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Spin-Lattice Relaxation of Hyperpolarized Metronidazole in Signal Amplification by Reversible Exchange in Micro-Tesla Fields

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Abstract

Simultaneous reversible chemical exchange of parahydrogen and to-be-hyperpolarized substrate on metal centers enables spontaneous transfer of spin order from parahydrogen singlet to nuclear spins of the substrate. When performed at sub-micro-Tesla magnetic field, this technique of NMR Signal Amplification by Reversible Exchange in SHield Enables Alignment Transfer to Heteronuclei (SABRE-SHEATH). SABRE-SHEATH has been shown to hyperpolarize nitrogen-15 sites of a wide range of biologically interesting molecules to a high polarization level ($P > 20\%$) in one minute. Here, we report on a systematic study of ^1H , ^{13}C and ^{15}N spin-lattice relaxation (T_1) of metronidazole- $^{13}\text{C}_2$ - $^{15}\text{N}_2$ in SABRE-SHEATH hyperpolarization process. In micro-Tesla range, we find that all ^1H , ^{13}C and ^{15}N spins studied share approximately the same T_1 values (*ca.* 4 s at the conditions studied) due to mixing of their Zeeman levels, which is consistent with the model of relayed SABRE-SHEATH effect. These T_1 values are significantly lower than those at higher magnetic (*i.e.* the Earth's magnetic field and above), which exceed 3 minutes in some cases. Moreover, these relatively short T_1 values observed below 1 micro-Tesla limit the polarization build-up process of SABRE-SHEATH—thereby, limiting maximum attainable ^{15}N polarization. The relatively short nature of T_1 values observed below 1 micro-Tesla is primarily caused by intermolecular interactions with quadrupolar iridium centers or dihydride protons of the employed polarization transfer catalyst, whereas intramolecular spin-spin interactions with ^{14}N quadrupolar centers have significantly smaller contribution. The presented experimental results and their analysis will be beneficial for more rational design of SABRE-SHEATH (i) polarization transfer catalyst, and (ii) hyperpolarized molecular probes in the context of biomedical imaging and other applications.

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Supporting Information. The following files are available free of charge. Table S1 providing numerical values and error bars for Figure 6. Table S2 providing numerical values and error bars for Figure 7. Figure S1 providing ^{15}N T_1 measurement at ~ 0.1 μT .

TOC GRAPHICS

INTRODUCTION

NMR hyperpolarization techniques^{1–10} enable amplification of nuclear spin polarization by several orders of magnitude resulting in the corresponding gains in the detected NMR signal and signal-to-noise ratio (SNR). This significant signal gain can be employed for a wide range of applications including biomedical imaging applications,^{7, 11–19} which have been the main driver behind the development and refinement of NMR hyperpolarization techniques.

In biomedical applications, a bolus of hyperpolarized (HP) fluid is typically prepared first,^{8, 20–21} which is then purified from other compounds facilitating hyperpolarization process (e.g. catalysts, radicals, etc.); finally it is injected^{22–23} in a living organism. The injected or inhaled²⁴ HP contrast agent can be imaged,^{25–27} and it can serve as a reporter probe or regional metabolism or function.^{7, 28–30}

Signal Amplification by Reversible Exchange (SABRE) is one of the most recent (*ca.* 2009) hyperpolarization techniques pioneered by Duckett and co-workers.^{31–34} This technique relies on a simultaneous chemical exchange process of parahydrogen (*para*- H_2) and to-be-hyperpolarized substrate compound on a metal complex, Scheme 1a. The spontaneous polarization transfer from parahydrogen-derived hydrides to nuclear spins of the to-be-hyperpolarized substrate³⁵ is the most efficient, when the difference in NMR frequencies is matched to a combination of J -couplings in the SABRE complex.³⁶ For protons, the matching condition corresponds to the field range of a several milli-Tesla (mT),^{31–33} whereas for heteronuclei (e.g. ^{15}N ,³⁷ ^{13}C ,^{38–39} etc.^{40–41}), the optimal matching field is on the order of 1 micro-Tesla (μT) or below, and the corresponding acronym SABRE-SHEATH (SABRE in SHield Enables Alignment Transfer to Heteronuclei) was coined.⁴² Hexacoordinate Ir-based complexes^{36, 43} have been shown to be the most potent in SABRE hyperpolarization, delivering the highest levels of polarization to date.^{44–46} Moreover, over the recent years, the SABRE technique has been expanded to a wide range of substrates,^{47–54} which will likely continue to grow rapidly. Furthermore, SABRE and SABRE-SHEATH have been demonstrated in an aqueous medium^{55–60} and on heterogeneous catalysts,^{61–63} the key requirements for production of pure aqueous HP fluids by SABRE technique. When combined, these developments will likely enable fast (within 1 minute)

production of HP injectable compounds using relatively simple hardware (compared to other HP techniques).

SABRE hyperpolarization of heteronuclei^{37, 64–66} is advantageous, because the lifetime of their HP state induced through the SABRE hyperpolarization process is at least several times longer than that of protons.⁴⁹ For example, the exponential decay constant of ¹⁵N sites can reach up to 20 minutes in some cases.^{67–70} ¹⁵N sites are also interesting, because they have a very wide dynamic range of chemical shifts, which is sensitive to the local micro-environment such as pH,^{71–72} ions,⁷³ etc., and therefore, HP ¹⁵N sites can potentially act as reporters of pH,^{71–72} hypoxia,⁴⁵ etc.⁷³ Until recently, efficient SABRE-SHEATH hyperpolarization of ¹⁵N directly interacting with Ir catalyst center (e.g. ¹⁵N₃ in Scheme 1b) has been observed.^{42, 45} However, we recently demonstrated that SABRE-SHEATH polarization can be relayed from these directly participating ¹⁵N sites to other ¹³C and ¹⁵N nuclei within the same molecule.⁷⁴ As a result, long-range ¹³C and ¹⁵N can be hyperpolarized via spin-½ relays (i.e. via J-coupling between nearly spin-½ sites).⁷⁴ Such spin relays may significantly expand the range of biologically relevant compounds amenable to SABRE and SABRE-SHEATH processes.⁷⁴

In our recent article, we have provided mechanistic evidences for the importance of spin ½ networks for the relayed polarization transfer.⁷⁴ The work presented here focuses on the systematic *T*₁ relaxation studies in the same biomolecule of metronidazole, an antibiotic that can be injected in large dose (~2 g per human⁷⁵). Moreover, this compound contains the same molecular motif of nitroimidazole moiety, which is frequently employed in ¹⁸F-labeled molecular probes of hypoxia sensing via Positron Emission Tomography (PET).^{76–80} Our focus on metronidazole is therefore determined by the potential use of this HP compound as a reporter probe of hypoxia.

METHODS

Bruker 9.4 T Avance III was used to record all NMR spectra. Most SABRE and SABRE-SHEATH experiments were performed using ~50% *para*-H₂. All enhancements reported were produced using 50% *para*-H₂. Some other experiments (for relaxation studies) were performed using ~80% *para*-H₂ produced by custom-made *para*-H₂ generator. The SABRE and SABRE-SHEATH procedure was performed using the setup and manipulation steps described in Figure 1. Metronidazole-¹⁵N₂-¹³C₂ (Millipore-Sigma P/N 32744-10MG) solutions in methanol-*d*₄ were placed in medium-walled 5 mm NMR tubes (Wilmad Glass, P/N 503-PS-9). These NMR tubes (9 inches long and 3.43-mm inner diameter) were equipped with a Teflon tube extension: ¼ inches outer diameter). The NMR spectra from ¹³C and ¹⁵N signal reference samples were obtained using standard-wall (0.38 mm) 5 mm tubes. Previously prepared IrIMes catalyst was used for the described studies.⁸¹

Methanol-*d*₄ solutions containing SABRE catalyst and metronidazole-¹⁵N₂-¹³C₂ or natural abundance (n.a.) metronidazole were prepared as described previously.⁷⁴ The following three samples were prepared and studied: (i) ~20 mM metronidazole-¹⁵N₂-¹³C₂ and ~1 mM catalyst ([IrCl(COD)(IMes)]), (ii) ~20 mM n.a. metronidazole and ~1 mM catalyst, and (iii) ~100 mM n.a. metronidazole and ~5 mM catalyst. Catalyst activation (achieved by *para*-H₂

bubbling via 1/16 in. OD Teflon capillary for >5 minutes (at 60 standard cubic centimeters (sccm) & 75 psi) in these samples was monitored by *in situ* ^1H NMR spectroscopy as described previously,^{56, 63} which typically shows the presence of intermediate hydride species during catalyst activation, and their disappearance upon completion of the catalyst activation. Approximately 0.6 mL of each solution was placed into an Argon-filled medium-walled 5 mm NMR sample tube. The tube was connected to the previously described setup⁵⁶ via 1/4 in. OD Teflon extension. The flow of *para*- H_2 was metered via the mass flow controller (MFC, Sierra Instruments, Monterey, CA, P/N C100L-DD-OV1-SV1-PV2-V1-S0-C0), Figure 1a and Figure 1b. The pressure inside the NMR tube was maintained using 75 psi safety valve.

NMR hyperpolarization

For SABRE and SABRE-SHEATH experiments, the *para*- H_2 flow rate (typically 60 sccm at 65 psi pressure) was controlled using a mass flow controller. The schematic of SABRE and SABRE-SHEATH experiments is shown in Figure 1a and Figure 1b respectively. For SABRE-SHEATH experiments, the Earth's magnetic field was attenuated by a three-layered mu-metal shield (Magnetic Shield Corp., Bensenville, IL, P/N ZG-206). The mu-metal shield was demagnetized using home-built setup. A custom-built solenoid coil placed inside demagnetized mu-metal shield and a power supply (GW INSTEK, GPRS series) with a variable-resistor bank connected in series with the magnet coil were employed to create weak (μT) magnetic field inside the shield ranging from $\sim 0.1 \mu\text{T}$ to $>5 \mu\text{T}$ (see corresponding Figures throughout the text). When *para*- H_2 bubbling was stopped (by opening the valve shown in Figure 1b), a sample tube was manually transferred from the shield to the Earth's magnetic field. Then the sample was manually transferred into NMR magnet for signal detection. Typically, the entire sample transfer procedure (from *para*- H_2 cessation to NMR signal detection) took 5–7 seconds with an average value of 6 ± 1 s. We note however, that the variation on the sample manipulation in the magnetic shield (where T_1 and T_b values are the shortest and therefore are more susceptible to error in sample manipulation times) was significantly better, i.e. within ~ 0.3 s. In all cases, the relative error in the timing of the sample manipulation adjusted for its T_1 value (i.e. t_1/T_1) was significantly lower than the relative shot-to-shot variation of the NMR signal. As a result, the data simulations for T_1 and T_b calculation are largely determined by the variation in the observed signal intensity, which is weighted in by the Origin 9.0 software employed for data analysis.

T_1 measurements

Spin-lattice relaxation T_1 decay constant was measured at several magnetic fields. Measurements at 9.4 T employed pseudo-2D experiments, where the spin polarization was sampled several times (on the same hyperpolarization run using small-angle excitation pulses) during polarization decay at 9.4 T. The relaxation measurements at other fields were performed differently. At μT field, once the *para*- H_2 flow was stopped, the sample was allowed for depolarization for time t_1 , and next it followed the sample transfer procedure described in the above paragraph. This time t_1 was varied for different hyperpolarization runs, and the corresponding decay curves were obtained. The signal decrease with t_1 was fitted with mono-exponential decay function resulting in good fits (the vast majority with

$R^2 > 0.95$). The examples of such data acquisition and analyses are shown in Figures 2b, 2d and 2f. The T_1 measurements at the Earth's field, fringe field (*ca.* 6 mT and 24 mT), and ~ 0.3 T (created by a permanent magnet) followed the same rationale as the ones at μ T regime (described above), i.e. previously hyperpolarized sample was transferred to such field and allowed for T_1 relaxation during variable time period t_1 .

T_b measurements

The build-up (T_b) constants were measured in the similar fashion. The sample was placed in the desired field (*e.g.* μ T, the Earth's field or other field) where the polarization build-up process was performed. The build-up process was controlled by the duration of *para*-H₂ bubbling (t_1). Once *para*-H₂ bubbling was stopped, the sample was transferred for NMR detection at 9.4 T as described above. The duration of t_1 was varied from one hyperpolarization run to another. The signal build-up was modeled by a mono-exponential function with typical fit quality of $R^2 > 0.95$. Examples of data acquisition and analyses are shown in Figures 2a, 2c and 2e.

Calculation of NMR Signal and Nuclear Spin Polarization Enhancements

In case of ^{15}N and ^{13}C experiments (especially at natural abundance level of ^{15}N isotope), the thermally polarized signals were typically too low for good-quality signal referencing. Therefore, external signal references (see corresponding Figures in Results and Discussion) were employed.⁷⁴ The signal enhancements were calculated as follows:

$$\varepsilon = \frac{I_{\text{HP}}}{I_{\text{REF}}} \times \frac{C_{\text{REF}}}{C_{\text{HP}}} \times \frac{A_{\text{REF}}}{A_{\text{HP}}} \times \frac{N_{\text{REF}}}{N_{\text{HP}}}$$

where I_{HP} and I_{REF} are NMR signals for hyperpolarized state and thermally polarized signal reference samples respectively, C_{REF} and C_{HP} are the effective isotope concentrations of thermally polarized signal reference and HP samples respectively, A_{REF} and A_{HP} are the solution cross-section in the NMR tube, and N_{REF} and N_{HP} are the number of symmetrical sites per molecule for the thermally polarized signal reference and HP samples respectively ($A_{\text{REF}}/A_{\text{HP}}$ was approximately 1.85 as described earlier^{37, 74}). In case of ^1H nuclei, the signal enhancements were computed by dividing the intensity of HP resonance and dividing it by the signal recorded from the same sample under condition of thermal polarization.

Percentage polarization ($\%P_{\text{H}}$, $\%P_{^{13}\text{C}}$ and $\%P_{^{15}\text{N}}$) was computed by multiplying the corresponding signal enhancement ε by the equilibrium nuclear spin polarization of a given spin (^1H , ^{13}C and ^{15}N) at 9.4T and 298 K: $3.2 \times 10^{-3}\%$ (^1H), $8.1 \times 10^{-4}\%$ (^{13}C), $3.3 \times 10^{-4}\%$ (^{15}N).

RESULTS AND DISCUSSION

General

We remind the reader that SABRE hyperpolarization experiments are typically performed in three different static magnetic field regimes to maximize polarization transfer efficiency. The first one is the few mT regime, when efficient $\text{H}(\text{hydride}) \rightarrow \text{H}(\text{substrate})$ polarization

transfer is achieved. The second regime is the sub- μ T regime, when efficient H(hydride) $\rightarrow$ X(substrate) polarization transfer is achieved (where X is ^{15}N , ^{13}C , etc.). Finally, the third regime is broadly defined from mT to sub-T, when efficient polarization transfer is achieved from parahydrogen-derived hydrides to pseudo-singlet states (e.g. ^{15}N - ^{15}N ⁶⁷ or ^{13}C - ^{13}C ^{38, 82}). The following papers provide more thorough consideration. 31–32, 34, 37, 42, 67

We note that various samples of metronidazole studied here contain catalyst (Scheme 1a and 1b). As a result, the reader is reminded that the relaxation measurements reported here were performed on a hyperpolarized mixture in a free and exchangeable Substrates' states (Scheme 1a). Hyperpolarized ^1H , ^{13}C , and ^{15}N resonances of the "free" state at 9.4 T (which have different resonance frequencies from the exchangeable and non-exchangeable states and therefore can be easily delineated at 9.4 T) were employed as the signal read-out. The reader should also be informed that performing ^{13}C and ^{15}N relaxation measurements without catalyst and in the thermally polarized state at these concentration is a practical challenge due to low detection sensitivity (and consequently insufficient NMR signal) of ^{13}C and ^{15}N nuclei. Moreover, ^{13}C and ^{15}N labeled metronidazole was available to us in small quantities due to its comparatively high cost > \$USD500 per 10 mg – as a result, preparation of highly concentrated samples (i.e. above 100 mM) was not possible to us at this time.

Relayed SABRE-SHEATH of ^{13}C and ^{15}N sites in metronidazole- $^{13}\text{C}_2$ - $^{15}\text{N}_2$ and natural abundance (n.a.) metronidazole

Figure 3a and Figure 3c show ^{15}N and ^{13}C NMR spectra of HP metronidazole- $^{13}\text{C}_2$ - $^{15}\text{N}_2$. As described previously,⁷⁴ performing *para*- H_2 bubbling in sub- μ T enables efficient polarization of the directly interacting (with Ir) $^{15}\text{N}_3$ site (see Scheme 1b), and polarization is then relayed via spin-spin coupling network to nearly $^{15}\text{N}_1$, $^{13}\text{C}_2$ and $^{13}\text{C}_2'$ sites.⁷⁴ Thermally polarized signal reference samples (see NMR spectra in Figure 3c and Figure 3d respectively) were employed to compute signal and polarization enhancements (ϵ). Their values ($\epsilon_{^{15}\text{N}_3} \sim 4,800$, $\%P_{^{15}\text{N}_3} \sim 1.6\%$, $\epsilon_{^{15}\text{N}_1} \sim 4,000$, $\%P_{^{15}\text{N}_1} \sim 1.3\%$, $\epsilon_{^{13}\text{C}_2} \sim 320$, $\%P_{^{13}\text{C}_2} \sim 0.26\%$, and $\epsilon_{^{13}\text{C}_2'} \sim 210$, $\%P_{^{13}\text{C}_2'} \sim 0.17\%$) were similar to those reported for this spin system earlier under identical conditions. Relayed SABRE-SHEATH produced sufficient Signal to Noise Ratio (SNR) for the relaxation studies of this sample. We also note that while n.a. metronidazole can be hyperpolarized at $^{15}\text{N}_3$ site,⁴⁵ we could not achieve sufficiently good SNR for $^{15}\text{N}_1$, $^{13}\text{C}_2$ and $^{13}\text{C}_2'$ resonances of this compound at 20 mM concentration; therefore, relaxation studies in n.a. metronidazole have focused only on $^{15}\text{N}_3$ site.

Proton SABRE in metronidazole- $^{13}\text{C}_2$ - $^{15}\text{N}_2$ and natural abundance (n.a.) metronidazole

Figure 4a demonstrates efficient SABRE hyperpolarization (at magnetic field of ~ 6 mT) of aromatic proton (shown in green color) of n.a. metronidazole. Comparison of signal intensity with thermally polarized sample (Figure 4b) yielded $\epsilon_{\text{H}} \sim 73$. The corresponding SABRE hyperpolarization of metronidazole- $^{13}\text{C}_2$ - $^{15}\text{N}_2$ (Figure 4d and Figure 4e) revealed smaller proton signal enhancement ($\epsilon_{\text{H}} \sim 21$) under similar conditions. Moreover, SABRE hyperpolarization of metronidazole- $^{13}\text{C}_2$ - $^{15}\text{N}_2$ below 1 μ T (Figure 4c) also yielded signal enhancement ($\epsilon_{\text{H}} \sim 15$). These observations may be explained by the presence of ^{15}N spin

(i.e. relayed network), and the proton T_1 relaxation at μT and mT regimes (see more thorough discussion below). We also note that the protons of $-\text{CH}_3$ group were hyperpolarized (Figure 4a), although at a significantly lower enhancement level ($\epsilon_{\text{CH}_3} < 10$). While proton signal enhancements were significantly lower than those on ^{13}C and ^{15}N sites, these HP resonances provided sufficient SNR for experimentations focused on nuclear spin relaxation.

Optimization of magnetic field and temperature for SABRE and SABRE-SHEATH experiments

Efficiency of SABRE hyperpolarization is sensitive to magnetic field and temperature, which is well documented in literature for both proton^{36, 83–84} and heteronuclei.^{71, 85} The sensitivity of polarization transfer to magnetic field is explained by the magnetic field matching conditions, whereas the sensitivity to temperature is explained by the modulation of the chemical exchange rates of *para*- H_2 and the substrate, which influence the overall efficiency of the SABRE polarization transfer process.^{32, 37, 86–87}

Optimization of temperature revealed that ^{13}C and ^{15}N SABRE-SHEATH (at $< 1 \mu\text{T}$) have optimal temperature around room temperature of 18–20 °C (Figure 5d and Figure 5f) for all ^{13}C and ^{15}N sites studied, whereas ^1H SABRE (at $\sim 6 \text{ mT}$) maximum was found at $\sim 6 \text{ }^\circ\text{C}$, Figure 5b for both proton sites studied.

Magnetic field optimization of ^1H SABRE yielded a maximum at $\sim 6 \text{ mT}$ (Figure 5a) in line with previous studies of other compounds employing the same SABRE catalyst.^{36, 83} $^{15}\text{N}_1$ and $^{15}\text{N}_3$ SABRE-SHEATH (Figure 5c) maxima were observed at $\sim 0.1 \mu\text{T}$, which is in agreement with previous studies.^{45, 71} $^{13}\text{C}_2$ and $^{13}\text{C}_2'$ SABRE-SHEATH exhibited the maxima at $\sim 0.2 \mu\text{T}$, Figure 5e.

T_1 relaxation times dependence on magnetic field

Figure 6 summarizes T_1 data (see Table S1 for details) for $^{15}\text{N}_1$, $^{15}\text{N}_3$, $^{13}\text{C}_2$, $^{13}\text{C}_2'$ and ^1H sites in metronidazole- $^{13}\text{C}_2$ - $^{15}\text{N}_2$ at 20 mM concentration in methanol- d_4 at five magnetic fields of interest: $< 1 \mu\text{T}$ (optimal field for ^{15}N and ^{13}C relayed SABRE-SHEATH), the Earth's magnetic field ($\sim 50 \mu\text{T}$), $\sim 6 \text{ mT}$ (optimal field for SABRE), 0.3 T (i.e. magnetic field regime, where ^{13}C and ^{15}N chemical shift anisotropy (CSA) is no longer a limiting factor for their T_1 ⁶⁷), and 9.4 T (high magnetic field, where ^{13}C and ^{15}N CSA typically limits their T_1 values). The most striking observation is the convergence of all ^{15}N , ^{13}C and ^1H T_1 to approximately 2.0–4.5 s range at magnetic field below 1 μT , where the Zeeman levels of all spins are mixed. Moreover, the T_1 values for four spin-spin coupled $^{15}\text{N}_1$, $^{15}\text{N}_3$, $^{13}\text{C}_2$, $^{13}\text{C}_2'$ sites are very well clustered in a range of $\sim 4.0 \text{ s}$ (Figure 7). These values are significantly lower than those at higher magnetic fields. Under these sub- μT magnetic field conditions, the nuclear spins with short relaxation times such as quadrupolar nuclei limit the relaxation times of the entire spin ensemble. Two likely explanations of this observation are the presence of quadrupolar Ir center of the catalyst (Scheme 1b), which is directly bound to $^{15}\text{N}_3$ site, and the presence of dihydride protons.^{91–93} The latter are known to have T_1 on the order of $\sim 3 \text{ s}$ even at high magnetic field.⁸¹ The presence of the relaxation center(s) reduces $^{15}\text{N}_3$ T_1 (and potentially T_1 s of metronidazole protons sites), and this effect is then spin-spin

relayed to other ^{13}C and ^{15}N sites within the molecule. A better proof of this explanation entails performing ^{15}N T_1 measurements of metronidazole- $^{13}\text{C}_2$ - $^{15}\text{N}_2$ below 1 μT , however such experiments are challenging, because labeled metronidazole was not available to us in sufficient quantities. However, we performed relaxation measurements with readily available neat ^{15}N -labeled pyridine (Figure S1), which yielded T_1 of 18.4 ± 4.3 s, which is significantly greater than T_1 of ~ 4.6 s measured in 20 mM solution of pyridine- ^{15}N in methanol- d_4 containing ~ 1.2 mM of the same catalyst (Figure 5A from Ref # ³⁷), *i.e.* at approximately the same concentration studied here with metronidazole compound (corresponding to similar T_1 values).

Performing similar experiments with n.a. metronidazole (see Figure 7 for details) revealed only somewhat lower $^{15}\text{N}_3$ T_1 value of 3.33 ± 0.67 versus 4.48 ± 0.44 s in metronidazole- $^{13}\text{C}_2$ - $^{15}\text{N}_2$ (see Table S2 for details), indicating that the effect of quadrupolar $^{14}\text{N}_1$ substitution (in n.a. metronidazole versus metronidazole- $^{13}\text{C}_2$ - $^{15}\text{N}_2$) is relatively negligible (in comparison to the effects induced by Ir quadrupoles below 1 μT), further supporting that it is quadrupolar Ir centers and/or dihydride protons that dominate T_1 at magnetic field below 1 μT .

Limitations of this study

While it was not possible to exactly pinpoint the source of ^{15}N , ^{13}C , and ^1H metronidazole efficient relaxation below 1 μT , we conclude that the relaxation centers are comprised of dihydride protons and/or Ir center. Future studies are certainly warranted to exactly delineate the mechanism of these relaxation processes, and theoretical simulations may be useful in particular. We note however if the dihydride protons would be the only source of such efficient relaxation, this effect would likely be observed at 50 μT , where the dihydride protons and metronidazole protons must be in the strong coupling regime already. Nevertheless, metronidazole ^1H T_1 at 50 μT is markedly greater than the one at <1 μT : 6.54 ± 1.67 s versus 1.96 ± 0.52 s, Table S1. This data indicates that dihydride protons alone may not necessarily fully explain the efficient relaxation of SABRE-SHEATH at magnetic fields below 1 μT .

We note that ^{15}N T_1 values were significantly greater than those of ^1H and ^{13}C nuclei in this compound at all magnetic field regimes besides that below 1 μT , Figure 6. At high magnetic fields, e.g. 9.4 T, CSA becomes the T_1 -limiting factor,⁴⁹ and ^{15}N were lower than those at the Earth's magnetic field, ~ 6 mT and ~ 0.3 T. ^{15}N T_1 values were the highest at ~ 0.3 T. Since the use of HP probes benefits from longer T_1 values (because it allows for a longer time window with HP contrast agent manipulation and biological uptake and bio-distribution), future experiments with this HP compound would therefore benefit from performing the experimentations at magnetic fields of ~ 0.3 T, where T_1 is the highest. We note that the detection sensitivity of HP compounds does not significantly depend on the read-out magnetic field in general,^{49, 94–95} while the difference in $^{15}\text{N}_1$ and $^{15}\text{N}_3$ T_1 values at 0.3 T and 9.4 T is almost three-fold, Figure 6.

Future consideration of more rational SABRE-SHEATH catalyst design

We note that for all nuclei studied T_1 below 1 μ T is significantly reduced (by a factor of more than three). If this reduction is due to Ir-induced quadrupolar relaxation (rather than due to relaxation caused by dihydride protons),^{96–99} and because % P_{15N} in SABRE-SHEATH is linearly proportional to the T_1 in <1 μ T (see Reference #³⁷ for supporting evidence), future development of SABRE-SHEATH catalyst without such relaxation-inducing centers may provide a convenient opportunity to boost ^{15}N T_1 and % P_{15N} by at least several fold.

Correlation of T_1 and T_b values is provided in Figure 7. Overall, we have observed that T_1 and T_b values were approximately the same (i.e. the general trend of increase of T_b with T_1) indicating that the rate constants of the SABRE-SHEATH polarization build-up process are indeed limited by the T_1 relaxation. This observation is in agreement with previous relaxation studies of SABRE process for protons in mT magnetic fields.^{81, 100}

The effect of intramolecular quadrupolar centers on ^{15}N and ^1H T_1 was examined by performing additional $^{15}N_3$ and ^1H T_1 measurements with n.a. metronidazole at four different magnetic field regimes: below 1 μ T, the Earth's field (~ 50 μ T), ~ 0.3 T and 9.4 T. N.a. metronidazole contains one extra quadrupolar ^{14}N site ($^{14}N_1$) versus metronidazole- $^{13}C_2$ - $^{15}N_2$), which contains only one quadrupolar ^{14}N site $-^{14}NO_2$. As already briefly discussed above, ^{15}N and ^1H T_1 values below 1 μ T did not significantly differ in n.a. metronidazole versus those in metronidazole- $^{13}C_2$ - $^{15}N_2$, because Ir-mediated efficient quadrupolar relaxation limited the overall T_1 of these nuclei. At the Earth's magnetic field (Figure 8), ^{15}N T_1 values of metronidazole- $^{13}C_2$ - $^{15}N_2$ and n.a. metronidazole are very distinct: 99 ± 5 s versus 35.1 ± 2.2 s respectively. At the same time at the Earth's magnetic field (Figure 8), ^1H T_1 values of metronidazole- $^{13}C_2$ - $^{15}N_2$ and n.a. metronidazole are nearly indistinguishable: 6.54 ± 1.67 s versus 7.7 ± 1.77 s respectively. We conclude that ^{14}N quadrupolar nuclei indeed contribute to the overall T_1 relaxation (via scalar relaxation of the second kind), and this effect is more pronounced for $^{15}N_3$ nuclei, because they have higher T_1 values than the proton studied, and because of the inverse nature of the T_1 contributions, i.e. $1/T_1(\text{n.a.-metronidazole}) = 1/T_1(\text{metronidazole-}^{13}C_2\text{-}^{15}N_2) + 1/T_1(^{14}N)$.

In case of ^{15}N nuclei, the $1/T_1(\text{metronidazole-}^{13}C_2\text{-}^{15}N_2)$ term is significantly smaller than the $1/T_1(^{14}N)$ term, and as a result, $1/T_1(\text{n.a.-metronidazole})$ is largely governed by $1/T_1(^{14}N)$ term. In case, of proton spin, the $1/T_1(\text{metronidazole-}^{13}C_2\text{-}^{15}N_2)$ term is significantly greater than the $1/T_1(^{14}N)$ term, and the $1/T_1(\text{n.a.-metronidazole})$ is therefore similar to $1/T_1(\text{metronidazole-}^{13}C_2\text{-}^{15}N_2)$.

At higher magnetic fields, i.e. ~ 0.3 T and 9.4 T compared to the Earth's magnetic field discussed in the above paragraph, ^{15}N T_1 values were similar in n.a. metronidazole and metronidazole- $^{13}C_2$ - $^{15}N_2$, clearly indicating that the effect of ^{14}N quadrupolar scalar relaxation of the second kind is negligible, because the latter scales almost linearly with the magnitude of the magnetic field (see Eq. 127 in Ref. #⁹⁶).

It should also be noted that the potential reasons why $^{15}N_3$ T_1 of metronidazole- $^{13}C_2$ - $^{15}N_2$ is lower at the Earth's magnetic field compared to that at ~ 0.3 T (Figure 8) is (i) due to the

presence of $^{14}\text{NO}_2$ quadrupolar center and (ii) due to residual quadrupolar relaxation from quadrupolar Ir centers (the latter likely scales differently from ^{14}N , because of ^{14}N and $^{191}\text{Ir}/^{193}\text{Ir}$ have different quadrupolar constants, spin numbers, and Larmor frequencies, which may contribute differently to T_1 relaxation at different fields, see Eq. 127 in Ref. # 96). From the practical standpoint, the use of ^{15}N HP metronidazole in the future should occur at sufficiently high magnetic field (on the order of a 1 mT or more) to minimize the polarization losses due to intramolecular quadrupolar relaxation. Alternative strategies may also benefit from the uniform ^{15}N isotopic labeling.

Spin-lattice relaxation of “long-lived spin states” in metronidazole- $^{13}\text{C}_2$ - $^{15}\text{N}_2$

Recent reports describing the preparation of long-lived spin states in near equivalent ^{15}N - ^{15}N ⁶⁷ and ^{13}C - ^{13}C ^{38, 82} pairs inspired us to prepare such states in metronidazole- $^{13}\text{C}_2$ - $^{15}\text{N}_2$. Figure 9a shows a spectrum of ^{15}N metronidazole- $^{13}\text{C}_2$ - $^{15}\text{N}_2$ hyperpolarized in mT magnetic field range. Note the 180°-degree phase shift of $^{15}\text{N}_1$ and $^{15}\text{N}_3$ NMR peaks due to pseudo singlet state nature of this spin state. The maximum signal enhancements ($\epsilon_{^{15}\text{N}_3} \sim 800$ and $\epsilon_{^{15}\text{N}_1} \sim 880$ via comparison of HP and thermal spectral intensities in Figure 9a and Figure 9b) obtained were a factor of 5–6 lower than those below μT via SABRE-SHEATH despite nominally greater ^{15}N T_1 at such high field (Figure 9d). This observation is explained by the deviation from the optimal condition ($J_{\text{HH}'} = J_{^{15}\text{N}-^{15}\text{N}'}$, see Ref. # 67 for details), because $J_{\text{HH}'} \gg J_{^{15}\text{N}-^{15}\text{N}'}$ in the case studied here. The optimization of the magnetic field revealed the maximum at ~ 0.1 T, Figure 9c. However, one of the key prerequisites for long-lived nature of such states (spin-spin coupling greater than the difference of the chemical shifts or $J/2\pi > \nu$ ^{101–102}) is not fulfilled at magnetic fields above 0.25 mT, because the two ^{15}N sites have rather large chemical shift difference of ~ 90 ppm with relatively small spin-spin coupling below 1 Hz. Indeed, Figure 9d provides the experimental evidence showing that the decay constants of ^{15}N - ^{15}N “LLSS” are not greater than those of Z-magnetization hyperpolarized via SABRE-SHEATH below 1 μT . We conclude that the structural motif of metronidazole does not provide a suitable platform for preparation of ^{15}N - ^{15}N long lived spin states.

On the relative efficiency of ^1H hyperpolarization via SABRE-SHEATH

Figure 4c and Figure 4d report on $\epsilon_{\text{H}} \sim 15$ and $\epsilon_{\text{H}} \sim 21$ ^1H proton signal enhancements via SABRE-SHEATH (at < 1 μT) and SABRE (at ~ 6 mT) for metronidazole- $^{13}\text{C}_2$ - $^{15}\text{N}_2$. While nominally SABRE-SHEATH produces lower polarization efficiency in this case, it is important to realize that ^1H T_1 at ~ 0.1 μT was 1.96 ± 0.52 s versus 19.7 ± 2.7 s at ~ 6 mT, i.e. it was approximately a factor of 10 ± 3 lower than that at ~ 6 mT. Because signal enhancement scales linearly with T_1 for SABRE,³⁷ ϵ_{H} is significantly reduced at ~ 0.1 μT because of such low T_1 values. In case if such T_1 limitation would be removed (i.e. through more rational catalyst design as described above), the potential ϵ_{H} value obtained via SABRE-SHEATH at ~ 0.1 μT would be a factor of 7 better than that via SABRE at ~ 6 mT in metronidazole- $^{13}\text{C}_2$ - $^{15}\text{N}_2$ (and would approximately be a factor of 2 greater than that obtained for n.a. metronidazole in Figure 4a). This analysis is important, because it may lead to significantly more efficient hyperpolarization of ^1H spins via the use of relayed SABRE-SHEATH.

CONCLUSION

We have reported on systematic relaxation study of ^1H , ^{13}C and ^{15}N spins in metronidazole- $^{13}\text{C}_2$ - $^{15}\text{N}_2$ and metronidazole in SABRE hyperpolarization process in a wide range of magnetic fields. ^1H , ^{13}C and ^{15}N spins studied have similar T_1 values of approximately 2–4 s at the conditions studied, which is consistent with the model of relayed SABRE-SHEATH effect.⁷⁴ The T_1 values at higher magnetic fields (*i.e.* the Earth's magnetic field and above) are significantly greater and exceed 3 minutes in some cases (e.g. ^{15}N sites at 0.3 T). Moreover, these relatively short T_1 values observed below 1 μT limit the polarization build-up process of SABRE-SHEATH—thereby, limiting maximum attainable ^{15}N polarization. The relatively short nature of T_1 values observed below 1 μT is primarily caused by intermolecular interactions with quadrupolar Ir centers or dihydride protons of the employed polarization transfer catalyst, whereas intramolecular spin-spin interactions with ^{14}N quadrupolar centers have significantly smaller contribution. The presented experimental results and their analysis will be useful for more rational design of SABRE-SHEATH (i) polarization transfer catalyst, and (ii) HP molecular probes in the context of biomedical imaging and other applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

The schematic of the setup and steps SABRE hyperpolarization process (a) and SABRE-SHEATH hyperpolarization process (b).

Figure 2.

HP signal build-up and decay at micro-Tesla magnetic field and mono-exponential modeling of hyperpolarization build-up and decay process to determine T_b and T_1 constants for a) nuclear spin polarization build-up of aromatic proton and T_b , b) nuclear spin polarization decay of aromatic proton and T_1 , c) nuclear spin polarization build-up of $^{13}\text{C}_2$ and T_b , d) nuclear spin polarization decay of $^{13}\text{C}_2$ and T_1 , e) nuclear spin polarization build-up of $^{15}\text{N}_3$ and T_b , f) nuclear spin polarization decay of $^{15}\text{N}_3$ and T_1 . Note that for experimentations shown in Figure 2a, it was experimentally challenging to deliver *para*- H_2 bubbling timings of less than 1 second.

Figure 3.

^{13}C and ^{15}N SABRE-SHEATH NMR spectra of 20 mM metronidazole- $^{15}\text{N}_2$ - $^{13}\text{C}_2$. a) HP ^{15}N NMR spectrum, b) ^{15}N spectrum of a thermally polarized reference sample, c) HP ^{13}C NMR spectrum, d) ^{13}C spectrum of a thermally polarized reference sample.

Figure 4.

^1H SABRE and SABRE-SHEATH NMR spectra. a) HP ^1H NMR spectrum of 20 mM natural abundance (n.a.) metronidazole sample hyperpolarized via SABRE at ~ 6 mT, b) corresponding thermally polarized ^1H spectrum for comparison with spectrum shown in (a), c) HP ^1H NMR spectrum of 20 mM metronidazole- $^{15}\text{N}_2$ - $^{13}\text{C}_2$ sample hyperpolarized via SABRE-SHEATH at ~ 0.1 μT , d) HP ^1H NMR spectrum of 20 mM metronidazole- $^{15}\text{N}_2$ - $^{13}\text{C}_2$ sample hyperpolarized via SABRE at 6.1 mT, e) corresponding thermally polarized ^1H spectrum for comparison with spectra shown in (c) and (d).

Figure 5.

Magnetic field and temperature dependencies of ^1H SABRE and ^{13}C and ^{15}N SABRE-SHEATH. a) magnetic field dependence of ^1H SABRE in mT range, b) temperature dependence of ^1H SABRE at ~ 6 mT, c) magnetic field dependence of ^{15}N SABRE-SHEATH in μT range, d) temperature dependence of ^{15}N SABRE-SHEATH at ~ 0.1 μT , e) magnetic field dependence of ^{13}C SABRE-SHEATH in μT range, f) temperature dependence of ^{13}C SABRE-SHEATH at ~ 0.2 μT . Experiments (a), (b), and (c) are performed at room temperature of ~ 18 $^{\circ}\text{C}$. The individual data points are connected by lines to guide the eye. It should be noted that H_2 solubility in methanol increases with temperature (by $\sim 18\%$ over 35 $^{\circ}\text{C}$ range),^{88–90} which can potentially modulate the observed signal in Figures 5b, 5d and 5e. This potentially minor correction factor is not taken into consideration in these corresponding Figures.

Figure 6.
 T_1 dependence on magnetic field of aromatic proton, $^{13}\text{C}_2$, $^{13}\text{C}_{2'}$, $^{15}\text{N}_1$ and $^{15}\text{N}_3$ sites.

Figure 7.

T_1 and T_2 correlation plot of aromatic proton, $^{13}\text{C}_2$, $^{13}\text{C}_{2'}$, $^{15}\text{N}_1$ and $^{15}\text{N}_3$ sites at 0.1 μT .

Figure 8.

T_1 dependence of aromatic proton and $^{15}\text{N}_3$ sites on magnetic field in natural abundance (n.a.) metronidazole and metronidazole- $^{13}\text{C}_2$ - $^{15}\text{N}_2$ at ~20 mM and ~1 mM catalyst concentrations.

Figure 9.

a) ^{15}N NMR metronidazole- $^{13}\text{C}_2$ - $^{15}\text{N}_2$ hyperpolarized via SABRE at ~ 0.1 T corresponding to the condition, when long-lived spin states (LLSS) may be created, b) ^{15}N NMR spectrum of thermally polarized signal reference sample, c) magnetic field dependence of pseudo LLSS (denoted as “LLSS”) created by spin-relayed⁷⁴ SABRE hyperpolarization, d) T_1 magnetic field dependence of $^{15}\text{N}_3$ and $^{15}\text{N}_1$ hyperpolarization resulting from polarization storage in z-magnetization (Zmag) and “LLSS” states.

Scheme 1.

a) Schematic of the SABRE-SHEATH Hyperpolarization Process of Metronidazole- $^{15}\text{N}_2$ - $^{13}\text{C}_2$ and Natural Abundance Metronidazole Using Transfer of Spin Order from parahydrogen on an Ir–IMes Hexacoordinate Complex.^{36, 43} SABRE-SHEATH is Accomplished via Spin–spin Couplings between Parahydrogen-derived Hydride Protons and Nuclear Spins of the Exchangeable (equatorial) Ligands. The Axial Ligands (occupied by Substrate) are Not Exchangeable. b) The Part of the Hexacoordinate Ir Complex Relevant for SABRE-SHEATH Polarization Transfer from Parahydrogen-derived Hydride Protons and Nuclear Spins of the Exchangeable (equatorial) Ligands.