

High resolution para-hydrogen induced polarization in inhomogeneous magnetic fields

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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 is carried out at the same field as the NMR experiment (PASADENA protocol) an antiphase signal is obtained, with a separation of the resonance lines of a few Hz. This imposes a stringent limit to the homogeneity of the magnetic field in order to avoid signal cancellation. In this work we detect the signal arising from hyperpolarized Hexene by means of a CPMG pulse train. After Fourier transformation the obtained J-spectra not only presents an enhanced spectral resolution but also avoids partial peak cancellation.

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1. Introduction

Nuclear Magnetic Resonance (NMR) has become a very powerful technique than can be applied in different fields encompassing physics, chemistry, biology and medicine among others. Despite its widely recognized advantages, NMR suffers from extremely low sensitivity due to the low polarization achieved by thermal equilibrium with an external magnetic field. Several methods have been implemented to obtain metastable states with polarization levels that can be orders of magnitude higher than thermal. Among these so called hyperpolarization methods, the hydrogenation of substrates with parahydrogen has grown significantly in the last years. Para-Hydrogen Induced Polarization (PHIP), involves a chemical reaction in which molecules of hydrogen gas enriched in the para-state ($p\text{-H}_2$) are deposited into an unsaturated precursor, prior to the NMR signal acquisition [1,2].

PHIP experiments have found different applications within the NMR community [3], including the study of kinetics of inorganic reactions [4–6], research of heterogeneous reactions [7] and the introduction of new MRI contrast agents [8–12]. Additionally, hyperpolarization transfer via special pulse sequences [13,14], by diabatic field cycling [15] or by transport through level avoiding

crossing have been implemented [16–18] along with the study of long lived states originated from $p\text{-H}_2$ [19–22].

The set of PHIP experiments is split into two subsets depending on the experimental protocol used. When the hydrogenation reaction and the NMR experiment are performed at the same magnetic field the process is referred to as PASADENA [1] (PArahydrogen and Synthesis Allow Dramatically Enhanced Nuclear Alignment). On the other hand, if the chemical reaction is performed at low magnetic field, followed by an adiabatic transport of the sample to a high magnetic field for signal detection, the process is called ALTA-DENA [2] (Adiabatic Longitudinal Transport After Dissociation Engenders Net Alignment). Polarization can be enhanced typically by several orders of magnitude with both methods.

Signals arising from PHIP hyperpolarization differ not only by the signal from those obtained from samples at thermal equilibrium at the same magnetic field. Consider, for instance, that both hydrogen nuclei that originally belong to the $p\text{-H}_2$ molecule (labelled 1 and 2) occupy chemically inequivalent sites in the target molecule, after a pairwise hydrogenation process has taken place. If their chemical shift difference, $\Delta\nu$, is much larger than the coupling constant J_{12} and we neglect coupling to other nuclei in the molecule, an isolated AX spin system is created. In PASADENA conditions, the resulting spectrum presents two anti-phase doublets with splitting equal to their coupling constant, whereas at thermal equilibrium two in-phase doublets are present. The anti-phase doublet in PASADENA is associated with the presence of a

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longitudinal two-spin order term in the density operator, of the form $I_1^z I_2^z$ [23,24].

The difference between anti- and in-phase doublets is crucial when the magnetic field homogeneity is limited. As the homonuclear coupling constants are of the order of few Hz in liquid samples, even relatively high homogeneous magnetic fields can produce partial signal cancellation in PASADENA experiments, thus diminishing the method's applicability. It is worth noticing that linebroadening not only refers to the homogeneity of the external magnetic field, but also to susceptibility changes introduced either by the sample itself or by the experimental setup.

The straightforward solution to overcome this drawback consists in performing the NMR experiments in extremely homogeneous magnetic fields, combined with sample setups that avoid susceptibility disturbances. This last condition imposes severe limitations in the variety of experiments that can be carried out in PASADENA conditions, such as MRI experiments with heterogeneous samples or the dilution of p-H₂ enriched gas via hollow-fiber membranes for instance [25].

At this point, a very important distinction between signal cancellation due to magnetic field inhomogeneities and initial absence of signal after a radiofrequency pulse must be made. Due to the structure of the PASADENA operator, the initial free induction decay (FID) is zero, and builds up due to evolution with J -couplings. If a waiting period, or J -coupling delay, is introduced after the excitation pulse the anti-phase doublet will evolve to an in-phase doublet. In other words, the intensity of the signal in the time domain will increase [24,26]. The introduction of a spin-echo, with a proper echo time, will refocus magnetic field inhomogeneities, therefore mitigating the attenuation of the top of the echo. Spin echoes in combination with J -coupling delays have been successfully implemented for instance in PHIP-MRI for monitoring chemical reactions [11], as contrast agents [12], in time domain NMR at low fields [27], to study relaxation time of hyperpolarized states [28] or in combination with selective frequency pulses for COSY experiments [29] among others. Nevertheless, no gain in spectral resolution can be achieved with these methods.

In the present work we apply the well-known J -Spectrum technique proposed by Freeman et al. in 1971 [30] to PHIP signals in PASADENA condition. The technique consists in the application of an echo train, where only the top of the echoes are acquired. By introducing suitable digital filters we show that not only partial signal cancelling is achieved, but also a significant enhancement of the spectral resolution for each individual multiplet in hyperpolarized samples can be obtained.

2. Theoretical background

Initially we consider an AX system subject to the application of a CPMG pulse train. By properly selecting the pulse repetition rate, as discussed below, the nonsecular part of the J -coupling Hamiltonian can be neglected and the secular part is unaffected by 180° pulses, thus the magnetization decay will appear modulated [24,31]. If the top of the successive echoes are recorded and Fourier transformed a J -spectrum is obtained, where the linewidth is proportional to $(\pi T_2)^{-1}$, essentially independent of magnetic field inhomogeneities. In the particular case of two isolated spins, the J -spectrum will consist of a doublet centred at zero frequency with a splitting equal to the J -coupling constant, because both doublets from the AX spin system collapse at the same frequency. In larger spin systems, detailed information of coupling constants can be obtained through a J -spectrum. For example, the determination of long range J -coupling constants of simple esters as methyl acetate, ethyl acetate, isopropyl acetate among others, were obtained with high accuracy [32].

In order to get pure J -spectra, with the resonance lines only related to the coupling constant, several conditions must be fulfilled: (i) the magnetic field must be strong enough to ensure first order approximation for the couplings, i.e. weak coupling limit, (ii) the pulse repetition rate must be small compared to the smallest chemical shift difference, (iii) the 180° pulses must be carefully adjusted, and (iv) any other possible source of modulation, e.g. chemical exchange, must be absent [30].

Violation of the conditions listed above lead to different consequences. For instance, if the spin system is strongly coupled, new frequencies will be present in the echo-train envelope. If the echo time is too long the resonance positions are shifted, whereas if the repetition rate is too high all J -modulations vanish. If the pulses are not set with enough accuracy, new resonances at frequencies related to the chemical shifts show up. In any of these cases the spectrum of the echo-train envelope can be classified as *spin-echo spectrum* instead of a J -spectrum [30]. The method is of course not restricted to simple AX systems, in fact, closed formulas for AB, A₂B, A₃B [33] and A_qBX_x spin systems [34] have been reported.

In the present work we restrict ourselves to the study of J -spectra. Even in this case, the response of multispin systems can become quite complex, nevertheless an elegant alternative was suggested by Freeman and Hill in 1971 [30]. In their work, they proposed to split the J -spectrum in subspectra by filtering the undesired frequency components during the acquisition of the echo maxima. The method consists in the application of hard 180° pulses centered at the resonance frequency of the desired multiplet, with digital filtered acquisition of the echo train. The authors have suggested to refer to this filtered data as *partial J-Spectra*.

3. Results and discussions

3.1. Partial J -spectra in inhomogeneous magnetic fields.

The setup performance was tested in ethanol, due to the simplicity of its NMR spectrum. The experiments were carried out with the excitation pulse set to 45° followed by an train of 180° pulses in order to reproduce the measurement conditions with p-H₂ hyperpolarized samples. The phases of the refocusing pulses were alternated in blocks of two as (yy $\bar{y}\bar{y}$). Possible artifacts in the spectra due to the use of the initial 45° instead the usual 90° pulse were tested by simulations, where off-resonance effects were incorporated to the pulses.

Fig. 1A displays a simulated NMR spectrum with high resolution, along with the partial J -spectra of the methylene and methyl group respectively. The partial J -spectra were simulated by evolving the density operator with the Hamiltonian $\mathcal{H} = \mathcal{H}^{\text{CS}} + \mathcal{H}^{\text{J}}$ where only the secular part of the J -coupling Hamiltonian was retained.

Experimental results are summarized in Fig. 1B–D, where the inhomogeneity of the magnetic field is systematically increased by gradually introducing a field gradient via the Z-coil of the shimm system. Horizontal lines in Fig. 1B indicate the center of irradiation and the 1 kHz digital bandwidth used for each multiplet. The expected increase of the spectral resolution in the partial J -spectra is evident. The agreement between the simulated and experimental J -spectra are independent of the field inhomogeneity as expected. Small artifacts due to off resonance effects in the 180° can be observed in the simulations as well as in the experimental data. For all experiments the echo time was set to $t_E = 8$ ms, resulting in a frequency window of 125 Hz, to ensure that the majority of artifacts due to pulse errors lie far from the region of interest.

3.2. Partial J -spectra in PHIP

We now turn our attention to the acquisition of partial J -spectra in PHIP hyperpolarized samples, in particular for the PASADENA

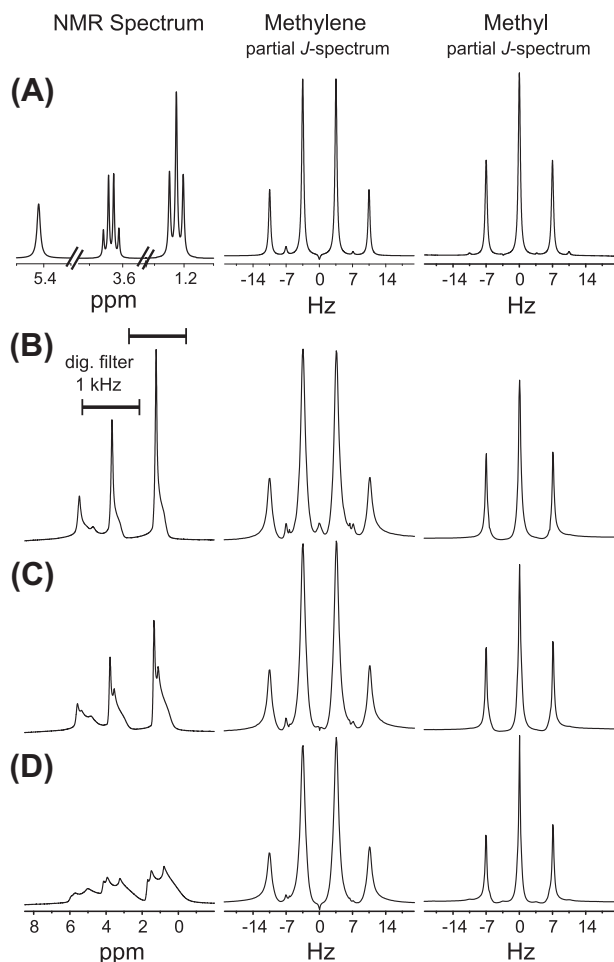


Fig. 1. (A) Simulated NMR spectrum of ethanol and J -spectra of the methylene and methyl groups. (B–D) Experimental spectra as a function of the magnetic field inhomogeneity. Horizontal bars in (B) indicate the width of the digital filter for each multiplet.

protocol. As detailed in the Experimental section, we deal with the hydrogenation of Hexyn resulting in Hexene. The former $p\text{-H}_2$ occupy inequivalent positions on the target molecule, as depicted in Fig. 2A.

Hyperpolarization is initially expected to be related to protons from the $p\text{-H}_2$ molecule, namely H_a and H_b . Nevertheless the strong coupling of H_b with H_c induces an appreciable amount of polarization transfer to this proton [10]. Additionally, J -couplings with H_d and H_e are strong enough to modulate the J -spectra, as shown in Table 1. Therefore, five spins were considered to build up the Hilbert space for the calculations.

The hydrogenation process was simulated using the protocol described in a previous publication [18]. The initial density operator, ρ , of the five spin system was created by the direct product of the density operator of the $p\text{-H}_2$ molecule with the thermal density operator of the target molecule, considered in this case as a three spin system. Further evolution is carried out with the Liouville–von Neumann equation [26]. Chemical reaction of different molecules are considered to occur during a time T in the presence of a 7 T magnetic field. We further assume that the reaction rate is faster than any molecular characteristic time ($T \gg (v_i)^{-1}, (J_{ij})^{-1}$), and that all molecules have reacted previous to the initial rf excitation. In this way, the initial density operator for the NMR experiment can be approximated by taking a time average of $\rho(T)$. Free evolution between pulses were performed with the Hamiltonian

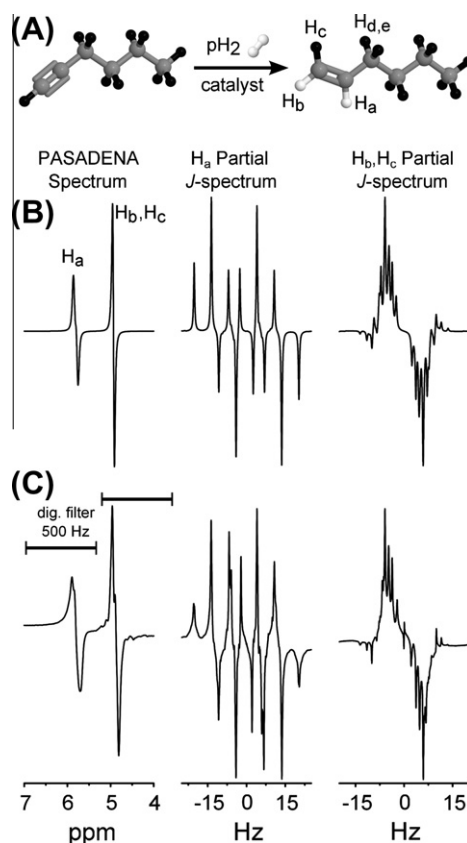


Fig. 2. (A) Scheme of pairwise hydrogenation of Hexene into Hexyne. (B) Simulated NMR spectrum with a spectral width of 900 Hz and partial J -spectra. (C) Experimental data, horizontal bars indicate irradiation frequency and digital filter width.

Table 1

Chemical shift and J -coupling values for the relevant protons in the Hexene for PASADENA experiments (see Fig. 2A).

	^1H C. S. (ppm)	H_a (Hz)	H_b (Hz)	H_c (Hz)	$H_{d,e}$ (Hz)
H_a	5.8	–	10.2	17	6.7
H_b	4.92	–	–	2.2	1.2
H_c	4.96	–	–	–	1.6
$H_{d,e}$	2.06	–	–	–	–

$\mathcal{H} = \mathcal{H}^{\text{CS}} + \mathcal{H}^{\text{J}}$. Off-resonance effects were implemented in the pulses by a suitable r.f. Hamiltonian [24,26].

Fig. 2B shows the results for simulations of a PASADENA spectrum from single 45° pulse and partial J -spectra with irradiation frequencies centred on proton H_a and protons H_b – H_c . A Lorentzian distribution of frequencies with FWHM = 15 Hz and 0.5 Hz respectively were used for the calculations.

Experimental results are presented in Fig. 2C. The irradiation frequencies and digital filter spectral width for the partial J -spectra acquisition are shown in the PASADENA spectrum. Echo times were set to $t_E = 8$ ms. An excellent agreement between experiments and simulations is observed, despite small deviations in the intensities. Data are normalized to its own maximum intensity as the signal intensity is not fully reproducible due to the ongoing chemical reaction from Hexene to Hexyne that is produced between successive experiments.

3.3. Partial signal cancellation

From Fig. 1 it can be clearly observed that the intensity of the J -spectra is insensitive to inhomogeneities in the external magnetic

field for the case of in-phase multiplets, while an increased broadening of the NMR spectrum is introduced.

Nevertheless, in a thermally polarized system the area of the NMR spectrum remains constant as it is related to the FID amplitude. This situation is very different in the case of anti-phase doublets, where not only spectral resolution is lost by linebroadening, but also partial peak cancellation occurs. As mentioned above, homonuclear coupling constants are of the order of few Hz in liquid samples, therefore small magnetic field inhomogeneities can produce partial signal cancellation in PASADENA experiments. Fig. 3A shows a single in-phase and an anti-phase doublet, obtained from simulations of an AX spin system, with $\Delta\nu = 2.5$ ppm and $J_{12} = 7$ Hz, at a magnetic field of 7 T. The different spectra were obtained by convolution with Lorentzians of full width at half maximum (FWHM) of 2, 10, 20 and 40 Hz.

As the total area of the spectrum in the anti-phase doublet is zero, we relate to the FID amplitude in order to analyze the impact of linebroadening in the signal intensity. Fig. 3B shows the maximum of the time domain signals for the in-phase and anti-phase doublets against the field inhomogeneity. The first point corresponds to the maximum signal for in-phase doublets, whereas

for the anti-phase doublet, a time evolution is needed for the maximum signal to be obtained, being thus influenced by T_2^* . For a FWHM of 2 Hz almost no signal cancellation is observed, whereas 80% of the signal is lost when FWHM = 20 Hz.

Thus, in the PASADENA experiments, the elimination of the influence of field inhomogeneities by the use of a CPMG train not only produces an increased spectral resolution, but also opens the possibility for the detection of PHIP signals in different scenarios where field homogeneity is not guaranteed. Such situations can involve samples or special setups that produce a degradation of the field homogeneity.

4. Experimental

4.1. NMR experiments

Experiments were carried out at 7 T with a Bruker Avancell console, equipped with a 10 mm probe for liquids. A modified CPMG pulse sequence was used, where the initial excitation pulse was set to 45° in order to maximize the PHIP signals. A pair-alternated phases, transversal to the excitation pulse, were used for the 180° ($yy\bar{y}\bar{y}$) [35], which showed a much better performance than the usual one of CPMG. A length of $28 \mu\text{s}$ was set for the 180° pulses. Influence of the radiofrequency field inhomogeneities were tested by measuring the J -spectra of ethanol as a function of the sample length. The best performance was found for a sample size of approximately half the length of the NMR birdcage coil, whereas for longer samples, strong baseline artifacts were observed. The sample lengths for all experiments were then set to 13.4 mm and only one scan was acquired to resemble the measuring conditions on hyperpolarized samples.

4.2. Sample preparation

A solution containing 0.62 g of Hexyne, 5.2 g of acetone- d_6 , and 0.016 g of the catalyst (Rhodium complex: CAS 79255-71-3), was prepared under controlled nitrogen atmosphere in 10 mm NMR tubes and then divided into a set of different samples. All components were acquired from Sigma Aldrich and used without further purification. Ethanol ($\text{CH}_3\text{CH}_2\text{OH}$) with 96% purity was purchased from Porta and used without further purification.

Parahydrogen was prepared by storing normal hydrogen at 77 K in activated charcoal during 30 min, and then stored in stainless steel cylinders (Swagelok, Germany) at 4 bars. Plastic 4 mm OD tubing was used for the transfer lines and electromagnetic valves (Festo KG, Esslingen, Germany) to control the gas delivery to the sample.

Bubbling of enriched $p\text{-H}_2$ gas in the sample was carried out during 15 s. and a waiting period of 5 s. was used to ensure no bubbles remained in the detection volume in all experiments.

5. Conclusions

In this work we applied the well known approach of J -spectra acquisition to PHIP hyperpolarized samples. The acquisition of the signal evolution prepared in PASADENA conditions with a CPMG echo train renders not only highly resolved spectra but also avoids peak cancellation due to inhomogeneities in the external magnetic field.

In terms of resolution, the linewidth is proportional to $(\pi T_2)^{-1}$ and further broadened by molecular diffusion. The influence of diffusion can naturally be overcome by shortening of the echo time, nevertheless the manipulation of this experimental variable is not always straightforward. In the present case we limit ourselves to a weak coupling limit in which the obtained J -spectra is

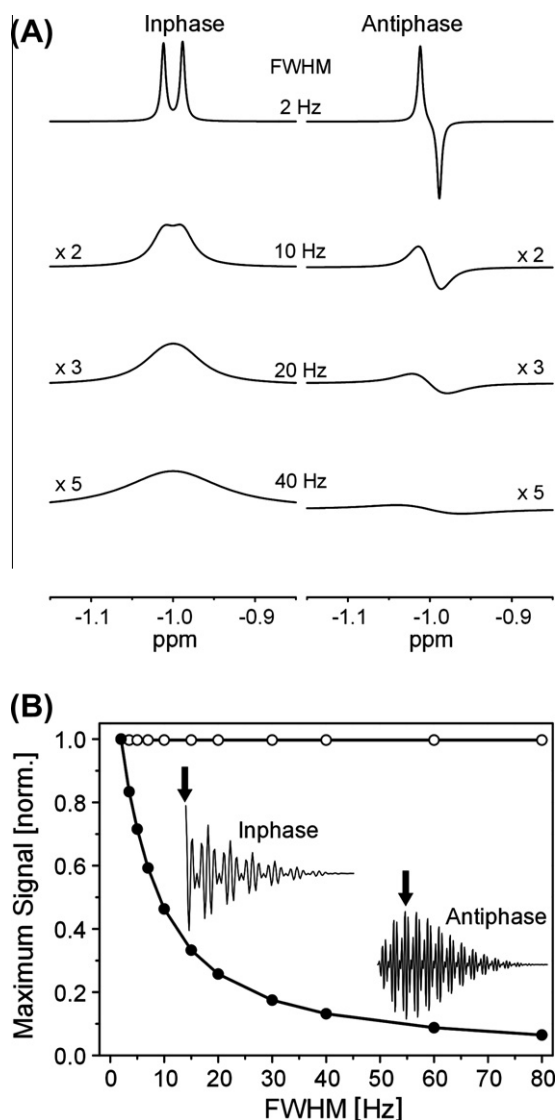


Fig. 3. In-phase and anti-phase doublets with $\Delta\nu = 700$ Hz and $J_{12} = 7$ Hz. (A) Spectra obtained by convolution with Lorentzians of FWHM = 2, 10, 20 and 40 Hz respectively. (B) Signal intensity as function of the inhomogeneity.

independent of the echo time. However, this situation changes if the system under study presents multiplets with strong couplings, or if the magnetic field is reduced. A systematic study of this last situation exceed the scope of this work, and will be presented in a future publication.

Concerning the signal cancellation the evolution of the magnetization with T_2 rather than T_2^* enables the preservation of PASADENA signals even in presence of strong inhomogeneities of the external magnetic field. This opens the possibility of the use of a wide range of experimental setups such as imaging in heterogeneous samples which can introduce susceptibility distortions or spectroscopy in systems where the enriched gas delivery is carried out with hollow-fiber membranes.

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