



Discrimination of parahydrogen induced polarization signals with *J*-spectra acquisition in poorly hyperpolarized samples



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ABSTRACT

The application of parahydrogen for the generation of hyperpolarization has increased continuously during the last years. When the chemical reaction that deposits the parahydrogen atoms into the target molecule is carried out following the PASADENA protocol an anti-phase signal is obtained, with a separation of the resonance lines of only a few Hz. Signal cancelation can be produced by line broadening due to magnetic field inhomogeneities or by the superposition with thermal signals. In this work we show that the acquisition of a *J*-spectrum has a double effect of enhancing spectral resolution and separating PHIP from thermal contributions even in systems where only a very small amount of hyperpolarization can be achieved.

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1. Introduction

Parahydrogen induced polarization (PHIP) is a powerful technique widely used in the last years in different fields like physics, chemistry and medicine. PHIP involves a chemical reaction that deposits the parahydrogen atoms into the target molecule, generating a metastable state with polarizations levels higher than the usual Boltzmann or thermal distribution. The versatility of this technique permits a number of applications in NMR, including the monitoring of inorganic reactions [1,2], highlighting a particular site of the target molecule [3] or its use as contrast agent in MRI [4,5]. Additionally, hyperpolarization can be transferred to other protons [6] or nuclei, usually ^{13}C [7,8], with special pulse sequences or field cycling techniques.

When the hydrogenation is carried out following the PASADENA protocol [9] (ParaHydrogen and Synthesis Allow Dramatically Enhanced Nuclear Alignment), a characteristic anti-phase NMR signal with a separation of the resonance lines of only a few Hz is observed. This line-shape is associated with the presence of a longitudinal two-spin order term ($I_{az}I_{bz}$) in the parahydrogen density operator, which is the dominant term for two weakly coupled spin systems (AX systems). Under this condition, even in high resolution NMR, partial cancelation is present, resulting in lower PHIP signals [10]. Additionally, anti-phase peaks can overlap with signals originated from thermally polarized protons, making the cancelation of PHIP signals even more pronounced.

Recently we have demonstrated that *J*-spectroscopy removes the evolution under magnetic field inhomogeneities to obtain high resolution PHIP spectra and simultaneously discriminates hyperpolarized signal from thermal signals [11]. The evolution of PHIP and thermal signals are remarkably different under this pulse sequence. Thus, the Fast Fourier Transform (FFT) algorithm used for the data processing produces the separation between both types of signals. We have suggested the acronym PhD-PHIP (Parahydrogen Discriminated-PHIP). In this work we show the performance of the method in a situation where only a small amount of hyperpolarization can be achieved, and is totally masked out by thermal signals.

2. Experimental

All experiments were performed at 7 T on a superconducting magnet with a Bruker Avance console. In order to use the maximum of PHIP signals available, the initial excitation pulse in the sequence was set to 45° with a length of 10 μs . The train of refocusing 180° pulses of 38.6 μs , with a delay of $t_E = 8$ ms were used, and 1024 echoes were acquired. The phase cycling of the refocusing pulses was as $yy\bar{y}\bar{y}$, as a better compared to the usual CPMG was observed in our system, resulting in *J*-spectra free of artifacts. In principle the same results could be obtained with the XY-8 phase cycle, however we preferred the MLEV-4 in order to keep the block shorter [12].

Sample length was set taking into account the effect of the r.f. pulse inhomogeneities. The influence of the sample length with respect to the Birdcage coil dimension is very important in order

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to obtain a high resolution J -spectrum. The optimal sample length was determined using a thermal sample (Ethanol 96.6%) with different filling factors in a 10 mm NMR tube. The best performance was achieved when the sample length was set to $\frac{1}{4}$ the length of the birdcage coil (data not shown).

The sample used for PHIP experiments consisted in a solution of 0.05 g of 1-hexyne, 0.1 g of hexene, 1.3 g of acetone- d_6 and 0.01 g of rhodium catalyst (CAS 79255-71-3) All the compounds were mixed in a controlled nitrogen atmosphere and then separated in three NMR 10 mm tubes. The hydrogenation of the sample (see Fig. 1) was carried out bubbling enriched $p\text{-H}_2$, prepared at 77 K in presence of activated charcoal, for 20 s inside the detection coil. Acquisition was performed after a waiting time of 10 s.

3. PhD-PHIP

To understand how this method works we consider the initial PHIP density operator of a weakly coupled spin system, A and B. As the hydrogenation is performed at high magnetic field (PASADENA protocol), the initial density PHIP operator is: $I_{az}I_{bz}$, which is not an NMR observable, unlike the usual case of thermal signals with the initial density operator being $I_{az} + I_{bz}$. Under a train of 180° pulses, only the J -coupling Hamiltonian ($2\pi J_{ab}I_{az}I_{bz}$) is considered, as the chemical shifts are refocused at the top of each echo. Following the evolution of each operator during the pulse sequence for n numbers of echoes, it can be obtained that the n -th echo intensity for PHIP is proportional to $(-1)^n \sin(\pi J_{ab} n t_E)$ whereas for thermal signals it is proportional to $\cos(\pi J_{ab} n t_E)$. Detailed calculations can be found in the Supplementary material of Ref. [11].

This odd–even effect (i.e., the prefactor $(-1)^n$) between successive echoes has a remarkable influence when an FFT of the time domain signal is carried out. Generally, the representation of a NMR signal after digitalization is a numerical series of N -numbers

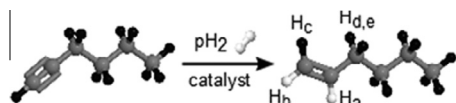


Fig. 1. Reaction of 1-hexyne to 1-hexene with enriched hydrogen gas in $p\text{-H}_2$ using a rhodium catalyst complex.

spaced by a dwell time dw , with a total acquisition time of $T = Ndw$. In J -spectroscopy, the dwell time is replaced by t_E and n by the number of acquired echoes. The FFT algorithm returns another numerical array with the same dimension ranging also from 1 to N , where the spectral width is $\nu_m = 1/t_E$. For a CPMG echo train corresponding to thermal signals, the FFT is,

$$F(\omega) = \sum_{n=0}^N F\left(\omega - \frac{n}{t_E}\right)$$

In the case of PHIP, the frequency domain signal can be written as,

$$F(\omega) = (1 - e^{-i2\pi\omega t_E}) \sum_{n=0}^N F\left(\left(\omega + \frac{n}{2t_E}\right) - \frac{n}{t_E}\right)$$

Besides a phase factor correction of order of unity, the main difference lies in a shift of the central frequency for PHIP signals by $n/(2t_E)$. Therefore, both signals will appear separated by half the spectral window in a J -spectrum. In standard NMR spectroscopy, a frequency shift of half of the spectral width is carried out, in the case of PhD-PHIP we do not perform this shift, thus PHIP signals appear on the center of the spectrum and thermal signals are present on the spectrum borders.

4. Results and discussion

In J -spectroscopy well-resolved multiplets can be obtained, however, chemical shift information is lost and all the multiplets appear at zero frequency. In the case of a complex sample with several groups of coupled spins, ambiguity in the J -spectrum is present. A spectrum can be simplified acquiring only one multiplet by performing the detection with a suitable digital filter (500 Hz in our work).

Fig. 2(a) shows the spectra and partial J -spectra corresponding to the H_a multiplet of the sample before hydrogenation with $p\text{-H}_2$. In the partial J -spectra the contribution from thermal signals is observed in the borders of the spectrum while no signal is present in the center. After 5 cycles of bubbling and detection, spectrum of Fig. 2(b) is obtained. Even though 33% of the sample consists of hexyne only a small fraction will be hyperpolarized, in fact hydrogenation can be observed even after 20 bubbling cycles due to the inefficiency of the on-going chemical reactions.

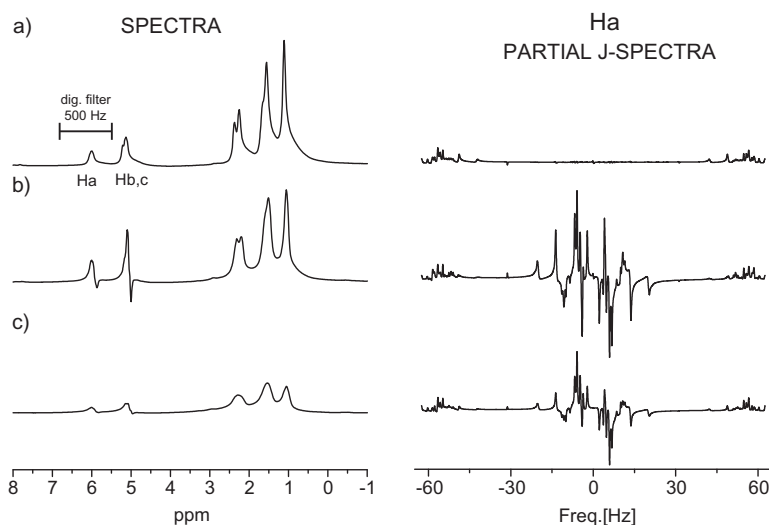


Fig. 2. Left column corresponds to thermal spectra and right column to J -spectra. (a) Spectra for 66% 1-hexene and 33% 1-hexyne. (b) Spectra after hydrogenation with $p\text{-H}_2$. (c) Spectra after 20 bubbling/measurement cycles, where a significant proportion of the sample has evaporated and most of the 1-hexyne has reacted increasing the proportion of 1-hexene.

Partial signal cancelation is depicted by the small antiphase resonance observed, however the PHIP *J*-spectrum appears clearly resolved in the center of the frequency domain. After 20 bubbling cycles the spectrum in Fig. 2(c) is obtained. A significant proportion of the sample has evaporated at this stage, which produces a change in the shimming of the magnetic field. In this situation the antiphase signals are barely observed in the spectrum whereas the *J*-spectrum maintains a great detail.

We have shown that *J*-spectroscopy is a powerful method to obtain high resolution in PHIP for very low hyperpolarization levels, even in the presence of inhomogeneous fields. A minor drawback is that in order to apply this method chemical shift information must be known beforehand in order to correctly set the digital filter window. In this respect, the use of Only Para-Hydrogen Spectroscopy (OPSY) [13] may seem as more advantageous if the magnetic field homogeneity is enough to resolve the individual multiplets. Perhaps the most important difference between both approaches resides in the use of the receiver gain; OPSY completely filters out thermal signals, so the receiver gain can be set to a maximum level to ensure proper digitalization of very small signals, whereas PhD-PHIP is limited by the presence of the thermal signal intensity. On the other hand, PhD-PHIP does not require the use of magnetic field gradients, and it opens new

exciting possibilities for the acquisition of PHIP in benchtop applications, were the high field homogeneity is not granted a priori, with spectral resolution.

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