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Direct enhancement of nuclear singlet order by dynamic nuclear polarization

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Abstract: Hyperpolarized singlet order is available immediately after dissolution DNP, avoiding need for additional preparation steps. We demonstrate this procedure on a sample of [1,2-^{13}C$_2$] pyruvic acid.

The large signal improvement provided by spin hyperpolarization methods have dramatically extended the *in vivo* potential of nuclear magnetic resonance (NMR).[1] Hyperpolarized nuclear spin order permits tracing of both endogenous and non-endogenous substances as they are transported through the blood vessels and organs and participate in metabolism.[2–4] A limitation of these techniques is the often short observation timescale imposed by nuclear spin relaxation. Hyperpolarized magnetization typically decays with the longitudinal relaxation time T_1, which is typically between a fraction of a second and one minute, and which can be particularly short *in vivo* due to the presence of paramagnetic species and high concentrations of other magnetic nuclei. Short decay times hinder the use of hyperpolarized substrates for imaging metabolism *in vivo* due to the relatively long times taken for substrates to reach the tissue of interest from the point of injection. While, for instance, hyperpolarized ^{129}Xe is used as a lung imaging agent (gas-phase MRI) due to its relaxation time of several hours in the gas phase, its potential as a tracer in blood is very limited; the T_1 relaxation time of ^{129}Xe reduces to only a few seconds when dissolved.[5]

A potential remedy to the limited hyperpolarization lifetime is the use of nuclear singlet states involving coupled spin-1/2 pairs. The lifetime of the nuclear singlet state $(|\alpha\beta\rangle - |\beta\alpha\rangle)/\sqrt{2}$ is capable of exceeding T_1, in some circumstances, since many common relaxation mechanisms are symmetric with respect to spin exchange and cannot induce singlet-triplet transitions.[6–9] In previous work we reported the potential of doubly ^{15}N-labeled nitrous oxide, (^{15}N$_2$O).[10,11] The ordinary T_1 lifetime of spin order is 30 seconds for ^{15}N$_2$O dissolved in human blood. The singlet decay constant in blood is ≈ 7 minutes, 15 times longer.[11]

In some cases, substances exhibiting hyperpolarized singlet order are accessible directly by chemical reactions of hydrogen enriched with the parahydrogen spin isomer.[12–14] An alternative approach is to prepare hyperpolarized magnetization, using methods such as dynamic nuclear polarization,[15,16] and follow by a conversion into singlet order. A variety of conversion methods is available:

- Resonant radio-frequency pulses are applied in high magnetic field to excite a 'precursor state'.[7] This transforms into singlet order upon adiabatic transport to low field;[17,18]

- Singlet order is prepared in high magnetic field by a radio-frequency pulse sequence;[19–21]

- An audio-frequency pulse sequence is applied in low magnetic field on a pre-polarized sample;[22]

- Chemical symmetry-switching reactions are exploited.[23]

All of these rely upon manipulations additional to spin hyperpolarization. In certain cases they may be difficult to implement, require extra hardware, or take up valuable time during an experiment.

In this communication we show these preparations may be circumvented by the availability of singlet spin order in the initial hyperpolarized state of coupled spins-1/2. This demonstrates 'direct' access, *i.e.* without extra manipulation to the sample, to hyperpolarized spin order that is potentially longer-lived than T_1. The following is a description of singlet order obtained during the dissolution dynamic-nuclear-polarization (DNP) technique,[15] but the principle should apply to other nuclear hyperpolarization methods as well.

The polarization p of an ensemble of non-interacting spins-1/2 is defined by

$$p = (n_\alpha - n_\beta)/2 \tag{1}$$

where n_α and n_β are the populations of the spin states with angular momentum parallel and opposite to the magnetic field, normalized so that $n_\alpha + n_\beta = 1$. The populations are therefore given in terms of the polarization by $n_\alpha = (1+p)/2$ and $n_\beta = (1-p)/2$. The thermal equilibrium polarization at temperature T and a magnetic field strength B^0 is given through the Boltzmann model by $p = p_{\text{thermal}} = \tanh(\hbar\gamma_{\text{nuc}}B^0/2k_BT)$. For ^{13}C, this is of the order of 10^{-5} at room temperature in the typical working field of an *in vivo* MRI magnet (≈ 4.7 T). Hyperpolarized samples exhibit a polarization $p \gg p_{\text{thermal}}$; the limiting situation is $|p| = 1$ which corresponds to complete polarization along the quantization axis. This represents the maximum population asymmetry $n_\alpha - n_\beta$, and the maximum sample magnetization.

In the case of chemically inequivalent spin-1/2 pairs in a strong magnetic field, the energy eigenstates are given by product states of the form $|\alpha_1\alpha_2\rangle = |\alpha_1\rangle \otimes |\alpha_2\rangle$, $|\alpha_1\beta_2\rangle = |\alpha_1\rangle \otimes |\beta_2\rangle$, *etc.*, for the coupled angular momenta of the nuclei. The populations of these states are simply products of those for each nucleus:

$$
\begin{aligned}
n_{\alpha_1\alpha_2} &= (1+p)(1+p)/4 \\
n_{\alpha_1\beta_2} &= (1-p)(1+p)/4 \\
n_{\beta_1\alpha_2} &= (1+p)(1-p)/4 \\
n_{\beta_1\beta_2} &= (1-p)(1-p)/4.
\end{aligned}
\tag{2}
$$

Longitudinal polarization in this case is the population difference $(n_{\alpha_1\alpha_2} - n_{\beta_1\beta_2})$, which again equals p.

Singlet order proportional to p^2 is generated from the imbalance between populations $(n_{\alpha_1\beta_2} + n_{\beta_1\alpha_2})$ and $(n_{\alpha_1\alpha_2} + n_{\beta_1\beta_2})$. In the dissolution DNP experiment,[15] the process occurs as follows: Nuclear polarization $p \approx p_e \gg p_{\text{thermal}}$ is obtained in high magnetic field at a temperature of about 1 K, through contact with a strongly polarized electron bath, ($p_e \approx \tanh(\gamma_e B^0/2k_BT) \approx 1$), in the presence of off-resonance microwave irradiation close to the electron

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Larmor frequency. The frozen sample is dissolved with a jet of hot solvent and collected as a room-temperature liquid in a region of low magnetic field outside the polarizer. During this transport, the high field eigenstates are adiabatically transformed into the nuclear singlet ($|S_0\rangle$) and triplet ($|T_M\rangle$) energy eigenstates in the low magnetic field, defined as follows

$$
\begin{aligned}
|S_0\rangle &= (|\alpha_1\beta_2\rangle - |\beta_1\alpha_2\rangle)/\sqrt{2} \\
|T_0\rangle &= (|\alpha_1\beta_2\rangle + |\beta_1\alpha_2\rangle)/\sqrt{2} \\
|T_{+1}\rangle &= |\alpha_1\alpha_2\rangle \\
|T_{-1}\rangle &= |\beta_1\beta_2\rangle
\end{aligned}
\tag{3}
$$

For a weakly-coupled spin pair with positive spin-spin coupling, gyromagnetic ratio, and chemical shift difference $\delta_1 - \delta_2$ the correspondence between the low-field and high-field states is[14,24]

$$
\begin{aligned}
|\alpha_1\beta_2\rangle \to |S_0\rangle \qquad |\beta_1\alpha_2\rangle \to |T_0\rangle \\
|\alpha_1\alpha_2\rangle \to |T_{+1}\rangle \qquad |\beta_1\beta_2\rangle \to |T_{-1}\rangle.
\end{aligned}
\tag{4}
$$

Nuclear singlet order corresponds to the mean singlet-triplet population difference. Neglecting relaxation losses during transport, this is given by

$$
p_S = n_{\alpha_1\beta_2} - \frac{1}{3}(n_{\alpha_1\alpha_2} + n_{\beta_1\alpha_2} + n_{\beta_1\beta_2}) = -p^2/3. \tag{5}
$$

The negative sign arises since strong polarization leads to an excess of population in the triplet, depleting the singlet state. Hyperpolarized singlet order is therefore available immediately after dissolution, albeit with significantly less intensity than the longitudinal order. The singlet polarization may nevertheless still be substantial compared to $p_{thermal}$. A 30%-polarized ensemble, ($p = 0.3$), for instance, should yield 3% negative singlet order ($p_S = -0.03$), which is 3 orders of magnitude larger than the thermal polarization.

In low magnetic field the singlet order decays with time constant T_S, which in suitable cases is longer than the magnetization relaxation time T_1. The long singlet lifetime may be exploited by introducing the singlet-hyperpolarized molecules into an object of interest. Apart from singlet relaxation, the singlet order persists as long as the subject remains in low field and the bond connecting the spin-coupled nuclei remains intact.

Eventual conversion of singlet order to an NMR observable is done by transporting the sample back into high magnetic field, following the reverse transformations of eq. 4 (see fig. 1a).[14] The resulting singlet *postcursor*[7] state corresponds to a population deficit in $|\alpha_1\beta_2\rangle$, and equal populations in the other states. A strong, nonselective radiofrequency pulse generates an NMR signal that comprises the outer doublet peaks with opposite amplitudes

$$
\pm \exp(-t/T_S) \times p_S \times \cos^2(\theta/2)\sin(\theta)/2, \tag{6}
$$

for a pulse flip angle θ and low-field waiting time t, and inner doublet peaks with opposite amplitudes

$$
\pm \exp(-t/T_S) \times p_S \times \sin^2(\theta/2)\sin(\theta)/2. \tag{7}
$$

Fig. 1b illustrates the spectra in the case of (i) a 90° pulse ($\theta = \pi/2$); (ii) a small flip angle pulse ($|\theta| \ll \pi/2$). The small flip angle gives peak intensities proportional to the population differences across the states. In this case, the two outer peaks occur with opposite amplitudes $\propto p^2/3$. The inner transitions are absent, since the singlet postcursor contains equal populations in the connected states.

A nonselective pulse applied to pure longitudinal order excites all transitions with equal amplitude

$$
\exp(-t/T_1) \times p \times \sin(\theta)/4. \tag{8}
$$

The singlet order therefore results in a spectrum where the area under each J-doublet is $(p_S/p)\exp[(1/T_1 - 1/T_S)t]$ times that from

Figure 1. Correlation between singlet population order in low magnetic field and the adiabatic postcursor state on which NMR readout is performed. The spectra in b are shown for (i) a strong 90 degree read pulse; (ii) a pulse of small flip angle θ, in the limit $|\sin(\theta)| \ll 1$. Labels apply to the case $(\delta_1 - \delta_2) > 0$, $\gamma > 0$ and $J > 0$. For simplicity, the population distribution assumes a pure singlet polarization $p_S = -1/3$.
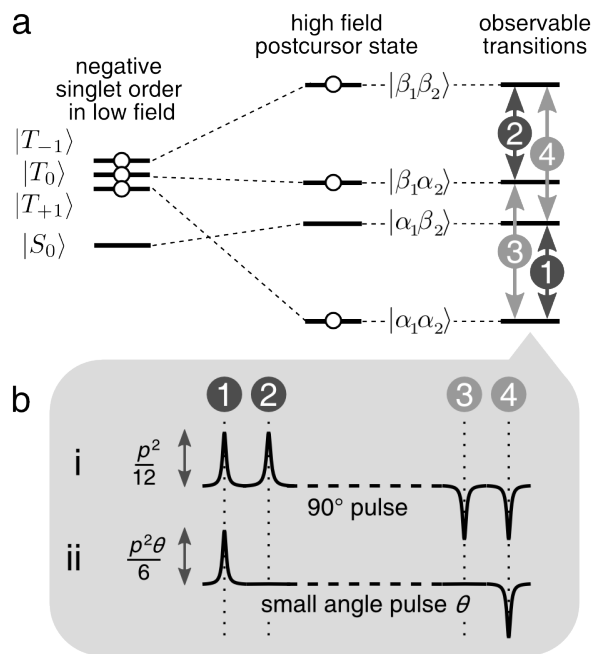
the longitudinal magnetization, after the same time in low field and pulse flip angle.

A natural target for hyperpolarization of singlet order is $^{13}C_2$-labeled pyruvate, ([1,2-$^{13}C_2$] 2-oxopropanoic acid), which is used for simultaneous real-time monitoring of pyruvate dehydrogenase and Krebs cycle metabolism in heart.[25] Using a previously described procedure,[4] labeled pyruvic acid was polarized in an alpha-prototype hyperpolarizer (GE Healthcare, Amersham, UK) working at 3.35 T. A mixture of 35 mg [1,2-$^{13}C_2$] pyruvic acid (95% purum, Sigma-Aldrich UK), 0.7 mg of the trityl radical OX063 (GE Healthcare, Little Chalfont, UK) and 1.2 mg of gadolinium chelate (Gadoteric acid, Dotarem®; Guerbet, Roissy, France) was cooled to 1.2 K in liquid He and irradiated with a 100 mW microwave source at *c*. 94 GHz. The build-up of ^{13}C polarization was monitored via the solid-state NMR signal. After approximately 1 hour of microwave irradiation the frozen material was dissolved with a jet of hot buffer solution (6 ml, heated to 180 °C, containing 100 mg/L EDTA, 30 mM NaCl, 94 mM NaOH and 40 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)) and the solution collected in a vial located outside the magnet.

To estimate the amplitudes of longitudinal polarization and the singlet order the hyperpolarized solution was divided in equal portions between two identical 10 mm o.d. NMR sample tubes, each already containing 2.0 ml D_2O. The final pyruvate concentration in each tube was $\approx$ 10 mM. The first tube was inserted immediately into an high-field NMR spectrometer (9.4 T, Varian Inova) and the ^{13}C spectrum recorded after a *c*. 6° flip angle pulse. The spectrum, which is shown in fig. 2a, is characteristic of the predominant longitudinal spin order.

The second tube, meanwhile, was inserted in a mu-metal cylinder and shaken for $\approx$ 5 seconds. This sample was then removed from the cylinder, inserted in the NMR spectrometer, and the spectrum taken using a 6° flip angle pulse. The shaking of the sample in the weak and sharply inhomogeneous magnetic field in the interior of the mu-metal chamber has the effect of rapidly equilibrating the

Figure 2. ^{13}C NMR spectra at 9.4 T using a 6° flip angle for detection recorded (a) on hyperpolarized $[1,2^{-13}C_2]$ pyruvate (single scan); (b) after erasing magnetization by shaking inside a mu-metal chamber, followed by transfer into high field (single scan); (c) later on the same sample in (a), thermally polarized (16 scans).
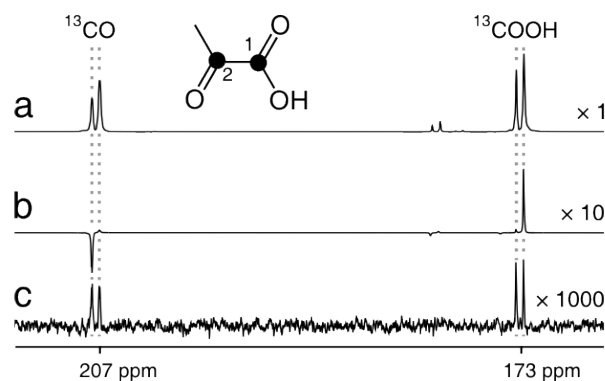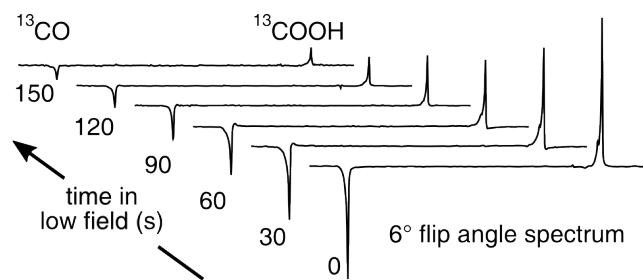

nuclear triplet populations, while leaving the singlet order intact, since the scalar singlet order is invariant to rotations. The shaking in the mu-metal chamber therefore leaves only a population asymmetry between the singlet and triplet states. The spectrum shown in fig. 2b displays the pattern predicted in fig. 1b(ii), demonstrating the presence of significant singlet order in low magnetic field. The presence of singlet order is also indicated through the asymmetry in the doublet peak components in fig. 2a.

By comparing the integral of the spectrum of the first tube with that of the same sample at thermal equilibrium, (fig. 2c), we obtain an estimated longitudinal polarization p of $26 \pm 1\%$ at the time of arrival in the detection magnet (≈ 15 s after dissolution). Assuming there is no relaxation of the sample during transport this value of p corresponds to an expected singlet polarization of 2.3%, which is roughly 12 times less than the longitudinal polarization. In our experiment the signal from the singlet order (a further 15 s after dissolution, see fig. 2b) was 20 times weaker than that obtained for the first tube, (fig. 2a), as deduced by comparing the integrals across the doublet at 173 ppm. This is reasonably consistent with the directly generated singlet order being $p/3$ times the longitudinal magnetization. The factor of 2 discrepancy is attributed to the relaxation during the additional low-field manipulations involved in the singlet NMR experiment.

Another experiment was performed to estimate the decay constant T_S of the $[1,2^{-13}C_2]$ pyruvate at low field, (≈ 0.5 mT). After exiting the hyperpolarizer the pyruvate solution was shaken in the mu-metal cylinder and then diluted on adding to a vial containing 15 ml D_2O. This solution was pipetted equally into six 10 mm o.d. NMR tubes, which were then inserted at 30 s intervals into the high field spectrometer where a small flip angle spectrum was recorded. Fig. 3 shows the spectra from the six tubes after the different low-field waiting times. The peak integrals were fit by an exponential

decay $\exp(-t/T_S)$ giving a decay constant T_S of 70 ± 2 seconds.

The T_1 of the same system was later measured on the thermally polarized sample using a field-cycled experiment. The sample was polarized to $p = p_{\text{thermal}}$ in the high field spectrometer magnet and then shuttled outside the magnet to rest for a time in low field (0.5 mT), for relaxation. Upon reinsertion in the magnet a 90° pulse was applied and the NMR spectrum recorded. Spectra were recorded for different low-field waiting times, and the integrals then fit by $\exp(-t/T_1)$ giving a decay constant T_1 of 36 ± 1 s for both carbons.

These results show singlet order in $[1,2^{-13}C_2]$ pyruvate persists twice as long as the conventional polarization, in low magnetic field. The ratio T_S/T_1 of only ≈ 2, however, is slightly disappointing, since it indicates the presence of relaxation mechanisms in pyruvate that have a strong effect on singlet relaxation. The nature of the mechanism is currently under investigation: intermolecular dipole-dipole interactions and spin-rotation couplings are both possible candidates.

To summarize, we have demonstrated that singlet order is available 'directly' from the initial hyperpolarized state of a sample containing coupled spin-1/2 pairs. Although the singlet order achieved is significantly less than the longitudinal polarization, this route eliminates the additional hardware and complexity involved in other singlet preparation methods.

Acknowledgement

Figure 3. Decay of singlet order on $[1,2^{-13}C_2]$ pyruvic acid in the laboratory magnetic field, fitted time constant $T_S = 70 \pm 2$ s.

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