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Pulse-Programmable Magnetic Field Cycling of Parahydrogen-Induced Polarization by Side Arm Hydrogenation

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Abstract

Among the hyperpolarization techniques geared toward *in vivo* magnetic resonance imaging, parahydrogen-induced polarization (PHIP) shows promise due to its low cost and fast speed of contrast agent preparation. The synthesis of ¹³C-labeled, unsaturated precursors to perform PHIP by side arm hydrogenation has recently opened new possibilities for metabolic imaging owing to the biological compatibility of the reaction products, although the polarization transfer between the parahydrogen-derived protons and the ¹³C heteronucleus must yet to be better understood, characterized, and eventually optimized. In this realm, a new experimental strategy incorporating pulse-programmable magnetic field cycling has been developed. The approach is evaluated by measuring the ¹³C polarization of ethyl acetate-1-¹³C, *i.e.* the product of pairwise addition of parahydrogen to vinyl acetate-1-¹³C, resulting from zero-crossing magnetic field sweeps of various durations, amplitudes, and step sizes. The results demonstrate (i) the profound effect these parameters have on the ¹H to ¹³C polarization transfer efficiency and (ii) the high reproducibility of the technique.

Graphical Abstract

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The hyperpolarization of nuclear spins can enhance the sensitivity of nuclear magnetic resonance (NMR) by several orders of magnitude when compared to thermal polarization levels.^{1–3} Recent advances in the hyperpolarization of ^{13}C -containing biomolecules have led to their use as injectable contrast agents to probe *in vivo* metabolism via magnetic resonance imaging (MRI).^{4–8} Clinical trials using hyperpolarized (HP) ^{13}C contrast agents such as pyruvate are now underway to probe cancer.^{9–11} Although dissolution dynamic nuclear polarization (d-DNP)^{12–13} is the leading HP technology at the moment, other alternative technologies have been garnering a lot of research attention lately.^{14–15}

Parahydrogen-induced polarization (PHIP), which is based on the pairwise parahydrogen (pH_2) addition to an unsaturated substrate,^{16–17} is one of these techniques. Several biomolecules have been hyperpolarized and tested *in vivo* using PHIP.^{18–23} The recent advent of the so-called side-arm hydrogenation (SAH) approach developed by Aime and co-workers^{24–25} has significantly expanded the range of biomolecules that can be hyperpolarized by means of PHIP.^{14, 26} This becomes possible by hydrogenation of the ester moiety, which contains an unsaturated $\text{C}=\text{C}$ or $\text{C}\equiv\text{C}$ bond. After the completion of pH_2 pairwise addition in the side arm and polarization transfer from pH_2 -derived protons to ^{13}C nucleus of the carboxyl group, the side arm (ester moiety) can be cleaved to obtain the final HP contrast agents prior to *in vivo* administration.²⁵ Many carboxyl groups can be hyperpolarized; for instance, esters of ^{13}C -pyruvate,^{25, 27–28} ^{13}C -lactate²⁹ ^{13}C -acetate^{24, 30–31} have been successfully demonstrated.^{32–33} The rationale for hyperpolarization storage of the carboxylic ^{13}C nucleus (despite polarization losses during the transfer) is the significantly enhanced lifetime of its HP state with *in vivo* T_1 on the order of a minute or more versus *in vivo* proton T_1 of a few seconds.

In the context of PHIP-SAHP, two approaches have been employed to transform the pH_2 -derived singlet spin order into magnetization of the spin-spin coupled carboxylic ^{13}C nucleus: magnetic field cycling (MFC)^{24–25} and radio-frequency (RF) pulse sequences.³⁴ While both approaches have their own merits, MFC only requires magnetic shielding to achieve an adiabatic passage at ultralow magnetic field, *i.e.* well below the microtesla, through the avoided level anticrossings (LACs) of the nuclear spin energy levels.^{35–37} To date, all ^{13}C -hyperpolarized demonstrations with PHIP-SAHP have been performed through manual MFC experiments, during which the sample is slowly pulled from the magnetic shield. Development of automated approaches and hardware for preparation of PHIP hyperpolarized contrast agents is however critical in the context of their biomedical applications to improve reproducibility and robustness. Levitt and co-workers have recently

reported on an automated MFC of ^{13}C -fumarate, a non-SAH molecule with a strongly coupled and highly symmetric spin system.³⁸ Although up to 100% ^{13}C polarization appeared theoretically feasible, a maximum of only ~1% was measured experimentally, leaving the door open to address important issues related to the programming of efficient adiabatic field sweeps or the reproducibility of the data.

Here, an automated – pulse-programmable – MFC study of the PHIP-SAH molecule ethyl acetate-1- ^{13}C (EA) is described (Scheme 1). Injections of non-HP acetate-1- ^{13}C (up to 10 g) have been demonstrated in brain metabolism studies,^{39–40} and HP acetate-1- ^{13}C has been used in studies of liver and brain metabolism.^{41–43} MFC of EA after pairwise pH_2 addition to vinyl acetate-1- ^{13}C (VA) has been studied in the laboratory^{25, 32–33, 44} and treated theoretically via the full density matrix approach.^{24, 45}

MATERIALS AND METHODS

A schematic of the apparatus is shown in Figure 1.

The setup is controlled by a low-field NMR spectrometer (Kea2, Magritek, New Zealand), which sends the RF excitation pulse, records the free induction decay (FID), modulates the waveforms applied to the field-cycling electromagnet, and administrates a series of transistor-transistor logic (TTL) outputs to activate – through solid-state relays (SSR)⁴⁶ – the power supply of the B_0 electromagnet (6060D, GW Instek, Taiwan), the degaussing circuit of the magnetic shields, and the solenoid valve (Series 331, Burkert, USA) delivering pH_2 to the solution. A gradient amplifier (7224-P, AE Techtron, USA) was employed due to the wide range of shaped pulses and precise timing possible as well as to their broad availability in the MRI community.

Three layers of μ -metal are used as an ultralow magnetic field chamber (ZG-209, Magnetic Shield Corp., USA) providing an attenuation of ~1500-fold of the Earth magnetic field (~32 μT). Within the shields, two solenoid electromagnets are placed concentrically: the external one (200 mm inner diameter (I.D.)) produces the time-dependent magnetic field and the inner one (80 mm I.D.) the static B_0 field (5.8 mT). A resistive attenuator (R) is mounted in series with the gradient amplifier. Two 3D-printed spacers are mounted to hold the B_1 saddle coils – orthogonal to each other and to B_0 – and to maintain the NMR tube at the center axis of the shields. The saddle coils, 100 mm in length and made of 64 turns of 28 AWG wire, were tuned to 78.8 kHz and matched to 50 Ω using capacitors (Series 25, Voltronics, USA) of 3970 pF (C_T) and 1000 pF (C_M) tuning and matching capacitances. The geometry was optimized with standard 5-mm NMR tubes filled with 0.5 mL of solution to minimize field inhomogeneity. The RF pulses were calibrated with an aqueous 10 mM CuSO_4 solution (^1H T_1 ~ 50 ms) and B_0 was tuned to proton resonance frequency by adjusting the amperage. The 90° pulse duration of ^{13}C was determined to be 358 μs at 1 W.

The pH_2 generator has been described elsewhere.⁴⁷ It enriches pH_2 with high-pressure, pulsed injections of normal hydrogen into a catalyst chamber maintained at ~20 K by a closed cycle cryocooler (expander: DE-204, compressor: ARS-4HW, Advanced Research Systems, USA). The generator has been slightly modified to produce more pH_2 (2 slm with

[pH₂] > 99%) and to operate with a closed loop water-cooling system (CoolPak, Advanced Research Systems, USA) that improves its mobility. A mass-flow controller (SmartTrak 50, Sierra Instruments, USA) regulates the pH₂ gas flow.

A key aspect to ensure a high degree of reproducibility of the experimental data relies in the strategy chosen for degaussing the μ -metal shields, which get magnetized by each MFC, *i.e.* when the gradient and detection field are applied. Although the degaussing circuitry employed here is standard, using three thermistors (9 Ω) in parallel instead of one offers the advantage of speeding up the time required for each thermistor to return to room temperature and its corresponding resistance between two runs.⁴⁸ A fan is installed to further improve this cooling. To avoid any possible artifact due to manual operation, degaussing is controlled through the Kea2 with 110 VAC pulses of 2 s duration.⁴⁸

The MFC assembly was used to apply sequences of events such as the one depicted in Figure 2. All the experiments were performed by pressurizing the NMR tube to 70 psig, heating at 80 °C, and bubbling pH₂ for 5 s with the mass flow controller set to a flow rate of 150 sccm. The hydrogenation reaction was performed at a negative magnetic field ($-32 \mu\text{T}$) so that the sample only experiences the zero-field once. The two diabatic (fast) transitions, from $-32 \mu\text{T}$ to $-B/2$ and from $+B/2$ to $+32 \mu\text{T}$, were of 10 ms duration (10 steps). The time evolution of the magnetic field was checked with the Physics Toolbox Sensor Suite application for smartphone (Vieyra Software, USA). After the B_0 field was turned on, a delay of 2 s was applied to let the electromagnet stabilize before sending the 90° excitation pulse for *in situ* detection of ¹³C polarization. Due to these constraints (stabilization for *in situ* detection / transport for 1.4 T detection), the systematic underestimation of the measured polarization levels due to relaxation was found similar for both detection methods.

Examples of spectroscopic acquisitions of ¹³C HP signal and ¹H thermal signal reference signal measured *in situ* at 5.8 mT are provided in Figure 3a and Figure 3b, respectively. A 1.4 T benchtop NMR spectrometer (SpinSolve Carbon, Magritek, New Zealand) was used in a complementary manner, *i.e.* to crosscheck the polarization enhancements measured *in situ* and to get better signal-to-noise ratio. An example of HP ¹³C spectrum is shown in Figure 3c along with a single-scan ¹³C thermal signal reference in Figure 3d. Standard deviations of $\pm 0.5\%$ and $\pm 0.4\%$ for the ¹³C polarization (*P*) measured *in situ* and by the 1.4 T spectrometer were obtained for five consecutive measurements, respectively. The total errors on ¹³C *P* can be estimated by adding a systematic contribution of $\pm 0.5\%$ originating from sample preparation.

RESULTS AND DISCUSSION

Hyperpolarization of ethyl acetate-1-¹³C.

The unsaturated precursor VA – synthesized in our laboratory previously^{26, 32} – and the catalyst (Bicyclo [2.2.1]hepta-2,5-diene)[1,4-bis(diphenylphosphino)butane] rhodium(I) tetrafluoroborate, CAS 82499-43-2, Sigma-Aldrich) were dissolved in CH₃OD to obtain a stock solution with concentrations of 40 mM and 4 mM, respectively. Argon gas was bubbled through this solution for 1–2 min to remove oxygen. The samples were prepared by filling 0.5 mL of the stock into standard 5 mm NMR tubes, which were subsequently sealed

under argon atmosphere (WG 1000–8, Wilmad, USA). The glassware was flushed with argon before and after the addition of the solution.

Effect of Gradient Step Rate ω .

The field sweeps were discretized by a number of gradient steps and a series of experiments were carried out to evaluate the effect of the step size and duration on the ^1H to ^{13}C polarization transfer efficiency. The results were obtained for linear sweeps of amplitudes $B = 4 \mu\text{T}$ and $10 \mu\text{T}$. They are shown in Figure 4 as a function of the gradient step rate ω expressed in units of steps per μT per second (steps/ $\mu\text{T/s}$). In both cases, a rate $\omega < 20$ steps/ $\mu\text{T/s}$ did not allow the spin system to reach its highest polarization transfer efficiency. On the other hand, increasing ω to values > 100 steps/ $\mu\text{T/s}$ was found fruitless and even too demanding for the gradient amplifier (due to limits of switch time) in the case of short sweep durations combined with large amplitudes. The influence of the resistive attenuator (R) was also evaluated with $R = 150$ and 1800Ω . As expected, no changes were observed in the measured ^{13}C polarization. The following experiments were performed with $R = 150 \Omega$ to limit the power of the gradient amplifier.

Effect of Sweep Duration t .

^{13}C P levels were measured as a function of sweep duration t with values ranging from 1 to 90 s. In these experiments, B was set to $4 \mu\text{T}$ (-2 to $+2 \mu\text{T}$) and ω to 50 steps/ $\mu\text{T/s}$. The results, obtained via *in situ* and 1.4 T detections, are shown in Figure 5. The data are characterized by a sharp rise of ^{13}C polarization from 0 to $7\text{--}7.5\%$ for ramps of 4 to 10 s, followed by a slower decay towards $\sim 1\%$ at 90 s. A bi-exponential model was adjusted to describe the experimental data:

$$P(t) = \frac{P_{\max}}{T_{PT}/T_{\text{relax}} - 1} \cdot \left(e^{-t/T_{PT}} - e^{-t/T_{\text{relax}}} \right) \quad \text{Eq. (1)}$$

where $P_{\max}$ is the maximum polarization without relaxation, T_{PT} the time constant for polarization transfer, and T_{relax} the time constant for relaxation. Values of $P_{\max} = 8.6 \pm 0.4\%$ and $9.1 \pm 0.3\%$, $T_{PT} = 1.9 \pm 0.3 \text{ s}$ and $2.0 \pm 0.2 \text{ s}$, $T_{\text{relax}} = 38 \pm 4 \text{ s}$ and $36 \pm 3 \text{ s}$ were thus determined for *in situ* and 1.4 T detection modes, respectively. These time constants need to be taken with caution because the polarization transfer only takes place at magnetic fields close to the LACs and not over the complete field sweep, while the observed relaxation is a combination of processes occurring from both ^1H and ^{13}C spin orders as well as the transition states in between.

Effect of Sweep Amplitude B .

Two series of experiments were performed to get insight into the effect of the sweep amplitude B on the polarization transfer efficiency. A fixed sweep duration of 8 s, *i.e.* near the effective $P_{\max}$ measured for $B = 4 \mu\text{T}$, was programmed and the ^{13}C polarization measured for B values ranging from $1 \mu\text{T}$ (-0.5 to $+0.5 \mu\text{T}$) to $20 \mu\text{T}$ (-10 to $+10 \mu\text{T}$) (Figure 6a). The results show that ^{13}C P rises from 0 to a maximum of nearly 10% for $B = 5 \mu\text{T}$, followed by a plateau at 7% for $B = 6$ to $20 \mu\text{T}$. The ^{13}C polarization was also measured as a function of t for different B values (Figure 6b). The data were fitted to

Equation 1 and the optimized parameters are summarized in Table 1. Overall, the results indicate that varying B can induce significant changes in the maximum polarization, *i.e.* from nearly 2 to 10%, and the relaxation time, *i.e.* from nearly 20 to 100 s, whereas the polarization transfer time remains apparently constant with an average value of 1.6 s.

Efficiency of Polarization Transfer from Nascent pH_2 -Derived Protons to ^{13}C Nucleus.

Under similar hydrogenation conditions, the ^1H P polarization was measured to be equal to $28 \pm 5\%$ (average of H_A and H_B polarizations) via ALTADENA spectral characterization.⁴⁹ The maximum ^{13}C polarization measured here (9.8%) corresponds to a ^1H to ^{13}C polarization transfer efficiency of about $35 \pm 6\%$ that falls on the upper side of the results of the MFC simulations neglecting relaxation (25–30%).^{24, 45} Over the course of the experiments, several manual MFC were performed. In this case, the ^{13}C polarization was systematically comprised between 5% and 7%, *i.e.* a polarization transfer efficiency of 18–25%. The difference between manual and automated MFC is therefore substantial but yet not spectacular. However, the increase of polarization observed with B amplitudes of 4 and 5 μT shows the existence of a dynamical bottleneck directed toward better polarization transfer efficiency. The key deliverable of this automated approach relies anyway in the high reproducibility of both the magnetic field cycling and the ^{13}C polarization measurements, which taken together allow for the technique to be sensitive enough for fine-tuning as well as for fundamental studies. Moreover, the design of more advanced pulse shapes may potentially boost the efficiency of polarization transfer and address the issue of dynamical bottlenecks. Finally, the use of high-pressure spray-injection PHIP hyperpolarizers^{21, 50–52} instead of the pH_2 bubbling setup employed here should in principle enhance the ^1H polarization levels and consequently automated MFC should deliver substantially higher ^{13}C polarization (ongoing work in our laboratory).

CONCLUSION

A new, automated approach for magnetic field cycling of PHIP via side arm hydrogenation has been developed with the main objectives of performing and detecting the ^1H to ^{13}C polarization transfer in a highly reproducible manner. The sensitivity of the technique is demonstrated through a systematic investigation over the effects of step size and sweep speed of the zero-crossing magnetic field ramps. The highest level of ^{13}C polarization ($\sim 10\%$) obtained at a relatively high concentration (~ 40 mM with complete conversion of the unsaturated precursor) is certainly suitable for potential biomedical applications. Future studies should address the following issues: (i) the role of single-triplet mixing during pairwise pH_2 addition and ways to maximize the proton polarization prior to polarization transfer via MFC, (ii) improving the degree of pairwise pH_2 addition, (iii) pulse shaping. The recent development of water-soluble PHIP catalyst of low toxicity⁵³ bode well for *in vivo* translation of EA and other ^{13}C HP biomolecules amenable by PHIP-SAH via MFC: pyruvate-1- ^{13}C ,²⁸ lactate-1- ^{13}C ,²⁹ and potentially many others.

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Figure 1. Schematic of the pulse-programmable magnetic field cycling experiment capable of performing PHIP-SAH and measuring ^{13}C polarization *in situ*.

Figure 2. Sequence of events to perform automated magnetic field cycling (MFC) of PHIP-SAH and detect ^{13}C polarization *in situ*.

Figure 3.

(a) *In situ* ^{13}C spectrum of HP ethyl acetate-1- ^{13}C after MFC; (b) *In situ* ^1H spectrum of the signal reference sample (water doped with ~ 10 mM CuSO_4) acquired at the same Larmor frequency than (a); (c) ^{13}C spectrum of HP EA after MFC obtained with the 1.4 T spectrometer; (d) ^{13}C spectrum of the signal reference sample (neat acetic acid-1- ^{13}C) for detection at 1.4 T.

Figure 4.

(a) ^{13}C polarization of EA measured with a 1.4 T spectrometer for a sweep amplitude of $B = 4 \mu\text{T}$, sweep durations of $t = 1, 2$, and 8 s , and sweep rates of $\omega = 10, 50$, and $250 \text{ steps}/\mu\text{T/s}$. (b) ^{13}C polarization of EA measured *in situ* as a function of gradient step rate ω for $B = 10 \mu\text{T}$ and $t = 5 \text{ s}$. The data in black were acquired with $R = 1800 \Omega$ and fitted by an exponential function. The green rectangle corresponds to $R = 150 \Omega$.

Figure 5.

^{13}C polarization in EA measured *in situ* (blue) and with the 1.4 T spectrometer (red) as a function of sweep duration t for $B = 4 \mu\text{T}$ and $\omega = 50 \text{ steps}/\mu\text{T/s}$. Data are fitted by a bi-exponential function (Eq. 1). Dashed lines represent 2σ confidence boundaries.

Figure 6.

(a) ^{13}C polarization in EA as a function of field amplitude B . The data were obtained for a sweep duration $t = 8 \text{ s}$ and a gradient step rate $\omega = 50 \text{ steps}/\mu\text{T/s}$. (b) ^{13}C polarization in EA as a function of sweep duration t for a series of B amplitudes performed with a gradient step rate $\omega = 50 \text{ steps}/\mu\text{T/s}$.

Scheme 1.

pH₂ (H_a-H_b) pairwise addition to vinyl acetate-1-¹³C (VA) resulting in production of ethyl acetate-1-¹³C (EA) with two nascent pH₂-derived protons (note the symmetry breaking of H_a and H_b); following MFC, the polarization is transferred from nascent pH₂ derived protons to ¹³C nucleus.

Table 1.

Fitted parameters $P_{\max}$, T_{PT} , and T_{relax} for different field amplitudes B (Eq. 1).

B (μT)	$P_{\max}$ / %	T_{PT} / s	T_{relax} / s
3	2.3 ± 0.3	1.3 ± 0.3	93 ± 23
4	8.9 ± 0.3	1.9 ± 0.2	37 ± 3
5	11.7 ± 0.4	1.6 ± 0.2	36 ± 3
6	7.9 ± 0.6	1.7 ± 0.3	29 ± 4
10	8.3 ± 0.4	1.5 ± 0.2	23 ± 2