Journal of Magnetic Resonance 262 (2016) 68–72

Contents lists available at ScienceDirect

Journal of Magnetic Resonance

journal homepage: www.elsevier.com/locate/jmr

Magnetic resonance imaging of ^{1}H long lived states derived from parahydrogen induced polarization in a clinical system

Dirk Graafen [a,b], María Belén Franzoni [a,*], Laura M. Schreiber [b,1], Hans W. Spiess [a], Kerstin Münnemann [a]

[a] Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany
[b] Department of Radiology, Johannes Gutenberg University Medical Center, Langenbeckstr. 1, 55131 Mainz, Germany

ARTICLE INFO

Article history:
Received 25 October 2015
Revised 5 December 2015
Available online 18 December 2015

Keywords:
Hyperpolarization
PHIP
Parahydrogen
Pulse sequence
Long lived state

ABSTRACT

Hyperpolarization is a powerful tool to overcome the low sensitivity of nuclear magnetic resonance (NMR). However, applications are limited due to the short lifetime of this non equilibrium spin state caused by relaxation processes. This issue can be addressed by storing hyperpolarization in slowly decaying singlet spin states which was so far mostly demonstrated for non-proton spin pairs, e.g. ^{13}C–^{13}C. Protons hyperpolarized by parahydrogen induced polarization (PHIP) in symmetrical molecules, are very well suited for this strategy because they naturally exhibit a long-lived singlet state. The conversion of the NMR silent singlet spin state to observable magnetization can be achieved by making use of singlet–triplet level anticrossings. In this study, a low-power radiofrequency pulse sequence is used for this purpose, which allows multiple successive singlet–triplet conversions. The generated magnetization is used to record proton images in a clinical magnetic resonance imaging (MRI) system, after 3 min waiting time. Our results may open unprecedented opportunities to use the standard MRI nucleus ^{1}H for e.g. metabolic imaging in the future.

1. Introduction

Nuclear magnetic resonance (NMR) has found many important applications not only in natural sciences [1] but also in the clinical routine setting [2]. However, important applications like, e.g. in vivo magnetic resonance spectroscopy are limited by the inherently low sensitivity of NMR at thermal equilibrium. Thus, the so-called hyperpolarization methods have been developed for generating a nuclear alignment far above the thermal polarization [3]. One of these promising techniques, known as parahydrogen induced polarization (PHIP) [4–7], is based on the pairwise catalytic transfer of molecular hydrogen, enriched in the nuclear singlet spin state (parahydrogen, pH_2), to an unsaturated precursor molecule. As an example, the hydrogenation of dimethyl acetylenedicarboxylate with pH_2 which generates ^{1}H hyperpolarized dimethyl maleate is depicted in Fig. 1a.

A drawback of all hyperpolarization methods is the limited lifetime of the hyperpolarized spin state mainly due to spin–lattice relaxation processes (typically on the order of seconds for ^{1}H to at best a few minutes for e.g. ^{13}C). A promising new approach to prolong the lifetime of the non-thermal spin alignment is to store it in a long-lived singlet spin state [8–13]. For symmetry reasons, these states do not relax via those relaxation mechanisms which are symmetric with respect to spin permutation, such as the dominant dipole–dipole relaxation mechanism in liquid state NMR. PHIP is especially well suited for the generation of singlet spin states because pH_2 is a singlet state already. The possibility to preserve the nuclear alignment generated by PHIP as a singlet state was already accomplished at low fields [14,15] and also at high fields [16–20]. A major benefit of using symmetrical molecules for the storage of singlet spin order is that they preserve the singlet state at nearly any magnetic field strength, thus greatly reducing experimental requirements. The singlet spin states themselves are NMR silent, since their total nuclear spin number is zero. Thus, for observation they need to be converted in a nuclear triplet spin state. If the singlet spin state is created among two spins which are chemically inequivalent or nearly equivalent, the singlet–triplet conversion is based on the chemical shift difference [10,11]. If the pair is chemically equivalent as in the case for the symmetrical molecule used in this study, this conversion can be performed in a controlled way due to symmetry breaking accessible by the *J*-coupling network. This was shown for the vinylene ^{1}H pair in dimethyl maleate [17,20], or the ^{13}C pair in diethyl oxalate–$^{13}C_2$ [21]. From a slightly different perspective, the symmetry of the

* Corresponding author at: FAMAF Universidad Nacional de Córdoba, IFEG CONICET, X5016LAE Córdoba, Argentina.
E-mail address: franzoni@famaf.unc.edu.ar (M.B. Franzoni).
[1] Present address: Comprehensive Heart Failure Center, Straubmühlweg 2a, 97078 Würzburg, Germany.

http://dx.doi.org/10.1016/j.jmr.2015.12.006
1090-7807/

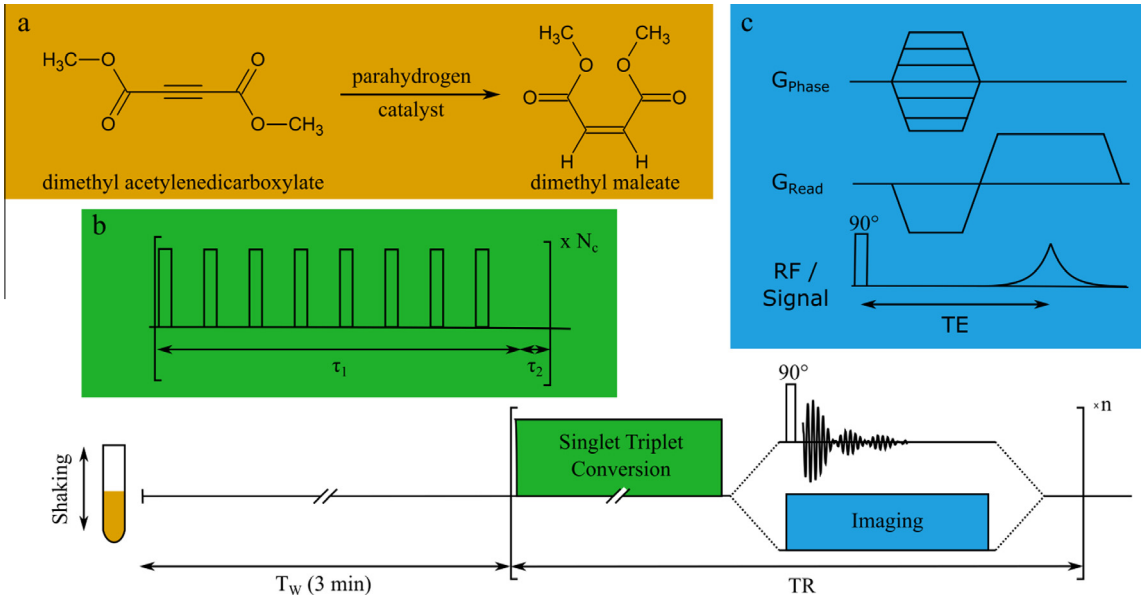

Fig. 1. Experimental procedure: Lower panel, steps of the complete sequence: After starting the hydrogenation (a), the acquisition is delayed by an initial waiting time of 3 min. Afterwards the singlet–triplet conversion is achieved by application of the CSS sequence (b). This was applied repetitively followed either by an FID acquisition or by an one phase encoding step of a gradient echo sequence (c).

molecule can be broken through a chemical reaction, revealing the singlet–triplet population imbalance [19,22,23].

The possibility to store hyperpolarization in singlet spin states was so far almost exclusively used for spectroscopy. Motivated by Feng et al. [24], it was only recently demonstrated for MRI by Pileio et al. [25,26]. In all three studies, a hyperpolarized ^{13}C–^{13}C spin pair was used for hyperpolarization storage. In Ref. [25], the authors introduced a recycling protocol in which the hyperpolarization can be used in full signal intensity and then is converted back to singlet spin order for further storage. By using this method they were able to acquire ^{13}C-MRI images for 30 min which can be extremely valuable for metabolic imaging of ^{13}C hyperpolarized substances. However, as clinical MRI systems are normally equipped with hardware optimized for the acquisition of protons, Feng et al. [24] converted the ^{13}C–^{13}C singlet spin state into ^{1}H magnetization. This, however, still requires the expensive usage of double ^{13}C–^{13}C labeled substrates, which represents a significant disadvantage. In contrast, MR images recorded by making use of proton singlet spin order originating from hyperpolarized signals should open the door toward widespread clinical application. Along these lines we reported the conversion of ^{1}H singlet to triplet spin order by rf irradiation achieved in a standard NMR spectrometer (7 T) but also in the 1.5 T field of a MRI scanner [18]. In concordance, Zhang et al. recently demonstrated the conversion in the 11.7 T field [20]. Here, we describe a low-power pulse sequence used for real time chemical shift scaling based on average Hamiltonian theory. The method allows multiple conversion of proton singlet order to observable hyperpolarized magnetization and subsequent MRI acquisition performed on a 1.5 T clinical MRI tomograph.

2. Theory

As we demonstrated in a previous contribution, the vinylene ^{1}H–^{1}H pair in dimethyl maleate has a singlet lifetime as long as 4 min at high magnetic fields [16], i.e. two orders of magnitude longer than its spin–lattice relaxation time T_1 and is thus well suited for MRI experiments. We showed that the singlet–triplet transition in this chemically equivalent proton spin pair can be achieved by exploiting an energy level anticrossing [17,27] at a well-defined magnetic field (B_R). Satisfying the condition:

$$\Delta\nu|_{B_R} \approx J_{VV'}, \tag{1}$$

where $J_{VV'}$ is the J-coupling between the two vinylene protons and $\Delta\nu|_{B_R} = \nu_V - \nu_M$ is the chemical shift difference between the vinylene and the methyl protons in dimethyl maleate at the magnetic field B_R. In our previous works, this condition was fulfilled by magnetic field cycling [16,17], i.e. letting the sample to evolve at the magnetic field B_R to generate the transition.

However, field cycling is not suitable for clinical purposes of MRI. Fortunately, there are other ways to fulfill condition (1) and, consequently, induce the singlet–triplet transition. The J-coupling is an inherent property of the molecule under study and cannot be manipulated. The experimenter can, however, control the chemical shift Hamiltonian under which the spin system evolves, in order to satisfy the mentioned condition. This can be achieved either by changing the static magnetic field B_0 to B_R or by scaling the chemical shift difference to the desired value by the application of rf pulse sequences. The application of a long constant rf pulse (continuous wave, CW) of a particular power and off-resonance position allows the desired scaling in the rotating frame [18,27]. With this, the singlet–triplet conversion can be performed inside the observation field with no need of physically moving the sample. However, a high rf pulse power and a long duration were necessary to achieve a satisfying conversion. Recently, a very efficient weak power rf pulse sequence to produce a spin-lock induced crossing (SLIC) of the spin singlet and triplet energy levels between nearly equivalent spins was demonstrated [28], and successfully applied to the chemically equivalent homonuclear 4-spin system [20].

In the present contribution we adapted the chemical shift scaling (CSS) sequence developed by Morris et al. [29], based on average Hamiltonian theory, to generate singlet–triplet conversion in dimethyl maleate and combined it with MRI. The implementation of the experiments in a clinical MRI system with its typical hardware imperfections (like magnetic field inhomogeneity and restrictions on the radiofrequency pulse power due to safety reasons) is considered in detail.

The CSS sequence [29] consists of a repetitive cycle of eight evenly distributed π pulses during a time τ_1 and an additional delay τ_2 (see Fig. 1b). The phases of the pulses follow an XY-16 supercycle $(x,y,x,y,y,x,y,x,-x,-y,-x,-y,-y,-x,-y,-x)$. The sequence generates an effective Hamiltonian which scales the chemical shift by a factor:

$$\lambda = \frac{\tau_2}{(\tau_1 + \tau_2)}. \tag{2}$$

In order to fulfil condition (1) for singlet–triplet conversion of the vinylene protons in dimethyl maleate, the following relation should be fulfilled:

$$\tau_1 = \left(\frac{\Delta\nu|_{B_0}}{J_{VV'}} - 1\right)\tau_2, \tag{3}$$

where $\Delta\nu|_{B_0} = \nu_V - \nu_M$ is the chemical shift difference at the observation magnetic field B_0.

3. Materials and methods

The experiments were performed on a 1.5 T clinical MRI system (Magnetom Sonata, Siemens). The samples used for the experiments consist of a mixture of 500 mg dimethyl acetylenedicarboxylate (99%, Sigma Aldrich) and 10 mg (0.23 mol%) of the hydrogenation catalyst [1,4-bis-(diphenylphosphino)butane] (1,5-cyclo-octadiene)rhodium(I)tetrafluoroborate, dissolved in 2.6 g of acetone-d 6 (99.9%D, Sigma Aldrich). They were prepared in an argon atmosphere and filled into 10 mm NMR pressure tubes sealed with a septum cap. Parahydrogen enriched to about 90% was prepared with a Bruker pH_2 generator operating at 36 K and stored in aluminum cylinders. The hydrogenation reaction was started by pressurizing the NMR tube with 4 bar of H_2 at earth magnetic field. Subsequently, the sample was rapidly transferred into the bore of the MRI system where it was shaken vigorously for 5 s. Finally, the sample was positioned inside a homemade transmit-receive solenoid coil optimized for 10 mm NMR tubes. After a delay of 3 min, which was chosen to ensure completion of the hydrogenation reaction, the singlet–triplet conversion was performed using the CSS sequence with 80 cycles and $\tau_1 = 12.56$ ms, and $\tau_2 = 1$ ms fulfilling the condition in Eq. (3).

As we have noted in previous studies [18,30] the application of the pulse sequence only results in partial singlet to triplet conversion. Therefore, it is possible to achieve several subsequent conversions by applying the CSS sequence repetitively requiring a time TR for each acquisition, see Fig. 1.

Following the procedure described in Refs. [18,30], but using the CSS sequence for conversion, an FID was acquired after each conversion of a multiple conversion train for different repetition times TR. The lifetime of the singlet state T_S and the singlet–triplet effective-conversion fraction ξ of the sequence (i.e. the fraction of the singlet state that is converted into measurable magnetization) were determined [20,30]. The signal S decays exponentially with the number of conversions n with a characteristic constant D_c which is a function of TR, T_S and ξ. Following the analysis in Refs. [18,30] the decay satisfies:

$$\frac{S_n}{S_0} = e^{-D_c n} \tag{4}$$

$$D_c = \frac{1}{T_S}\mathrm{TR} - \ln(1-\xi). \tag{5}$$

The experiments were performed with repetition times TR of 6.6 s, 11.6 s, 21.6 s, 31.6 s, and 51.6 s using 20, 20, 15, 10, and 10 conversions, respectively.

Additionally the multi-conversion train is exploited for MRI. For this purpose, each conversion is used to scan one k-space line of a gradient echo sequence (flip angle: 90°, TR: 1.7 s, FOV: 20×20 mm^2, 16×16 pixel), see Fig. 1c. Five different echo times TE were applied: 3 ms, 5.5 ms, 8 ms, 11.5 ms, and 14 ms. A centric sampling scheme was used to detect the maximum of signal intensity in the middle of the k-space.

4. Results

4.1. Singlet lifetime and conversion fraction

In Fig. 2 the spectrum obtained after the conversion at the observation field of 1.5 T is shown. In good agreement with theory [17] and previous experiments [16,18] the signal corresponding to the vinylene group exhibits a 180° phase shift with respect to that of the methyl groups.

In Fig. 3a the amplitude of the vinylene peak as a function of the number of conversions for a repetition time TR = 21.6 s is plotted, showing a monoexponential decay. The same experiment was repeated for different repetition times and monoexponential decays as a function of the number of conversion were observed in all cases. The exponential decay parameter (D_c) is plotted as a function of the repetition time, TR, in Fig. 3b. After a linear fit, and according to Eqs. (4) and (5), the lifetime of the singlet state was confirmed as $T_S = 4 \pm 1$ min and an effective conversion fraction ξ of the used CSS sequence was determined as $\xi = 10 \pm 2\%$.

4.2. Imaging

After a waiting time of 3 min, to ensure the generation of a well-defined spin state independent of the initial hydrogenation reaction, ^{1}H MRI images were acquired. It is important to remark that image recording, after these long waiting times, was only possible because the non-thermal proton spin order was stored as a singlet spin state and only later converted to observable magnetization for the MRI acquisition.

In Fig. 4 images of a 10 mm NMR tube containing the hyperpolarized substance acquired with different echo times TE are shown. Due to different chemical shifts and the J-coupling network in the dimethyl maleate molecule, the echo time dependence is not a single exponential but shows oscillations. Moreover, due to the unusual spin state which is recognizable by the 180° phase shift of the vinylene and the methyl group peaks, the maximum MRI signal is not obtained for a minimal echo time, as was shown before by our group for the PHIP hyperpolarized molecule 1-hexene [31]. As this strongly differs from the behavior of thermal polarization, this feature can be used as a contrast mechanism to distinguish the hyperpolarized molecules from a large thermally polarized background signal. For the gradient echo sequence the echo time dependency of the signal amplitude in the images agrees with the temporal evolution of the FID (see inset in Fig. 4).

5. Discussion

The measured singlet state lifetime is in full agreement with the values obtained using the field cycling [16], the CW methods [18] and the SLIC method [20]. This confirms the good performance of the CSS sequence for singlet–triplet conversion. Moreover, all these experiments correctly determine the lifetime of the singlet state independently of the details of the singlet to triplet conversion.

Signal enhancements estimated from the maximum of the thermal spectrum relative to the spectrum after the singlet–triplet conversion were only around 5, which is mainly due to the rather small chemical conversion of the hydrogenation reaction. This

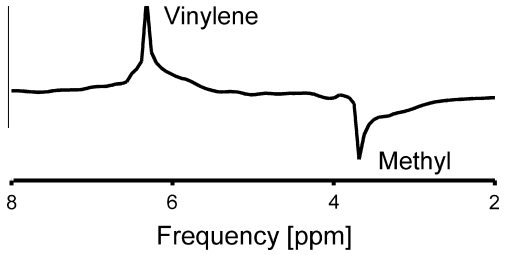

Fig. 2. ^{1}H spectrum of hyperpolarized dimethyl maleate at 1.5 T acquired after the singlet–triplet conversion achieved by the CSS sequence.

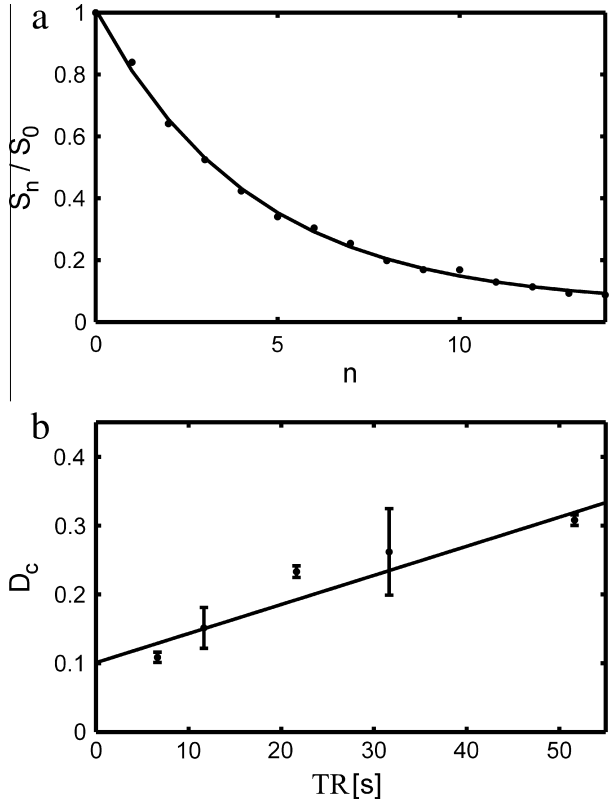

Fig. 3. (a) Exponential decay of the vinylene group signal of dimethyl maleate for multiple successive singlet–triplet conversions shown exemplarily for TR = 21.6 s. The signal was normalized to the first integral value. (b) Linear fit of the decay parameters D_c yields the singlet state lifetime T_S and the conversion fraction ξ.

could be improved by using e.g. a polarizer equipped with an optimized reaction chamber [32] and probably by optimizing the chemical structure of the catalyst. However, the optimization of the initially achieved PHIP hyperpolarization was not the purpose of the present study.

In comparison to the continuous wave (CW) pulses method used in a previous study [18], the CSS method obtains higher conversion fraction (CSS: 10%, CW: 7%) using a five times shorter sequence duration (CSS: 1 s, CW: 5 s). The efficiency of conversion to detectable magnetization is therefore even higher since the duration in which the converted triplet state can decay by relaxation processes is shorter. By applying the SLIC sequence to the same system studied in the present paper, Zhang et al found a slightly higher conversion fraction (13.9%) with a weak power rf sequence [20]. Evidently, there are many sequences available to achieve singlet to triplet conversion. Here, we compared those which have been applied to the same molecule. The experimentalist can thus decide which method is most suitable for the specific case of interest. For example, even though the conversion fraction

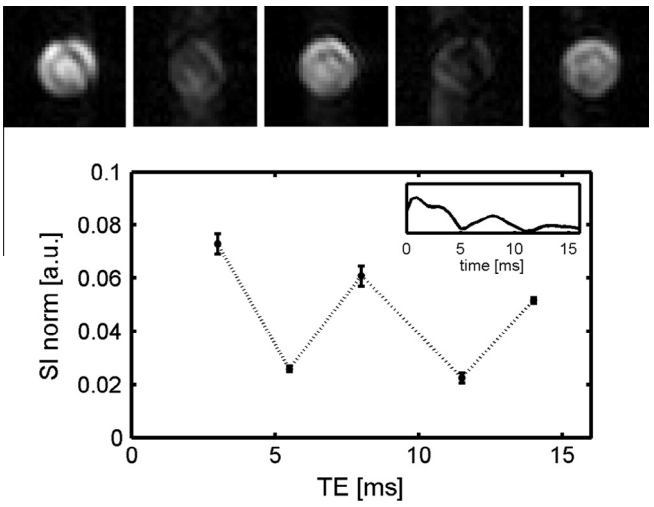

Fig. 4. Images normalized to the initial amount of hyperpolarization for five different echo times. In the bottom the corresponding signal intensities are shown. The inset in the plot at the bottom shows the magnitude of an FID of hyperpolarized substance.

for the CSS is slightly lower than that for the SLIC sequence the CSS sequence has a broader excitation bandwidth, because it uses 180° pulses, which might be beneficial for MRI. For this reason the CSS sequence was employed in this study in order to show the potential of combining singlet–triplet conversions to generate hyperpolarized proton spin pairs with imaging sequences. In the MR images signal inhomogeneities inside the NMR tube are visible. These are caused by undersampling of the abrupt susceptibility change at the tube border (Gibbs ringing artifact) [2]. The exponential decay of the signal amplitude in combination with the centric reordering scheme leads to a blurring of the image since it is associated with a Lorentzian shape of the point spread function (PSF). Correction of this exponential decay reduces the width of the PSF. However, it reduces also the signal-to-noise ratio. The next step in development would be to combined the singlet–triplet conversion with single shot MRI sequences such as e.g. EPI (echo planar imaging) [33] or RARE (rapid acquisition with relaxation enhancement) [34]. However, care has to be taken to avoid distortions generated by chemical shift effects, especially for the EPI technique. This can be addressed either by choosing suitably high bandwidth or alternatively by using molecules for which the singlet–triplet conversion is realized by heteronuclear J couplings to spins like ^{19}F or D. The use of single shot techniques has the advantage that the hyperpolarization can be used more efficiently and the number of phase encoding steps is not limited by the exponential decay of the multiple conversions. Moreover, it opens up the possibility to use multiple conversions for the acquisition of time resolved image series.

6. Conclusion

In this study, a long-lived proton singlet state was generated via PHIP in a symmetric molecule taking advantage of the fact that the singlet spin state is directly generated from the enriched parahydrogen employed. The ^{1}H–^{1}H singlet spin state was converted into measurable hyperpolarized magnetization in a clinical MRI system by a low-power radiofrequency pulse sequence with carefully chosen parameters. The possibility of combining these singlet–triplet conversions with an imaging sequence was shown. This technique might be applied in future studies to use the standard MRI nucleus ^{1}H, e.g. for metabolic imaging without the necessity of additional

hardware or costly isotope labeling. In vivo hyperpolarized MRI or MRS is usually performed with heteronuclei like ^{13}C or ^{15}N exploiting the negligible natural background signal and the longer T_1 relaxation times. However, this requires costly isotope labeling of the applied substances, adaption of pulse sequences as well as special instrumentation like double resonant coils and broadband amplifiers. In contrast, hyperpolarized ^{1}H MRI can be performed with conventional pulse sequences and hardware, and does not require isotopically-enriched substances. Moreover, the ^{1}H spin order derived by PHIP provides a natural contrast method because the initial state and evolution differ from those of the thermal ^{1}H signals in the background. In this paper ^{1}H images were acquired after a 3 min. waiting time. This shows that the storage of the ^{1}H nuclear spin order in a long-lived nuclear singlet spin state is a promising tool to avoid the problem of short T_1 relaxation times for in vivo applications of ^{1}H hyperpolarization, where long waiting times before acquisition are unavoidable.

Acknowledgments

The authors gratefully acknowledge financial support from the Max Planck Society, the Max Planck Graduate center with Johannes Gutenberg-University Mainz (MPGC) and the Federal Ministry of Education and Research within the program "Validierung des Innovationspotenzials wissenschaftlicher Forschung – VIP" (VIP0327).

References

[1] R.R. Ernst, G. Bodenhausen, A. Wokaun, Principles of Nuclear Magnetic Resonance in One and Two Dimensions, Clarendon Press, 1990.
[2] M.F. Reiser, W. Semmler, H. Hricak, Magnetic Resonance Tomography, Springer, 2007.
[3] C. Witte, L. Schröder, NMR of hyperpolarised probes, NMR Biomed. 26 (2013) 788–802.
[4] C.R. Bowers, D.P. Weitekamp, Transformation of symmetrization order to nuclear spin magnetization by chemical reaction and nuclear magnetic resonance, Phys. Rev. Lett. 57 (1986) 2645–2648.
[5] C.R. Bowers, D.P. Weitekamp, Parahydrogen and synthesis allow dramatically enhanced nuclear alignment, J. Am. Chem. Soc. 109 (1987) 5541–5542.
[6] K. Münnemann, H.W. Spiess, Nuclear magnetic resonance the art of signal enhancement, Nat. Phys. 7 (2011) 522–523.
[7] R.A. Green, R.W. Adams, S.B. Duckett, R.E. Mewis, D.C. Williamson, G.G.R. Green, The theory and practice of hyperpolarization in magnetic resonance using parahydrogen, Prog. Nucl. Magn. Reson. Spectrosc. 67 (2012) 1–48.
[8] M. Carravetta, O.G. Johannessen, M.H. Levitt, Beyond the T_1 limit: singlet nuclear spin states in low magnetic fields, Phys. Rev. Lett. 92 (2004).
[9] M. Carravetta, M.H. Levitt, Theory of long-lived nuclear spin states in solution nuclear magnetic resonance. I. Singlet states in low magnetic field, J. Chem. Phys. 122 (2005).
[10] G. Pileio, M. Carravetta, M.H. Levitt, Storage of nuclear magnetization as long-lived singlet order in low magnetic field, Proc. Natl. Acad. Sci. USA 107 (2010) 17135–17139.
[11] M.C.D. Tayler, M.H. Levitt, Singlet nuclear magnetic resonance of nearly-equivalent spins, Phys. Chem. Chem. Phys. 13 (2011) 5556–5560.
[12] M.H. Levitt, Singlet nuclear magnetic resonance, Annu. Rev. Phys. Chem. 63 (2012) 89–105.
[13] G. Stevanato, J.T. Hill-Cousins, P. Hakansson, S.S. Roy, L.J. Brown, R.C.D. Brown, G. Pileio, M.H. Levitt, A nuclear singlet lifetime of more than one hour in room-temperature solution, Angew. Chem., Int. Ed. 54 (2015) 3740–3743.
[14] D. Canet, S. Bouguet-Bonnet, C. Aroulanda, F. Reineri, About long-lived nuclear spin states involved in para-hydrogenated molecules, J. Am. Chem. Soc. 129 (2007) 1445–1449.
[15] E. Vinogradov, A.K. Grant, Hyperpolarized long-lived states in solution NMR: three-spin case study in low field, J. Magn. Reson. 194 (2008) 46–57.
[16] M.B. Franzoni, L. Buljubasich, H.W. Spiess, K. Münnemann, Long-lived 1H singlet spin states originating from para-hydrogen in Cs-symmetric molecules stored for minutes in high magnetic fields, J. Am. Chem. Soc. 134 (2012) 10393–10396.
[17] L. Buljubasich, M.B. Franzoni, H.W. Spiess, K. Münnemann, Level anti-crossings in ParaHydrogen Induced Polarization experiments with Cs-symmetric molecules, J. Magn. Reson. 219 (2012) 33–40.
[18] M.B. Franzoni, D. Graafen, L. Buljubasich, L.M. Schreiber, H.W. Spiess, K. Münnemann, Hyperpolarized 1H long lived states originating from parahydrogen accessed by rf irradiation, Phys. Chem. Chem. Phys. 15 (2013) 17233–17239.
[19] Y.N. Zhang, P.C. Soon, A. Jerschow, J.W. Canary, Long-lived 1H nuclear spin singlet in dimethyl maleate revealed by addition of thiols, Angew. Chem., Int. Ed. 53 (2014) 3396–3399.
[20] Y. Zhang, K. Basu, J.W. Canary, A. Jerschow, Singlet lifetime measurements in an all-proton chemically equivalent spin system by hyperpolarization and weak spin lock transfers, Phys. Chem. Chem. Phys. 17 (2015) 24370–24375.
[21] Y. Feng, R.M. Davis, S.W. Warren, Accessing long-lived nuclear singlet states between chemically equivalent spins without breaking symmetry, Nat. Phys. 8 (2012) 831–837.
[22] W.S. Warren, E. Jenista, R.T. Branca, X. Chen, Increasing hyperpolarized spin lifetimes through true singlet eigenstates, Science 323 (2009) 1711–1714.
[23] V.V. Zhivonitko, K.V. Kovtunov, P.L. Chapovsky, I.V. Koptyug, Nuclear spin isomers of ethylene: enrichment by chemical synthesis and application for NMR signal enhancement, Angew. Chem., Int. Ed. 52 (2013) 13251–13255.
[24] Y.S. Feng, T. Theis, X.F. Liang, Q. Wang, P. Zhou, W.S. Warren, Storage of hydrogen spin polarization in long-lived C13 singlet order and implications for hyperpolarized magnetic resonance imaging, J. Am. Chem. Soc. 135 (2013) 9632–9635.
[25] G. Pileio, S. Bowen, C. Laustsen, M.C.D. Tayler, J.T. Hill-Cousins, L.J. Brown, R.C. D. Brown, J.H. Ardenkjaer-Larsen, M.H. Levitt, Recycling and imaging of nuclear singlet hyperpolarization, J. Am. Chem. Soc. 135 (2013) 5084–5088.
[26] J.N. Dumez, J.T. Hill-Cousins, R.C.D. Brown, G. Pileio, Long-lived localization in magnetic resonance imaging, J. Magn. Reson. 246 (2014) 27–30.
[27] K.L. Ivanov, A.N. Pravdivtsev, A.V. Yurkovskaya, H.M. Vieth, R. Kaptein, The role of level anti-crossings in nuclear spin hyperpolarization, Prog. Nucl. Magn. Reson. Spectrosc. 81 (2014) 1–36.
[28] S.J. DeVience, R.L. Walsworth, M.S. Rosen, Preparation of nuclear spin singlet states using spin-lock induced crossing, Phys. Rev. Lett. 111 (2013) 173002.
[29] G.A. Morris, N.P. Jerome, L.Y. Lian, Real-time chemical-shift scaling in high-resolution NMR spectroscopy, Angew. Chem., Int. Ed. Engl. 42 (2003) 823–825.
[30] D. Graafen, S. Ebert, O. Neudert, L. Buljubasich, M.B. Franzoni, J.F. Dechent, K. Münnemann, 1H NMR spectroscopy and MR imaging with hyperpolarised substances, in: Annual Reports on NMR Spectroscopy, Elsevier, 2014, pp. 167–215.
[31] J.F. Dechent, L. Buljubasich, L.M. Schreiber, H.W. Spiess, K. Münnemann, Proton magnetic resonance imaging with para-hydrogen induced polarization, Phys. Chem. Chem. Phys. 14 (2012) 2346–2352.
[32] J.B. Hövener, E.Y. Chekmenev, K.C. Harris, W.H. Perman, L.W. Robertson, B.D. Ross, P. Bhattacharya, PASADENA hyperpolarization of C-13 biomolecules: equipment design and installation, Magn. Reson. Mater. Phys. 22 (2009) 111–121.
[33] K.V. Sharp, R.J. Adrian, Transition from laminar to turbulent flow in liquid filled microtubes, Exp. Fluids 36 (2004) 741–747.
[34] J. Hennig, A. Nauerth, H. Friedburg, RARE imaging: a fast imaging method for clinical MR, Magn. Reson. Med. 3 (1986) 823–833.