Communication

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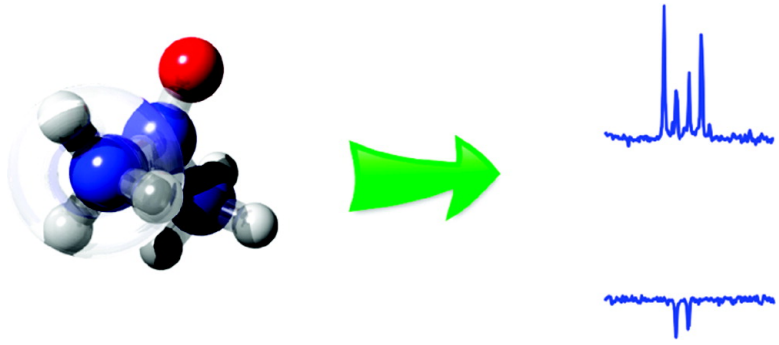

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Optimizing the Signal Enhancement In Cryogenic ex situ DNP−NMR Spectroscopy

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Dynamic nuclear polarization (DNP) is often carried out at low temperatures to utilize the high spin polarization of the radical electrons, which reaches 23% at 1.5 K and with a magnetic field strength of 3.35 T. As samples solidify to form fixed paramagnetic centers in a glass state, the solid effect and the thermal mixing effect become active, allowing efficient polarization.[1,2] At low temperatures, an additional gain in sensitivity arises from the higher nuclear population difference as a result of the Boltzmann distribution.

In experiments where samples were polarized at low temperature (∼1.5 K) followed by dissolution with hot solvent and transfer to an NMR magnet to record a ^{13}C NMR spectrum,[3] we discovered a significantly higher ^{13}C polarization efficiency for microwave frequencies of $\omega_e + \omega_N$ versus $\omega_e - \omega_N$ whenever substances possessing methyl groups were polarized.

Even for targets without methyl groups, the higher microwave frequency gave an increased DNP polarization after 1 h in solvents which carry methyl groups, such as DMSO and acetone.

This effect cannot be attributed to the buildup of Boltzmann polarization, which has been shown to yield a higher overall polarization for the lower frequency.[4] To investigate the origin of these phenomena, we measured the polarization buildup for acetone and DMSO when samples are cooled to ∼1.5 K for 1 h in the absence of a radical and without microwave irradiation followed by dissolution and transfer to the NMR magnet. An acetone/DMSO mixture is commonly used for such experiments because the mixture forms a glass state, whereas DMSO does not glass alone. In this experiment, a significant carbon polarization is observed for the methyl signals of acetone and DMSO. This polarization has the opposite sign compared to the polarization arising from DNP with a microwave frequency of $\omega_e - \omega_N$ (see Supporting Information). In this communication, we discuss a possible mechanism for this observation and show how this effect on methyl carbons can be exploited to optimize DNP experiments for small molecules.

Figure 1 shows a dissolution spectrum for a mixture of acetone and DMSO placed in the polarizer for 1 h at 1.5 K in the absence of radical and without microwave irradiation. If the sample is kept at low temperature for increasing durations, the absolute value of the polarization increases reaching a maximum after approximately 120 min followed by a slow decay (Figure 2). Using deuterated acetone and DMSO, no signals are observed under the same conditions. This suggests that the polarization observed in this experiment arises from the methyl groups and is possibly associated with the rotational tunneling energy levels of the methyl protons at low temperature. Since ^{13}C spectra were recorded, magnetization must have been transferred from protons to carbons, most likely by a heteronuclear−nuclear cross-relaxation effect.

To further investigate the effect of solvent methyl groups on DNP, we have used oxaloacetate, a common metabolite in blood samples, to elucidate determinants of optimal polarization. NMR spectra of oxaloacetate in water/acetone/DMSO (1:1:1) show a significantly larger polarization buildup when using a microwave

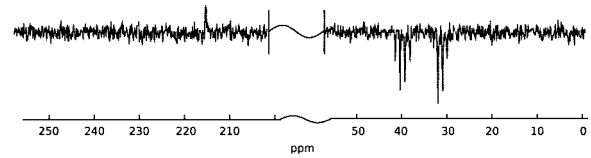

Figure 1. Acetone/DMSO placed in the polarizer for 1 h at 1.4 K with no radical or microwave irradiation. The two negative quartets correspond to the DMSO (39.5 ppm) and acetone (29.9 ppm) methyl signals. The signal at ∼216 ppm is the thermal polarization for the acetone carbonyl carbon.

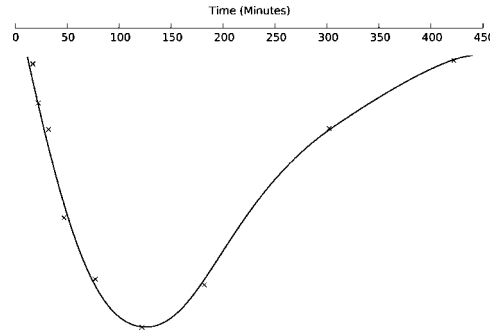

Figure 2. Acetone methyl signal intensity against time where acetone/DMSO were placed at 1.5 K for several durations. No microwave irradiation or radical was used for these experiments, so this polarization is independent of the DNP effect. The trend line is a fit to eq 2.

frequency of $\omega_e + \omega_N$ rather than $\omega_e - \omega_N$ (Figure 3a,b). This suggests that that polarization arising from the methyl groups can interfere destructively with polarization built up via the DNP effect for a microwave frequency of $\omega_e - \omega_N$ while the two mechanisms interact constructively for a microwave frequency of $\omega_e + \omega_N$. For longer polarization times, the effect arising from methyl groups decreases. Panels c and d of Figure 3 show that after 18 h of polarization the largest polarization is observed for a microwave frequency of $\omega_e - \omega_N$. This can be attributed to the positive contribution from Boltzmann polarization and the apparently transient nature of the negative methyl effect. Spectra in Figure 3e,f show the same pattern as in c and d, but without the methyl effect which was eliminated by deuteration.

The secondary effect for oxaloacetate in solvents with methyl groups requires a mechanism, most likely spin diffusion within the ^{1}H or ^{13}C network, to transfer magnetization between spins of the solvent and oxaloacetate. For diffusion of magnetization between methyl groups, spin symmetry diffusion (SSD) has been described as an alternative effect where paired transitions between tunnel splittings carry magnetization across the sample.[5] SSD can be associated with a flip of proton spins thus creating Zeeman polarization for protons.[6] The high density of acetone and DMSO methyl groups in the solvent of 50% DMSO/50% acetone (∼12 M acetone/DMSO) allows for an efficient polarization transfer to the more dilute solute (1 M). Deuteration of solvents will not only

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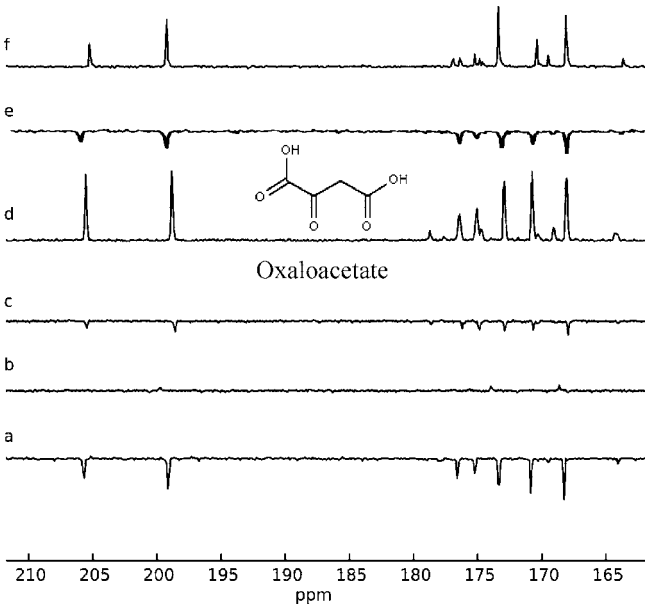

Figure 3. (a) Oxaloacetate (1 M) in water/acetone/DMSO polarized at a frequency of $\omega_e + \omega_N$ for 60 min. (b) Oxaloacetate (1 M) in water/acetone/DMSO polarized at $\omega_e - \omega_N$ for 60 min. (c) Oxaloacetate (1 M) in water/acetone/DMSO polarized at $\omega_e + \omega_N$ for 18 h. (d) Oxaloacetate (1 M) in water/acetone/DMSO polarized at $\omega_e - \omega_N$ for 18 h. (e) Oxaloacetate (1 M) in water/acetone-d_6/DMSO-d_6 polarized at $\omega_e + \omega_N$ for 60 min. (f) Oxaloacetate (1 M) in water/acetone-d_6/DMSO-d_6 polarized at $\omega - -\omega_N$ for 60 min. N.B. The larger than expected number of signals originates from keto−enol tautomerization in oxaloacetate and hydrate formation.[7]

eliminate effects arising from methyl groups but also reduce relaxation effects which affect efficient spin diffusion.

The results presented in this communication suggest a primary role for methyl groups in the context of DNP experiments carried out at very low temperature. These observations are reminiscent of an effect first described by Haupt which generates dipolar−polarization resulting from transitions between quantum rotor states driven by a large change in temperature.[8] The Haupt effect requires weakly hindered methyl groups since the temperature step must cause a significant change between quantum rotor populations. Coupling to the Zeeman reservoir is required to generate Zeeman polarization. Haupt reported a proton polarization on the order of 10 000-fold for γ-picoline and a temperature jump of 30 K[8,9] and showed a maximum for the proton enhancement after approximately 10 min for a temperature change from 55 to 4 K.[10] In experiments reported in this communication, the temperature change was significantly larger (from ~300 to 1.5 K) and the maximum ^{13}C polarization for the acetone or DMSO methyl signal is observed after approximately 2 h. Tunneling phenomena in acetone and DMSO were previously studied, and tunneling frequencies were determined for acetone and DMSO.[11,12] However, the experiments reported here add a level of complexity because ^{13}C is observed rather than protons, and polarizations were observed not only for pure samples in the absence of microwaves and radicals but also in conjunction with DNP experiments. Whether the observed effect is a Haupt effect or a related effect arising from magnetic properties of methyl groups remains an enigma.

Nevertheless, the rate of polarization buildup for the methyl signal of acetone (Figure 2) in the absence of microwaves and radical can be estimated using a simple model assuming polarization buildup and decay along with polarization transfer from ^{1}H to ^{13}C nuclei, most likely via a dipolar interaction such as a heteronuclear−nuclear Overhauser effect (NOE).

This can be expressed as a partial differential equation

$$\frac{\partial \rho_c}{\partial t} = \frac{\rho_c}{c} + k\sigma(e^{-t/a} - e^{-t/b}) \quad (1)$$

or in its integrated form:

$$\rho_c = k_{AMP}(k_1 e^{-t/a} - k_2 e^{-t/b} - k_3 e^{-t/c}) \quad (2)$$

where the constants k_{AMP}, k_1, k_2, k_3, and k are constants composed of ρ_c, the ^{13}C polarization, σ, the heteronuclear cross-relaxation rate, the ^{1}H polarization buildup rate, a, the ^{1}H relaxation rate, b, and the ^{13}C T_1 relaxation rate c (see Supporting Information). The resulting triple exponential function fits to the time course of the polarization shown in Figure 2, although the system is underdetermined to yield reliable rates.

These results show that for desirable short polarization times optimal polarization can be achieved when DNP is carried out at $\omega_e + \omega_N$ where an effect arising from methyl groups and DNP interfere constructively. We assume that the effect observed for methyl groups is associated with polarization arising from rotational tunneling similar to the mechanism described by Haupt. Beyond optimizing DNP, this mechanism alone may also provide an additional route to achieve nuclear hyperpolarization.

Application of this effect enabled us to obtain DNP enhanced spectra for a series of small molecules which could otherwise not be polarized efficiently. This allowed the application of DNP−NMR for metabolites such as oxaloacetate, glucose, and citrate. In the case of glucose, the most significant enhancement was achieved in perdeuterated DMSO/water (see Supporting Information).

These observations have significant implications for DNP−NMR and may open up a new chapter for NMR of small molecules and the application of DNP for in situ metabolomics.[13,14]

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Supporting Information Available: Spectra and a mathematical description of the polarization buildup on ^{13}C due to the methyl effect. This material is available free of charge via the Internet at http://pubs.acs.org.

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