Toward ^{13}C hyperpolarized biomarkers produced by thermal mixing with hyperpolarized ^{129}Xe

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The ^{13}C NMR signal of acetic acid 1- ^{13}C -AcH is enhanced by polarization transfer from hyperpolarized ^{129}Xe using a thermal mixing procedure. 1- ^{13}C -AcH acid and hyperpolarized ^{129}Xe are mixed as gases to disperse ^{129}Xe in the acetic acid. The mixture is frozen with liquid N_2 at 0.5 T. The magnetic field is then momentarily dropped to allow for exchange of spin polarization between ^{13}C and ^{129}Xe . After polarization exchange the magnetic field is raised to its original value and the mixture is thawed, resulting in a solution of polarization enhanced 1- ^{13}C -AcH. A ^{13}C nuclear spin polarization enhancement of 10 is observed compared to its thermal polarization at 4.7 T. This polarization enhancement is approximately three orders of magnitude lower than that predicted by theory. The discrepancy is attributed to the formation of either an inhomogeneous solid matrix and/or spin dynamics during polarization transfer. Despite the low polarization enhancement, this is the first report of polarization transfer from ^{129}Xe to ^{13}C nuclear spins achieved by thermal mixing for a proton-containing molecule of biomedical importance. If future work can increase the enhancement, this method will be useful in hyperpolarizing a wide range of ^{13}C enriched compounds important in biomedical and biophysical research. © 2009 American Institute of Physics. [DOI: 10.1063/1.3181062]

I. INTRODUCTION

Carbon is an essential element and is directly and intimately involved in nearly all biochemical pathways. Nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI) can be used to noninvasively explore structure and properties of ^{13}C compounds. Compared to other nuclei, ^{13}C has large chemical shift dispersion; for example, the ^{13}C chemical shift range is about 30-fold greater than that of protons. When ^{13}C enriched compounds are used, the NMR spectrum is simpler to interpret since only the NMR signals originating from the administered compounds and corresponding metabolites are observed. However, the analytical potential of ^{13}C MR has not been exploited to its full capacity because of poor sensitivity. The low sensitivity of ^{13}C NMR/MRI originates from the inherently low natural abundance (1.1%) and small gyromagnetic ratio, $\gamma_{^{13}\text{C}} = \gamma_{^1\text{H}}/4$. Thus, ^{13}C nuclear spin polarization (fraction of nuclear spins contributing to MR signal), is only 4×10^{-6} at 4.7 T. For comparison, the polarization produced by optical methods is close to unity. The low thermal ^{13}C spin polarization can be enhanced by polarization transfer to carbon from more abundant protons. A number of approaches has been developed to enhance the thermal ^{13}C polarization by as much as a factor of 4. However, the transfer of thermal polarization leads to only a minor improvement and leaves the sensitivity inad-

equate for the majority of *in vivo* applications.

An alternative approach to enhance ^{13}C NMR signal is to create nonthermal polarization, often referred to as *hyperpolarization*. Hyperpolarization methods include optical pumping of noble gases,¹ parahydrogen and synthesis allow dramatically enhanced nuclear alignment (PASADENA)^{2,3} and dynamic nuclear polarization (DNP).^{4,5} These methods can potentially create a nonthermal polarization close to unity, thereby increasing ^{13}C NMR signals by a factor of 10^4 – 10^5 . The PASADENA effect relies on the molecular addition of parahydrogen to a double bond in a substrate molecule via catalytic hydrogenation. The spin polarization of adjacent protons can be transferred to ^{13}C by various methods.^{6,7} DNP is based on polarizing nuclear spins in the solid state at cryogenic temperatures and utilizes the transfer of spin polarization from electrons to the nuclear spins. This mechanism requires the presence of unpaired electrons, which are added to the sample as organic free radicals. Both DNP and PASADENA have been shown to be particularly useful for enhancing the signals of some carbon compounds.⁸ Despite recent successes of PASADENA^{7–11} and DNP,^{12–15} these methods have significant limitations. For example, the PASADENA method is limited to unsaturated molecular precursors with the $\text{C}=\text{C}$ bond that allow molecular addition of parahydrogen to produce hyperpolarized molecular agents. DNP requires high field magnets, expensive cryogenic equipment, and several tens of minutes to produce hyperpolarized samples. Moreover, DNP and PASADENA utilize toxic chemicals (free radical or catalyst, respectively), which re-

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quire filtration for use *in vivo*.

The nuclear spin polarization of noble gases, ^3He and ^{129}Xe , can be enhanced by several orders of magnitude (up to a factor of 10^5) by optical pumping methods. Hyperpolarized noble gases are produced by transferring the angular momentum from a beam of circularly polarized laser light to the atom's nuclear spins via an intermediate alkali metal vapor step.^{16–20} Removal of the alkali metal from the hyperpolarized gas is easily accomplished by a simple freeze out, which is a part of polarization procedure. Importantly, this can be accomplished with virtually no loss of polarization. In the case of PASADENA and DNP filtering out toxic substances requires an extra step upon which some polarization losses might occur. Hyperpolarized noble gases are easily administered via inhalation and therefore have crucial advantage for *in vivo* applications, most importantly for human studies. Hyperpolarized noble gas MRI has allowed visualization of the sinuses,²¹ perfusion in the brain^{22,23} and the lung,^{24–32} with main research effort being devoted to the imaging of lung structure and function.

Hyperpolarized ^{129}Xe can also be used to enhance ^{13}C polarization by spin polarization-induced nuclear Overhauser effect (SPINOE)^{33,34} or low-field thermal mixing (TM).^{4,5,35,36} SPINOE is the result of cross relaxation between hyperpolarized xenon and other nuclear species. The low-field TM is performed in a frozen mixture of the hyperpolarized xenon and ^{13}C -containing compound. TM is driven by the dipolar interactions between the nuclear spins and requires a uniform distribution of ^{13}C spins in a solid ^{129}Xe matrix. When the applied magnetic field (referred to as a “mixing field”) is comparable to the local dipolar fields, nuclear spins equilibrate their polarizations. Although several attempts to transfer polarization from hyperpolarized ^{129}Xe to ^{13}C spins in the solid state by TM have been implemented,^{35,36} the measured transfer efficiency has always been substantially lower than that predicted by theory. A major requirement of the transfer techniques is the formation of a homogeneous phase by ^{129}Xe and ^{13}C species. As a result, prior methods have been limited to compounds that dissolve well in liquid xenon. Thus, the reported TM studies have all used carbon disulphide ($^{13}\text{CS}_2$) due to its good solubility in liquid xenon. The majority of biomolecules, however, have poor xenon solubility, which poses a severe limitation for biologically relevant applications.

Here, we utilize the TM procedure to transfer spin polarization from hyperpolarized ^{129}Xe to acetic acid $1\text{-}^{13}\text{C}\text{-AcH}$. We mix the two species in the *gas phase*, after which the mixture is frozen with liquid N_2 . This approach overcomes the problem of limited ^{13}C -species solubility in xenon. This report is the first study of the polarization transfer from ^{129}Xe to ^{13}C achieved by TM for a proton-containing molecule of biological relevance. The ^{13}C NMR polarization was enhanced by tenfold compared to the equilibrium Boltzmann polarization at 4.7 T. We discuss experimental design and limitations as well as optimization of the technique. The results presented here show the potential for producing polarization enhanced ^{13}C biomarkers for use in biological research and biomedicine.

II. MATERIALS AND METHODS

Hyperpolarized ^{129}Xe is produced by spin-exchange optical pumping, a method originally described by Driehuys *et al.*¹⁸ and optimized by Ruset *et al.*¹⁹ A mixture of 1.5% ^{129}Xe , 25.5% N_2 , and 73% He (Spectra Gases) at 500 Torr enters a polarizing cell and flows through a spiral with rubidium pools (Alfa Aesar) maintained at 140 °C. The Rb vapor is optically pumped with circularly polarized light from a 90 W diode laser array (Coherent). After leaving the optical cell, the gas mixture containing polarized xenon enters a small spiral cell emerged in liquid N_2 , surrounded by a permanent magnet that provides a holding field of 0.2 T. Here xenon freezes on the cell's walls, while the buffer gases (He and N_2) are vacuumed out. A detailed description of the polarizer is given in Ref. 19. Hyperpolarized xenon is transferred to a Tedlar bag (Jensen Inert) and delivered to the facility where the NMR measurements are performed. The delivery is performed using a suitcase equipped with a solenoid coil and fitted with a stainless steel case. This device creates a relatively uniform magnetic field (~ 30 G) and minimizes the polarization loss during the transfer.³⁷ The total time between optical pumping and mixing with the acetic acid is ~ 15 min. The polarization of xenon measured at this point varies from 30% to 45% in our experiments.

A critical requirement for the experiment is to perform polarization transfer on the time scale shorter than T_1 relaxation of the interacting species. The choice of acetic acid ($1\text{-}^{13}\text{C}\text{-AcH}$) for the polarization transfer from ^{129}Xe is determined by the following factors: (i) $1\text{-}^{13}\text{C}\text{-AcH}$ is involved in important biochemical pathways, (ii) the partial pressure of $1\text{-}^{13}\text{C}\text{-AcH}$ is sufficiently high to achieve micromolar concentrations of the compound in the gas phase at normal conditions,³⁸ and (iii) $1\text{-}^{13}\text{C}\text{-AcH}$ has a relatively long T_1 in solution (~ 30 s). At the same time, ^{129}Xe has a T_1 of 2.6 h in the gaseous phase,^{39,40} and several minutes in the liquid and solid states in the presence of a modest magnetic field.⁴¹

Prior to TM, the fringe field of the 4.7 T magnet is mapped with a gaussmeter and the locations that correspond to the field strengths of 0.5 and 0.02 T are identified. These field strengths are used for condensation and field compensation, respectively. Liquid $1\text{-}^{13}\text{C}\text{-AcH}$ (99% ^{13}C isotopically enriched, Isotec) is deposited in a Tedlar bag (~ 50 mg) and evacuated for ~ 1 min. A similar bag filled with hyperpolarized xenon is connected to the bag with $1\text{-}^{13}\text{C}\text{-AcH}$ and ~ 150 mL of gaseous hyperpolarized ^{129}Xe is transferred to the bag with the acetic acid. $1\text{-}^{13}\text{C}\text{-AcH}$ evaporates and is mixed with xenon gas for ~ 1 min, Fig. 1. All parts, connectors, tubing, and the condensation finger (a piece of 5 mm NMR tube) shown in Fig. 1 are evacuated for ~ 5 min to a residual pressure of ~ 1 m Torr prior to the mixing of the compounds to remove the residual oxygen from the system. At first, valve no. 1 is opened (see Fig. 1) to deliver the gaseous mixture of $1\text{-}^{13}\text{C}\text{-AcH}$ and ^{129}Xe to the condensation finger after which the setup is brought to the fringe field of 0.5 T. The condensation finger, Fig. 1, is subsequently placed in the tight-fitted copper tubing and bathed in liquid N_2 , Fig. 2(a). Xenon and $1\text{-}^{13}\text{C}\text{-AcH}$ condense without a visible liquid phase. Copper tubing is used to improve the

FIG. 1. Condensation apparatus.

thermal conductivity between the condensation finger and liquid N_2 . We refer to the condensation finger inserted into the copper tube as a condensation setup in further discussion. It took 40 s to complete condensation.

The transfer of spin polarization is the most efficient when the mixing field is comparable to the local dipolar fields, the latter varying in the range of several gauss. At this field strength, the T_1 relaxation becomes very efficient due to both dipolar and quadrupolar mechanisms.^{41,42} The latter occurs because ^{131}Xe impurities relax the ^{129}Xe polarization in sufficiently low magnetic fields. Thus, T_1 relaxation competes with polarization transfer. As a compromise, the condensation setup is kept at a magnetic field strength of 0.5 T, which exceeds the dipolar and quadrupolar fields by several orders of magnitude during, prior, and after condensation. It

FIG. 3. ^{13}C NMR spectrum of hyperpolarized 1- ^{13}C -AcH (red) vs the spectrum acquired from the same sample at equilibrium Boltzmann polarization (blue). The splitting of the spectral line is likely due to magnetic field inhomogeneities.

is exposed to the mixing field only for a short period of time, required for polarization transfer. The mixing field is generated by compensating the fringe field with a custom-built electromagnet. Because of technical limitations, the maximum compensating field strength is 0.02 T. Consequently, the condensation setup is physically relocated after condensation from 0.5 to 0.02 T for the polarization transfer.

The condensed sample is collected in a 5 mm NMR tube at the fringe field of 0.5 T and the condensation setup is quickly transferred to a home-built electromagnet situated in the fringe field of 0.02 T [Fig. 2(a)]. The 0.02 T fringe field is compensated by dc electromagnet with the resulting mixing field, B_{mix} , ranging from 0.1 to 0.5 mT [Fig. 2(b)]. The electromagnet is turned on to maintain B_{mix} for 0.1–0.3 s. The characteristic time required for polarization transfer is proportional to the inverse of the dipolar coupling constant between the spins in the solid state and is expected to be in the 0.1–100 ms range. Thus, our range of B_{mix} is sufficient even for weakly coupled spins to exchange polarizations. We also anticipate B_{mix} to be comparable to the local dipolar fields between ^{129}Xe , ^{131}Xe , ^1H , and ^{13}C spins. The electromagnet switch is controlled by LABVIEW (National Instruments, Austin, TX). Following the TM, the sample is transferred back to the 0.5 T field, where it is thawed for 30–40 s, Fig. 2(c). Upon thawing, liquid 1- ^{13}C -AcH remains in the 5 mm NMR tube, while gaseous xenon expands in the Tedlar bag of the condensation setup, Fig. 2. The ^{13}C spectrum of liquid hyperpolarized 1- ^{13}C -AcH is collected approximately 5–8 s after thawing using a single resonance home-built NMR probe tuned to ^{13}C resonance frequency (50 MHz). The detected hyperpolarized NMR signal is compared to the thermal signal of the same sample, at 4.7 T, Fig. 3.

FIG. 2. The diagram of the experimental setup used for TM experiment. (a) The gaseous mixture of 1- ^{13}C -AcH and ^{129}Xe is condensed in liquid N_2 at 0.5 T. (b) Quick sample transfer to 0.02 T, where TM is performed. (c) The setup is transferred back to 0.5 T field, where 1- ^{13}C -AcH is melted and the signal is detected at 4.7 T.

III. RESULTS AND DISCUSSION

The observed enhancement values, expressed as a ratio of integrated signal intensity of the hyperpolarized signal relative to that of the reference thermal spectrum, ranged

from 3 to 5, Fig. 3, depending on the initial xenon polarization (30%–45%) and the ^{129}Xe :1- ^{13}C -AcH molar ratio (the approximate range from 10:1 to 50:1). It should be noted that at least 45 s elapsed between the end of TM and ^{13}C signal detection. Because ^{13}C T_1 is ~ 30 s in the liquid state as we measured, we estimate that approximately half of the initial polarization is lost due to relaxation. Therefore, the ^{13}C polarization enhancement factor achieved during TM is extrapolated to ~ 10 . We found no significant dependence of ^{13}C hyperpolarization on both mixing time (0.1–0.4 s range) and B_{mix} (0.1 mT to 0.5 mT range).

In the hyperpolarized ^{129}Xe /1- ^{13}C -AcH matrix with a molar ratio of 1:1, the final carbon polarization is predicted to be about half of the initial xenon polarization. Thus, for a ^{129}Xe polarization of 30%, the upper limit for the final carbon polarization is 15%, which would correspond to the enhancement factor of about 10^4 , three orders of magnitude higher than the observed experimental enhancement. The polarization transfer mechanism in TM is nonselective by its nature and occurs with respect to all nuclear spins that are present in the system including ^1H , ^{13}C , and quadrupolar impurities such as ^{131}Xe . The latter can potentially depolarize ^{129}Xe , ^{13}C , and ^1H in the solid matrix. To determine whether the presence of ^{131}Xe impurity is the factor that limits the efficiency of our polarization transfer experiment, the polarization of ^{129}Xe is measured under conditions identical to those in TM procedures. ^{129}Xe was depolarized from 30% to 10%, which corresponds to T_1 relaxation of xenon in the solid state. Therefore, we conclude that quadrupolar impurities are not the dominant source of polarization loss in the solid matrix.

The noteworthy discrepancy between the theoretical limit and the experimental results is likely due to formation of an inhomogeneous solid matrix and/or relaxation processes in the solid state. Since the melting temperatures T_{melt} of ^{129}Xe and 1- ^{13}C -AcH are substantially different ($T_{\text{melt}} = 159$ K for ^{129}Xe and $T_{\text{melt}} = 290$ K for 1- ^{13}C -AcH), it is conceivable that the acetic acid condenses first, so that the two species become microscopically separated and do not establish the dipolar contact in the solid state. To explore this hypothesis, the condensation is also performed in the mixture of ^{129}Xe and 1- ^{13}C -ethanol (10:1 molar ratio). The melting temperature of 1- ^{13}C -ethanol is $T_{\text{melt}} = 170$ K, comparable to that of xenon. However, no enhancement in ^{13}C polarization is observed in TM of 1- ^{13}C -ethanol. This result suggests that the transfer efficiency of spin polarization is not limited by the species separation in the solid phase. While the possibility of an inhomogeneous matrix formation cannot be excluded, it is not due to a difference in melting points between 1- ^{13}C -AcH and ^{129}Xe .

Aside from quadrupolar interactions with ^{131}Xe , there are two competing processes of polarization transfer from ^{129}Xe to ^1H and ^{13}C . Protons have ~ 4 times greater gyromagnetic ratio γ than the ^{13}C and the internuclear distance between a ^1H – ^{129}Xe pair is significantly shorter than that for ^{13}C – ^{129}Xe . Since dipolar interactions are proportional to γ and inversely proportional to the cube of the distance between the interacting spins, the couplings of ^{129}Xe with proton lattice are expected to be about one to two orders of

magnitude greater than those with ^{13}C . As a result, we anticipate ^{129}Xe polarization to be transferred to the ^1H lattice rather than to ^{13}C . Since the T_1 losses of ^{129}Xe polarization are minimal, we consider other contributions to polarization loss that can occur via ^1H as well as ^{13}C spin lattice relaxation processes. While magnetic fields of <1 mT are necessary for the nuclear spins to exchange polarizations, the dipolar energy reservoir makes the relaxation mechanisms more efficient at low magnetic fields,⁴² thereby compromising the net efficiency of the process. For example, ^1H T_1 values in the millisecond range were reported for methyl⁴³ and ammonium⁴⁴ protons at low temperatures and low magnetic field. Such fast relaxation is governed by the dynamics of methyl and ammonium groups. If proton T_1 is reduced to the millisecond range, it would result in efficient relaxation of polarization via the ^1H lattice during TM, thereby explaining the relatively low experimental enhancements of ^{13}C polarization. Future work will focus on minimizing the polarization losses in the solid state. Moreover, cross polarization from ^{129}Xe is another viable alternative for polarization transfer to ^{13}C molecules, albeit with more demanding hardware requirements.

IV. CONCLUSIONS

TM is a potentially useful method to transfer noble gas hyperpolarization via direct dipolar interactions between nuclear species. It does not require sophisticated hardware and can be applied to a wide range of molecules. Moreover, the condensation procedure utilized here does not require dissolving ^{13}C enriched compounds in liquid xenon and thus overcomes the problem of limited solubility of biological molecules. We demonstrated that the ^{13}C nuclear polarization of 1- ^{13}C -AcH can be enhanced by polarization transfer from hyperpolarized ^{129}Xe in the solid state using TM. The observed experimental maximum enhancement is likely a result of competing polarization transfers from ^{129}Xe to ^{13}C and ^1H and relaxation. The results presented here demonstrate that TM is an experimentally viable approach to enhance ^{13}C polarization in proton containing molecules. We believe this work will stimulate future developments of methodology for ^{13}C polarization enhancement using hyperpolarized ^{129}Xe .

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