Deuteron-decoupled singlet NMR in low magnetic fields: Application to the hyperpolarization of succinic acid

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The reaction of unsaturated substrates with hydrogen gas enriched in the para spin isomer leads to products with a high degree of nuclear singlet spin order. This leads to greatly enhanced NMR signals, with important potential applications such as magnetic resonance imaging (MRI) of metabolic processes. Although parahydrogen-induced polarization has the advantage of being cheap, compact, and mobile, especially when performed in ultralow magnetic fields, efficiency is lost when more than a few protons are involved. This strongly restricts the range of compatible substances. We show that these difficulties may be overcome by a combination of deuteration with the application of a sinusoidally modulated longitudinal field as well as a transverse rotating magnetic field. We demonstrate a six-fold enhancement in the 13C hyperpolarization of [1-13C, 2,3-d2]-succinic acid, as compared with standard hyperpolarization methods, applied in the same ultralow field regime.

Magnetic Resonance Imaging (MRI) represents a versatile, non-invasive diagnostic tool, but suffers from low sensitivity, due in part to the very low levels of equilibrium nuclear spin polarization. Very large (~ 105) polarization enhancements are available by hyperpolarization techniques [1-10], leading to the possibility of imaging low-concentration metabolites, with important clinical applications [1-9]. Hyperpolarization is particularly advantageous for 13C-labelled substances, since 13C nuclei often have relatively long relaxation times compared to the abundant protons, allowing the hyperpolarization effect to persist longer, as well as a large chemical shift range, facilitating compound-specific imaging. For example, hyperpolarization of 13C-labelled metabolites such as pyruvate, fumarate and succinate (1,4-butanedioic acid, see figure 1) allow monitoring of the tricarboxylic acid (TCA) cycle, exposing metabolic anomalies which are indicative of disorders such as cancer [2-10].

Most applications of hyperpolarized compounds for in vivo metabolic imaging currently use the Dynamic Nuclear Polarization (DNP) technique, for which commercial apparatus exists [11-10]. However, the DNP technique suffers from high capital and running costs, low throughput, large footprint and technical complexity. This has stimulated interest in compact, mobile and less expensive methods such as parahydrogen-induced polarization (PHIP) [11, 12]. Although less general than DNP, PHIP-based techniques do not require high magnetic fields or cryogenic equipment, and also have high potential throughput.

Parahydrogen-induced polarization of a 13C-labelled target substance is achieved by catalytic hydrogenation of a suitable precursor with para-enriched hydrogen gas (pH2). The proton pairs in the reaction product acquire a high degree of nuclear singlet order. The non-magnetic nuclear singlet order is converted into observable 13C magnetization by the application of suitably modulated magnetic fields, leading to large enhancements of the 13C NMR signals [13-15].

The conversion of proton singlet order into 13C magnetization is a crucial step. Methods exist for implementing this transformation either in the high magnetic field of an NMR magnet, or in low magnetic field. Low-field procedures are preferred for real-world applications, since they allow the use of relatively inexpensive, compact, and mobile equipment [17-21]. Recently, purified solutions of highly polarized [1-13C]-fumarate have been produced using a low magnetic field sweep for singlet-to-magnetization conversion [21]. However, [1-13C]-fumarate is a favourable case since it has a very simple spin system, containing only three coupled spins-1/2.

The application of such procedures to more complex spin systems is problematic. Every additional spin doubles the spin dynamical complexity and decreases the transformation efficiency. For example, [1-13C]-succinate contains 5 coupled spins-1/2, and has 4 times as many quantum states as [1-13C]-fumarate. In general field-sweep methods perform poorly for multiple-spin systems, since it is hard to maintain control of the highly complex nuclear spin dynamics, especially through the multiple level crossings associated with field sweeps [19].

The number of coupled protons may be reduced by selective chemical replacement of protons by deuterons. An example is

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Fig. 1 Chemical structure of [1-13C, 2,3-d2]-succinic acid. The spin-spin couplings have been reported previously [16], except for those involving deuterium, which are estimated. The labile protons are ignored.

<table>
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<tr>
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shown for $[1^{13}C, 2,3-d_2]$-succinic acid in figure 1. Although this leads to a beneficial simplification of the proton spin system, deuteration introduces problems in the context of ultralow-field NMR, where the spin-spin couplings are of the same order of magnitude as nuclear Larmor frequencies. In this regime, the rapid quadrupolar relaxation of the deuterium nuclei induces rapid decoherence of the entire spin system, resulting in substantial polarization losses [22,24]. Furthermore, singlet-triplet mixing effects induced by heteronuclear couplings lead to further losses [25,28]. Conventional heteronuclear decoupling is not only decouple disruptive deuterium nuclei, but also suppress singlet-triplet mixing effects, in the regime of ultralow magnetic fields.

In this article we demonstrate efficient singlet-to-magnetization conversion in the micro-Tesla regime, while simultaneously achieving deuteron decoupling and suppression of singlet-triplet mixing. This is done by applying two periodically modulated fields at the same time. The first field is a sinusoidally modulated longitudinal field, as in the recently described Weak Oscillating Low Field (WOLF) technique [29]. The second field rotates in a plane perpendicular to the first, as in the recently described Singlet-Triplet Oscillations through Rotating Magnetic Fields (STORM) method [30]. We show that the combination of these two fields achieves selective polarization transfer with suppression of deuterium-induced relaxation. We achieve a $^{13}C$ polarization of $\approx 6.1\%$ for a $50 \text{mM}$ solution of $[1^{13}C, 2,3-d_2]$-succinic acid, in the case of a modest parahydrogen enrichment level of $50\%$. This result is encouraging for the application of low-field parahydrogen-induced polarization to a wide range of deuterated molecular systems.

To demonstrate the technique, 30 mg (0.25 mmol) of $[1^{13}C, 2,3-d_2]$-fumaric acid (Sigma-Aldrich) and 18.1 mg (0.025 mmol) of the $[\text{Rh(dpdp})(COD)]BF_4$ catalyst were dissolved in methanol-$d_4$ (5 mL). 50%-para-enriched $H_2$ was prepared by passing hydrogen gas at a pressure of 10 bar over iron oxide at 77 K. Each experimental run started by transferring 250 $\mu$L of the stock solution to a high-pressure NMR tube and heating the sample for an interval of duration $\tau_{H}$, in the presence of a static bias field of 50 $\mu T$, in order to separate the Larmor frequencies of the different isotopes, helping preserve the proton singlet order. The magnetic field was then swept linearly from $\approx 0 \mu T$ to $\approx 1 \mu T$ over a duration $\tau_T$, to induce the singlet-to-magnetization transfer. As shown in table 1 experimental optimization of the bubbling time ($\tau_H = 7$ s) and the transfer time ($\tau_T = 90$ ms) resulted in a $^{13}C$ polarization of $p = 0.98\% \pm 0.04\%$ for the $[1^{13}C, 2,3-d_2]$-succinic acid reaction product. A representative spectrum for protocol (a) is given in figure 3a. The moderate level of $^{13}C$ polarization level is not surprising, since the conventional field-ramp protocol is subject to interference from both deuterium quadrupolar relaxation and singlet-triplet mixing.

Protocol (b) in figure 2 employs the rotating-field STORM technique [30] for the polarization transfer. After bubbling with para-enriched $H_2$, the transverse rotating STORM field was applied for a duration $\tau_T$ in the presence of a longitudinal 6 $\mu T$ bias field. The amplitude of the rotating STORM field was $B_{\text{STORM}} = 3.9 \mu T$. The rotation frequency of the STORM field was $\omega_{\text{STORM}}/2\pi = 72.5$ Hz. As discussed in the supplementary material, this choice of field parameters Tactics the proton spins from the deuteron spins during the transfer step. Figure 2b shows the resulting $^{13}C$ spectrum for an optimal STORM duration of $\tau_T = 100$ ms. The final $^{13}C$ polarization was estimated to be $p = 1.70\% \pm 0.22\%$, which is significantly higher than that for protocol (a). Since the bubbling protocols are identical in procedures (a) and (b), we attribute the increase in the $^{13}C$ polarization level to the robustness of the STORM pulse with respect to interference from fast deuteron relaxation.

Although the use of a rotating-field STORM suppresses the deleterious effects of deuteron relaxation during the singlet-to-magnetization transfer, the problem of singlet-to-triplet mixing remains. In the current context of very low magnetic fields, this mixing is caused by differences in the $J$-couplings of the two protons to heteronuclei such as $^{13}C$ and $^2H$ (see the coupling network in figure 1). As a result of these couplings, proton singlet state is not a Hamiltonian eigenstate for the succinic acid product. Reaction of the fumaric acid precursor with para-hydrogen therefore initiates coherent oscillations of the spin density operator. Since the hydrogenation reaction is relatively slow, these
For the experimental implementation of protocol (c) we kept the STORM pulse parameters identical to protocol (b), but added a WOLF pulse with amplitude $B_{WOLF} = 20 \, \mu T$ and frequency $\omega_{WOLF}/2\pi = 800 \, Hz$ during the hydrogenation interval. The WOLF pulse parameters were based on the analysis given in the supplementary information. The pH, bubbling period was optimized to $\tau_B = 15 \, s$ for protocol (c). The resulting $^{13}C$ spectrum is shown in figure 3c, which corresponds to a $^{13}C$ polarization of $p = 6.13\% \pm 0.07\%$. As highlighted in table 1, this represents a factor of 6 improvement over a conventional field sweep procedure.

The molar polarization (product of polarization level and concentration) has been proposed as a more useful hyperpolarization metric than the polarization level alone [21]. The molar polarization achieved in the current experiment is estimated to be $6.12\% \times (49 \pm 1) \, mM = 3.00 \pm 0.06 \, mM$ (see Supporting Information).

Figure 4 compares the observed $^{13}C$-polarization as a function of bubbling time $\tau_B$ for protocols (b) and (c). Since these protocols use the same singlet-to-magnetization conversion procedures, differences in performance may be attributed to the chemical and spin dynamics during the hydrogenation. The markers represent experimental data, whereas the solid curves represent numerical simulations. For protocol (b), which has no special intervention during the hydrogenation, the $^{13}C$-polarization quickly reaches a plateau at a relatively low level. For the WOLF+STORM approach (protocol (c)) the build-up of $^{13}C$-
polarization continues to longer times with a plateau reached at \( t_\text{d} = 15 \) s. No significant reduction in \( ^{13}\text{C}-\)polarization was observed even beyond 20 seconds of bubbling. These data support the hypothesis that the simultaneous WOLF+STORM fields suppress singlet-triplet mixing during hydrogenation.

The solid lines in figure 4 show simulations derived by a Monte-Carlo procedure which combined many spin dynamical trajectories initiated at random times, according to a statistical model of the chemical kinetics. Details are given in the Supporting Information. An acceptable match between experiment and simulation is attained for both protocols (b) and (c), assuming an effective hydrogenation rate constant given by 

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k_{\text{eff}} = 0.05 \pm 0.01 \ \text{s}^{-1}
\]

and a singlet order decay time constant \( T_2 \) of at least 60 seconds. This indicates that singlet spin order accumulates on the proton pair during the hydrogenation under WOLF+STORM irradiation.

To summarize, we have demonstrated that it is possible to hyperpolarize the \( ^{13}\text{C} \) nuclei of isotopically labelled succinate using a compact, low-cost apparatus which exploits para-enriched hydrogen gas, a cheap and readily available substance. The achieved level of \( \sim 6\% \) \( ^{13}\text{C} \)-polarization could readily be increased to \( \sim 18\% \) by using pure para-H\(_2\) gas. This polarization level is competitive with dissolution-DNP procedures but is achieved far more rapidly and with much less cost. This suggests the feasibility of this method for producing hyperpolarized \( ^{13}\text{C} \)-succinate for metabolic imaging applications. Further improvements in the yield and throughput are likely to be possible, for example by operation at higher pressure and temperature.

The demonstration described here combines several chemical and spin dynamical methodologies: (1) the use of a deuterated and \( ^{13}\text{C} \)-labelled fumarate precursor, in order to generate a deuterated succinate with a simplified proton spin system; (2) novel magnetic field modulation methodologies applied under ultralow-field conditions, which induce efficient singlet-to-magnetization transfer even in the presence of the rapidly relaxing deuteron nuclei, while also suppressing singlet-triplet mixing effects during the hydrogenation.

We anticipate that this proof-of-concept experiment should open up the technique of ultralow-field parahydrogen-induced polarization to a much wider range of compounds than is currently accessible. Related hyperpolarization techniques such as side-arm hydrogenation [31, 32] are also likely to benefit, allowing the use of deuteration to simplify the spin systems and improve spin-dynamical efficiency, while avoiding the problems associated with rapid deuteron relaxation.

**Conflicts of interest**

There are no conflicts of interest to declare.

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**References**


