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# DNP enhanced NMR with flip-back recovery

Snædís Björgvinsdóttir, Brennan J. Walder, Arthur C. Pinon, Jayasubba Reddy Yarava, Lyndon Emsley *

*Institut des Sciences et Ingéniere Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland*

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ABSTRACT

DNP methods can provide significant sensitivity enhancements in magic angle spinning solid-state NMR, but in systems with long polarization build up times long recycling periods are required to optimize sensitivity. We show how the sensitivity of such experiments can be improved by the classic flip-back method to recover bulk proton magnetization following continuous wave proton heteronuclear decoupling. Experiments were performed on formulations with characteristic build-up times spanning two orders of magnitude: a bulk BDPA radical doped *o*-terphenyl glass and microcrystalline samples of theophylline, L-histidine monohydrochloride monohydrate, and salicylic acid impregnated by incipient wetness. For these systems, addition of flip-back is simple, improves the sensitivity beyond that provided by modern heteronuclear decoupling methods such as SPINAL-64, and provides optimal sensitivity at shorter recycle delays. We show how to acquire DNP enhanced 2D refocused CP-INADEQUATE spectra with flip-back recovery, and demonstrate that the flip-back recovery method is particularly useful in rapid recycling regimes. We also report Overhauser effect DNP enhancements of over 70 at 592.6 GHz/900 MHz.



## 1. Introduction

Cross-polarization magic angle spinning (CP MAS) NMR is the cornerstone experiment in solid-state NMR [1,2]. The sensitivity of conventional CP MAS experiments depends on the proton spin-lattice relaxation time, as it is the proton spin reservoir from which the rare nuclei draw their polarization. Many organic solids, however, have long proton spin-lattice relaxation times, which for rigid compounds can approach one hour. When combined with the low natural abundance and gyromagnetic ratio of important rare nuclei such as $^{13}C$ and $^{15}N$ this severely limits the sensitivity of CP MAS experiments. The acquisition of one-dimensional spectra with usable signal-to-noise ratios is particularly difficult in such cases, and multi-dimensional experiments are usually precluded.

In the early days of CP, resolution of the rare spin spectrum was improved by decoupling using spin locking of the proton magnetization during acquisition with a high power "continuous wave" (cw) rf field [1]. After acquisition, a significant fraction of the original proton magnetization remained, dephasing only upon release of the spin locking field. Since the very inception of cross-polarization, it was recognized that the residual proton magnetization could be further utilized to improve the signal-to-noise ratio of the rare spin spectrum, and to this end strategies such as multiple contact/acquisition schemes were proposed [1]. The method of using a $\pi/2$ pulse to return the residual proton magnetization to the *z*-axis after acquisition to allow a shorter recovery period was introduced by Tegenfeldt and Haeberlen in 1979 [3]. Owing to its facile implementation, use of this flip-back recovery method increased throughout the subsequent decades, with examples including work on zeolites [4], bacteriorhodopsin [5] and multidimensional $^{13}C$ tensor correlation experiments on saccharides [6,7].

With the introduction of TPPM decoupling in 1995 [8], followed by other modern heteronuclear decoupling methods such as SPINAL-64 [9] it became more difficult to lock the proton magnetization during decoupling [10], and the flip-back experiment largely fell into disuse. However, the flip-back pulse element remains essential to schemes such as those used in quantitative cross-polarization methods [11], RELOAD-CP [12], dissolution DNP [13], and the suppression of water signals in solution NMR [14]. Recently, classic flip-back has been shown to be useful in the context of fast MAS, where efficient heteronuclear decoupling at sample rotation rates > 50 kHz can be provided by low power cw spin locking fields [15,16].

Solid-state magic-angle-spinning dynamic nuclear polarization (DNP) can provide exquisite enhancements in overall sensitivity for a range of materials from frozen solutions to microcrystalline powders [17–20]. Most strategies involve the generation of hyperpolarization close to a radical source and transportation of this polarization to the target substrate by spontaneous proton spin dif-

* Corresponding author.
*E-mail address:* lyndon.emsley@epfl.ch (L. Emsley).

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fusion [21,22]. This is followed by conventional cross-polarization transfer of the enhanced proton polarization from the proton bath to the rare spins. For example, the use of incipient wetness impregnation DNP for microcrystalline systems can enhance the sensitivity of CP MAS experiments by factors of 100 or more at temperatures of 100 K [19]. However, since the polarization must be transported, the overall sensitivity of relayed DNP experiments is throttled by the long build up times needed to optimize sensitivity.

Here we show that flip-back methods can significantly improve the overall sensitivity of MAS DNP experiments.

## 2. Experimental

Salicylic acid, L-histidine monohydrochloride monohydrate and theophylline were obtained from Sigma Aldrich. The samples were ground by hand with a mortar and pestle for 5 min and subsequently wetted with a 16 mM solution of TEKPol [23] in 1,1,2,2-tetrachloroethane (TCE), [24] which dissolves the radical but is a non-solvent for the powders [18]. The formulation ratio was 10 μL of radical solution to 40 mg of powdered solid. The wet powder was packed in a 3.2 mm sapphire rotor and centered with a polytetrafluoroethylene insert.

All experiments on microcrystalline samples were performed on a widebore 9.4 T Bruker Avance III solid-state NMR spectrometer coupled with a 263 GHz microwave source [25]. The samples were spun in a 3.2 mm low-temperature MAS probe at a rate of 12.5 kHz at temperatures near 90 K. To improve enhancements, the samples were deoxygenated with three thawing cycles by repeated ejection and insertion of the sample in the NMR probe [26]. Polarization enhancement factors of the TCE protons were estimated from comparing proton spectra at a 5 s recycle delay with and without microwave irradiation, indicating enhancements over 200 for L-histidine monohydrochloride monohydrate and salicylic acid. The enhancement of the theophylline system could not be estimated in this way because of substantial recovery of the theophylline protons due to their short intrinsic proton $T_1$. On the basis of CP spectra with and without microwave irradiation (Fig. S1), the TCE enhancement of the theophylline system is similar to that of the other two microcrystalline systems.

BDPA (α,γ-bisdiphenylene-β-phenylallyl) was obtained from Sigma Aldrich and dissolved in partly deuterated ortho-terphenyl (95:5 OTP-$d_{14}$:OTP). The resulting 75 mM solution was melted in a 3.2 mm rotor, topped with a polytetrafluoroethylene insert and rapidly inserted into a cold NMR probe.

Overhauser effect DNP experiments [27] on BDPA in OTP [28] were carried out at 21.14 T using a Bruker Avance Neo spectrometer. The frequency of the microwave beam could be lowered to reach the Overhauser effect condition from the optimum binitroxide cross effect condition by changing the temperature of the resonant cavity (Fig. S2). The OTP sample was spun at a rate of 12.5 kHz in a low-temperature MAS probe. The signal enhancement factor, $\varepsilon_C$, as indicated by the ratio of signal with and without microwave irradiation was 73 (Fig. S3).

Unless otherwise specified, a 93 kHz radio-frequency field amplitude was used for the heteronuclear decoupling fields. For XiX decoupling [29] the pulse widths used were 640 μs. CP contact time was 2 ms for the microcrystalline solids and 4 ms for the OTP glass. The $^1$H rf field amplitude was ramped from 90% to 100% during CP for all compounds. The acquisition time for the microcrystals was 26 ms and 4 ms for the OTP system. In order to reach a steady state condition, a number of dummy scans, roughly equal to 10% of the scans used during data accumulation, were implemented for the flip-back experiments. The experiments were performed in order of high to low values of recycle delay, further minimizing the change in steady state condition between consecutive experiments.

For the INADEQUATE experiments, 20 complex $t_1$ points at an indirect sampling interval of $\Delta t_1$ = 0.16 ms were acquired at effective 512 scans each for a total experiment time of ~14 h. A 150 kHz radio-frequency field amplitude was used for decoupling.

We simulate the diffusion of polarization from the impregnating solution to the microcrystal using numerical simulations as described in detail elsewhere [22]. The resulting polarization is integrated over the microcrystal volume. The polarization is then scaled by the retention factor and propagated again during the subsequent polarization period. This procedure is repeated for the number of experimental scans. The resulting accumulated signal is then scaled by the number of loops, and by the square root of time, to obtain a sensitivity curve as a function of the recycle delay. This procedure is repeated for all recycle delays, numbers of scans, and the only variable parameter between the "with flip-back" case and "without flip-back" case is the retention factor.

## 3. Results and discussion

The fundamentals of bulk proton magnetization recovery using a flip-back pulse, as shown in Fig. 1a, and an excellent overview of the benefits of the sequence were given in the seminal work of Tegenfeldt and Haeberlen [3]. In a thermodynamic framework, the heat capacity of the abundant $^1$H spin reservoir far exceeds that of the rare $^{13}$C spin reservoir, particularly when the latter is present at natural abundance. As CP MAS NMR is usually practiced today, the proton magnetization is destroyed during acquisition by heteronuclear decoupling methods such as TPPM or SPINAL. Consequently, the magnetization must recover over a recycle delay $\tau_{rd}$ prior to the execution of the following scan. In a simple exponential model of recovery from zero intensity, the degree to which the magnetization is restored during $\tau_{rd}$ is given by

$$M_z(\tau_{rd}) = M_\infty \left[1 - \exp\left(-\frac{\tau_{rd}}{T_B}\right)\right]. \quad (1)$$

We introduce the build-up time constant, $T_B$, in this expression to acknowledge that in DNP the magnetization does not usually recover according to the intrinsic spin-lattice relaxation time, $T_1$, of the substrate [30]. Only for conventional NMR of a pure solid analyzed in the absence of the exogenous source of polarization is $T_B = T_1$. This is important, since in many DNP experiments $T_B < T_1$, and this is already a source of increased overall sensitivity for DNP enhanced solid-state NMR experiments [31–33].

At temperatures around 100 K or below, where MAS DNP experiments are today most efficient, there is often a more or less pronounced loss in spectral resolution due to freezing out molecular motions [34,35]. The cw method of heteronuclear decoupling can thus often be used with only a modest loss of resolution (*vide infra*). In this case, proton magnetization can be locked during decoupling and is depleted primarily through $T_{1\rho}$ relaxation (and, to a lesser extent, direct polarization transfer to $^{13}$C nuclei). Upon flip-back, the longitudinal component of the proton magnetization, $M_z$, resumes its build up toward $M_\infty$, but at a significant nonzero initial amplitude. Under steady state conditions, this implies that

$$M_z(0) = f_0 M_z(\tau_{rd}). \quad (2)$$

where $f_0$, the retention factor, is the ratio of bulk proton magnetization before and after the CP experiment. $f_0$ is an empirical parameter that depends on many factors in the experiment, including notably $T_{1\rho}$, the efficiency of the pulses, off resonance effects, and the decoupling sequence used. For decoupling methods such as TPPM, $f_0$ is usually zero, and the initial level of magnetization available for subsequent scans is also zero.