Extension of the singlet state lifetime of N₂O via alteration of temperature and viscosity of dissolving solvent
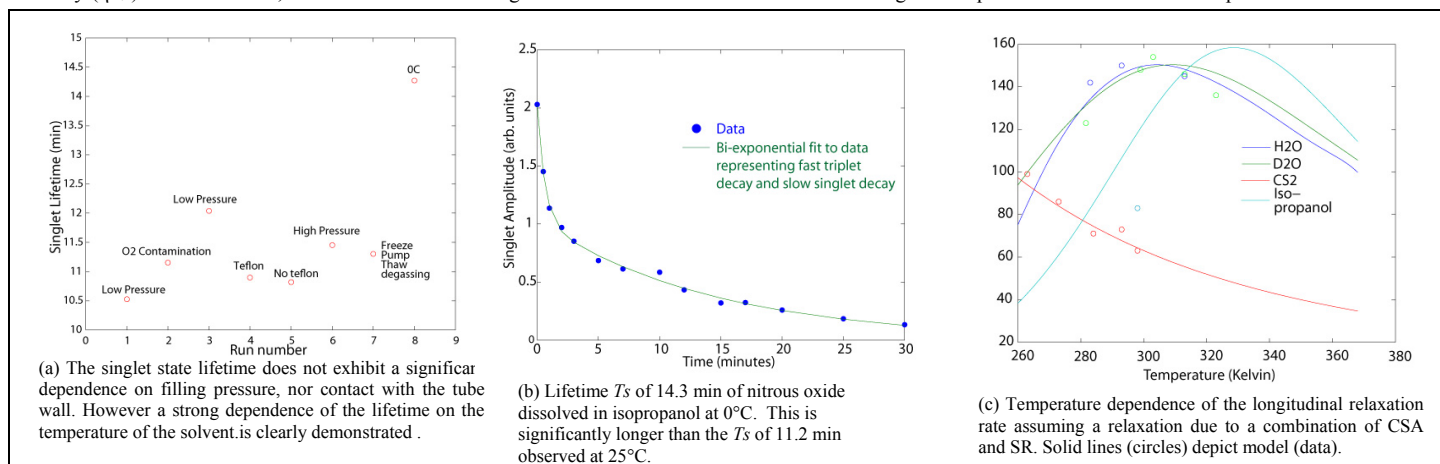
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Introduction: Recent advances in the field of hyperpolarization physics allow dramatically increased nuclear spin polarization of labeled substrates by several orders of magnitude over the equilibrium Boltzmann polarization. The enhanced sensitivity offers the potential for detection of numerous diseases such as cancer, but only if the hyperpolarized substrate can be delivered to the target organ of interest and metabolized before the hyperpolarization has decayed. However, short polarization lifetime limits many attractive applications of hyperpolarized substrates in humans. Recently, substantial increases in polarization lifetimes have been demonstrated by transferring nuclear spin polarization to singlet states [1]. In the present work we investigate the relaxation processes of the singlet state of dissolved nitrous oxide. We quantify contributions of spin rotation and paramagnetic impurities to the relaxation rate of singlet state. We describe techniques and conditions under which the lifetime of the singlet state "Ts" in nitrous oxide can be further extended by altering the properties of the dissolving solvent. We note that although the singlet state is nonmagnetic, conversion by adiabatic transport to distinct, anti-phase lines at imaging-strength fields make it a suitable target for high-sensitivity MRI.

Methods: Doubly ¹⁵N-labeled N₂O was dissolved in various solvents in 5-mm NMR tubes (Wilmad Glass) of different thicknesses. Solutions were degassed under N₂ prior to introduction of N₂O. Additionally, 100 μM EDTA was added to aqueous solutions (H₂O and D₂O) as a metal ion chelating agent. N₂O was introduced by cryopumping a calibrated volume into a section of the NMR tube which was in contact with liquid N₂. The tube was then flame-sealed, inverted several times, and allowed to equilibrate to room temperature, after which the dissolved N₂O was in equilibrium with a gas pressure of between 5 and 40 bar. To investigate the effects of paramagnetic oxygen, different schemes for degassing such as freeze pump thaw cycles, degassing under N₂, and deliberate contamination with oxygen were applied during sample preparation. The effect of paramagnetic contamination in the tube wall was investigated by modifying some samples with a Teflon liner, and further purifying the solvents with chelex (bio-rad) for 10 hrs before filtration.

NMR experiments were performed on a Varian 11.7 T vertical bore magnet. After the sample was fully thermally polarized, the N₂O singlet state was populated using a two-step process. First, a single, long (3.3 ms) square π pulse centered on one ¹⁵N resonance was applied. The length was chosen to have a node at the adjacent ¹⁵N frequency and leave it unaffected. Subsequent adiabatic transport of the NMR tube from the bore of the 11.7 T magnet to the center of a magnetically shielded cylinder resulted in over- (under-) population of the *m*=0 singlet (triplet) states. After a set time interval the sample was reintroduced into the bore of the magnet. A non-selective π/2 pulse was applied to both nuclei to read out the polarization. The procedure was repeated allowing a wait of 5 times the longer of the two individual nuclear T_1's to allow for thermal re-polarization of the sample. Systematic error in overestimating the amplitude of the peaks utilizing the Lorentzian fit was reduced by integrating correlations between points in the spectra separated by the well-measured J-coupling prior to fitting to a single Lorentzian lineshape. The majority of experiments were carried out at 25°C. Experiments at 0°C in low field were carried out by placing the samples in low field in an insulated ice bath at the triple point of water. The 11.7 T NMR probe was chilled to 0°C as well during these experiments.

Results and Discussion: The lifetime of the singlet state of nitrous oxide is largely dominated by relaxation due to the spin rotation interaction (SR) at low magnetic fields of approximately 1G [2]. The contribution of paramagnetic impurities in either gaseous form or contamination on the NMR tube walls is small compared to the overall relaxation rate (Fig.1a). The temperature dependence of the longitudinal relaxation rate at high field can be sufficiently described by assuming the relaxation is due to a combination of spin rotation, chemical shift anisotropy (CSA) and trace level paramagnetic impurity (Fig. 1c). At low fields the contribution from CSA is negligible as the strength of the CSA drops as a function of the square of the magnetic field. Thus at low field the relaxation is expected to be dominated by SR. Converse to the CSA and dipolar interactions, relaxation due to SR decreases at both lower temperatures and when the product of the hydrodynamic radius and viscosity (ηr_h^3) increases. Thus, if SR is the dominant longitudinal relaxation mechanism then lowering the temperature should increase the polarization lifetime of

(a) The singlet state lifetime does not exhibit a significant dependence on filling pressure, nor contact with the tube wall. However a strong dependence of the lifetime on the temperature of the solvent.is clearly demonstrated .

(b) Lifetime Ts of 14.3 min of nitrous oxide dissolved in isopropanol at 0°C. This is significantly longer than the Ts of 11.2 min observed at 25°C.

(c) Temperature dependence of the longitudinal relaxation rate assuming a relaxation due to a combination of CSA and SR. Solid lines (circles) depict model (data).

the singlet state as well (Eq. 1). This effect has been unambiguously observed in N₂O dissolved in CS₂ or isopropanol (Fig. 1b). Lowering the temperature to 0°C resulted in a subsequent increase in Ts from 11.2 min to 14.3 min in the sample dissolved in isopropanol, and from 8.5 min to 11.0 min for the sample dissolved in CS₂. Furthermore the ratio of the singlet lifetimes in the various solvents to (ηr_h^3) is constant as expected for relaxation dominated by spin rotation (Eq. 1) assuming r_h is an effective hydrodynamic radius equated from a Wilke and Chang diffusion model [3].

	η(cP)@25°C	r_h (nm)	T_S (min.)	$T_S/(\eta\, r_h^3)$
isopropanol	1.960	0.074	11.2±1.0	13000
H₂O	0.890	0.13	22.8±0.9	11700
D₂O	1.132	N/A	22.0±7.5	N/A
CS₂	0.351	0.12	8.5±1.2	14000

Table 1: Measured Ts for N₂O in four solvents at 25°C, as well as the dependence on the product of the viscosity and cube of hydrodynamic radius.

$$(T_s^{sr})^{-1} \sim 4\kappa_B T(C_j-C_k)^2 \hat{I}^2/(24\pi\hbar^2\eta r_h^3) \qquad (1)$$

Conclusion: The singlet lifetime of N₂O is dominated by spin rotation relaxation. By lowering the temperature or increasing (ηr_h^3), properties of the solvent and its interaction with the singlet agent, the singlet lifetime can be significantly lengthened. This leads to a simple strategy for further extending the spin polarization lifetime of hyperpolarized compounds in the singlet state which may have benefit for imaging *in-vivo*.

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