Communication

Direct enhancement of nitrogen-15 targets at high-field by fast ADAPT-SABRE

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Signal Amplification by Reversible Exchange (SABRE) is an attractive nuclear spin hyperpolarization technique capable of huge sensitivity enhancement in nuclear magnetic resonance (NMR) detection. The resonance condition of SABRE hyperpolarization depends on coherent spin mixing, which can be achieved naturally at a low magnetic field. The optimum transfer field to spin-1/2 heteronuclei is technically demanding, as it requires field strengths weaker than the earth’s magnetic field for efficient spin mixing. In this paper, we illustrate an approach to achieve strong 15N SABRE hyperpolarization at high magnetic field by a radio frequency (RF) driven coherent transfer mechanism based on alternate pulsing and delay to achieve polarization transfer. The presented scheme is found to be highly robust and much faster than existing related methods, producing ~3 orders of magnitude 15N signal enhancement within 2 s of RF pulsing.

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1. Introduction

Despite the huge success of NMR in a wide assortment of research fields ranging from structural material characterization to the imaging of internal human organs, it is still regarded to be underexploited based on its theoretical potential [1,2]. Most of the successes of NMR and MRI applications have been achieved utilizing the thermal level of nuclear spin polarization which is only of the order of 10−5 at room temperature in a standard high field spectrometer [2]: only one spin in 30,000 contributes to the NMR signal for protons in a 9.4 T magnet. Improving this poor sensitivity would make NMR and MRI more widespread and cost-efficient. The solution to this challenge is offered by hyperpolarization naturally at a low magnetic field. The optimum transfer field to spin-1/2 heteronuclei is technically demanding, as it requires field strengths weaker than the earth’s magnetic field for efficient spin mixing. In this paper, we illustrate an approach to achieve strong 15N SABRE hyperpolarization at high magnetic field by a radio frequency (RF) driven coherent transfer mechanism based on alternate pulsing and delay to achieve polarization transfer. The presented scheme is found to be highly robust and much faster than existing related methods, producing ~3 orders of magnitude 15N signal enhancement within 2 s of RF pulsing.

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Here we show that, the recently published ADAPT (Alternating Delays Achieve Polarization Transfer) sequence [26] can be applied to transfer polarization from singlet hydrides to target $^{15}$N nuclei in a repetitive fashion. It takes only few seconds to build up strong $^{15}$N hyperpolarized signal whilst the transfer mechanism is fully exploited all of the combinations of $\alpha$, $\Delta$ and $m$ we tested produced a return [26].

ADAPT remains robust even in the case of uncertainty about the value of the heteronuclear $J_{123}$ (simulations not shown).

2.2. Initial state and trajectories

When the $^{14}$N-hydrogen and the substrate bind to the catalyst, the initial state can be represented as an overpopulation of the $^1$H scalar singlet order. We can define a set of orthogonal operators $I_{13}^x, I_{13}^y, I_{13}^z$ with cyclic commutation relationships $[I_{13}^x, I_{13}^y] = iI_{13}^z$, for each of the 2 x 2 subspaces in Eqs. (2) and (3). The initial state and the observable $I_{13}$ $^{15}$N-transverse magnetization are related to the following single transitions operators:

\[
\frac{I^x_{13}}{2} \propto -\frac{1}{2}(I_{13}^{\theta} - I_{13}^{\phi})
\]

(4)

From the set of Eq. (4), it is apparent that polarization transfer can be obtained, for example, when a sequence of events invert the sign of the operator $I_{13}^\theta$ while maintaining the sign of the operator $I_{13}^\phi$. ADAPT achieves this by performing a $\pi$ rotation in the subspace $H^{a,b}$ (see Fig. 2).

2.3. Analogy with the LIGHT-SABRE and the LAC approach

A LAC [27] occurs when (i) the energy level relative to a pair of states $|m\rangle$ and $|n\rangle$ is equal $E_{mn} = E_{0m}$ and (ii) there is a matrix element $V_{mn} = \langle m|V|n\rangle$ for some operator $V$ that splits them. Essentially, the LIGHT-SABRE and LAC methods share the same clever idea: a resonance condition between the spin populations of the subspace $H^{a,b}$ and $H^{a,d}$ can be established upon spin locking on the heteronuclear channel. The Hamiltonian operator for a constant RF heteronuclear irradiation is $H_{RF} = 2\pi \nu_{RF} I_{15}$.
The modulation used in the LAC method can be included by introducing a time dependency in the term $m_{RF}$. For simplicity, here we disregard the time dependency $m_{RF}$ as in the LIGHT-SABRE protocol [23]. The matrix representation of the Hamiltonian operator $H = H_J + H_{RF}$ transforms Eqs. (2) and (3) into:

$$\hat{H}^{a,b} = \langle a | \left( -\frac{3\omega_d}{4} - \pi\nu_{RF} \right) - \frac{\omega_a}{4} + \pi\nu_{RF} \rangle | b \rangle$$  \hspace{1cm} (5)

$$\hat{H}^{c,d} = \langle c | \left( -\frac{\omega_d}{4} - \pi\nu_{RF} \right) - \frac{\omega_a}{4} + \pi\nu_{RF} \rangle | d \rangle$$  \hspace{1cm} (6)

In Eqs. (5) and (6), when $\nu_{RF} = J_{12} = E_a = E_{cd} = E_a = E_d$ so that a level crossing occurs. More importantly, in presence of magnetic inequivalence $\omega_a \neq 0$, and a LAC can be established for each subspace in Eqs. (5) and (6). As a result, the heteronuclear J-coupling imbalance removes the degeneracy and promotes spin population transfer in each subspace $\hat{H}^{a,b}$ and $\hat{H}^{c,d}$. The term $\omega_a$ is completely analogous to $V_{mn}$. The duration of the optimal spin lock is inversely proportional to $\omega_a$.

Fig. 2. ADAPT induced rotations in the subspaces $H^{a,b}$ and $H^{c,d}$ upon application of ADAPT for $\Delta = 8$ ms and $m = 5$ for $J_{12} = 8$ Hz, $J_{13} = J_{23} = 25.5$ Hz.

Fig. 3. $^1$H PHIP NMR spectrum of $^{15}$N-ethylnicotinate measured in a 500 MHz spectrometer, showing enhanced hydride region. The coupling constants presented in Fig. 1a can be calculated from the spectrum. The ‘down-field’ region (0–10 ppm) is vertically multiplied by 8 compared to the ‘up-field’ (hydride) region.

Fig. 4. Experimental timings and RF sequence for the high-field ADAPT-SABRE experiment. The solution was bubbled by $p$-$H_2$ gas for the duration of $\tau_b$; after that an appropriate waiting time ($\tau_w$) was provided to settle down the solution before applying the ADAPT pulse sequence (as shown in Fig. 1b) in $^{15}$N channel on-resonance and selectively exciting the equatorial bound peak. The ADAPT block was repeated $n$ times before a final 90° hard pulse detects the signal. A low-powered continuous wave (CW) pulse was applied on $^1$H channel throughout the experiment on-resonance to the hydrides region.
proportional to $2^{p}$ \( D \). To complete the analogy with the ADAPT method, it has also been noted by Theis et al. [23], that the effect of the CW irradiation is to produce a pulse which transfers population between the hydrogen singlet population to the \( ^{15}N \) transverse magnetization: precisely the same transformation achieved by ADAPT in the subspace of Eq. (3) (see Fig. 2).

3. Experimental results and discussions

All the measurements were carried out with a 500 MHz Bruker Avance III spectrometer equipped with a broad-band (BBO) probe at 298 K. The sample was prepared in a 5 mm NMR tube by mixing 10 mM of [IrCl(COD)(IMes)] (IMes = 1,3-bis(2,4,6-trimethylphenyl) imidazole-2-ylidene, COD = cycloocta-1,5-diene) pre-catalyst and 50 mM of \(^{15}N\)-ethyl nicotinate [29] in 0.6 ml methanol-\( d_4 \) solution. A valve-controlled para-hydrogen flow PTFE (polytetrafluoroethylene) tube was immersed inside the NMR tube to bubble the solution with para-hydrogen originating from a para-hydrogen generator with 90% enrichment. To accurately assign the resonances of the concerned spins and measuring their coupling constants within the network, we first performed a standard in-magnet PHIP experiment by bubbling p-H\(_2\) inside the spectrometer and recording a \(^1H\) spectrum upon applying a 45° pulse on the proton channel. A large and transient antiphase spectrum is observed in the hydride region reflecting the hydrogenation product, resulting from reaction with p-H\(_2\). Both the hydrides (spin-1 and -2) show overlapping resonances at -22.66 ppm as shown in Fig. 3. The \(2J_{HH}\) and \(cis-2J_{NH}\) coupling constants were simply measured from the hydride region of the spectrum. Whilst \(trans-2J_{NH}\) could not be observed it is expected to be negligible (<0.5 Hz) in these types of system [12,27].

Next we performed a standard SABRE-SHEATH experiment [22] by shaking the solution with p-H\(_2\) inside a \(\mu\)-metal can and then quickly recording a \(^{15}N\) spectra upon a 90° detection pulse. The purpose of this experiment was to find out the \(^{15}N\) resonance frequencies for the different forms of the substrate; the substrate can be free from the catalyst and also bound to the catalyst. Two different forms of bound substrate exist here (equatorial and axial position). In our study, we observe \(^{15}N\) free resonance at 303.22 ppm and equatorial-bound resonance at 256.60 ppm whilst the axial-bound resonance remains undetected due to insufficient detectable polarization. The parameters obtained from these initial screening experiments were sufficient to optimize the ADAPT pulse sequence numerically (see Fig. 1b).

![Fig. 5. ADAPT-SABRE derived \(^{15}N\) spectra showing free substrate peaks with increasing numbers (n) of ADAPT block (see Fig. 4). A maximum enhancement of 940-fold was achieved at n = 40.](image)

![Fig. 6. \(^{15}N\) NMR spectra of \(^{15}N\)-ethyl nicotinate showing hyperpolarized spectra acquired by ADAPT-SABRE method in seconds and (b) corresponding thermal spectra obtained by 90° hard pulses over 400 transients with 120 s of recycle delays (total experimental time of 13.5 h). The free \(^{15}N\) resonance peak was observed at 303.2 ppm whilst the ‘equatorial-bound’ peak was found at 255.6 ppm.](image)
The high-field SABRE experiments were performed according to the experimental protocol depicted in Fig. 4. After a suitable relaxation delay, p-H2 was bubbled through the solution for a duration of τg (typically 10 s) followed by a short waiting period (τw = 1 s) for the solution to settle. The optimized ADAPT pulse sequence immediately followed with a total duration of τf (≈ A × m × n), selectively exciting the bound-15N resonance without affecting the free resonance. The offset for 15N channel was set on the bound-peak (256.60 ppm) whilst the band-width (BW) of the RF pulse was kept at 500 Hz (≈10 ppm). After n number of repeated ADAPT blocks, a hard 90° pulse was applied to detect the magnetization. During the RF sequence, a continuous-wave (CW) pulse of 1 kHz band-width was applied in proton channel resonant with the hydride region to enforce chemical equivalence between the hydride protons. However, when performed without any CW pulse in proton channel, we observe polarization transfer, albeit with a 30–40% of less enhancement than earlier.

Using the numerically optimized parameters (see Section 2), the ADAPT sequence efficiently transfers singlet-order polarization of hydrides into longitudinal polarization of 15N nuclei that are connected to the network. An optimum level of polarization can be realized for ADAPT30 with A = 8 ms and m = 5 in a total pulse duration of only 40 ms. Using these parameters, one ADAPT block achieves 196-fold enhancement factor (ε) for the free 15N target. The enhancement factor (ε) of hyperpolarization was calculated as the ratio of the integral for the hyperpolarized signal and the integral for the thermal signal divided by the number of thermal scans. As SABRE is an exchangeable process, the polarization transfer rate is limited by the residence time of both the hydrides and the free substrate, which are in the range of 100–200 ms under standard SABRE conditions. As a result, hydrogenation with p-H2 and therefore the polarization transfer does not occur simultaneously across the entire sample volume. For this reason, the ADAPT block has to be repeated multiple times to build up 15N polarization. In practice, one block of ADAPT sequence transfers only a fraction of available polarization into 15N magnetization. But as long as fresh p-H2 keeps exchanging together with non-polarized substrates, we observe accumulation in 15N magnetization by repeated application of the ADAPT block. Fig. 5 presents a gradual build-up in free 15N magnetization with increasing numbers of ADAPT blocks. A maximum enhancement of 940-fold was achieved after 40 ADAPT blocks of total duration ~1.6 s. Ultimately, spin relaxation together with p-H2 consumption take over in the magnetization build-up process and ε starts decreasing with larger loop counts. The ε is also limited by several other factors e.g. RF inhomogeneity and imperfections caused by mixing and diffusion [30,31].

In Fig. 6 we show the success of the ADAPT-SABRE method in driving polarization from singlet-hydrides to bound 15N nuclei of ethyl nicotinate and enhancing the free 15N response. The earlier parameters were used to achieve the 1-shot ADAPT-SABRE spectra in Fig. 6a. The corresponding thermal signal in Fig. 6b was acquired by averaging 400 scans with a recycle delay of 120 s (T1(15N) = 21.5 ± 0.5 s), taking over 13.5 h. The bound peaks remain undeTECTable in the thermal measurements even after many scans signifying greater relative enhancements as predicted previously [25]. The lack of a thermal signal for the bound peaks can be attributed to their broad line shapes, they are visible after 2000 scans.

4. Conclusions

In summary, we have used ADAPT-SABRE to generate 15N hyperpolarization at high magnetic field without the requirement of below-earth field sample mixing. The conversion is robust and faster than previously reported methods: it took only 1.6 s to reach nearly 3 orders of signal enhancement for a 15N target. This method has several advantages over the low-field SABRE mechanism, e.g. constant field shuttling, unnecessary signal losses during transport. The presented scheme whilst being inherently simple, can be easily augmented to any SABRE active species and their 13C, 19F and 31P nuclei. We are currently working on its further optimization via exchange rate, sample concentration and additive-dependence studies. In the future, we plan to examine how the dynamics of SABRE exchange can be harnessed to improve this ADAPT process. We believe it will be of particular importance in terms of achieving in vivo hyperpolarization, where sample transport poses a significant challenge to hyperpolarization based experiments.

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underwent palladium mediated hydrogenation to give the desired compound. Full experimental procedures and characterisation data is available from the York Data Catalogue.

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