

High-Resolution Hyperpolarized *J*-Spectra with Parahydrogen Discrimination

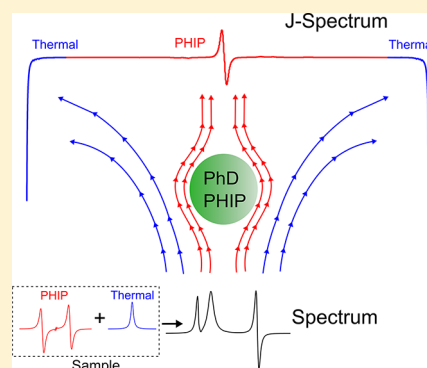
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S Supporting Information

ABSTRACT: Parahydrogen-induced polarization (PHIP) has become a powerful tool not only to overcome the low intrinsic sensitivity of nuclear magnetic resonance (NMR) but also as a probe for catalytic reactions, as a contrast agent in magnetic resonance imaging (MRI), or in analytic chemistry. In complex systems, the antiphase signals coming from parahydrogen in a PASADENA (parahydrogen and synthesis allow dramatically enhanced nuclear alignment) experiment can be partially canceled by the presence of large thermally polarized signals. In the present work, we present a simple method to separate the thermal and hyperpolarized contributions by taking advantage of their very different evolution during a modified CPMG sequence. The separation is obtained in combination with a property of the fast Fourier transform algorithm (FFT). The technique is experimentally demonstrated for a mixture of hyperpolarized 1-hexene and a large amount of CH_2Cl_2 .

SECTION: Spectroscopy, Photochemistry, and Excited States



The use of parahydrogen-induced polarization^{1,2} (PHIP) in NMR has greatly increased in the last years, not only as a way of reducing experimental times but mostly due to its potential applications in analytical chemistry. For instance, a particular site of the target molecule can be highlighted,³ a chemical reaction process can be monitored,^{4–6} hyperpolarization can be transferred to other protons^{7,8} or heteronuclei, usually ^{13}C ,^{9–13} among other applications. Additionally, the target molecule can be used as a contrast agent in MRI.^{14–18}

Nowadays, PHIP is a rather straightforward method to achieve hyperpolarization in NMR. Enhancement of polarization is produced in general by direct hydrogenation in which molecules of hydrogen gas enriched in the para-state (*p*- H_2) are deposited into an unsaturated precursor before the NMR signal acquisition is carried out, resulting in a product molecule with a specific hyperpolarized site. In the case that the hydrogenation reaction and the NMR experiment are performed at the same high magnetic field, the process is referred to as PASADENA (parahydrogen and synthesis allow dramatically enhanced nuclear alignment).¹ In this type of experiment, the main feature of the spectra is the antiphase character of the signal, associated with the presence of a longitudinal two-spin order term initially present in the parahydrogen density operator (i.e., a term as $I_1^z I_2^z$).^{19,20} This is usually the dominant term for weakly coupled spins or AX systems. If the former parahydrogen are strongly coupled to a third spin in the product molecule, extra longitudinal two-spin order terms arise associated with hyperpolarization transfer. In both cases, the coupling constants are on the order of a few Hz, and thus partial signal cancellation might be present even in the case of high-resolution NMR.

The usual approach to remove the evolution under magnetic field inhomogeneities is the use of a spin echo or a spin echo train, namely, the acquisition of the time domain signal at the top of the successive spin echoes. The experiment consists of the application of a train of 180° pulses separated by a time interval t_E that generate multiple spin echoes in a modified Carr–Purcell–Meiboom–Gil (CPMG)²¹ sequence. However, *J*-coupling evolution is not refocused by the 180° radio-frequency (r.f.) pulses. Spin echoes in combination with *J*-coupling delays have been successfully used for PHIP-MRI^{16,17} and low-field time domain NMR.²² Recently,²³ we have shown that *J*-spectroscopy can be applied to PHIP in PASADENA conditions in order to achieve highly resolved spectra in the presence of inhomogeneous magnetic fields. A modified CPMG sequence was introduced for data acquisition, enabling not only partial peak cancellation removal but also rendering high-resolution partial *J*-spectra,²⁴ that is, an individual spectrum for a specific multiplet can be acquired with the aid of a selective digital filter.

A second aspect to be taken into consideration is the potential overlap of PHIP signals with those arising from thermal polarization that could even produce a complete cancellation of an antiphase resonance line. An elegant solution was provided by Aguilar et al. with the introduction of the OPSY (only parahydrogen spectroscopy) sequence, which relies on the use of a pair of unbalanced pulsed field gradients with ratio 1:2 to filter thermal signals from PHIP-enhanced

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NMR spectra^{25,26} However, partial signal cancellation cannot be dealt for with this method.

The present work addresses the problem of acquisition of highly resolved multiplets in the presence of magnetic field inhomogeneities when there is a frequency overlap of thermal and hyperpolarized signals. We show theoretically and experimentally that the evolution of the PHIP signals during a modified CPMG sequence, in combination with the properties of the fast Fourier transform (FFT) algorithm, produces a natural separation, which allows the discrimination of thermal and hyperpolarized signals. We refer to this as the parahydrogen discriminated-PHIP spectrum (PhD-PHIP).

The antiphase character of PHIP signals produces a modulation of the top of the echoes in a train of refocusing pulses that differs considerably with the evolution of thermal signals.²⁷ We start by monitoring the evolution of the density operator under the action of a CPMG sequence. For simplicity, we assume an isolated two-spin system, which is weakly coupled. Two different density operators are considered for the description of the evolution of the thermal and PHIP signals. The initial thermal operator is $\rho_T(0) \propto I_1^z + I_2^z$, whereas the one corresponding to PHIP is $\rho_P(0) \propto 2I_1^z I_2^z$. The last expression is obtained by taking a time average during a long reaction period.⁸ The signal at the top of the echoes is calculated by considering only J -coupling evolution as Hamiltonians linear on spin operators as the chemical shift or magnetic field inhomogeneities are refocused at those particular time values. Evolutions are described by the usual rotations in the subspaces spanned by $(I_1^z, 2I_1^z I_2^z, 2I_1^y I_2^z)$ for thermally polarized protons and $(2I_1^z I_2^z, I_1^y, 2I_1^x I_2^z)$ for PHIP^{20,28} (see the Supporting Information for detailed calculations). For simplicity, we consider the evolution of one spin only, as schematized in Figure 1, because the calculations are exactly the same for the other spin. The density operator immediately after the first 45° r.f. pulse is shown in the second row of the figure.

This tip angle rather than 90° is applied in order to achieve maximum PASADENA signal. Note that for thermal spins, the state is proportional to I_1^z , giving rise to a maximum signal immediately after the r.f. pulse. On the other hand, the state of the PHIP protons is proportional to $2I_1^z I_2^z$, which is not an NMR observable; therefore, the signal intensity is initially zero. A maximum will be obtained when the operator is aligned with the I_1^x axis. It is clear that evolution under the influence of magnetic field inhomogeneities will reduce the intensity of PHIP spectra. Before the application of the first refocusing pulse, both types of operators evolve in their respective plane under the action of the J -coupling Hamiltonian during a time $t_E/2$. The 180° pulse introduces the first important difference in the evolutions. As I_1^x commutes with the pulse operator and $2I_1^z I_2^z$ changes its sign twice, the thermal operator is “transparent” to the application of the 180° r.f. pulses. On the other hand, the evolution of the PHIP operator is shifted by π on its evolution plane because I_1^z and $2I_1^z I_2^z$ undergo a sign change (see instant (2) in Figure 1). The signal intensity corresponding to the thermal operator is proportional to $\cos(\pi J t_E)$, whereas for PHIP, it is proportional to $-\sin(\pi J t_E)$ at the top of the echo (3). Continuing this line of reasoning, the thermal operator at the top of the second echo has the usual cosine phase ($\cos(2\pi J t_E)$); in contrast, the PHIP operator presents an inverted phase with respect to the first echo (i.e., $+\sin(2\pi J t_E)$). In other words, an operator arising from a PHIP reaction evolves not only under the J -coupling interaction but also under the influence of the rotations imposed by the r.f. pulses,

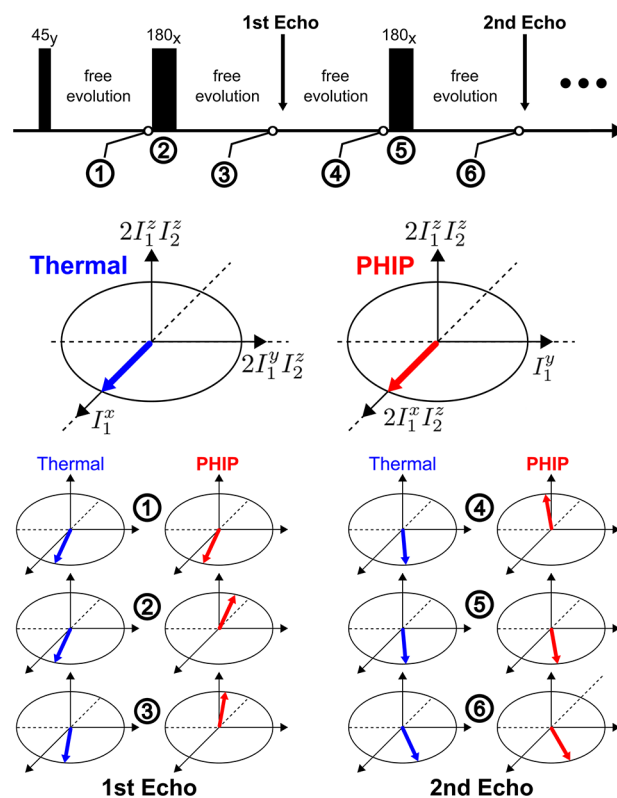


Figure 1. (Top) Pulse sequence and time events considered. (Center) Subspaces spanned by the product of the spin operators corresponding to scalar couplings; rotations are considered positive in the right-handed sense. (Bottom) Evolution of a single spin operator during the formation of two consecutive echoes; a phase alternation on the PHIP signals is observed.

inverting the signal upon the application of each pulse, resembling the signal evolution of a Carr–Purcell (CP) sequence,²⁹ as shown schematically in Figure 2A.

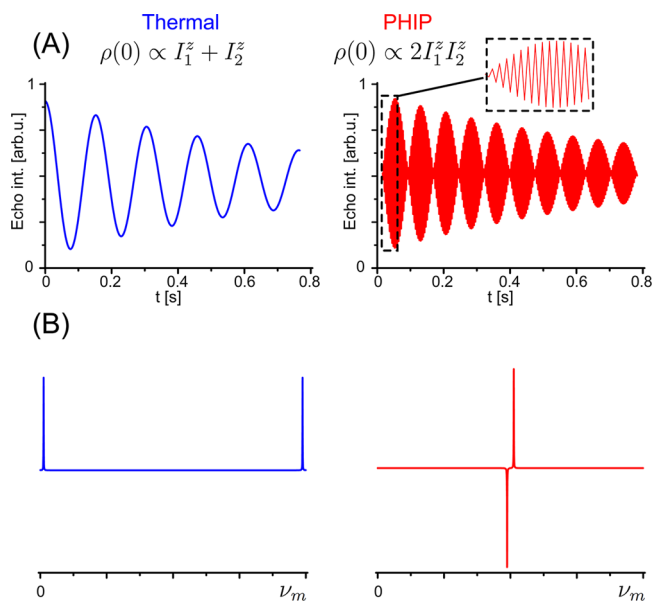


Figure 2. (A) Simulated thermal and PHIP signals at the top of the echoes during a CPMG echo train (only the real part is shown). (B) Spectra obtained without a frequency shift on the FFT.

The odd–even behavior has a dramatic influence when a FFT of the time domain signal is carried out, rendering spectra with a frequency offset of $N/2$, with N as the number of acquired data points^{30,31} (see the Supporting Information for detailed calculation). If resonance lines corresponding to thermal operators appear *on*-resonance, signals arising from PHIP operators will be on the borders of the spectral window. A common mathematical operation included in the software of most NMR spectrometers is a shift of the spectrum by $N/2$ points and reassignment of the frequencies to range from $-\nu_m/2$ to $\nu_m/2 - 1$, where $\nu_m = 1/t_E$ for the particular case of a J -spectrum. In Figure 2B, the simulated data are presented without the standard shift after fast Fourier transformation, to exemplify the situation. When the J -spectrum of the thermally polarized spins present resonances at the edges of the frequency windows, the J -spectrum of the PHIP spins appear centered. This provides a mechanism for signal discrimination. Notice that this effect is independent of the echo time, that is, independent of the chosen spectral resolution.

This effect of course is not restricted to AX spin systems, which are discussed here for the sake of clarity. The idea can be extended to larger spin systems if the conditions of J -spectroscopy are fulfilled.²³ As an experimental validation, we have used the hydrogenation of 1-hexyne resulting in 1-hexene (Figure 3A) due to its complex J -coupling network, which is suitable to demonstrate the robustness of the method.

A small amount of CH_2Cl_2 was added to the sample tube prior to the incorporation of $p\text{-H}_2$ to have a large thermally polarized peak interfering with the hyperpolarized peaks in 1-hexene. The relevant part of the spectrum after hyperpolarization is shown in Figure 3B, where a strong distortion in the antiphase character of the resonance lines is observed.

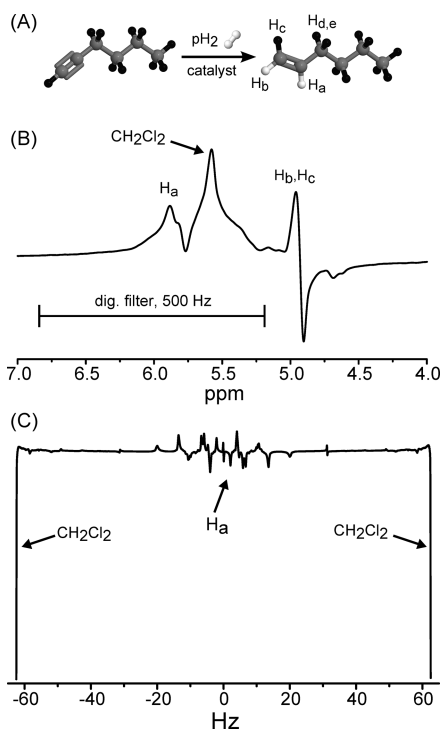


Figure 3. (A) Scheme of pairwise hydrogenation of hexene into hexyne with a mixture of CH_2Cl_2 . (B) NMR spectrum for a 45° excitation pulse. (C) J -spectrum corresponding to the digital filter shown in (B).

The negative part of the antiphase peak corresponding to proton H_a is completely canceled, while the peak corresponding to protons H_b and H_c is less distorted. A partial J -spectrum was obtained (see Figure 3C) with a digital filter of 500 Hz and the frequency irradiation set to acquire the H_a multiplet (denoted by the horizontal bar in Figure 3B). For the sake of clarity, the frequency axis is presented with the usual zero frequency in the center of the frequency span; nevertheless, the FFT was applied without a frequency shift, as discussed previously. The central section of the J -spectrum corresponds to the PHIP-induced signals, while the border regions correspond to the CH_2Cl_2 signals, clearly showing the separation of thermal from PHIP-induced signals.

A zoom of the PhD-PHIP spectra of 1-hexene and the 1-hexene/ CH_2Cl_2 mixture is shown in Figure 4. Both partial J -

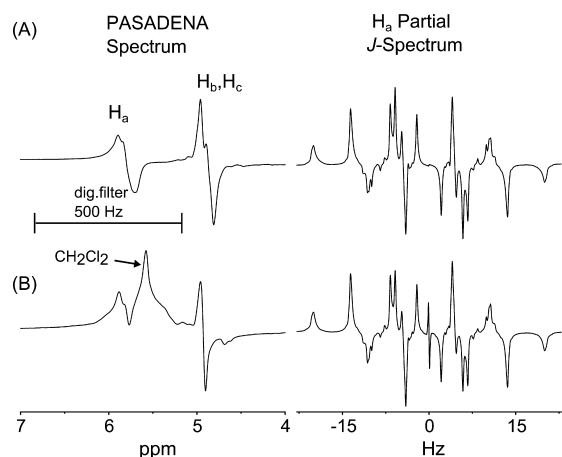


Figure 4. Experimental spectra for (A) hexene and (B) a hexene/ CH_2Cl_2 mixture. The left column corresponds to 1H spectra acquired at 300 MHz after a 45° pulse, while the J -spectrum of the column on the right corresponds to PhD-PHIP spectra.

spectra show excellent agreement, despite small zero-frequency artifacts observed only in the mixture J -spectrum. It must be emphasized that the shown spectra correspond to a single shot acquisition. The usual NMR spectra were acquired with a line width of 50 Hz, while the resulting J -spectra obtained by PhD-PHIP present a line width of 0.6 Hz.

Summarizing, we have presented the feasibility of acquiring highly resolved J -spectra of PHIP hyperpolarized nuclei practically free of contamination with signals arising from thermally polarized nuclei. The method can be applied straightforwardly and does not depend on any particular instrumental setup. It must be observed, however, that prior knowledge of the resulting time domain spectra of the product molecule is necessary in order to correctly set the irradiation frequencies and the digital filters. It also must be clear that the receiver gain in PhD-PHIP, which is related to the experimental sensitivity, is imposed by the largest signal present in the sample, which could be either thermal or PHIP. In a situation where sensitivity becomes a relevant aspect, a combination of an OPSY filter of thermal signals combined with PhD-PHIP detection appears as a promising approach for a boost in sensitivity and resolution in the field of hyperpolarization with parahydrogen.

The possibility to acquire highly resolved spectra with PHIP discrimination opens the way to different new applications in the field of hyperpolarization; for example, in large molecules,

interpretation of complicated spectra strongly relies on the line width. Additionally, the thermal signal can interfere destructively with hyperpolarized ones. The PhD-PHIP method provides a simple solution to both issues.

EXPERIMENTAL SECTION

A solution of 0.15 g of 1-hexyne, 1.3 g of acetone-*d*₆, and 0.01 g of catalyst (rhodium complex: CAS 79255-71-3) was prepared under a controlled nitrogen atmosphere and then separated in three NMR tubes (10 mm diameter). The hexyne/CH₂Cl₂ mixture was prepared as before with the addition of 33 μL of dichloromethane just before the hydrogenation process. All components were acquired from Sigma Aldrich and used without further purification. Parahydrogen was prepared by storing normal hydrogen at 77 K and afterward was transferred into aluminum cylinders. Bubbling of enriched *p*-H₂ gas was carried out during 15 s inside of the detection coil, which was placed in the bore of a 7 T superconducting magnet. A waiting time of 10 s was introduced between the end of bubbling and the NMR experiments. A 10 mm Bruker probe for liquids and a Bruker Avance II console operating at 300 MHz were used. The initial excitation pulse in the sequence was set to 45° in order to maximize the PHIP signals, with a length of 10 μs. A length of 38.6 μs was set for the train of 180° pulses, with an interpulse delay of $t_E = 8$ ms, and 1024 echoes were acquired. The phase cycling *yyyy* was used for the 180° pulses, which showed a much better performance than the usual CPMG,^{32,33} resulting in spectra free of spin-locking artifacts that, in general, give rise to strong zero-frequency resonance lines. Sample lengths for all experiments were set to 10 mm, approximately half of the length of the NMR birdcage coil in order to improve the accuracy of the refocusing pulses.²³ All of the experiments were acquired in a single scan.

ASSOCIATED CONTENT

Supporting Information

Detailed calculations of the evolution of density operators for thermal and PHIP protons and the fast Fourier transform of the time domain signals are included. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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