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Facile Removal of Homogeneous SABRE Catalysts for Purifying Hyperpolarized Metronidazole, a Potential Hypoxia Sensor

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Abstract

We report a simple and effective method to remove IrIMes homogeneous polarization transfer catalysts from solutions where NMR Signal Amplification By Reversible Exchange (SABRE) has been performed, while leaving intact the substrate's hyperpolarized state. Following microTesla SABRE hyperpolarization of ^{15}N spins in metronidazole, addition of SiO_2 microparticles functionalized with 3-mercaptopropyl or 2-mercaptoethyl ethyl sulfide moieties provides removal of the catalyst from solution well within the hyperpolarization decay time at 0.3 T (T_1>3 mins)—and enabling transfer to 9.4 T for detection of enhanced ^{15}N signals in the absence of catalyst within the NMR-detection region. Successful catalyst removal from solution is supported by the inability to "re-hyperpolarize" ^{15}N spins in subsequent attempts, as well as by ^{1}H NMR and ICP-MS. Record-high ^{15}N nuclear polarization of up to ~34% was achieved, corresponding to >100,000-fold enhancement at 9.4 T, and approximately 5/6th of the ^{15}N hyperpolarization is retained after ~20-second-long purification procedure. Taken together, these results help pave the way for future studies involving *in vivo* molecular imaging using agents hyperpolarized via rapid and inexpensive parahydrogen-based methods.

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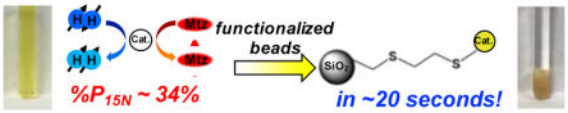
[‡]Author Contributions: B.E.K. and J.L.G. contributed equally.

TOC Graphic



Introduction

MRI is a powerful imaging method not only because its ability to distinguish anatomical boundaries of soft tissues without ionizing radiation, but also because of its potential to spectrally discern among different biochemical species and physiological states.[1, 2] However, the need for much greater detection sensitivity for dilute species has led to growing interest in hyperpolarization[3–5]—the generation of highly non-equilibrium population distributions of nuclear spins to dramatically increase the detectable magnetization. Various methods for hyperpolarization have been developed with an eye toward biomedical applications, including Spin-Exchange Optical Pumping (SEOP),[6, 7] dissolution Dynamic Nuclear Polarization (d-DNP),[8–11] ParaHydrogen Induced Polarization (PHIP),[12–14] and Signal Amplification By Reversible Exchange (SABRE).[15–17] In particular, SABRE—along with its microTesla variant dubbed “SABRE-SHEATH” (for SABRE in SHield Enables Alignment Transfer to Heteronuclei)[18, 19–22]—have garnered increasing attention because they are rapid and inexpensive to perform, scalable, and do not require major instrumentation. A wide range of biomolecules is amendable to SABRE-SHEATH.[4, 23] However, reliance on a heavy-metal (Ir-based) catalyst to mediate polarization transfer to a biocompatible substrate presents an obstacle to envisioned clinical applications because of its presence in the same solution as the hyperpolarized (HP) agent. Efficient removal of the catalyst while preserving the HP state of the substrate is thus likely necessary before studies with human subjects can be considered.

Generating a pure HP substrate via SABRE necessitates that the catalyst is either heterogeneous (enabling ready separation of the catalyst from the dissolved substrate), or that the homogeneous catalyst is removed somehow from the solution post-hyperpolarization transfer to target nuclei. While progress has been made,[24–29] each approach demonstrated so far has drawbacks and none have enabled the production of catalyst-free solutions with agents possessing nuclear spin polarizations of several percent. Indeed, such approaches are further complicated by the necessity of the HP nuclei to retain their HP state during (and following) catalyst removal, enabling it to survive all the way through separation, agent administration, and ultimate MRI detection. The present work is thus motivated by the desire for simple methods to efficiently remove standard SABRE catalysts from solution while retaining the HP state of the substrate.

Experimental/Background

Metronidazole, a molecule that has a ^{15}N T_1>3 min at 0.3 T,[30] is relatively easy to hyperpolarize,[31, 32] and possesses biological significance (it is an FDA-approved antibiotic of interest for probing tissue hypoxia),[31,33] was utilized in the present study. Indeed, we note

that metronidazole can be administered in relatively large dose (~2 g per patient[34]). Moreover, metronidazole contains a nitroimidazole moiety, a structure which is frequently employed in positron emission tomography (PET) molecular probes for hypoxia sensing.[33, 35–38] Correspondingly, we anticipate that when hyperpolarized, this agent will potentially be able to distinguish between hypoxic and normoxic tissues via the ^{15}N chemical shift differences that are expected for structures in healthy versus pathological tissues.

Each sample utilized here contains a 20 mM methanol-d_4 solution of metronidazole and 1 mM Ir-catalyst precursor [IrCl(COD)(IMes)] (where IMes = 1,3-bis(2,4,6-trimethylphenyl)-imidazol-2-ylidene and COD = cyclooctadiene)[39,40]; ~75–85% parahydrogen (at 75 psi) is administered via bubbling through a 5 mm NMR tube with a flow rate of 150 sccm using an experimental setup described elsewhere.[41] The catalyst was activated by bubbling for at least ~5–10 mins prior to initial NMR acquisition. Additional experimental details can be found in the Supporting Information (SI) document.

Results and Discussion

A SABRE-SHEATH mixing field of ~1 μT for ^{15}N was found to result in ^{15}N nuclear spin polarization of up to ~34% for the Ir-binding nitrogen in the free substrate (Figure 1), which corresponds to an enhancement factor (ε) of ~103,000-fold at 9.4 T and 300 K.

To our knowledge, such a value represents the greatest ^{15}N polarization yet reported amongst all hyperpolarization methods (moreover, if nearly 100% $p H_2$[42] were employed, and assuming an original fraction of ~85%, P of ~42% and ε of ~129,000 would be achieved[4]). At ^{15}N natural abundance (0.364%), we also observed HP ^{15}N signals from that nitrogen in catalyst-bound species and the adjacent imidazole nitrogen of free species (8% and 0.6% polarization, respectively; see Table S1 for a summary of calculations of polarization enhancement). With a long hyperpolarization lifetime[30] and high ^{15}N polarization values, metronidazole represents an ideal candidate to attempt catalyst removal post-hyperpolarization. We also note that SABRE hyperpolarization of this compound in organic solvent (vs. that in aqueous medium) may be desirable, because of metronidazole and parahydrogen have significantly greater solubility in organic solvents enabling preparation of highly concentrated and highly polarized liquids; such a HP liquid prepared in this fashion potentially can be diluted with isotonic buffer in a manner suitable for in vivo injection (e.g. 5 mg/ml solution).[31, 41]

The functionalized SiO_2 microparticles (3-mercaptopropyl and 2-mercaptoethyl ethyl sulfide) investigated here are commercially available (Sigma 538086 and Sigma 745111), and do not require post-synthetic modifications. The surface functional moieties are terminated with strongly binding sulfur atoms that in principle can rapidly remove the catalyst from solution (ideally, on a timescale $\ll T_1$). To provide a rough estimate of the amount of functionalized SiO_2 microparticles necessary to complete catalyst removal, ^1H SABRE enhancement (several hundred-fold) as a function of time (post-SiO_2 particle addition) was studied first, using 40 mM solutions of a test substrate (pyridine, also containing 4 mM catalyst in methanol-d_4; Figure S1). These preliminary experiments were initiated by activating the catalyst with ~5–10 min. of parahydrogen bubbling, performing

SABRE at ~10 mT, and then measuring the SABRE enhancement at 9.4 T prior to particle addition; once a ^{1}H enhancement baseline was established, functionalized (3-mercaptopropyl) or non-functionalized (control) SiO_2 microparticles were added to the solution. ^{1}H SABRE experiments were then repeated and the decreasing ^{1}H SABRE enhancement was recorded over time. Indeed, subsequent ^{1}H SABRE spectra showed drastic reductions in enhancement as a function of time for solutions containing functionalized SiO_2 microparticles, where the fastest decay (within ~250 s) occurred for microparticle:catalyst molar ratios of 30:1 (Figure S2). However, corresponding solutions of non-functionalized SiO_2 microparticles did not show a change in enhancement over ~2,000 s following an initial loss likely attributable to physisorption of the catalyst.

Informed by the above results (which indicated the need for large molar ratios of surface functionalization to catalyst), the experimental procedure used for obtaining enhanced ^{15}N spectra from metronidazole with catalyst removal is summarized in Figure 2.

To rapidly remove homogeneous catalyst species from solution, microparticle:catalyst molar ratios of ~170:1 (3-mercaptopropyl) and ~142:1 (2-mercaptoethyl ethyl sulfide) were respectively used. Immediately following hyperpolarization transfer to ^{15}N at ~1 μT, the solution is transferred to a 0.3 T storage field (where the ^{15}N T_1 is > 3 min[30]), depressurized to 1 atm, and then ~85 mg of the functionalized or non-functionalized SiO_2 microparticles were added to the solution. The solution was bubbled with parahydrogen for ~5 s to ensure good mixing, and then immediately transferred to 9.4 T for detection. This entire process takes ~20 s and can likely be significantly accelerated (and even automated) with a more streamlined apparatus.

Results obtained with metronidazole (naturally abundant in ^{15}N spins, hyperpolarized via SABRE-SHEATH) are summarized in Figure 3 and Figures S3, S4; all enhancement calculations are summarized in Table S2 of the SI.

First, enhanced ^{15}N spectra obtained prior to particle addition for three different runs are shown in Figures 3a, Figure S3a, and Figure S4a. Figures 3b, Figure S3b, and Figure S4b show significant retention of ^{15}N hyperpolarization after each type of SiO_2 microparticles were added. Importantly, the functionalized microparticles (Figure 3b and Figure S3b) took on a pale-yellow color similar to the catalyst while the supernatant liquid became clear (see photos in Figure 2), consistent with rapid removal of catalyst molecules from the solution as a result of binding to the microparticles. The decreases in signal intensity (and hence hyperpolarization level) are likely due to T_1 losses; for example, Figure 3 demonstrates that ~84% of the initial ^{15}N polarization was retained after the ~20-second-long purification procedure. After detection at 9.4 T, we attempted to “re-hyperpolarize” the ^{15}N spins of the substrate, but observed no detectable ^{15}N enhancement for the two samples containing functionalized SiO_2 microparticles, again supporting the effective absence of catalysts in the supernatant solution. However, we were still able to achieve similar hyperpolarization levels for the sample containing non-functionalized SiO_2 microparticles (SI). These results are also corroborated by corresponding ^{1}H SABRE studies with these types of samples (Figure S5). Moreover, high-resolution ^{1}H NMR of the supernatant liquid following addition of 3-mercaptopropyl-functionalized microparticles does not contain signals from the SABRE

catalyst (Figure S6). Finally, ICP-MS elemental analysis of solutions obtained using similar concentrations and approach (and with volumes scaled up by ~25-fold) found that >98% of the catalyst had been removed (see SI)—consistent with the NMR results.

Conclusion

In summary, a simple and effective method is reported for removal of the most potent homogeneous IrIMes SABRE catalyst from solutions containing HP agent. The method uses inexpensive and commercially available microparticles, 5 mm NMR tubes, and it is sufficiently rapid ($\ll T_1$) to enable detection of NMR signals from substrates with intact HP states, in the apparent absence of dissolved catalysts (maintaining ^{15}N polarization levels of up to >84% of the initial value). We note that the entire procedure from beginning of hyperpolarization to the end of the purification process requires less than 1.5 minutes. We envision that the purified HP metronidazole organic solution can be transferred from 5-mm-NMR-tube-based setup into a syringe partially filled with isotonic saline buffer via a catheter and particle filter for subsequent *in vivo* injection. Moreover, larger volumes with higher concentrations catalyst, parahydrogen, and agent (as well as agent isotopic labeling) should be easily amenable to the present approach—along with the use of other agents (including our recent demonstration of SABRE-hyperpolarized cleavable metabolic agents[43]). Current work is also focusing on developing cartridges or other devices that can be integrated with our setups to increase the efficiency of the process while minimizing polarization losses during catalyst removal and/or agent separation. These results, combined with observation of record ^{15}N polarization of up to 34% in metronidazole, bode well for a wide range of envisioned *in vivo* molecular imaging applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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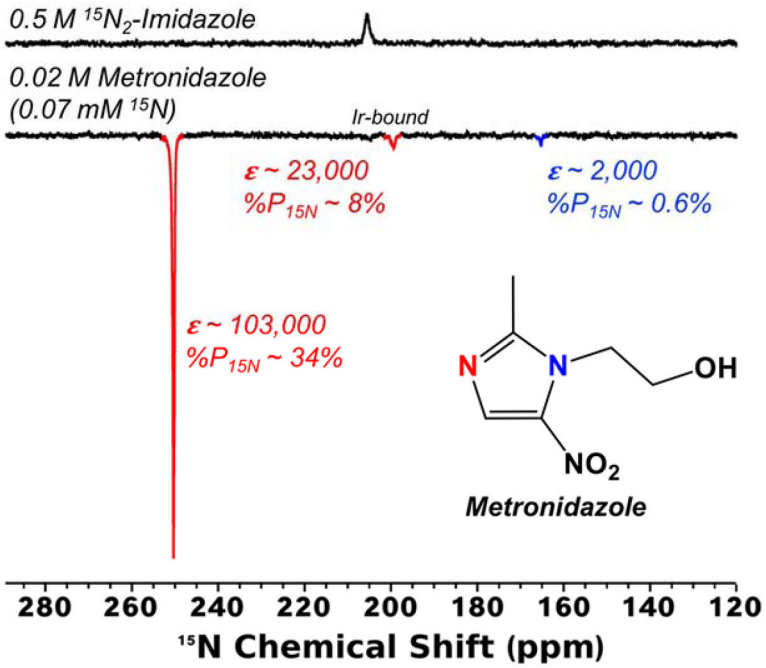

Figure 1.

Bottom: ^{15}N spectrum from metronidazole exhibiting 34% ^{15}N polarization achieved via SABRE-SHEATH. The Ir-binding nitrogen site of the free substrate exhibited the greatest polarization; the resonance for that nitrogen site for the Ir-bound substrate and the adjacent imidazole nitrogen were polarized 8% and 0.6%, respectively. *Top*: ^{15}N spectrum from thermally polarized $^{15}N_2$-imidazole, used as a reference for calculating polarization enhancement.

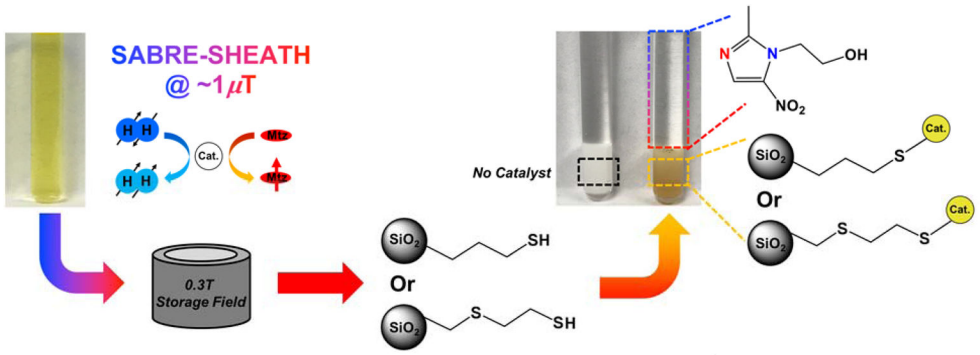

Figure 2.

Schematic of the experiment. After polarization transfer to ^{15}N of metronidazole (Mtz) at ~1 μT (within a magnetic shield, not shown), the solution is transferred to a 0.3 T storage field, where the ^{15}N T_1 is long. The sample is depressurized and functionalized (3-mercaptopropyl or 2-mercaptoethyl ethyl sulfide) or non-functionalized (control) SiO_2 microparticles are added, which in the former cases, cause the catalyst to be removed from solution while retaining high levels of the substrate's ^{15}N polarization.

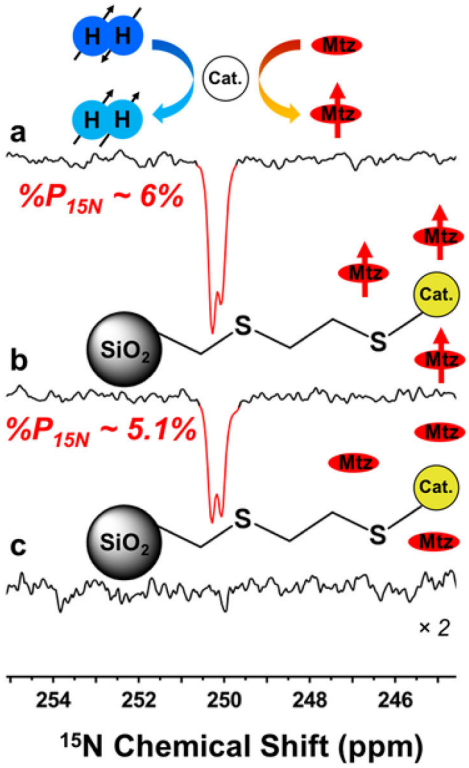

Figure 3.

^{15}N SABRE-SHEATH spectra from metronidazole (naturally abundant in ^{15}N spins) at different stages: First, high levels of ^{15}N polarization prior to microbead addition was seen (a). ^{15}N hyperpolarization is retained after addition of 2-mercaptoethyl ethyl sulfide functionalized silica (b). Any attempt to re-hyperpolarize the solutions yielded no enhancements for the functionalized SiO_2 solutions (c).