concentration the buildup time increases. Previous $^{13}$C DNP experiments at 5 T and 1.2 K with $Gd^{3+}$ (~2 mM) doping of trityl showed a similar, but smaller, increment in polarization (~30%) for [1-$^{13}$C] sodium acetate, while the buildup time increased from ~4800 to ~6500 s[31]. Gadolinium doping also resulted in a decrease of $\Delta_{DNP}$ of 12 MHz. Therefore, Ho-DOTA doping produces not only significantly more polarization than gadolinium doping at 5 T, but also reduces the buildup time by ~40%, a factor similar to the total increase in enhancement (~45%).

Table 1 Spacing between two polarization peaks in the $^{13}$C DNP spectra ($\Delta_{DNP}$), and buildup times ($T_{bu}$) for various concentrations (*c*) of Ho-DOTA

| *c* (mM) | $\Delta_{DNP}$ (MHz) | $T_{bu}$ (s) |
|---|---|---|
| 0 | 78.6 | 4460 ± 250 |
| 0.5 | 54.0 | 3540 ± 440 |
| 1 | 51.6 | 2840 ± 110 |
| 2 | 44.4 | 2830 ± 250 |
| 3 | 39.6 | 3450 ± 540 |
| 5 | 36.0 | 4120 ± 170 |

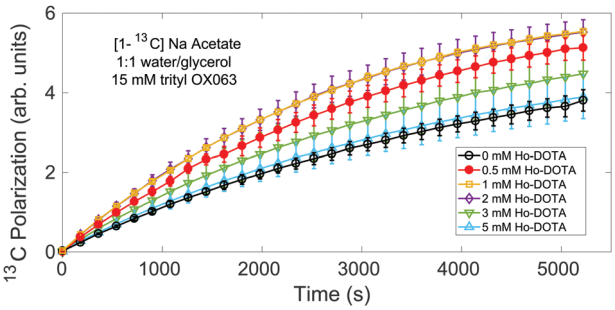


Fig. 2 $^{13}$C polarization buildup curves of [1-$^{13}$C] sodium acetate in 1 : 1 (v/v) water/glycerol solution for different concentrations of Ho-DOTA (0, 0.5, 1, 2, 3, 5 mM) at the P(+) maximum peak position.

Similar DNP experiments[32] at 4.6 T and 1.15 K with Gd doping and trityl radicals demonstrated a small beneficial effect on the $^{13}$C polarization without any effect on the buildup time. At 7 T and ~1.4 K, the polarization enhances by only 6%[33] upon addition of 1 mM $Gd^{3+}$. Yoshihara *et al.*[34] also observed a slight boost in polarization upon adding 1.5 mM $Gd^{3+}$ at 7 T and 1.0 K. Note that in another study, the $Gd^{3+}$ dopant at 7 T and 3.7 K has a negative effect on the polarization.[24]

It is widely acknowledged that the trityl radical produces DNP enhancements for $^{13}$C *via* the thermal mixing mechanism almost exclusively at 3.35 T and 1.4 K[35], though experiments at higher temperatures result in a more complicated interplay between mechanisms (Fig. 3).[36] The situation is less clear at 5 T, where the $^{13}$C Larmor frequency should closely match the ESR linewidth of the trityl radical. Previously, the measured experimental homogeneous ESR linewidth for 15 mM trityl radicals at 3.35 T and 1.2 K was reported to be around 63 MHz,[14] which according to theory meets the requirements of the TM mechanism ($\delta\omega_{ESR} > \omega_{0I}$) at 5 T. We would not expect the ESR linewidth to decrease at 5 T.

Wenckebach[37,38] has recently generalized the thermal mixing (TM) theory of Provotorov[39] to low temperatures. In this case, the DNP spectrum can be obtained by knowing only the ESR spectrum and the electron spin–lattice relaxation time $T_{1e}$. Data from that paper shows that the reduction of $T_{1e}$ brings down the polarization and increases the buildup time, in partial agreement with the experimental data here. Note that in the modified TM model $T_{1e}$ variation doesn't have any influence on the DNP microwave sweep positions. Although it seems that the DNP mechanism should be TM for our system in the absence of holmium, the existence of the optimum $T_{1e}$, assuming that Ho-DOTA exclusively acts as an electronic relaxation agent, and reduction in $\Delta_{DNP}$ upon increasing Ho-DOTA concentration provide more evidence of the presence of the cross effect (CE) mechanism.[24,40,41] To be sure that CE is in fact a major mechanism in the system, we will conduct more DNP experiments for lower and higher-$\gamma$ nuclei such as $^{15}$N and $^{31}$P, respectively, as was suggested earlier.[15,35,42,43] Therefore, given our data, and the data of Kiswandhi,[31] we believe that further theoretical investigations of the DNP phenomena under these experimental conditions is also justified.

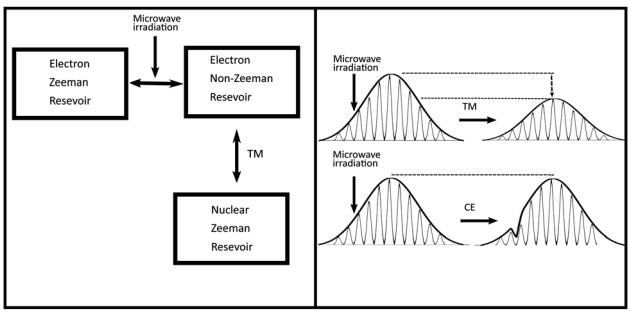


Fig. 3 Left panel: Schematic diagram of thermal mixing (TM), where a model of three interdependent spin baths is invoked. Right panel: The difference between the TM (complete saturation of the ESR line) and the cross effect (CE), where electron spectral diffusion is limited.

## 4 Conclusions

In summary, we have shown that a small amount of $Ho^{3+}$ paramagnetic centers enhances the $^{13}$C DNP and reduces the buildup time at 5 T and 1.2 K. At this field strength, the spacing between maximum polarization peaks in the $^{13}$C DNP spectra reduces upon addition of $Ho^{3+}$. Furthermore, the experimental observations seem to indicate the TM and CE mechanisms are in operation simultaneously, but likely only upon $Ho^{3+}$ doping. In addition, the optimum electron relaxation time is achieved at the ~2 mM Ho-DOTA concentration in the presence of 15 mM OX063 radical. These results are at the same field used for the current clinical polarizer, and hence we believe they can have impact on clinical practice.

## Conflicts of interest

There are no conflicts to declare.

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