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# Microwave frequency modulation to enhance Dissolution Dynamic Nuclear Polarization

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Dedicated to To Martial Rey, as a token of appreciation.

ABSTRACT

Hyperpolarization by Dissolution Dynamic Nuclear Polarization is usually achieved by monochromatic microwave irradiation of the ESR spectrum of free radicals embedded in glasses at 1.2 K and 3.35 T. Hovav et al. (2014) have recently shown that by using frequency-modulated (rather than monochromatic) microwave irradiation one can improve DNP at 3.35 T in the temperature range 10–50 K. We show in this Letter that this is also true under Dissolution-DNP conditions at 1.2 K and 6.7 T. We demonstrate the many virtues of using frequency-modulated microwave irradiation: higher polarizations, faster build-up rates, lower radical concentrations, less paramagnetic broadening, more efficient cross-polarization, and less critical frequency adjustments.



## 1. Introduction

Hyperpolarization methods aim at enhancing the nuclear spin polarization well beyond Boltzmann equilibrium. Since the sensitivity of NMR and MRI is directly proportional to the nuclear spin polarization, it can be enhanced considerably. Dissolution Dynamic Nuclear Polarization (D-DNP) [1,2] can provide dramatic enhancements up to four or five orders of magnitude for a broad variety of molecules and nuclear spins. Many novel applications have emerged thanks to the improved sensitivity afforded by DNP, ranging from the detection of reaction intermediates in chemistry [3,4] to the real-time metabolic imaging of tumors in medicine [5]. Since its invention in 2003, D-DNP has been generally performed under similar experimental conditions. Free radicals, embedded in a glassy matrix together with the substrate or metabolite of interest, are normally irradiated with monochromatic microwaves in the vicinity of the electron spin resonance (ESR) frequency at 1.2 K and 3.35 T [6]. Depending on the offset between the microwave frequency and the center of the ESR line, the polarization of one or several nuclear spin species can either be enhanced or depleted (positive or negative DNP, leading to positive or negative spin temperatures). The effect can arise from different DNP mechanisms, namely Thermal Mixing (TM) [7,8], the Cross Effect (CE) [9–11] or the Solid Effect (SE) [12]. Most of the theory of DNP was developed in the 1960's. The recent renaissance of DNP has led to improvements of the theory which has also become more comprehensible [13–20].

While TM is best performed with a monochromatic microwave irradiation, it has been shown recently by Thurber et al. [21], Cassidy et al. [22], and most recently by Hovav et al. [23] that DNP by CE and SE can be greatly improved by using either field-modulation or frequency-modulated microwave irradiation. We show in this Letter that the same approach is also beneficial at lower temperatures $T = 1.2$ K and at a higher magnetic field $B_0 = 6.7$ T. The effect of frequency modulation is pronounced and substantial gains in polarization by factors up to $\varepsilon_{fm} > 3$ can result in absolute $^1$H polarization levels in excess of 60%, as reported in this Letter. Another great advantage of frequency modulation is the acceleration of the DNP build-up times by factors up to $\kappa_{fm} \sim 10$. Finally the use of frequency modulation enables a reduction in the concentration of free radicals by a factor up to 2 without hindering the final DNP efficiency. Such a reduction in radical concentration results in narrower $^1$H NMR widths and longer $T_2$ and $T_{1\rho}$ at 1.2 K, which in turn significantly improves the efficiency of Cross

*Abbreviations:* D-DNP, Dissolution Dynamic Nuclear Polarization; PA, polarizing agent.

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Polarization (CP) to transfer magnetization from $^1$H spins to low gamma nuclear spins such as $^{13}$C that have long $T_1$'s in solution, in view, for example, of metabolic imaging experiments. After direct $^1$H DNP, which may be combined with $^1$H → $^{13}$C CP, dissolution can be performed in a standard manner, and the sample can be transferred to a liquid-state NMR spectrometer or MRI machine while retaining a large fraction of its hyperpolarization.

## 2. Results and discussion

All DNP experiments reported herein were performed on a home-built DNP polarizer at $T$ = 1.2 K in a static magnetic field $B_0$ = 6.7 T. The polarizer was modified from its original version [24–26] to accommodate an improved NMR circuit including double resonance on both $^1$H and $^{13}$C frequencies, resonating at $f$ = 285.23 and $f$ = 71.73 MHz respectively. The innovative design of the DNP insert allows one to perform $^1$H → $^{13}$C CP-DNP experiments during continuous microwave irradiation [27]. When using the free radical 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL) as a polarizing agent, CP-DNP has shown great potential for yielding polarizations in excess of $P(^{13}C)$ >70% in a record time using a doubly tuned solenoidal NMR coil with a 50 μL volume [28,29]. The horizontal solenoidal coil can be replaced by a saddle coil with a 1 mL volume to allow vertical access for rapid dissolution of large sample volumes. Such a compromise leads to a decrease in *rf* efficiency and homogeneity, but polarizations as high as $P(^{13}C)$ = 45% could nevertheless be achieved [30]. Using TEMPOL as a polarizing agent, $^1$H → $^{13}$C CP-DNP allows one to achieve higher $P(^{13}C)$ polarizations than direct $^{13}$C DNP, we shall therefore mostly focus on $^1$H DNP in this Letter.

Proton DNP was investigated for samples (1), (2) and (3) containing 10, 25 and 50 mM TEMPOL, respectively, in a 10:40:50 (v/v/v) $H_2O$:$D_2O$:glycerol-$d_8$ mixture, at $T$ = 1.2 K. Figure 1a shows the effect of microwave frequency modulation on the $^1$H DNP build-up behavior of sample (2) for positive or negative DNP performed at the optimal monochromatic frequencies $f_{\mu w}$ = 187.85 and 188.3 GHz. For sample (2), the amplitude of the frequency modulation was set to $\Delta f_{\mu w}$ = 100 MHz with a modulation frequency $f_{mod}$ = 10 kHz. A scheme explaining these frequency modulation parameters is presented in Figure 1b. Sinusoidal and triangular frequency modulation had identical efficiencies. According to Figure 1a, frequency modulation provides a drastic way of increasing the proton polarization $P(^1H)$, while simultaneously increasing the DNP build-up rate $R_{DNP}(^1H) = 1/\tau_{DNP}(^1H)$. Table 1 gives the final proton polarization $P(^1H)$ and the corresponding build-up rates $R_{DNP}(^1H)$ with and without frequency modulation for positive and negative DNP effects, and for three different radical concentrations. The effect of frequency modulation is hardly remarkable at a high radical concentration of 50 mM, but it is much more pronounced as the radical concentration is decreased to 25 or 10 mM.

Increasing the radical concentration enhances electron–electron dipolar couplings which enable rapid spectral spin diffusion within the broad inhomogeneous ESR line of TEMPOL. As a consequence, a larger fraction of the electron spins can contribute to the DNP process. Usually, in absence of microwave frequency modulation, the radical concentration needs to be carefully optimized. If the radical concentration is too low, only a very small fraction of electron spins will contribute to DNP, which will translate in low nuclear spin polarizations and very long build-up times. On the other hand, if the electron spin concentration is too high, the ESR line will tend to be homogeneously broadened well beyond its inhomogeneous width, which will translate into fast build-up rates, but with poor nuclear spin polarizations. In practice, the best radical concentration was found to be around 50 mM at 1.2 K and

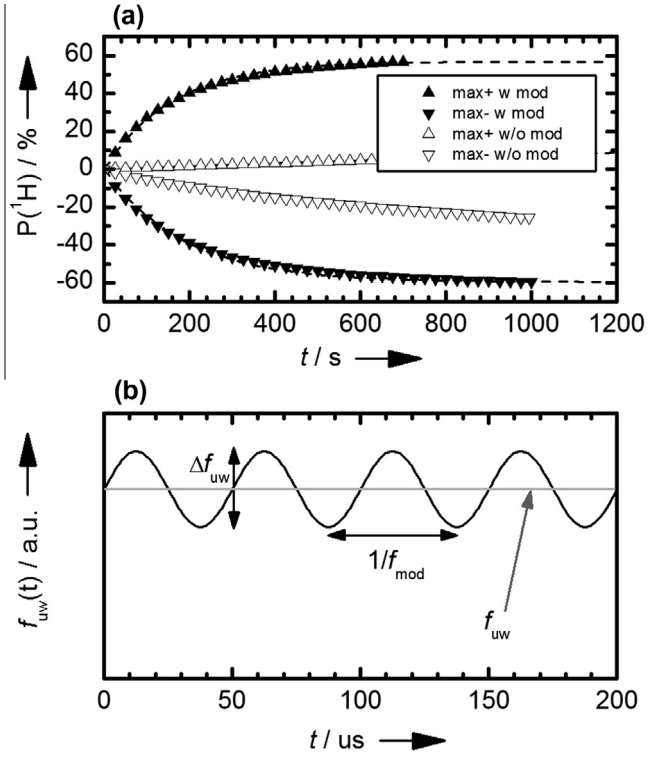


**Figure 1.** (Top) Negative and positive $^1$H DNP build-up curves measured at $T$ = 1.2 K and $B_0$ = 6.7 T, with and without frequency modulation, in sample 2 (a 10:40:50 (v/v/v) $H_2O$:$D_2O$:glycerol-$d_8$ mixture with 25 mM TEMPOL). The optimal frequencies $f_{\mu w}$ = 187.85 and 188.3 GHz were set for positive or negative DNP respectively, with a microwave power $P_{\mu w}$ = 87.5 mW. An amplitude $\Delta f_{\mu w}$ = 100 MHz was used for frequency modulation. (Bottom) Scheme illustrating the frequency modulation method. The microwave frequency typically varies in a sinusoidal fashion according to $f_{\mu w}(t) = f_{\mu w} + ½\ \Delta f_{\mu w} \sin(2\pi f_{mod}\ t)$ where $f_{\mu w}$ is the average frequency, $\Delta f_{\mu w}$ the amplitude of the frequency modulation, and $f_{mod}$ the modulation frequency.

**Table 1**
Proton polarization and build-up times at $T$ = 1.2 K and $B_0$ = 6.7 T for different radical concentrations in a 10:40:50 (v/v/v) $H_2O$:$D_2O$:glycerol-$d_8$ mixture, with and without frequency modulation.

| [PA]/mM | Modulation | $P^+(^1H)$ (%) | $\tau^+_{DNP}(^1H)$ (s) | $P^-(^1H)$ (%) | $\tau^-_{DNP}(^1H)$ (s) |
|---|---|---|---|---|---|
| 10 | With | 14.5* | 2600 ± 1000** | −21.1* | 2500 ± 1000** |
| | Without | 0.9* | NA*** | −1.2* | NA*** |
| 25 | With | 57.3 | 159 ± 1.8 | −60.7 | 185 ± 2 |
| | Without | 9.3* | 9000 ± 2000** | −29.5 | 625 ± 11 |
| 50 | With | 61.3 | 108 ± 1.6 | −63.3 | 152.2 ± 2 |
| | Without | 21.9 | 338 ± 7 | −43.7 | 218 ± 4 |

* DNP maximum was not reached; the polarization shown was achieved after 20 min of microwave irradiation.
** Fits have large uncertainties because only the first 20 min of the DNP build-up curve were recorded.
*** Estimates of the build-up time not available because of poor fits.

6.7 T in our laboratory [29]. When frequency modulation is used, the optimization of the electron concentration can be largely dispensed with. The fraction of the ESR line where DNP is effective is no longer related to the radical concentration. In fact, frequency modulation can play a similar role as spectral spin diffusion. A more detailed theoretical explanation supported by numerical simulations is given by Hovav et al. [23].

(Figure 2a) shows the ESR line-shape of TEMPOL measured in our DNP polarizer at $T$ = 1.2 K and $B_0$ = 6.7 T by longitudinally detected ESR (LODESR) with a home-built apparatus inspired by