Microwave-gated dynamic nuclear polarization†

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Dissolution dynamic nuclear polarization (D-DNP) has become a method of choice to enhance signals in nuclear magnetic resonance (NMR). Recently, we have proposed to combine cross-polarization (CP) with D-DNP to provide high polarization $P(13\text{C})$ in short build-up times. In this paper, we show that switching microwave irradiation off for a few hundreds of milliseconds prior to CP can significantly boost the efficiency. By implementing microwave gating, $^{13}\text{C}$ polarizations on sodium $[1-^{13}\text{C}]$acetate as high as 64% could be achieved with a polarization build-up time constant as short as 160 s. A polarization of $P(13\text{C}) = 78\%$ could even be reached for $[^{13}\text{C}]$urea.

Introduction

Dynamic nuclear polarization (DNP) 1,2 has become a method of choice to enhance signals in nuclear magnetic resonance (NMR). DNP usually consists of transferring electronic spin polarization to the surrounding nuclear spins via microwave irradiation in frozen glasses doped with paramagnetic polarizing agents (PAs) at suitable temperatures and magnetic fields. Enhancements in polarization $P(13\text{C})$ in the liquid-state at room temperature by factors exceeding 10 000 are possible using the so-called dissolution-DNP (D-DNP) technique. 3 Here, the DNP sample is polarized as a frozen organic glass at low temperatures and in moderate magnetic fields (typically $1.2 < T < 4.2 \text{ K}$ and $B_0 = 6.7 \text{ T}$ in our laboratories) where the electron spin polarization approaches unity, partly saturated by suitable microwave irradiation, dissolved and brought to room temperature with superheated water, and finally transferred to NMR or MRI apparatus for detection. The method has found applications in the field of medical imaging, where hyperpolarized [1-$^{13}\text{C}$]pyruvate or other metabolites can be used to monitor enzymatic conversion rates. This allowed real-time localized $^{13}\text{C}$ spectroscopy for tumour characterization in patients. 4

The D-DNP experiment was initially designed to polarize directly low-gamma nuclear spins, mainly $^{13}\text{C}$, using radicals such as trityl that have narrow ESR lines. There are many advantages in polarizing $^{13}\text{C}$ nuclear spins as (i) they can be enriched, (ii) there is no significant background signal, (iii) and the longitudinal relaxation times can be as long as $T_1(13\text{C}) > 50 \text{ s}$ in carboxylic, carbonyl, or quaternary sites. This allows a significant part of the hyperpolarized magnetization of metabolites such as [1-$^{13}\text{C}$]pyruvate to survive the transfer from the polarizer to the detecting magnet, including infusion into animals or patients.

Recently, we have proposed to combine D-DNP with cross-polarisation (CP). 5–7 The abundant proton spins are first rapidly polarized using PAs with broad ESR lines such as TEMPO. The proton polarization can be as high as $P(H) > 90\%$ at $T = 1.2 \text{ K}$ and $B_0 = 6.7 \text{ T}$. This can then be subsequently transferred to low-gamma spins such as $^{13}\text{C}$. This indirect strategy provides high polarizations $P(13\text{C})$ in short build-up times.

In this paper, we show that by gating the microwave irradiation off prior to CP, the electron polarization returns to its highly polarized Boltzmann thermal equilibrium $P_{\text{eq}}(e)$ on the time-scale of $T_1(e)$ which is on the order of 100 ms in our systems. As a result, the proton relaxation time in the rotating frame in the presence of a radiofrequency field, $T_{\text{rel}}(\text{H})$, can be extended by as much as an order of magnitude, while $T_{\text{rel}}(13\text{C})$ also increases, albeit by a smaller factor. This allows the CP contacts to be extended, thus significantly improving the CP efficiency. By gating the microwaves, a polarization $P(13\text{C}) = 64\%$ could be achieved in [1-$^{13}\text{C}$]acetate with build-up time constants as short as 160 s. A record polarization of $P(13\text{C}) = 78\%$ could be reached in $[^{13}\text{C}]$urea, albeit with a somewhat longer build-up time constant of 470 s.

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Paramagnetic relaxation

Under conditions that are suitable for DNP, a significant contribution to the relaxation rate constant in the rotating frame $1/T_{1p}(^1\text{H})$ can arise from the presence of PAs. This has been extensively studied in the presence and absence of spin diffusion, albeit only in the high temperature approximation. The spin-locked magnetization of a nuclear spin $I$ at a distance $r$ from an electron spin $S = 1/2$ relaxes with the following rate constant:

$$\frac{1}{T_{1p}} = \frac{C}{\hbar^2} \frac{\tau}{1 + \omega_1^2 \tau^2}$$  \hspace{1cm} (1)

with $\omega_1$ being the nuclear nutation frequency in the rotating frame, $\tau$ being the correlation time of the fluctuations of the electron dipolar field seen by the nuclear spins, and $C$ being a constant (see the ESI for details on $\tau$ and $C$). This equation is however valid only in the high-temperature approximation where the electron spin polarization is negligible.

Microwave irradiation can shorten $T_{1p}$

Typically, at low temperatures and in high fields, the electron spin polarization in thermal equilibrium $P_e^{\text{TE}}$ can be close to unity, which leads to an attenuation of the transition rates within the electron spin manifold by a factor

$$\kappa = 1 - P_e^{\text{TE}}$$  \hspace{1cm} (2)

where $P_e^{\text{TE}}$ is the partly saturated electron spin polarization in the presence of microwave irradiation. This leads to an attenuation of the relaxation rate constant in the rotating frame:

$$\frac{1}{T_{1p}} = \frac{C}{\hbar^2} \frac{\kappa \tau}{1 + \omega_1^2 (\kappa \tau)^2}$$  \hspace{1cm} (3)

Under magic angle spinning (MAS) conditions, typically $T = 100$ K and $B_0 = 9.4$ T, the electron spin polarization in thermal equilibrium is relatively low with $P_e^{\text{TE}} = 6.3\%$, which translates into $0.996 < \kappa < 1$, so that $1/T_{1p}$ remains essentially the same with or without electron spin saturation by microwave irradiation. However, under D-DNP conditions, typically at $T = 1.2$ K and $B_0 = 6.7$ T, the electron spin polarization in thermal equilibrium is close to unity with $P_e^{\text{TE}} = 99.8\%$, which translates into $2.1 \times 10^{-3} < \kappa < 1$. Therefore, one can expect an increase in $1/T_{1p}$ by orders of magnitude when microwave irradiation is applied to saturate the electron spin transition.

To verify this, $T_{1p}(^1\text{H})$ relaxation curves of the proton spins were measured with a suitable pulse sequence (Fig. 1a) at $B_0 = 9.4$ T and $T = 100$ K (Fig. 1b) and $B_0 = 6.7$ T and $T = 1.2$ K (Fig. 1c) with and without microwaves at 263 and 188.3 GHz, respectively (in the latter case, we applied frequency modulation over a range $\Delta f_{\text{mod}} = 50$ MHz at a rate $f_{\text{mod}} = 10$ kHz), in a frozen glassy sample containing 3 M [1-13C]acetate in H$_2$O:D$_2$O:glycerol-d$_8$ (v:v:v = 1:4:5) doped with 40 mM TEMPO as PA. As expected, the effect of microwave irradiation on $1/T_{1p}$ is insignificant at $B_0 = 9.4$ T and $T = 100$ K, but is dramatic at $B_0 = 6.7$ T and $T = 1.2$ K.

![Figure 1](image-url) (a) Pulse sequence used to measure $T_{1p}(^1\text{H})$ where the proton spins are saturated by a train of 90° pulses, and subsequently evolve during a fixed delay $t_{\text{bup}} = 2$ s, either relaxing to their equilibrium if there is no microwave irradiation, or building up towards their DNP steady-state in the presence of microwave irradiation. After $t_{\text{bup}}$ has elapsed, a half-chirp pulse (swept from $-100$ kHz to the centre of the line in 175 μs, with a sweep rate of 0.57 kHz μs$^{-1}$ and an amplitude $\gamma B_0/2\pi = 20$ kHz) is applied to the protons to bring their magnetization to the transverse plane. This is followed by a spin-lock pulse with the same amplitude of 20 kHz and a variable duration $t_{\text{SL}}$. Finally, the magnetization is flipped back towards the longitudinal axis with a half-chirp pulse (swept from the centre of the line to $-100$ kHz in 175 μs, all other parameters being identical) and the proton NMR signal is observed following a 10° excitation pulse. (b) Proton $T_{1p}$ decay measured at $B_0 = 9.4$ T and $T = 100$ K in a frozen glassy sample containing 3 M [1-13C]acetate in H$_2$O:D$_2$O:glycerol-d$_8$ (v:v:v = 1:4:5) doped with 40 mM TEMPOOL with (●) and without (○) microwave irradiation with $f_{\text{mw}} = 4$ W and $f_{\text{mw}} = 263$ GHz. (the lines are drawn to guide the eye). (c) Proton $T_{1p}$ decay measured at $B_0 = 6.7$ T and $T = 1.2$ K in the same sample, with (●) and without (○) microwave irradiation with $f_{\text{mw}} = 87.5$ mW and $f_{\text{mw}} = 188.3$ GHz modulated over a range $\Delta f_{\text{mod}} = 50$ MHz at a rate $f_{\text{mod}} = 10$ kHz (the lines are fitted to eqn (4)).

**Numerical simulations**

Simulations of the relaxation curves were performed using Matlab. The model was implemented using a finite element routine for the description of the space and time domains,
assuming a spherically symmetric system with one electron spin located at the centre, and surrounded by a spherical medium of radius \( c \) containing nuclear spins. Nuclear spins closer than 1 nm to the electron were considered to be inside a “diffusion barrier” across which no spin diffusion can occur. Nuclear spins within this diffusion barrier are subjected to strong dipolar couplings to electrons leading to large splittings or frequency shifts; they are therefore considered as ‘NMR invisible’ and consequently can be safely neglected.\(^{12}\) Such a system can be described by a partial differential equation:\(^{8}\)

\[
\frac{\partial P(r, t)}{\partial t} = -\frac{1}{J_p} P(r, t) + \beta P(r, t) + D\nabla^2 P(r, t) \tag{4}
\]

where \( P \) is the proton polarization, \( D \) is the diffusion constant, and \( \beta \) is a leakage rate that describes all sources of relaxation other than paramagnetic effects due to the unpaired electrons of TEMPO. The initial polarization at time \( t = 0 \) can be arbitrarily set to \( P(r, 0) = 1 \) and the boundary conditions at the edges of the system can be set to \( \partial P(r, t)/\partial r = 0 \). The equation was integrated numerically over space using trapezoidal steps, yielding the total proton polarization as a function of time. Experimental relaxation curves were fitted using the least-squares method. Best fits were obtained with fixed parameters for the diffusion barrier \( b = 1 \text{ nm} \) (see ref. 13 for discussion), the diffusion constant \( D = 10 \text{ nm}^2 \text{s}^{-1} \), and the non-paramagnetic leakage relaxation rate \( \beta = -8.6 \text{ s}^{-1} \), and with free parameters that converged robustly to a sphere radius \( c = 2 \text{ nm} \), \( C = 2.3 \times 10^{-8} \text{ \mu m}^6 \text{s}^{-2} \), and \( \tau = 2.769 \times 10^{-4} \text{ s} \). In the absence of microwave irradiation, the thermal equilibrium polarization \( P_e^{\text{TE}} \) depends only on the sample temperature, i.e., \( P_e^{\text{TE}} = 99.89\% \) at 1.2 K. In the presence of microwave irradiation, the electron polarization \( P_e^{\text{MW}} \) was treated as an adjustable temperature-dependent parameter and was estimated to be \( P_e^{\text{MW}} = 48\% \) at 1.2 K, well above \( P_e^{\text{MW}} = 0\% \) that would describe complete saturation. Even a partial

\[P_e = P_e^o \cdot \exp(-t/t_1) \quad \text{and} \quad P_e = P_e^o \cdot \exp(-t/t_2) \]

for varying microwave power, where \( t_1 \) and \( t_2 \) are characteristic times. The plots show \( P_e(t) \) as a function of microwave power for different sets of parameters. The plots are labeled with different microwave powers (6.25 mW, 12.5 mW, 25 mW, 50 mW, 87.5 mW) and times (1 ms, 2 ms, 4 ms, 10 ms).

(a) \( T_1 \) curves varying microwave power

(b) Average electron spin polarization under microwave irradiation

(c) Estimated electron spin polarization varying \( \tau_{\text{gate}} \)

Fig. 2  (a) \( T_1(\text{H}) \) decay of the proton magnetization measured with the sequence of Fig. 1a as a function of the applied microwave power \( P_{\text{MW}} \) (with \( f_{\text{MW}} = 188.3 \text{ GHz}, \Delta f_{\text{MW}} = 50 \text{ MHz}, f_{\text{mod}} = 10 \text{ kHz} \)) in 3 M [1-\text{13C}]acetate in \( \text{H}_2\text{O}:\text{D}_2\text{O}:\text{glycerol-d}_8 \) (\( v:v:v = 1:4:5 \)) doped with 40 mM TEMPO measured at 1.2 K and 6.7 T (the lines are fitted to eqn (4) with \( P_{e}^{\text{MW}} \) as the only free parameter). This behaviour reflects varying degrees of saturation of the electron polarization. (b) Average electron spin polarisations under microwave irradiation \( P_{e}^{\text{MW}} \) (averaged over the whole ESR line) as a function of the power \( P_{\text{MW}} \) of the microwave irradiation, estimated by numerical fits to eqn (4) (the line is drawn to guide the eye).

Fig. 3  (a) Pulse sequence used to determine \( T_1(\text{H}) \) with microwave gating. (b) Proton signal integrals in 3 M [1-\text{13C}]acetate in \( \text{H}_2\text{O}:\text{D}_2\text{O}:\text{glycerol-d}_8 \) (\( v:v:v = 1:4:5 \)) doped with 40 mM TEMPO measured at \( B_0 = 6.7 \text{ T} \) and \( T = 1.2 \text{ K} \) after spin locking \((\Delta f_{\text{SL}} = 20 \text{ kHz}, \tau_{\text{SL}} = 5 \text{ ms})\) as a function of the gating interval \( \tau_{\text{gate}} \) with the microwave parameters \( P_{\text{MW}} = 87.5 \text{ mW}, f_{\text{MW}} = 188.3 \text{ GHz}, \Delta f_{\text{MW}} = 50 \text{ MHz}, \) and \( f_{\text{mod}} = 10 \text{ kHz} \) (a line was drawn to guide the eye). (c) Simulated recovery of the electron spin polarization \( P_e \) in the range \( P_e^{\text{MW}} < P_e < P_e^{\text{TE}} \) as a function of the interval \( \tau_{\text{gate}} \) by fitting \( T_{\text{se}} \) to match the experimental data in (b); the mono-exponential curve has a characteristic time constant \( T_{\text{se}} = 48 \text{ ms} \).
After spin-locking for more than 90% of the spin-locked proton magnetization survives microwave power from the fits are reported in Fig. 2b as a function of the applied resonance, and hence the electron–spin polarization under microwave irradiation. Displacements the proton signal integral measured after spin locking parameters for the protons are the same as in Fig. 1a). Fig. 3b shows the spin-locking sequence with microwave gating (the pulse parameters for the protons are the same as in Fig. 1a). Fig. 3b displays the proton signal integral measured after spin locking ($\gamma B_\text{e}(1H)/(2\pi) = 20$ kHz, $\tau_{\text{SL}} = 5$ ms) as a function of the microwave gating interval $\tau_{\text{gate}}$. Clearly, the relaxation time $T_{1p}(1H)$ is extended as the electron spin polarization relaxes back to its highly polarized state after switching off the microwave irradiation. As a result, the proton signal that survives the spin locking experiment increases when the gating period is extended, until it reaches a plateau. The electron spin polarization $P_e$ was simulated using eqn (4) as a function of $\tau_{\text{gate}}$, with $t = 5$ ms, $b = 1$ nm, $c = 2$ nm, $D = 10$ nm² s⁻¹, $C = 2.3 \times 10^{-8}$ μm$^6$ s⁻², $\tau = 2.7690 \times 10^{-4}$ s and $\beta = -8.6$ s⁻¹ (Fig. 3c). A comparison between experiments and simulations allowed us to estimate the electron spin–lattice relaxation time $T_{1e} = 48 \pm 1$ ms. We systematically used a microwave gating interval $\tau_{\text{gate}} = 500$ ms ($\gg T_{1e}$) in all subsequent experiments.

The advantages of microwave gating are substantial at low temperatures

Fig. 4 shows the $T_{1p}$ relaxation curves measured for different temperatures with the pulse sequence of Fig. 3a, with continuous microwave power $P_{\text{mw}}$.

**Fig. 4** $T_{1p}(1H)$ decay of the proton magnetization in 3 M [1-13C]acetate in H$_2$O:D$_2$O::glycerol-d$_2$ (v:v:v = 1:4:5) doped with 40 mM TEMPOL with continuous or gated microwaves ($P_{\text{mw}} = 87.5$ mW, $f_{\text{mw}} = 188.3$ GHz, $\Delta f_{\text{mw}} = 50$ MHz, $\tau_{\text{mw}} = 10$ kHz) as a function of the sample temperature, i.e., as function of the electron Boltzmann polarization $P_e^B$. The fast decays observed with continuous microwaves (lower curves) reflect varying degrees of saturation of the electron polarization, while the behaviour with gated microwaves (upper curves) reflects the thermal electron spin polarization $P_e^T$ that depends on the sample temperature (lines are numerical fits to eqn (4)).
Microwave gating improves the efficiency of cross polarization

We have recently shown that D-DNP can be boosted by performing $^1$H → $^{13}$C cross-polarization (CP) in combination with DNP. Here we show that the microwave gating strategy can greatly improve the CP efficiency. Fig. 5a shows the pulse sequence used for CP with gated microwaves which is simply an extension of the sequence of Fig. 3a except that spin locking is also applied on $^{13}$C to allow the Hartmann–Hahn contact between $^1$H and $^{13}$C (pulse parameters for $^1$H and $^{13}$C are the same as in Fig. 1a, except that a 50% → 100% ramp with an average $\gamma B_0(1H)/(2\pi) = 20$ kHz rather than a rectangular pulse is applied to $^{13}$C during $t_{CP}$). Fig. 5b shows the $^{13}$C magnetization as a function of the CP contact time $t_{CP}$, without and with microwave gating. With continuous microwaves, the optimum CP contact time lies around $t_{CP} = 1.5$ ms before the decay of the $^1$H magnetization (see Fig. 4a) compromises the transfer of polarization to $^{13}$C. When the microwaves are gated, CP contact times can be much longer (in this example with an optimum beyond $t_{CP} = 20$ ms), thanks to the extended relaxation time $T_{1H}(1H)$ (see Fig. 4), leading to a significantly improved CP efficiency (an improvement of a factor 2.5 in our sample). Note that this improvement is expected to be even more important for systems where the magnetization is transferred to $^{13}$C from remote protons and necessitates extended CP contact times (for example, for deuterated molecules immersed in a protonated solvent).

In a D-DNP experiment, one aims at building up the highest possible $^{13}$C polarization prior to dissolution. We have shown that the $^{13}$C polarization can be maximized by applying multiple CP contacts at intervals $t_{CP} \sim t_{DNP}(1H)$. The time interval $t_{CP}$ allows for the proton polarization to be replenished by DNP before being drained again during the next CP contact. The use of microwave gating in this context has two favourable outcomes: (i) reducing losses of proton magnetization during spin locking (as shown in Fig. 4) and (ii) improving the CP transfer efficiency (shown in Fig. 5). Altogether, the efficiency of the multiple CP sequence is greatly improved by microwave gating, as shown in Fig. 6 where the evolution of both $^1$H and $^{13}$C polarizations is followed using small nutation angle pulses in the course of a multiple CP experiment. With a fixed interval $t_{CP} = 160$ s, we applied optimal CP contact durations $t_{CP} = 16$ ms with gating and $t_{CP} = 1.5$ ms without gating which led to $P(13C) = 64\%$ with an effective build-up time constant $t_{DNP}^{CP}(13C) = 168$ s with microwave gating. This compares favourably with $P(13C) = 38\%$ and $t_{DNP}^{CP}(13C) = 190$ s with continuous microwaves. Using microwave gating thus enhances the build-up of the final $^{13}$C polarization by a factor of 1.7.

As the losses of $P(1H)$ during CP contacts are greatly reduced by microwave gating, the proton polarization in the course of a CP-DNP sequence with gated microwaves almost reaches the same value as when CP is not used. In samples containing methyl groups, such as sodium pyruvate or sodium acetate, the steady state is typically limited to $P(1H) = 70\%$ because of relaxation induced by the rotation of the CH₃ groups. Using non-methylated DNP samples, such as [13C]urea dissolved in H₂O: D₂O: glycerol-d₈ (v:v:v = 1:4:5) with 40 mM TEMPOL, $P(1H)$ builds up to 90% under our DNP conditions at 1.2 K. Using CP with microwave gating, it was possible to reach a polarization of $P(13C) = 78\%$ for this sample.

![Fig. 6](image-url) (a) Build-up of polarization $P(13C)$ during a multiple CP pulse sequence (see Fig. 5a) applied every 2.5 minutes with continuous (●) or gated (○) microwave irradiation ($P_{mw} = 87.5$ mW, $f_{mw} = 188.3$ GHz, $A_{mw} = 50$ MHz, $f_{noa} = 10$ kHz) in 3 M sodium [1-13C]acetate with 40 mM TEMPOL at 1.2 K and 6.7 T. (b) DNP build-up of proton polarization $P(1H)$ in the same sample and conditions without any CP (●) or during multiple CP applied every 2.5 minutes with continuous microwave irradiation (●) or during multiple CP with gated microwave irradiation (○) (all lines are drawn to guide the eye).
Conclusions

Low temperature DNP relies on the steady-state saturation of the electron spins of paramagnetic polarizing agents through microwave irradiation. We have shown in this paper how this saturation leads to a significant deleterious shortening of the nuclear spin relaxation times in the rotating frame $T_1^{1H}$. This is supported by numerical simulations using a simple paramagnetic relaxation model. The shortening of $T_1$ is obviously detrimental to cross-polarisation from $^1H$ to $^{13}C$ or other low-gamma nuclei. However, we demonstrate that this can be avoided by briefly gating off the microwave irradiation prior to cross-polarisation. For $^1H \rightarrow ^{13}C$ cross-polarization, the final $^{13}C$ polarization can be boosted by 70% (i.e., increased by a factor of 1.7), resulting in $P(^{13}C) = 64\%$ in $[1-{^{13}C}]$acetate with a build-up time constant of 160 s, and $P(^{13}C) = 78\%$ in $[^{13}C]$urea with a time constant of 470 s. The characteristic time constant of the return of the electron spins to their thermal equilibrium can be determined by simulations and was found to be $T_{1e} = 48\ ms$ for the system under investigation at 1.2 K.

Abbreviations

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<tr>
<td>DNP</td>
<td>Dynamic nuclear polarization</td>
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<td>D-DNP</td>
<td>Dissolution dynamic nuclear polarization</td>
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<td>CP</td>
<td>Cross polarization</td>
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<td>TEMPO</td>
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