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The influence of Ho^{3+} doping on ^{13}C DNP in the presence of BDPA†

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

Polarization transfer from unpaired electron radicals to nuclear spins at low-temperature is achieved using microwave irradiation by a process broadly termed dynamic nuclear polarization (DNP). The resulting signal enhancement can easily exceed factors of 10^4 when paired with cryogenic cooling of the sample. Dissolution-DNP couples low temperature polarization methods with a rapid dissolution step, resulting in a highly polarized solution that can be used for metabolically sensitive magnetic resonance imaging (MRI). Hyperpolarized [1-^{13}C]pyruvate is a powerful metabolic imaging agent for investigation of *in vitro* and *in vivo* cellular metabolism by means of NMR spectroscopy and MRI. Radicals (trityl OX063 and BDPA) with narrower EPR linewidths typically produce higher nuclear polarizations when carbon-13 is the target nucleus. Increased solid-state polarization is observed when narrow line radicals are doped with lanthanide ions such as Gd^{3+}, Ho^{3+}, Dy^{3+}, and Tb^{3+}. Earlier results have demonstrated an incongruence between DNP experiments with trityl and BDPA, where the optimal concentrations for polarization transfer are disparate despite similar electron spin resonance linewidths. Here, the effects of Ho-DOTA on the solid-state polarization of [1-^{13}C]pyruvic acid were compared for 3.35 T (1.4 K) and 5 T (1.2 K) systems using BDPA as a radical. Multiple concentrations of BDPA were doped with variable concentrations of Ho-DOTA (0, 0.2, 0.5, 1, and 2 mM), and dissolved in 1 : 1 (v/v) of [1-^{13}C] pyruvic acid/sulfolane mixture. Our results reveal that addition of small amounts of Ho-DOTA in the sample preparation increases the solid-state polarization for [1-^{13}C] pyruvic acid, with the optimum Ho-DOTA concentration of 0.2 mM. Without Ho-DOTA doping, the optimum BDPA concentration found for 3.35 T (1.4 K) is 40 mM, and for 5 T (1.2 K) system it is about 60 mM. In both systems, inclusion of Ho-DOTA in the ^{13}C DNP sample leads to a change in the breadth (Δ_{DNP}) of the extrema between the P(+) and P(−) frequencies in microwave spectra. At no combination of BDPA and Ho^{3+} did polarizations reach those achievable with trityl. Simplified analysis of increased polarization as a function of decreased electron T_{1e} used to explain results in trityl are insufficient to describe DNP with BDPA.

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1 Introduction

Nuclear magnetic resonance (NMR) is a non-invasive and versatile technique that is associated with high specificity in molecular and structural studies of materials. In the past, the low sensitivity associated with NMR made magnetic resonance spectroscopy for biological specimens using ^{1}H detection challenging, and nearly impossible for low gyromagnetic ratio nuclei such as ^{13}C, ^{15}N, and ^{17}O. Insensitivity associated with these low gyromagnetic ratio nuclei in NMR can be addressed by dynamic nuclear polarization (DNP).[1–6] At moderate magnetic field strengths (>2.5 T), and low temperatures (~1 K), the electron polarization associated with paramagnetic centers doped into a sample can approach unity. Irradiation with microwaves close to the electron Larmor frequency results in polarization transfer to surrounding nuclei, with solid state polarizations of carbon-13 readily reaching 50%.[7] Dissolution DNP (d-DNP) uses superheated water to rapidly melt the hyperpolarized (HP) target, resulting in HP solutions suitable as metabolic contrast agents for use with magnetic resonance imaging (MRI). When paired with isotopic enrichment of the target, the sensitivity of the experiment compared to thermally polarized, natural abundance carbon-13 spectroscopy is easily increased by 5 orders of magnitude. The increased sensitivity allows biochemical reactions in functioning tissues to be observed with 1 s time resolution. Because the HP magnetization decays towards the equilibrium polarization

[a] *Department of Biochemistry and Molecular Biology, University of Florida College of Medicine, Gainesville, USA. E-mail: matthewmerritt@ufl.edu; Tel: +1 352 2948398*
[b] *Department of Physics, University of Florida, Gainesville, USA*
[c] *Advanced Imaging Research Center, UT Southwestern Medical Center, Dallas, TX, USA*

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with the normal T_{1e} relaxation time, ^{13}C enriched small molecules utilized in central carbon metabolism have been the substrates of choice for development of this new class of metabolic contrast agents. These include acetate,[8–10] butyrate,[11] and most importantly pyruvate,[12–17] all of which display long ^{13}C T_{1e} relaxation times when labelling is restricted to the non-protonated carbons.

Maximal polarization enhancement plays a major role in completing ^{13}C NMR or MRI HP experiments successfully. Therefore, optimization of DNP conditions are warranted. The DNP enhancement is affected by several factors that include sample composition and concentration, types of radicals used, glassing agents with or without deuteration,[10,18] lanthanide as a relaxation agent,[7,9,10,13,19–21] microwave power and frequency, temperature, and magnetic field.[22–25] So far, several radicals with either wide or narrow ESR linewidths have been reported to enhance polarization of different types of targeted nuclei.[7,22,26–30] The carbon centered tris 1-benzo(1,2-*d*:4,5-*d*)-bis(1,3)dithiole-4-ylmethyl sodium salt (trityl OX063) has been the DNP agent of choice, producing polarizations in excess of all other free radical centers.[7,9,21,22,31] The ESR linewidth of the trityl radical exceeds the Larmor frequency of ^{13}C (36 MHz) at 3.35 T, a field commonly used for these experiments. In this experimental condition, DNP is generally considered to occur by a three spin effect involving two electrons and a dipolar coupled nucleus (the cross-effect, CE) or by thermal mixing (TM). TM is readily described using the Borghini spin temperature model, which has been recently extended to high polarizations by Wenckebach.[32] However, other work suggested that a third mechanism, the electron-nuclear two-spin solid effect (SE) might also contribute at 3.35 T and at temperatures 10 K or higher.[25] It is not clear however that this description holds at temperatures close to 1 K, as another report showed a strict conservation of spin temperature between ^{13}C and ^{89}Y, an observation that would normally be used as strong evidence of being exclusively in the thermal mixing regime.[6] While trityl is very effective as a polarization agent, its production is also very expensive, limiting its ready availability to some research groups. The free radical 1,3-bisdiphenylene-2-phenylallyl (BDPA) has an ESR linewidth even more narrow than that of trityl, and is more readily available. It also has the added advantage of being readily removed from the sample prep after dissolution, as it is insoluble in water and can be easily filtered.[28] To first approximation, the linewidth of the radical is equivalent to the heat capacity of the electron dipolar system in TM.[33] Therefore, with the condition that the ESR linewidth exceeds the nuclear Larmor frequency, a more narrow line should produce greater nuclear polarization. BDPA displays almost no *g*-anisotropy, and produces a microwave frequency sweep spectrum even narrower than trityl.[22] With this rationale, BDPA should produce superior ^{13}C polarization in comparison to trityl. Instead, BDPA has consistently produced lesser polarization than trityl in the experimental regime used for d-DNP.[28] In addition, the optimal concentration for polarization enhancement for BDPA at 3.35 T was demonstrated to be around 40 mM, as compared to the 15 mM concentration commonly used for trityl. Notably, a water soluble derivative of BDPA produced polarizations equivalent to that of trityl, but again the concentration of the radical was 40 mM.[34]

Previously, many groups have also reported that doping samples with lanthanide metal ions such as Gd^{3+} and Ho^{3+} into either trityl OX063, BDPA or 4-oxo-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) is associated with increasing solid-state polarization of some ^{13}C-enriched biomolecules.[7,20,35] Inclusion of 1 to 2 mM Gd^{3+} in DNP samples containing 15 mM trityl as a radical is a well-established protocol for many groups.[7,9,19–21,36] Holmium has recently gained attention for producing polarizations in excess of those achieved with Gd^{3+}, presumably because of modulation of T_{1e}.[9,19,20] If the source of divergent results between BDPA and trityl is related to the T_{1e}, a proper choice of relaxation agent should be able to produce congruent polarization between the two agents. The goal of this project was to determine if an optimal mixture of BDPA and a holmium based relaxation agent could produce enhancements similar to those of trityl. In addition, experiments were carried out at 3.35 T and 5 T, a field strength now emerging as the standard of operation. The optimum concentration of BDPA alone at 5 T is 60 mM in these conditions. We found that addition of 0.2 mM of Ho^{3+} produces the highest solid-state nuclear polarization at both magnetic fields. Experiments using 15 mM BDPA and various concentrations of Ho^{3+} failed to produce polarizations similar to that of higher BDPA concentrations. We conclude from this that the need for higher concentrations of BDPA to produce optimal nuclear polarization is unlikely to be related to T_{1e} effects. Furthermore, the observed frequencies of maximum DNP enhancement are not readily predicted by simple application of TM, CE, or SE theories.

2 Materials and methods

2.1 Materials

All chemical used in this report were purchased and used without further purification from commercial sources:

BDPA from Sigma-Aldrich, [1-^{13}C] pyruvic acid from Cambridge Isotope Lab, Sulfolane from Alfa-Aesar. Ho-DOTA was synthesized as reported previously.[37]

2.2 Sample preparation

All samples were prepared on the same day as the DNP experiments, as decreasing polarization was observed in samples stored in as little as 24 hours. BDPA solutions (15 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, and 100 mM) were made by adding the calculated BDPA mass to 1 : 1 v/v [1-^{13}C] pyruvic acid : sulfolane. Due to the difficulty in weighing out correct weights of BDPA, we prepared the stock solutions of 120 mM and/or 240 mM of BDPA in sulfolane and added appropriate amounts of [1-^{13}C] pyruvic acid to achieve the desired concentrations. To maintain equal numbers of ^{13}C spins, the Ho-DOTA was added in 1 : 1 v/v [1-^{13}C]pyruvic acid : sulfolane as well. The sample sizes were 25 μl for both 3.35 T and 5 T magnet systems. Structures for BDPA and Ho-DOTA discussed in this study are shown in ESI,† Fig. S1.

2.3 Solid-state polarization measurement

All DNP experiments were performed at the University of Florida in 3.35 T (HyperSense) and 5 T (homebuilt) systems. The HyperSense polarizer (Oxford Instruments, UK) operates at 1.4 K in the cryostat sample space. The microwave power was set at 100 mW using an ELVA microwave source (ELVA-1 millimeter Wave Division, RU) that has a 400 MHz sweepable frequency range. For microwave sweep spectra at 3.35 T, 25 μL aliquots of DNP samples were polarized for 5 min at each frequency. The data from the HyperSense was analyzed by MATLAB (MathWorks Inc., Natick MA). In the 5 T DNP home-built system, the NMR signals were collected using a TecMag (APOLLO) spectrometer at 1.2 K. The microwave frequency sweeps were accomplished with irradiation for 3 minutes at each time point. To monitor the build-up polarizations, a low flip-angle pulse ($\sim 5^\circ$) was applied every 3 min. All DNP enhancement curves as a function of time were recorded with the microwave frequency set to the maximum P(+) enhancement. The frequency difference between P(+) and P(−) peak positions was used to calculate Δ_{DNP}. Error in the buildup curves is $\sim\pm 5\%$ of the total polarization, based on repeated buildup curves of a single sample. For both systems, hard pulse trains were applied to destroy polarization before measuring the next frequency step.

The solid-state NMR signal enhancement "ε_S" was calculated using the equation:

$$\varepsilon_S = P_{hp}/P_{th} \quad (1)$$

where P_{hp} is the polarization from solid-state DNP experiment and is given as $P_{hp} = \tanh(h\nu_n/2k_BT_s)$, the Brillouin function. Similarly, P_{th} is the polarization correspond to thermal experiment and expressed as $P_{th} = \tanh(h\nu_n/2k_BT_L)$. Here "$h$" is the Planck's constant, "k_B" is Boltzmann constant, "ν_n" is nuclear Larmor frequency, "T_s" and "T_L" are the given spin and lattice temperatures, respectively.

The liquid-state NMR signal enhancement "ε_L" was calculated as:

$$\varepsilon_L = (I_{hp}/I_{th})(\sin\theta_{th}/\sin\theta_{hp})\,(C_{th}/C_{hp})\exp(T_r/T_{1e}) \quad (2)$$

"I" denotes the integrated area, "$\sin\theta$" is the RF flip angle for NMR, "C" is the concentration of [1-^{13}C] pyruvic acid, and "T_r" is the repetition time used in NMR experiment.[6] The solid and liquid-state NMR signal enhancements were utilized to determine percentage polarization for 5 T and 3.35 T, respectively. The liquid state enhancement was used to estimate the total polarization, as the signal to noise ratio (SNR) of the thermally polarized solid-state NMR spectrum was low and unsuitable for normalization.[38]

3 Results and discussion

The optimal DNP frequency as a function of concentration of BDPA and Ho-DOTA in 1 : 1 (v/v) of sulfolane/[1-^{13}C]pyruvic acid for 3.35 T displayed almost no variance (Fig. 1 and Table 1). For BDPA, Δ_{DNP} has a strong dependence on the field strength. Δ_{DNP}

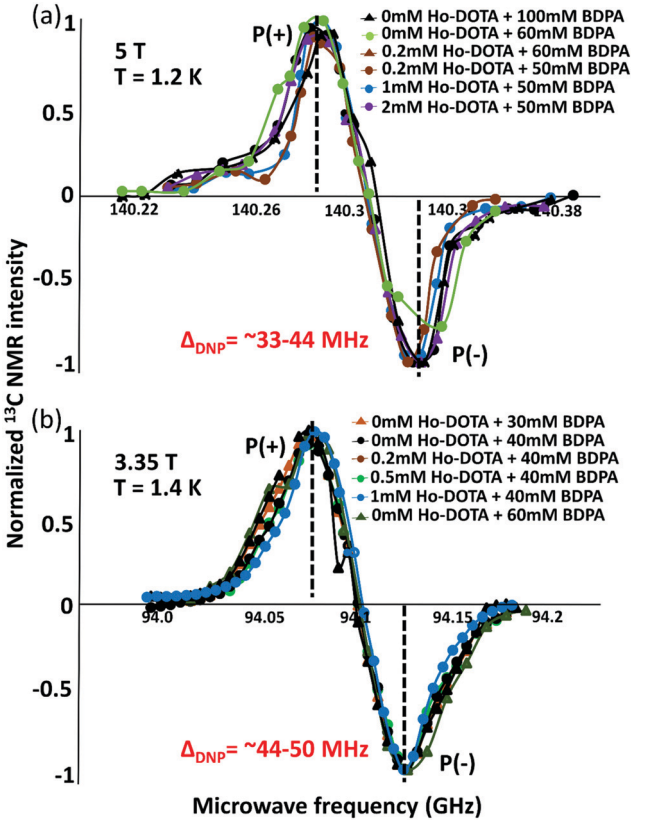

Fig. 1 Normalized ^{13}C DNP microwave sweeps of 1 : 1 (v/v) sulfolane/[1-^{13}C]pyruvic acid in the presence of variable concentrations of BDPA radical and Ho-DOTA. (a) At 5 T, (50 or 60 or 100 mM) BDPA doped with 0, 0.2, 1, 2 mM of Ho-DOTA. (b) At 3.35 T with 30 or 40 or 60 mM BDPA doped with 0, 0.2, 0.5, and 1 mM Ho-DOTA. Note the slight shift in the locations of P(+) and P(−) towards each other with increasing concentrations of Ho-DOTA for both systems.

is narrower at 5 T (35 MHz) as compared to 3.35 T (45–50 MHz). At both fields, the addition of Ho-DOTA also seems to narrow Δ_{DNP} (Table 1), though in greater magnitude at 5 T. However, variable concentrations of BDPA (without Ho-doping) do not significantly alter Δ_{DNP} at either field strength. Increasing concentrations of Ho-DOTA also produce an apparent narrowing of both the P(+) and P(−) manifolds above 0.2 mM (Fig. 1).

In contrast to the similarity of the microwave sweep data, the DNP buildup curves as a function of time showed strong dependencies on both the BDPA and Ho-DOTA concentrations (Fig. 2). Since all of these ^{13}C DNP experiments were performed with similar experimental conditions that include freshly prepared samples, constant volume (25 μL), similar temperatures (1.2 K for 5 T and 1.4 K for 3.35 T), the same sample cup, and the solvent system, their polarizations can be quantified relative to each other.

At 3.35 T, the optimal concentration for BDPA in 1 : 1 (v/v) sulfolane/[1-^{13}C]pyruvic acid was found to be 40 mM, as previously reported.[7,22] However, the optimal BPDA concentration at 5 T is more difficult to establish (Fig. 2a). BDPA concentrations of 40, 50, and 60 mM produced increasingly shorter buildup rate constants, but should plateau at essentially

Table 1 The Δ_{DNP} at 5 T and 3.35 T for pyruvic acid samples prepared with BDPA in sulfolane

5 T system at 1.2 K			3.35 T system at 1.4 K		
Concentration of Ho-DOTA (mM)	Concentration of BDPA (mM)	Δ_{DNP} in MHz for 5 T	Concentration of Ho-DOTA (mM)	Concentration of BDPA (mM)	Δ_{DNP} in MHz for 3.35 T
0	100	42.0	0	30	50.0
0	60	43.8	0	40	48.0
0.2	60	33.6	0.2	40	47.0
0.2	50	37.9	0.5	40	45.0
1	50	36.0	1	40	44.5
2	50	34.8	0	60	48.0

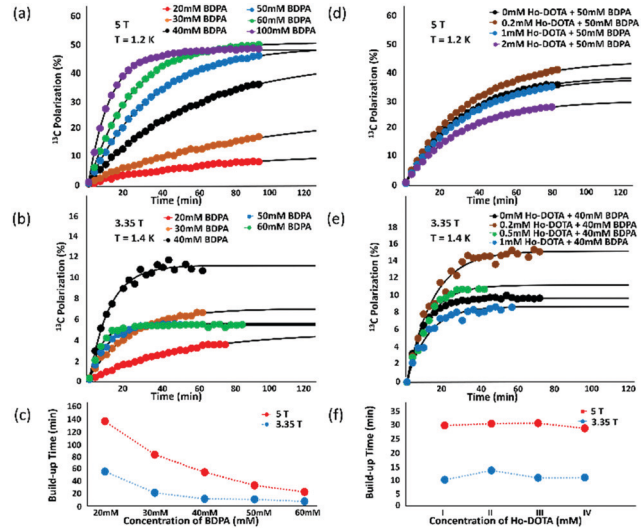

Fig. 2 Time dependent ^{13}C DNP polarization of 25 μl of 1 : 1 (v/v) sulfolane/[1-^{13}C]pyruvic acid (solid state) in the presence of variable concentrations of BDPA radical (20, 30, 40, 50, 60 mM) at (a) 5 T and (b) 3.35 T. (c) DNP buildup times as extracted from fitting with a single exponential for various concentrations of BDPA. Buildup times decrease with increasing concentration of BDPA at both field strengths. Similarly, Fig. 2d and e, and f are ^{13}C DNP polarization buildups for 1 : 1 (v/v) sulfolane/[1-^{13}C]pyruvic acid with BDPA and various concentrations of Ho-DOTA (0, 0.2, 0.5, 1 mM) (d) 50 mM BDPA for 5 T system. Note the 50 mM BDPA curve with no Ho-DOTA does not match the observed polarization in (a) as the samples were prepared in different batches. With such high concentrations of radical in limited volumes, excursions in absolute polarizations were nearly unavoidable. (e) 40 mM BDPA for 3.35 T system (f) Buildup times as a function of Ho-DOTA concentration with 40 (at 3.35 T) or 50 mM (at 5 T) BDPA radical. The samples were polarized at the positive polarization peak with a 100 mW microwave power source.

equivalent levels of polarization at long times, as evidenced by the fits to the data (solid lines). The build-up time always decreases with increasing concentration of BDPA at both field strengths, though the drop in rate is more precipitous at 5 T (Fig. 2c). To simplify the research plan, 50 mM BDPA was chosen for experiments assessing the effects of Ho-DOTA on the polarization efficiency. Addition of Ho-DOTA to BDPA samples was expected to mimic results of a similar study with the trityl radical. As compared to the trityl radical at 5 T, addition of Ho-DOTA to BDPA samples produced an increase in polarization (~13%) at only the lowest assayed concentration (0.2 mM) and a rapid fall in polarization at 1 and 2 mM (Fig. 2d). At 3.35 T, 0.2 mM Ho-DOTA produced an almost 50% increase in polarization, but addition to 0.5 mM produced only a 10% increase (Fig. 2e). At 1 mM, the polarization decreased below that of the 40 mM BDPA only (control) sample. The polarization build up times did not change as a function of [Ho-DOTA] (Fig. 2f). Previous measurements of T_{1e} for BDPA compared to the trityl radical showed a shorter longitudinal relaxation time for BDPA, which helped rationalize a stronger enhancement of signal for trityl as a function of Gd^{3+} concentration.[22] In essence, due to the longer T_{1e} of trityl at 15 mM concentration, addition of an electron relaxation agent could modulate the T_{1e} more effectively as compared to BDPA. If the Ho-DOTA enhances DNP efficiency primarily through a T_{1e} shortening effect, then the shorter T_{1e} of BDPA as compared to trityl "leaves less room" for the agent to improve DNP efficiency before the T_{1e} becomes so short as to prevent significant saturation of the electron dipolar system.[7,22] Similar DNP studies were performed by Kiswandhi[9] at 3.35 T (1.4 K) for sodium [1-^{13}C]acetate with 15 mM Trityl, doped with 2 mM Ho-DOTA, and demonstrated an enhancement of 2.7 times better ^{13}C nuclear polarization. Our group performed the same experiment in 5 T (1.2 K) system and found ~1.5 times better enhancement on sodium [1-^{13}C]acetate nuclear polarization.[19] Several other groups reported a small beneficial effect on ^{13}C solid state polarization for Gd^{3+} doped DNP samples with trityl in different magnetic fields, with temperature ranges from 1.4 to 3.7 K.[7,17,26,39]

Moreover, we have also evaluated the effects of 0.2 mM Ho-DOTA with variable concentrations of BDPA (ESI,† Fig. S2). For 5 T (ESI,† Fig. S2a), 60 mM BDPA doped with 0.2 mM of Ho-DOTA show higher solid-state polarization than for lower BDPA concentrations. In contrast, for 3.35 T, the optimal BDPA concentration shifted (ESI,† Fig. S2b) to 30 mM when doped with 0.2 mM Ho DOTA. The ability of Ho-DOTA addition to lower the concentration of BDPA, optimizing solid-state polarization, does not translate from 3.35 T to 5 T.

As a final attempt to replicate the experimental conditions that produce optimal polarization for trityl, samples prepared with 15 mM BDPA were doped with variable concentrations of Ho-DOTA (Fig. 3a and b). At 5 T, 0.2 mM Ho-DOTA produced about 85% increase in polarization with a similar 70% increase for 3.35 T. However, concentrations of Ho-DOTA above 0.2 mM display different dependencies on field strength. At 5 T, a rapid fall in polarization was observed at 0.5 and 1 mM (Fig. 3a) that lie below the control (*i.e.* 15 mM BDPA only). But the polarization for the 0.5 and 1 mM lie above the control at 3.35 T (Fig. 3b). The build-up times for various concentrations of

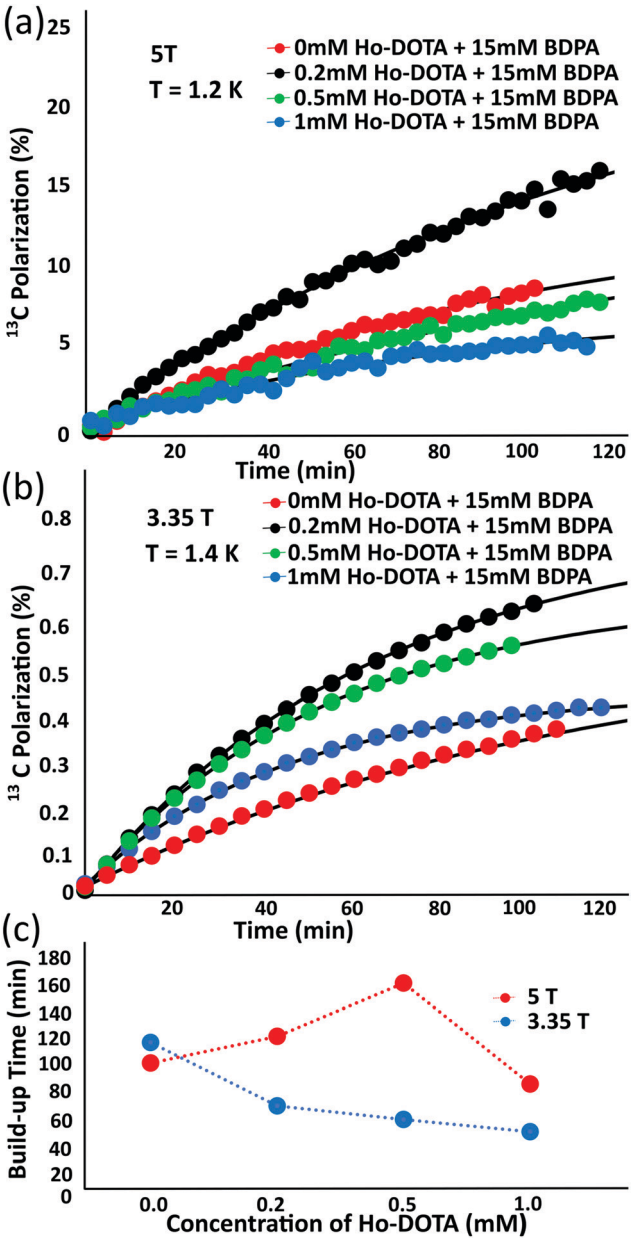

Fig. 3 ^{13}C DNP polarization of 25 μl of 1 : 1 (v/v) sulfolane/[1-^{13}C]pyruvic acid in the presence of 15 mM BDPA radical doped with various concentrations of Ho-DOTA (0, 0.2, 0.5, 1 mM) (a) at 5 T (b) at 3.35 T (c) buildup times for various concentrations of Ho-DOTA with 15 mM BDPA radical. The samples were polarized at the positive polarization peak with a 100 mW microwave source. At 3.35 T, the buildup time decreases with increase in concentration of Ho-DOTA. At 5 T, after an initial increase in buildup time, there is a sharp decrease at 1.0 mM Ho-DOTA.

Ho-DOTA with 15 mM BDPA radical are also field dependent (Fig. 3c). For 3.35 T, build-up time decreased with increasing concentration of Ho-DOTA, but initially rose, and then fell at 5 T.

Fig. 2 and 3 demonstrate that ^{13}C DNP using BDPA has complicated, field dependent effects. The addition of Ho-DOTA improves the polarization, but it is difficult to rationalize the observed effects using logic based on our current understanding of the SE, CE, and TM mechanisms. As is well known, and recapitulated here, optimal BDPA concentrations for ^{13}C signal enhancement peak at values 2 or even 3 times greater than those needed for trityl. A higher concentration for BDPA is necessary to achieve maximum solid state enhancements at 5 T than at 3 T. In the absence of Ho-DOTA, DNP by BDPA is likely well described by the thermal mixing mechanism at 3.35 T and 1.4 K.[28,40] According to the thermal mixing theory at very low temperatures (high polarization), as given by Wenckebach, the DNP spectrum can be predicted with the ESR spectrum and an estimate of the inverse spin temperature of the electron non-Zeeman reservoir.[32] However, this theory applies strictly when the ESR line is broadened by *g*-value anisotropy (for example, TEMPO), as opposed to our case for BDPA. At W-band, the ESR linewidth for BDPA is substantially more narrow and shows less anisotropy than trityl.[22] The observed Δ_{DNP} for BDPA at 3.35 T matches the expected ESR linewidth, suggestive of TM as the dominant mechanism. However, at 5 T, Δ_{DNP} drops by ~10% and continues to decline as Ho-DOTA concentration increases. The observation of a drop in Δ_{DNP} is usually equated with a transition to the CE mechanism.[31,41] Transition from TM to CE should however be accompanied by a switch of Δ_{DNP} to close to the Larmor frequency. At 5 T, Δ_{DNP} is less than the ^{13}C Larmor frequency (53 MHz). With the observed narrowing of the DNP sweep spectrum, it seems that contributions of the SE should be discounted, as Δ_{DNP} would be expected to approach ~106 MHz (2× Larmor frequency) in this limit.

At the 15 mM target concentration for BDPA, adding Ho-DOTA systematically lowers the buildup time constant at 3.35 T (Fig. 3c), consistent with a T_{1e} shortening effect and thermal mixing. But, the absolute polarization is strikingly low *versus* higher concentrations of BDPA, and is 30 to 40 times less *versus* that of optimal trityl formulations.[35] If T_{1e} shortening alone was sufficient to produce polarizations equivalent to higher concentrations of BDPA, the array of concentrations for Ho-DOTA used should have been able to produce polarizations close to 12% and 50% at 3.35 T and 5 T respectively. The 15 mM concentration of BDPA is significantly worse than higher concentrations at 5 T, but still manages to exceed 15% at long build up times. The precipitous drop in polarization levels at 3.35 T and lower concentrations suggest that a precondition for thermal mixing (a single, well coupled electron dipolar system) are somehow not met at lower BDPA concentrations.

Addition of Ho^{3+} has numerous effects compared to the addition of Gd^{3+} on polarization with BDPA. Ho^{3+} doping does not significantly shorten the ^{13}C T_{1e} after dissolution, unlike Gd^{3+}.[9] For the trityl radical, Gd^{3+} still produces superior gains compared to Ho^{3+} in solid state polarization at 3.35 T.[9] However, Gd^{3+} doping produces only a ~16% increase in polarization for BDPA at 3.35 T, as compared to ~52% for Ho^{3+}.[7] Like Gd^{3+}, the gains in polarization for Ho^{3+} are more subtle at 5 T. We also note a recent report that showed ligand design can dramatically affect the polarization realized in the case of magic-angle-spinning DNP.[42]

4 Conclusions

Many factors suggest that BDPA should provide superior polarization to the trityl radical. Here, both BDPA concentrations

and the effects of lanthanide doping were assayed simultaneously to ascertain a sample formulation that would deliver superior polarization to trityl without success. BDPA represents a significant experimental challenge, as the high concentrations of radical needed present difficulties in preparing the samples consistently, even using a larger volume stock solution that enabled larger weights of radical to be used. These solutions must be prepared daily, as degradation of polarization after overnight storage was a guaranteed effect. Instead of identifying a superior sample formulation, a series of physical observations that seem to contradict current dogma surrounding DNP mechanisms is reported. We suggest that current theory must be augmented to correctly explain these observations.

Conflicts of interest

There are no conflicts to declare.

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References

1 A. Abarca, P. Gómez-Sal, A. Martín, M. Mena, J. M. Poblet and C. Yélamos, *Inorg. Chem.*, 2000, **39**, 642–651.
2 V. K. Michaelis, T. C. Ong, M. K. Kiesewetter, D. K. Frantz, J. J. Walish, E. Ravera, C. Luchinat, T. M. Swager and R. G. Griffin, *Isr. J. Chem.*, 2014, **54**, 207–221.
3 R. G. Griffin and T. F. Prisner, *Phys. Chem. Chem. Phys.*, 2010, **12**, 5737–5740.
4 B. Corzilius, A. A. Smith, A. B. Barnes, C. Luchinat, I. Bertini and R. G. Griffin, *J. Am. Chem. Soc.*, 2011, **133**, 5648–5651.
5 A. B. Barnes, G. D. Paëpe, P. C. van der Wel, K. N. Hu, C. G. Joo, V. S. Bajaj, M. L. Mak-Jurkauskas, J. R. Sirigiri, J. Herzfeld, R. J. Temkin and R. G. Griffin, *Appl. Magn. Reson.*, 2008, **34**, 237–263.
6 L. Lumata, A. K. Jindal, M. E. Merritt, C. R. Malloy, A. D. Sherry and Z. Kovacs, *J. Am. Chem. Soc.*, 2011, **133**, 8673–8680.
7 L. Lumata, M. E. Merritt, C. R. Malloy, A. D. Sherry and Z. Kovacs, *J. Phys. Chem. A*, 2012, **116**, 5129–5138.
8 A. Flori, M. Liserani, F. Frijia, G. Giovannetti, V. Lionetti, V. Casieri, V. Positano, G. D. Aquaro, F. A. Recchia, M. F. Santarelli, L. Landini, J. H. Ardenkjaer-Larsen and L. Menichetti, *Contrast Media Mol. Imaging*, 2015, **10**, 194–202.
9 A. Kiswandhi, P. Niedbalski, C. Parish, P. Kaur, A. Martins, L. Fidelino, C. Khemtong, L. Song, A. D. Sherry and L. Lumata, *Phys. Chem. Chem. Phys.*, 2016, **18**, 21351–21359.
10 A. Flori, M. Liserani, S. Bowen, J. H. Ardenkjaer-Larsen and L. Menichetti, *J. Phys. Chem. A*, 2015, **119**, 1885–1893.
11 D. R. Ball, B. Rowlands, M. S. Dodd, L. Le Page, V. Ball, C. A. Carr, K. Clarke and D. J. Tyler, *Magn. Reson. Med.*, 2014, **71**, 1663–1669.
12 S. E. Day, M. I. Kettunen, M. K. Cherukuri, J. B. Mitchell, M. J. Lizak, H. D. Morris, S. Matsumoto, A. P. Koretsky and K. M. Brindle, *Magn. Reson. Med.*, 2011, **65**, 557–563.
13 L. Friesen-Waldner, A. Chen, W. Mander, T. J. Scholl and C. A. McKenzie, *J. Magn. Reson.*, 2012, **223**, 85–89.
14 R. E. Hurd, Y. F. Yen, D. Mayer, A. Chen, D. Wilson, S. Kohler, R. Bok, D. Vigneron, J. Kurhanewicz, J. Tropp, D. Spielman and A. Pfefferbaum, *Magn. Reson. Med.*, 2010, **63**, 1137–1143.
15 M. I. Kettunen, D. E. Hu, T. H. Witney, R. McLaughlin, F. A. Gallagher, S. E. Bohndiek, S. E. Day and K. M. Brindle, *Magn. Reson. Med.*, 2010, **63**, 872–880.
16 L. Lumata, Z. Kovacs, C. Malloy, A. D. Sherry and M. E. Merritt, *Phys. Med. Biol.*, 2011, **56**, N85–N92.
17 H. A. Yoshihara, E. Can, M. Karlsson, M. H. Lerche, J. Schwitter and A. Comment, *Phys. Chem. Chem. Phys.*, 2016, **18**, 12409–12413.
18 Y. Zhang, P. J. Baker and L. B. Casabianca, *J. Phys. Chem. B*, 2016, **120**, 18–24.
19 A. A. Sirusi, E. H. Suh, Z. Kovacs and M. E. Merritt, *Phys. Chem. Chem. Phys.*, 2018, **20**, 728–731.
20 J. W. Gordon, S. B. Fain and I. J. Rowland, *Magn. Reson. Med.*, 2012, **68**, 1949–1954.
21 E. Ravera, D. Shimon, A. Feintuch, D. Goldfarb, S. Vega, A. Flori, C. Luchinat, L. Menichetti and G. Parigi, *Phys. Chem. Chem. Phys.*, 2015, **17**, 26969–26978.
22 L. Lumata, Z. Kovacs, A. D. Sherry, C. Malloy, S. Hill, J. van Tol, L. Yu, L. Song and M. E. Merritt, *Phys. Chem. Chem. Phys.*, 2013, **15**, 9800–9807.
23 E. V. Kryukov, K. J. Pike, T. K. Tam, M. E. Newton, M. E. Smith and R. Dupree, *Phys. Chem. Chem. Phys.*, 2011, **13**, 4372–4380.
24 E. V. Kryukov, M. E. Newton, K. J. Pike, D. R. Bolton, R. M. Kowalczyk, A. P. Howes, M. E. Smith and R. Dupree, *Phys. Chem. Chem. Phys.*, 2010, **12**, 5757–5765.
25 D. Banerjee, D. Shimon, A. Feintuch, S. Vega and D. Goldfarb, *J. Magn. Reson.*, 2013, **230**, 212–219.
26 J. H. Ardenkjaer-Larsen, S. Macholl and H. Johannesson, *Appl. Magn. Reson.*, 2008, **34**, 509–522.
27 U. L. Günther, *Top. Curr. Chem.*, 2013, **335**, 23–69.
28 L. Lumata, S. J. Ratnakar, A. Jindal, M. E. Merritt, A. Comment, C. Malloy, A. D. Sherry and Z. Kovacs, *Chem. – Eur. J.*, 2011, **17**, 10825–10827.
29 P. Niedbalski, C. Parish, A. Kiswandhi and L. Lumata, *Magn. Reson. Chem.*, 2016, **54**, 962–967.
30 L. Lumata, M. E. Merritt, C. Khemtong, S. J. Ratnakar, J. van Tol, L. Yu, L. Song and Z. Kovacs, *RSC Adv.*, 2012, **2**, 12812–12817.
31 S. A. Walker, D. T. Edwards, T. A. Siaw, B. D. Armstrong and S. Han, *Phys. Chem. Chem. Phys.*, 2013, **15**, 15106–15120.

32 W. T. Wenckebach, *J. Magn. Reson.*, 2017, **277**, 68–78.
33 S. Goertz, *Nucl. Instrum. Methods Phys. Res., Sect. A*, 2004, **526**, 28–42.
34 J. L. Muñoz-Gómez, E. Monteagudo, V. Lloveras, T. Parella, J. Veciana and J. Vidal-Gancedo, *Org. Biomol. Chem.*, 2015, **13**, 2689.
35 J. H. Ardenkjær-Larsen, S. Macholl and H. Johannesson, *Appl. Magn. Reson.*, 2008, **34**, 509–522.
36 Andhika Kiswandhi, Bimala Lama, Peter Niedbalski, Mudrekh Goderya, Joanna Long and L. Lumata, *RSC Adv.*, 2016, **6**, 38855.
37 J. F. Desreux, *Inorg. Chem.*, 1980, **19**, 1319–1324.
38 J. J. Miller, L. Cochlin, K. Clarke and D. J. Tyler, *Magn. Reson. Med.*, 2017, **78**, 2082–2094.
39 H. Jóhannesson, S. Macholl and J. H. Ardenkjaer-Larsen, *J. Magn. Reson.*, 2009, **197**, 167–175.
40 L. Lumata, M. E. Merritt and Z. Kovacs, *Phys. Chem. Chem. Phys.*, 2013, **15**, 7032–7035.
41 C. F. Hwang and D. A. Hill, *Phys. Rev. Lett.*, 1967, **19**, 1011.
42 G. Stevanato, D. J. Kubicki, G. Menzildjian, A. S. Chauvin, K. Keller, M. Yulikov, G. Jeschke, M. Mazzanti and L. Emsley, *J. Am. Chem. Soc.*, 2019, **141**, 8746–8751.