Ultra-Low Temperature Nuclear Magnetic Resonance

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Because NMR spectroscopy requests more sensitivity and more resolution, high-frequency and high-power microwave irradiation of electron spins in a magnetic field, Dynamic Nuclear Polarization (DNP) is becoming a common partner for fast sample spinning NMR experiments. Currently, this technics is performed at minimum sample temperatures \(~100\) K, using cold nitrogen gas to pneumatically spin and cool the sample. The desire is to improve NMR by providing ultra-low temperatures, using cryogenic helium gas. It is shown that stable and fast spinning can be attained for sample temperatures down to 30 K using a cryostat developed in our laboratory. Using this cryostat to cool a closed-loop of helium gas results in spinning frequencies that can greatly surpass those achievable with nitrogen gas. It results in substantial sensitivity enhancements (~ 600) and according experimental time-savings by 2 to 4 orders of magnitude. Therefore, access to this temperature range is demonstrated to be both viable and highly pertinent.

1. Introduction

Much progress has been made since the demonstration [1] that the intrinsically larger polarization of electron spins in a magnetic field can be transferred to the much less polarized nuclear spins [2]. This hyperpolarization of nuclei, known as dynamic nuclear polarization (DNP), has only recently started to be routinely exploited [3–7]. This is due to the technological breakthroughs resulting from the intra-MIT collaboration and perseverance of Griffin, Temkin, and coworkers [8,9], as well as the subsequent commercialization of the necessary hardware [10]. Nowadays, high-resolution spectra, recorded under magic angle sample spinning (MAS) conditions, can be acquired in a fraction of the previously required experimental time for a large variety of systems ranging from biomolecules [4,5] to functional materials [6,7]. The boundaries of the technique still strive to be pushed further. This article presents our work towards one of the possible futures of so-called MAS-DNP. The first MAS-DNP experiments were performed at room temperature and low static magnetic field strengths (1.4 T) [11]. Extending this technique to the larger magnetic fields required for high-resolution solid state ssNMR studies occurred with the introduction of higher frequency and high-power continuous-wave microwave (\(\mu W\)) sources [8] and their combination with hardware providing low temperature samples under MAS [12], both facilitating saturation of the electron spin resonance and accordingly improving MAS-DNP efficiencies. Presently, commercial equipment supplying a full experimental setup of MAS-DNP is available at magnetic field strengths of 9, 14, and 19 T, with sample temperatures down to ~100 K. We [13,31], as well as others [12,14–16], are working to demonstrate the further improvement in sensitivity and resolution by performing MAS-DNP at even lower temperatures. It implies the use of helium gas to cool down the spinning sample and technical solutions are far from trivial.
2. Practical aspects for high-field MAS-DNP

The recent revival of DNP for ssNMR has ultimately been a result of technological advancements in interplay with theoretical improvements. Gyrotrons require large static magnetic fields (generally of the same order or half the strength of the NMR magnet), and superconducting magnets are therefore required for gyrotrons implemented in high-field DNP-enhanced NMR studies. This puts a large expense on the experimental setup. Moreover, the gyrotron must be placed at a certain distance from the NMR magnet and coupled to the sample by a waveguide transmission line [10] (only 30% efficient). Furthermore, NMR probes for MAS-DNP are predominantly designed to achieve low sample temperatures as this increases spin relaxation times, making the DNP process much more efficient [17,18]. A design similar to the one used in the commercial system to achieve low temperature (~100 K) MAS is detailed in Ref. [21]. Essentially, a large flow of nitrogen gas, required for fast MAS, is passed through a heat exchanger, which cools it to the desired temperature before it can be used to rotate the sample at the chosen frequency. This heat exchanger is composed of a coil, in which the high-pressure gas passes through, placed in a closed chamber of liquid and gaseous N2. The pressure of this chamber has to be kept above the pressure of the gas within the coil to avoid condensing the MAS gas, as this would result in unstable sample spinning. Unfortunately, using closed chambers of N2 to cool N2 gas implies a minimum sample temperature above the temperature of liquid N2 (88 K at 2 bars above atmospheric pressure). Accordingly, the minimum operating temperature for most MAS-DNP experiments is ~100 K, considering the thermal losses in the transfer lines between the heat exchanger and the NMR probe, as well as in the probe itself. Moreover, the speed of sound in N2 gas, which is the current limiting factor for the maximum sample rotation frequency, decreases with temperature. The maximum attainable spinning frequency is therefore strongly decreased by going to low temperature (cf. 23 kHz at 300 K and 15 kHz at 100 K for a 3.2 mm outer diameter sample rotor).

3. Practical aspects for “ultra”-low temperature high-field MAS-DNP

3.1. Contemporary approaches to “ultra”-low temperature MAS

Innovative studies by Yannoni and coworkers showed that cold helium (He) gas can successfully be used to cool spinning samples at temperatures <<100 K [23,24], others followed the idea with their own technological designs [22]. The construction of a device to access this “ultra”-low temperature spinning (ULTMAS) regime is far from straightforward and specific limitations as cost and loss of (precious) He or low kinematic viscosity of He gas inhibiting stable MAS [25]. However, the benefits are numerous, embracing an increased Boltzmann distribution (cf. ~0.1% 1H polarization at 10 K, ~0.003% at 300 K, all at 10 T), reduced Johnson-Nyquist noise in the radio frequency (RF) circuit, in addition of being able to study low-temperature phenomena at an atomic scale [22,26]. Hardware for ULT-MAS combined with DNP at temperatures <<100 K, has been developed in the teams of R. Griffin at the Massachusetts Institute of Technology (MIT), USA [12], R. Tycko in Bethesda (NIH), USA [14], and T. Fujiiwara in Osaka, Japan [16]. The MIT design from 1997 employed He as both the spinning and cooling gases and gave substantial DNP enhancement, on/off (the ratio of the intensities of the NMR signal detected in the presence and absence of microwave irradiation suitable for DNP) It was stated that the DNP enhancement dramatically improved by lowering the temperature from 100 (1H on/off ~5) to 25 (1H on/off ~20) to 55 K (1H on/off ~100) [12]. The Bethesda design differs from the others in that it uses cold He gas for the VT only, with the bearing and drive being supplied with cold N2 gas [27]. This requires the use of elongated 4 mm rotors and restriction of the VT gas to the center area of the rotor to avoid mixing of the N2 and He gases, which would result in liquefying/solidifying N2 and consequently in significant sample spinning instabilities. This inventive design has the strong advantage of only consuming a small amount of liquid He (3 Lh-1), and enables MAS-DNP experiments to be performed at MAS frequencies up to ~7 kHz at 20 K [20]. 1H on/off ~25 have been obtained. The Osaka design for MAS-DNP using cold He for both bearing and drive gases (no VT) can achieve 30 to 90 K for >10h at MAS frequencies up to 3 kHz [16]. The produced cold He gas passes through needle valves used to control independently the flow rate of each gas line, and proceeds to the NMR. Since the maximum He flow rates depend upon the Dewar pressure, maximum MAS frequencies are kept low for safety reasons. The Osaka design has recently been upgraded for sustainable ULT-MAS using a closed-loop cooling circuit, which involves the implementation of GM-coolers [25]. This design allows minimum sample temperatures of 35 K for MAS frequencies up to 4000 Hz using 3.2 mm rotors [25]. Experiments can be sustained for weeks, with associated running costs only including electricity.
cryostat is cooled down to ~80 K using cold N2 gas boiled off from a liquid N2 Dewar. This process cools the cryostat cold, thanks to a series of valves and a He gas circuit that bypasses the heat exchanger. When needed, the NMR probe can be warmed up with a flow of heated (300 K) He gas, while keeping and spun to the desired MAS frequency with the NMR probe being at any temperature (30–300 K). There are additional heaters for each of the lines inside the base of the probe if required. NUMOC has very efficient heat transfer with only 8 W of thermal losses throughout the liquid He Dewar, the transfer line connecting the He Dewar to NUMOC, the heat exchanger, the He gas transfer lines, and the inlet to the probe. The lowest sample temperature for “safe” spinning conditions is currently ~30 K. This regime is illustrated by the DNP enhanced NMR spectrum recorded at MAS frequencies of 13, 17.5 and 25 kHz for sample temperatures of 36, 50 and 90 K (see spinning rate curve in Fig. 3), that we recently reported [13,31]. It has to be reminded here that the maximum possible MAS frequency with N2 gas at the minimum possible sample temperature of 100 K is only 15 kHz using the same probe. After having been used to spin the rotor, the exhausted gas from the stator is recollected, and serves first to cool the RF circuitry inside the probe (i.e., RF coil, inductors, resistors, capacitors, and cables) and then to thermally shield the incoming bearing, drive, and VT lines (see Fig. 2a). This return gas is then heated to RT, compressed, separated into the three lines, and fed back into the heat exchanger where it is re-cooled for further cooling and spinning of the sample, forming thus a closed-loop system. Samples can be pneumatically inserted/ejected directly into/from the stator using He gas and spun to the desired MAS frequency with the NMR probe being at any temperature (30–300 K). When needed, the NMR probe can be warmed up with a flow of heated (300 K) He gas, while keeping the cryostat cold, thanks to a series of valves and a He gas circuit that bypasses the heat exchanger. The cryostat is cooled down to ~80 K using cold N2 gas boiled off from a liquid N2 Dewar. The cryostat is then cooled in <30 mins to 6 K with cold He gas boiled off from a liquid He Dewar. Both the liquid N2 and liquid He Dewars can be concomitantly connected to NUMOC, the selection being ensured through a series of valves. Once cold, the cryostat can remain cold for days when not used. For routine use, the cryostat temperature is maintained by cold He gas, which is continuously boiled off from the liquid He Dewar using an internal heater at a rate of 5-25 L h⁻¹, depending on the final desired sample temperature and spinning rate. Once the cryostat is cold, a significant flow rate of He gas in the closed-loop system is passed through the heat exchanger to the NMR probe and back to cooling down the probe from RT to 30 K in ~15 mins. A suitable compressor along with PID controllers that can regulate the desired flow rates for each line ensures stable and fast sample spinning. The MAS and temperature stability of the setup as a function of time are illustrated in Fig. 4. Obviously the temperature of the bearing and VT lines slightly decreases over time, due to improved line insulation as the return gas also slightly decreases in temperature. Careful heating of the drive gas was used as a compensation to maintain a constant MAS frequency and sample temperature. NUMOC is a prototype device constructed to demonstrate the feasibility to perform fast MAS-DNP experiments at ULT and also to dimensionalize a fully autonomous system that can be used for completely sustainable ULT-MAS. Like the new Osaka design [25] (see above), our fully autonomous system, named SACRYPAN (SmAll CRYostat for Proof of AutoNomy), employs cyro-coolers to complete a fully closed loop system with zero consumption of liquid He (see Fig. 2b). SACRYPAN is currently under testing and should be able to produce the same specifications as NUMOC (up to 15 kHz at 30 K and >25 kHz at >90 K, for a 3.2 mm rotor) (see Fig. 5).
Figure 1. Pictures of the cryostat named NUMOC. Overview of the whole setup DNP-NUMOC (top left) installed in our laboratory in Grenoble and the control system of our full automatized process (top right). Vessel of the counter-current heat exchanger (bottom left), specific cryogenic transfer line and the coupling device to the NMR probe (bottom right).

Figure 2. Simplified schematic representation of the experimental setups used for MASDNP with helium sample spinning using a closed-loop system with NUMOC (a) and a fully autonomous system using SACRYPAN (b).

Figure 3. Plot illustrated the MAS frequency raised as a function of temperature in DNP experiments and @ ULT spinning only.
Figure 4. Plot illustrating the stability of the temperature measured at the base of the ULT-MAS-DNP probe and the MAS frequency as a function of time. The sample temperature and spinning frequency (±50 Hz, measured with no signal averaging) are kept constant by applying slight heating to the drive gas.

Figure 5. Pictures of the cryostat named SACRYPAN under assembling and testing in our laboratory in Grenoble.

4. Results from high-field ULT-MAS-DNP

The MIT [12] and previous Osaka [16] probe designs had the tuning and matching capacitors outside of the He environment to help avoid electrical discharge arcing, which can result from high power RF irradiation. As stated above, the Grenoble design uses the cold He gas exhausted from the stator to cool some of the RF electronics, including the matching and tuning capacitors. Nevertheless, there are no signs of electrical breakthrough and discharge arcing. The absence of observable arcing is attributed to the high mass flow rate of He gas used to spin and cool the sample. This lack of arcing allows high-resolution spectra to be recorded. The work from MIT [12] and Osaka [16] showed that reducing the sample temperature tends to increase the DNP-enhancement, as expected. We investigate further this effect at high field (9.4 T) and high-power μw irradiation by combining NUMOC with a Bruker Biospin ULT-MAS-DNP prototype probe and the commercial DNP-NMR spectrometer in Grenoble [13,31]. Using the nitroxide biradical AMUPol [19], one of the current best-performing polarizing agents, DNP-enhancements $1H_{\text{on/off}} \approx 300$ are commonly achieved for model systems under standard MAS-DNP conditions, i.e. at 9.4 T and at 110 K with N2 gas for sample cooling and spinning. Fig. 7 shows the effect of reducing the temperature of such a model sample from ~110 K to ~55 K, using cold He gas produced by NUMOC. Notably, $1H_{\text{on/off}}$ increases continuously, with $1H_{\text{on/off}} \approx 680$ at 55 K. $1H_{\text{on/off}}$ is very easy to measure, as long as some signal can still be detected in the absence of μw irradiation. It is thus a valuable parameter to judge the DNP effect alone. The maximum theoretical signal enhancement attainable through DNP is given by $\gamma_e/\gamma_n$, which translates to a theoretical limit of 658 for $1H$. The experimental value of $1H_{\text{on/off}} \approx 680$ obtained at 55 K thus clearly demonstrates that $1H_{\text{on/off}}$ does not directly relate to the theoretical enhancement. The reason for this has been thoroughly described in a recent publication [28], which followed the initial inspired work by Thurber and Tycko [29]. The ratio $\gamma_e/\gamma_n$ can only then be related to the theoretical maximum when both the electron and nuclear thermal steady-state polarizations are at their respective Boltzmann equilibriums in the absence of irradiation. Indeed, the nuclei can in such conditions be in a de-polarized state in absence of μw irradiation, i.e. a polarization state lower than the Boltzmann equilibrium, leading to an effective polarization difference between electron spins and protons much greater than the expected factor of 658. It has been shown that more than half the proton polarization can be depleted at ~110 K [28] and up to ~80 % at 25 K [29]. Therefore, $\gamma_e/\gamma_n$ can also dramatically over exaggerate the gain from DNP. To judge the pertinence of using MAS-DNP as an analytical tool, the gain in absolute sensitivity, defined as the signal-to-noise ratio returned per unit square root of time, should be chosen as the best criterion.
Figure 6. (a) $\text{H}^1$ Hahn-echo NMR spectrum recorded with (red) and without (blue) $\mu\text{w}$ irradiation on a 13C-urea model solution containing 5 mM AMUPol recorded at 9.4 T, with a sample temperature of 55 K, and using a MAS frequency of 10 kHz. (b) The measured DNP-enhancement ratio as a function of sample temperature for the sample from (a). Adapted with permission from Ref. [13].

The advantage of going to lower temperatures for MAS-DNP in terms of absolute sensitivity is highlighted in Fig. 8. For this organic semiconductor system under structural study in our laboratory [30], performing an experiment at ~50 K results in a sensitivity increase of a factor of 5.6 compared to the same experiment recorded at ~110 K and corresponds to a 30-fold reduction in required experimental time. Fig. 7 shows that even at ~50 K it is possible to record DNP enhanced NMR spectra with sample spinning at 17.5 kHz.

Figure 7. DNP-enhanced {1H-}13C CP spectra of cyclo-diphenylalanine (top right) recorded at sample temperatures of ~108 K (orange) and ~50 K (purple) using MAS frequencies of 12.5 kHz. Also shown (gray) is a similar spectrum recorded at ~50 K but using a MAS frequency of 17.5 kHz. Adapted with permission from Ref. [22] - Published by The Royal Society of Chemistry.

The performance of our ULT-MAS-DNP system in terms of temperature, MAS frequency, and RF translates into global stability and sustainability allowing the acquisition of multi-dimensional experiments, which are crucial for detailed atomic scale structural analysis. Experiments recorded with $\text{H}^1\text{enon/loff} > 300$ at a sample temperature of ~55 K, and with a MAS frequency of 8 kHz demonstrated this ability on two different types of 2D 13C homonuclear.

Conclusions
This Paper has described a promising but challenging new direction for high-field MAS-DNP that involves sample temperatures much below of 100 K and faster MAS. Thanks to the efforts of the few
teams working on ULT-MAS, the challenge of spinning NMR samples at temperatures well below 100 K is becoming realizable and realistic, and its combination to DNP seems to really fulfill the expectations of significant increases in sensitivity. In addition, we expect that this new regime of temperatures will open NMR spectroscopy and DNP to new fields of investigation involving specific chemical and physical properties. The numerous benefits of lower temperatures for MAS-DNP were highlighted with experimental results obtained at high magnetic field (9.4 T) and high-power μW irradiation (>1 W), by combining the NUMOC setup with a Bruker Biospin ULTMAS-DNP functional prototype probe and a commercial DNP-NMR spectrometer. With this setup, unprecedented DNP enhancements as well as absolute sensitivity gains could be obtained, leading to reductions in experimental times by a further factor of 30 compared to MAS-DNP at 100 K. The NUMOC setup is currently only semi-closed-loop, still requiring consumption of liquid He to cool the cryostat but the fully autonomous apparatus with zero He consumption, named SACRYPAN, already under cryogenics testing should be connected to the DNP setup in few weeks.

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