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Rapid-melt Dynamic Nuclear Polarization

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ABSTRACT

In recent years, Dynamic Nuclear Polarization (DNP) has re-emerged as a means to ameliorate the inherent problem of low sensitivity in nuclear magnetic resonance (NMR). Here, we present a novel approach to DNP enhanced liquid-state NMR based on rapid melting of a solid hyperpolarized sample followed by 'in situ' NMR detection. This method is applicable to small (10 nl to 1 μl) sized samples in a microfluidic setup. The method combines generic DNP enhancement in the solid state with the high sensitivity of stripline ^{1}H NMR detection in the liquid state. Fast cycling facilitates options for signal averaging or 2D structural analysis. Preliminary tests show solid-state ^{1}H enhancement factors of up to 500 for $H_2O/D_2O/d_6$-glycerol samples doped with TEMPOL radicals. Fast paramagnetic relaxation with nitroxide radicals, In nonpolar solvents such as toluene, we find proton enhancement factors up to 400 with negligible relaxation losses in the liquid state, using commercially available BDPA radicals. A total recycling delay (including sample freezing, DNP polarization and melting) of about 5 s can be used. The present setup allows for a fast determination of the hyper-polarization as function of the microwave frequency and power.

Even at the relatively low field of 3.4 T, the method of rapid melting DNP can facilitate the detection of small quantities of molecules in the picomole regime.

1. Introduction

Nuclear magnetic resonance (NMR) is the spectroscopic modality of choice for many analyses due to its high specificity, and its ability to provide information on structure and dynamics. However, one factor that limits the applicability of NMR in many experiments is its inherent insensitivity, caused by the low energy of the nuclear spin in a magnetic field. Dynamic Nuclear Polarization (DNP) is a concept that was proposed in the 1950s [1,2], where microwave irradiation is used to transfer part of the much higher polarization of doped electron spins to nuclei in the sample.

A variety of approaches to DNP have been developed over the years, differing by the temperature-regime of operation and the choice of state for NMR analysis (solid or liquid). In the solid phase, three main mechanisms for DNP have been identified: a two-spin (electron–nuclear) interaction, coined Solid Effect DNP [3–5], a three spin (two electron, one nuclear) interaction or Cross Effect DNP [6] and a many spin interaction or Thermal Mixing DNP [7]. These polarization transfer mechanisms have been the subject of many studies and are elegantly treated elsewhere [8–14]. In the liquid state and in metals, the Overhauser DNP mechanism is based on translational dynamics that can introduce a net electron-nuclear cross relaxation [1,2,15]. The maximum achievable DNP polarization enhancement depends on the mechanism at hand, but is fundamentally limited by the ratio of electron and nuclear gyromagnetic constants (660 for protons, 2600 for ^{13}C nuclei). Commercially available DNP systems rely on continuous wave microwave irradiation at cryogenic temperatures, followed either by fast dissolution and NMR detection in the liquid state [16] or by in situ solid-state NMR detection using a cryogenic Magic Angle Spinning set-up. In dissolution DNP, the nuclear spin system is polarized at ultra-low temperatures (1–2 K) using a Solid Effect or Thermal Mixing transfer induced by low power microwaves of 95–190 GHz (3.4–6.9 T). Using a fast dissolution step, the frozen solid is dissolved in hot solvent and transferred to a separate liquid-state NMR spectrometer where a single (or few) scan experiment can be performed. As the available time window is limited by the T_1 of the nucleus, the most common detection is based on ^{13}C NMR [17], although more recent experiments attempt to include direct [18] or indirect ^{1}H detection [19]. MAS-DNP in its present implementation relies on in situ DNP using a cryogenic MAS probe, with high power microwave irradiation produced by gyrotrons and with optimized biradicals to profit from Cross-Effect polarization transfers.

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Both methods can achieve spectacular enhancements of the NMR signal, up to 20,000 for the dissolution version, allowing single scan detection of systems that would otherwise take many hours or days of continuous signal accumulation. Part of the enhancement is related to the DNP process itself, while a substantial contribution is due to the cryogenic cooling, adding a Bolzmann enhancement factor (up to 200). Dissolution DNP is the method of choice for in vivo or in vitro NMR/MRI where fast metabolic processes can be followed in real time. MAS-DNP is mostly used for structure elucidation in large bio-molecules, although interesting developments arise in the field of materials sciences, in particular related to the study of catalytic surfaces.

Despite this spectacular progress in the field of DNP it is fair to say that both commercialized methods also have their drawbacks. Proton relaxation is generally too fast to allow ^{1}H NMR detection with dissolution DNP methods. Also, the quasi-single scan nature prevents the use of standard 2D NMR methods. Dissolution DNP requires a long preparation time, including cooling, DNP polarization and sample transfer, which can amount to several hours. This may not be a big problem for MRI where multiple specimens can be polarized in parallel, but it does restrict the applications for common high resolution NMR spectroscopy. A minor setback is related to the dissolution itself, where the final detection is performed in a larger sample volume with an inevitably lower concentration and thus a reduced NMR detection sensitivity.

MAS-DNP is an in situ method that does not suffer from most of these drawbacks. However, at the relatively high radical concentrations, there can be a negative impact on the spectral resolution and it may not be trivial to obtain superior information content compared to a more common multiple averaging approach with thermal polarization levels. Detection takes place at low T, which can also have a negative effect on resolution if the frozen glassy state leads to variation in local conformations. Also, the method requires substantial investment by adding another magnet cryostat to house the gyrotron source and operating costs increase due to the cryogenic liquids required for the cryo-MAS.

In this paper we propose a fast melting method to allow liquid-state NMR detection combined with generic solid-state DNP. This method can be characterized by the following hardware elements: (a) A liquid nitrogen cooled microwave resonator or nonresonant concentrator coupled to a high power microwave source. With the microfluidic sample in this position, it is rapidly frozen and proton polarization of the sample matrix can be achieved in a matter of seconds. (b) A fast shuttling mechanism driven by a linear motor allows fast transfer (within typically 20 ms) of the frozen (hyper-polarized) sample to a melting zone, where hot nitrogen gas (at 70 °C) is used to melt the sample. (c) A second shuttling step then transports the capillary containing the liquid sample into the room temperature NMR detector, which is a stripline NMR chip with free axial access. After the NMR experiment, which runs analogous to common liquid-state NMR, where all regular pulse sequences are possible, the sample is transported back into the cryogenic zone and the process is repeated. As we avoid sample dilution, repetitive operation becomes possible, allowing for extensive averaging or multidimensional spectroscopy. Second, the transition and transport time to go from frozen solid DNP to liquid NMR can be done in a few hundreds of milliseconds or less, rather than the (tens of) seconds that are needed for dissolution. Moreover, the sample remains at high field so that relaxation is minimized. This makes NMR experiments on rapidly relaxing nuclei (such as protons) feasible. Protons tend to have a faster build-up time than ^{13}C [20]. Indeed, one could aim to take advantage of the benefits of proton polarization even when doing ^{13}C detection by implementing cross polarization sequences after the DNP step, while the sample is still in the solid state (as is common with CP MAS-DNP [21]). The NMR is performed in the liquid state at room temperature in order to benefit from the higher resolution attainable with liquid-state NMR spectroscopy without recourse to sample spinning. Scaling the assembly down from a typical 5 mm NMR tube, containing around half a ml of sample, to volumes on the order of 10 nl to 1 µl can also bring a significant improvement in SNR. In general, for a given sample amount the SNR of an NMR coil scales inversely with detector radius [22] if the sample can be concentrated accordingly. The stripline NMR detector is a lithographically defined structure that provides high sensitivity with low static field distortion and that is cheap to produce. It is also very convenient for the present purpose, as it provides an open axial access along the static field axis [23].

Other groups have already investigated in situ, temperature jump DNP procedures – notably the Griffin group at MIT – by using a CO_2 laser to rapidly melt a small sample inside a MAS rotor [25]. Here, we use a jet of warm gas directed at the capillary in a heating stage situated between the DNP and NMR centers to perform the rapid melt. Because of the low thermal mass of nl sized samples and good heat conductivity of thin walled quartz capillaries, melting can be achieved in a relatively short time. If required, dielectric RF heating or IR laser illumination can be used to assist the sample-melting process immediately prior to the NMR experiment.

In the following experimental section we will first describe the general set-up of the rapid melting DNP probe. The results obtained with aqueous and nonpolar solutions are described in Section 3. A more detailed analysis of the obtained DNP enhancements will follow in a future publication. In Section 5 we will discuss the potential of the method for future applications.

2. Instrumentation

A schematic representation of the system is provided in Fig. 1. The core elements of the system are a sample contained within a micro-capillary, of typically 360 µm outer diameter and 150 µm inner diameter, which is moved by an electronic actuator between three main system-regions: (1) a low temperature region at the

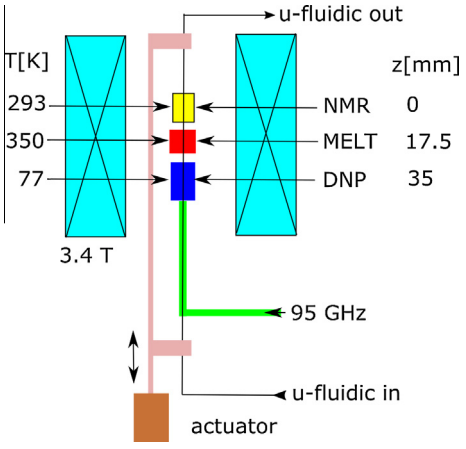

Fig. 1. Schematic diagram of the system set-up (not to scale) showing the major components within the magnet bore: the cooling region where microwave irradiation occurs, the melt region, where heated nitrogen gas is directed onto the frozen sample, and finally, the stripline NMR detection region. Both NMR and DNP sections are located within the homogeneous region of the magnet, as indicated by the distance z, relative to the center of the magnet. A linear actuator is used to move the capillary through the system, thereby moving the sample between system components. 95 GHz Microwaves are introduced from the bottom of the probe, as are cryogens and heated nitrogen gas.