Published in final edited form as:

J Magn Reson. 2012 December ; 225: 52–57. doi:10.1016/j.jmr.2012.10.002.

Frequency Dependence of Electron Spin Relaxation Times in Aqueous Solution for a Nitronyl Nitroxide Radical and Perdeuterated-Tempone between 250 MHz and 34 GHz

Joshua R. Biller^{a,b}, Virginia M. Meyera^{a,b}, Hanan Elajaili^{a,b}, Gerald M. Rosen^{b,c,d}, Sandra S. Eaton^{a,b}, and Gareth R. Eaton^{a,b}

^aDepartment of Chemistry and Biochemistry, University of Denver, Denver, CO 80208

^bCenter for EPR Imaging in Vivo Physiology, University of Denver, Denver, CO 80208 and University of Maryland, Baltimore, Baltimore, MD, 21201

^cCenter for Biomedical Engineering and Technology, University of Maryland, Baltimore, Baltimore, MD, 21201

^dDepartment of Pharmaceutical Sciences, University of Maryland, Baltimore, Baltimore, MD, 21201

Abstract

Electron spin relaxation times of perdeuterated tempone (PDT) **1** and of a nitronyl nitroxide 2-(4-carboxy-phenyl)-4,4,5,5-tetramethylimidazoline-3-oxide-1-oxyl) **2** in aqueous solution at room temperature were measured by 2-pulse electron spin echo (T_2) or 3-pulse inversion recovery (T_1) in the frequency range of 250 MHz to 34 GHz. At 9 GHz values of T_1 measured by long-pulse saturation recovery were in good agreement with values determined by inversion recovery. Below 9 GHz for **1** and below 1.5 GHz for **2**, $T_1 \sim T_2$, as expected in the fast tumbling regime. At higher frequencies T_2 was shorter than T_1 due to incomplete motional averaging of g and A anisotropy. The frequency dependence of $1/T_1$ is modeled as the sum of spin rotation, modulation of g and A -anisotropy, and a thermally-activated process that has maximum contribution at about 1.5 GHz. The spin lattice relaxation times for the nitronyl nitroxide were longer than for PDT by a factor of about 2 at 34 GHz, decreasing to about a factor of 1.5 at 250 MHz. The rotational correlation times, τ_R are calculated to be 9 ps for **1** and about 25 ps for **2**. The longer spin lattice relaxation times for **2** than for **1** at 9 and 34 GHz are due predominantly to smaller contributions from spin rotation that arise from slower tumbling. The smaller nitrogen hyperfine couplings for the nitronyl **2** than for **1** decrease the contribution to relaxation due to modulation of A anisotropy. However, at lower frequencies the slower tumbling of **2** results in a larger value of $\omega\tau_R$ (ω is the resonance frequency) and larger values of the spectral density function, which enhances the contribution from modulation of anisotropic interactions for **2** to a greater extent than for **1**.

© 2012 Elsevier Inc. All rights reserved.

Corresponding Author: Gareth R. Eaton, Department of Chemistry and Biochemistry, University of Denver, Denver, CO 80208, geaton@du.edu, Phone: 303-871-2980, Fax: 303-871-2254.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

The widespread application of electron paramagnetic resonance (EPR) of nitroxides, including spin labeling of proteins [1,2], has led to extensive studies of nitroxide electron spin relaxation times over a wide range of motional regimes [3–6]. The focus of this paper is on the rapid tumbling regime with rotational correlation times, τ_R , of tens of picoseconds, as expected for small spin probes *in vivo*. Hyde and co-workers reported spin lattice relaxation times for 0.5 mM aqueous solutions of tempone (2,2,6,6-tetramethyl-4-piperidone-1-oxyl) and CTPO (3-carbamoyl-2,2,5,5-tetramethyl-3-pyrroline-1-oxyl) that decreased monotonically from 0.92 and 1.58 μ s, respectively, at 34.6 GHz to 0.33 and 0.25 μ s, respectively, at 2.54 GHz [7,8]. If this tendency toward shorter T_1 at lower frequency were to continue to 250 MHz or 1 GHz where *in vivo* studies are commonly performed [9,10], it would make pulsed EPR *in vivo* studies very difficult. To design molecules for pulsed EPR of nitroxides *in vivo* it is important to extend the measurements of relaxation times to lower frequencies and to model the relaxation processes to predict relaxation rates as structural and experimental conditions are varied.

There is interest in finding spin probes with longer T_1 to facilitate *in vivo* studies. For nitronyl nitroxides the nitrogen hyperfine couplings are smaller than in piperidinyloxy or pyrrolidinyloxy due to the delocalization of the unpaired spin density over two nitrogens [11]. It was therefore of interest to determine the extent to which the smaller nitrogen hyperfine couplings would result in longer spin lattice relaxation times. Nitronyl nitroxides are used in identifying nitric oxide [12], dynamic nuclear polarization [13–15], and spintronics [16,17]. After the completion of this work, we received a pre-publication copy of [18], which reports the temperature dependence of relaxation for another nitronyl nitroxide in toluene at X-band, which complements the work reported here.

The goals of this work are to determine (i) to what extent the small nitrogen nuclear hyperfine splitting of a nitronyl nitroxide results in slower electron spin relaxation than for piperidinyl nitroxides, (ii) whether the trend to shorter T_1 at lower resonance frequency reported in ref. [7] continues as frequency is decreased, (iii) whether the spin-rotation and modulation of g and A relaxation mechanisms (Eq. 1 – 4) that were sufficient to model the relaxation of PDT at X-band in aqueous solution are also sufficient to explain relaxation at lower frequencies, and (iv) whether a thermally activated process (Eq 5) makes a significant contribution in the fast tumbling regime.

Spin rotation [19] is a frequency-independent contribution to $1/T_1$ that depends only on g anisotropy and the rotational correlation time, τ_R , as described by Eq. (1). Its importance for a wide range of organic radicals in the rapid tumbling regime has been recognized for many years [3].

$$\frac{1}{T_1^{SR}} = \frac{\sum_{i=1}^3 (g_i - g_e)^2}{9\tau_R} \quad (1)$$

where $i = x, y, z$ and $g_e = 2.0023$, the free electron g value.

In the relatively rapid tumbling regime, modulation of nitrogen hyperfine anisotropy, also called the END (electron nuclear dipolar mechanism) makes a substantial contribution to T_1 [5,6,20–22] for nitroxides.

$$\frac{1}{T_1^A} = \frac{2}{9} I(I+1) \sum_i (A_i - \bar{A})^2 J(\omega) \quad (2)$$

where A_i is a component of the nitrogen hyperfine in angular frequency units, $\bar{A}$ is the average nitrogen hyperfine, $I = 1$ is the nitrogen nuclear spin, ω is the resonance frequency in angular units, and $J(\omega)$ is the spectral density function, which usually is taken to be the Bloembergen, Purcell, and Pound (BPP) spectral density function Eq. (3) [4,23].

$$J(\omega) = \frac{\tau_R}{1 + (\omega\tau_R)^2} \quad (3)$$

Modulation of g anisotropy also contributes to $1/T_1$ as described by Eq. (4) [20]. For nitroxide radicals this contribution is negligible at 9 GHz, but is significant at 34 GHz and higher frequencies.

$$\frac{1}{T_1^g} = \frac{2}{5\hbar^2} (\mu_B B)^2 \left\{ \frac{(\Delta g)^2}{3} + (\delta g)^2 \right\} J(\omega) \quad (4)$$

where B is the external magnetic field, μ_B is the Bohr magneton, $\Delta g = g_{zz} - \frac{1}{2}(g_{xx} + g_{yy})$, and $\delta g = \frac{1}{2}(g_{xx} - g_{yy})$.

The spin lattice relaxation rates in aqueous solution for three perdeuterated nitroxides, including perdeuterated tempone (PDT) at X-band [22] are consistent with predictions based on Eq. (1) – (4). In studies of tempol (4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl) in water:glycerol mixtures at 1.9, 3.1, and 9.2 GHz it was observed that for $\tau_R > \sim 1$ ns, $1/T_1$ was faster than predicted by contributions from mechanisms described by Eq. (1), (2), and (4). Relaxation in the slower tumbling regime was dominated by a frequency-dependent process that was attributed to the same thermally-activated process that had been observed for tempol doped into a solid host [24]. This process is described by Eq. (5)

$$\frac{1}{T_1^{therm}} = C_{therm} \left(\frac{\omega}{\omega_{ref}} \right) \frac{\tau_{therm}}{1 + (\omega\tau_{therm})^2} \quad (5)$$

where $\tau_{therm} = \tau_c^0 \exp(E_a/RT)$, E_a is the activation energy, τ_c^0 is the pre-exponential factor, C_{therm} is the coefficient for the contribution of the thermally activated process, and $\omega_{ref} = 9.5$ GHz. The values of E_a (1100 K) and τ_c^0 (2.5×10^{-12} s) were assumed to be the same in the water:glycerol solutions as in the doped solid. The assumption that E_a is independent of τ_R , seems reasonable for an intramolecular process. These values of E_a and τ_c^0 give $\tau_{therm} = 1.0 \times 10^{-10}$ s at 295 K, which predicts a maximum effect of this process at $\omega = 1/\tau_{therm}$, or $\nu = 1.6$ GHz.

Most prior studies of EPR relaxation processes in fluid solution have varied rotational correlation times by changing temperature [20,21,25–27] and/or by changing solvent [5,21]. Relaxation rates as a function of frequency are particularly useful for characterizing relaxation processes that depend on ω [5–8], including modulation of g and A anisotropy and thermally-activated processes. The frequency dependence of T_1 and T_2 at five frequencies that span more than three orders of magnitude, 250 MHz to 34 GHz, is reported in aqueous solution for two nitroxides that are of interest for in vivo EPR studies, PDT **1** and nitronyl nitroxide, **2** (Fig. 1). PDT was selected for comparison with the nitronyl because its narrow lines give improved signal-to-noise for in vivo studies and because its relaxation rates have been successfully modeled at X-band.

Motion is a major factor in electron spin relaxation in fluid solution. As discussed below, the values of τ_R are 9 and 25 ps for **1** and **2**, respectively. For both radicals, $\omega\tau_R < 1$ at low frequencies and $\omega\tau_R > 1$ at 34 GHz. Thus the range of frequencies studied includes two limiting regimes for the spectral density function, Eq. 3, which provides a rigorous test of the model for the contribution of modulation of g and A to spin relaxation and a check on the values of τ_R .

Methods

Sample Preparation

PDT (**1**) was prepared as described previously [22]. Solutions were 0.5 mM in water. This concentration was selected to give adequate signal-to-noise for a range of frequencies and resonators. Although Heisenberg exchange makes a detectable contribution to T_2 , and to a lesser extent to T_1 , at this concentration [22], these contributions are independent of frequency and will not alter conclusions concerning frequency-dependent contributions to relaxation.

Preparation of 2-(4-Acetoxymethoxycarbonylphenyl)-4,4,5,5-tetramethylimidazoline 3-oxide 1-oxyl

To a DMSO (5 mL) solution of 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline 3-oxide 1-oxyl (0.25 gm, 0.9 mmol), prepared as described in the literature [28,29], was added anhydrous K_2CO_3 (0.25 gm, 1.8 mmol). This mixture was stirred at room temperature for 5 min, then bromoethylacetate (0.15 gm, 0.1 mL, 1 mmol, Aldrich Chemical Company, Milwaukee, WI), was added. This mixture was stirred at room temperature for 5 hr. Thereafter, water (100 mL) and chloroform (100 mL) were added. The organic solution was washed four more times with water (100 mL). Then, the chloroform solution was dried over anhydrous Na_2SO_4 , filtered and reduced to dryness on a rotary evaporator. The remaining residue was chromatographed using silica gel (230–400 mesh, EMD, distributed by VWR International, Suwanee, GA), initially eluting with petroleum ether:Et₂O (10:3), which removed a red solid that was discarded. Continued elution with this mixed solvent system afforded the desired product, a purple solid, mp = 103–104°C, recrystallized from hexane, 0.2 gm, 65%. IR (CHCl₃): 1746 cm⁻¹ (C=O). Mass spectrum (M^+) calcd. For C₁₇H₂₁N₂O₆: 349.37. Found 349.10.

The ester of 2-(4-Acetoxymethoxycarbonylphenyl)-4,4,5,5-tetramethylimidazoline 3-oxide 1-oxyl was hydrolyzed to anion **2** (Fig. 1) by dissolving the acetoxy ester in 2 mM NaOH. Sonication was used to speed dissolution. Samples for EPR spectroscopy were 0.5 mM in 2 mM NaOH.

Oxygen Removal

The procedure for solution deoxygenation depended on sample geometry. The effectiveness of oxygen removal was monitored by measuring T_2 . Deoxygenation was continued, or repeated, until a maximum value of T_2 was observed. *Method 1- nitrogen purge of resonator or tube:* The Q-band samples were contained in 0.3 mm id thin-wall Teflon tubing supported in a 1.6 mm o.d. quartz tube that was open at both ends. The resonator and cryostat were purged with nitrogen for a minimum of 1 hr. At X-band the sample was in 0.97 mm i.d. Teflon tubing that was doubled over to give 2 lengths of tubing supported in a 4 mm quartz tube. At S-band the 0.97 mm i.d. tubing could be folded three times for six lengths of sample in a 6 mm quartz tube. Nitrogen was purged through the 4 (or 6) mm tube via an additional thin-wall Teflon tube. *Method 2- Freeze-Pump-Thaw (FPT).* L-band samples were contained in heavy or medium wall 4 mm quartz tubes. A length of empty 0.97 mm i.d. Teflon tubing was placed in the sample to relieve the strain caused by water expansion

during freezing and thawing. Seven FPT cycles were applied and the vacuum gauge reading on the last cycle was 5–10 mTorr, before the sample was flame sealed. *Method 3 – nitrogen bubbling:* For 250 MHz, nitrogen was bubbled through samples in 25 mm tubes to displace oxygen. The head space above the sample was purged with N₂ for 20 minutes, followed by 15 minutes N₂ bubbling in the sample, and an additional 20 minute purge of the head space before flame sealing. The bubbling was slow enough that evaporation was not detectable.

Spectroscopy

Measurement of spin-spin relaxation times (T_2) by two pulse spin echo and of spin lattice relaxation times (T_1) by inversion recovery were performed at ca. 22°C at 34.5 GHz (Q-band) on a Bruker E580, and at 9.5 GHz (X-band) [30], 3.0 GHz (S-band), 1.5 GHz (L-band) [31], and 250 MHz (VHF) [32] on locally designed and built spectrometers. The resonators were Q-band, Bruker ER5107 dielectric pulse resonator; X-band, Bruker ER4119-X5MS; S-band, copper cross-loop resonator [31,33]; L-band, loop-gap resonator [34]; 250 MHz, copper cross-loop resonator [35]. The 250 MHz resonator uses Q-spoiling to decrease the dead-time and facilitate measurement of short relaxation times. Saturation recovery measurements at X-band were performed on a locally designed and built spectrometer [36] with pump times that were long relative to T_1 (T_{1e}) or T_{1N} . Values of T_1 and T_2 were measured in triplicate for each hyperfine line of **1** and for $M_I = -1, 0$, and 1 of **2**. Due to the lower intensities of the $M_I = \pm 2$ lines of **2**, these were not studied.

Data fitting to obtain relaxation times

The spin echo decay curves and saturation recovery curves fit well with a single exponential, using a least squares criterion. At X-band and lower frequencies, the inversion recovery curves fit well with a single exponential, as expected in the regime where $\omega\tau_R \sim 1$ [21]. For these radicals at 34 GHz, $T_{1N} < T_1$ and two components were observed, as expected [37,38], for the inversion recovery curves; the longer relaxation time is assigned as T_1 . The uniform penalty (UPEN) fitting procedure [39,40] was used to confirm the presence of two contributions to the exponential decays at 34 GHz. Values of T_1 measured by saturation recovery at 9.5 GHz were in good agreement with the values obtained by inversion recovery, which confirmed the validity of the inversion recovery measurements.

Results

The values of T_1 and T_2 for the nitrogen $m_I = -1, 0$, and $+1$ lines for **1** and $M_I = -1, 0$, and $+1$ for **2** in deoxygenated aqueous solution at 22°C at the five frequencies studied are summarized in Tables 1 and 2. The data show that the trend to shorter relaxation times between 34 and 3 GHz does not continue as frequency is decreased further. At frequencies up to 9 GHz for **1** and up to 3 GHz for **2** $1/T_1 \sim 1/T_2$ as expected in the rapid tumbling regime where motional averaging of g and A anisotropy is essentially complete and T_1 is the dominant contribution to T_2 . At 34 GHz the higher resonant field enhances the contribution to T_2 from incomplete motional averaging of g anisotropy, and $1/T_2$ is larger than at lower fields/frequencies. The increase in $1/T_2$ at 34 GHz is greater for **2** than for **1**, because of the slower tumbling of **2**, which is discussed in the following section. Since T_1 dominates T_2 in water over most of the frequency range studied, the focus of this paper is on the frequency dependence of T_1 .

For **1**, the systematically longer values of T_1 and T_2 for $m_I = +1$ and 0 than for $m_I = -1$ are similar to observations at X-band for **1** and other nitroxides in aqueous solution [22]. For **2**, the longest values of T_1 , and T_2 at all frequencies except 34 GHz, are for $M_I = 0$. Since current models for nitroxide T_1 do not include a dependence on nitrogen nuclear spin, the data for $m_I (M_I) = 0$ are shown in Fig. 2 and analyzed in the following sections.

Analysis of the frequency dependence of $1/T_1$ for PDT 1

The literature values for T_1 of 0.5 mM tempone in water: 0.25 μ s at 2.54 GHz, 0.32 μ s at 3.45 GHz, 0.49 μ s at 9.2 GHz, and 0.92 μ s at 34.6 GHz [7] agree within better than 10% with the values in Table 1 at 9.1 and 34 GHz and are shorter by about 40% at lower frequencies. Since the literature values are for natural isotope abundance tempone and our studies were for the perdeuterated analog, this agreement is consistent with prior studies that reported relatively small H/D isotope effects on nitroxide T_1 in solution [5,41].

The literature g values for **1** and **2** are shown in Table 3. The tumbling correlation time for **1**, determined from the m_I dependence of T_2 [42,43] in H_2O at X-band, is 9 ps [22]. The value of $1/T_1^{SR}$, calculated using Eq. (1) and the g values in Table 3 is shown as a solid line in Fig. 3. The calculation agrees well with the experimental data at 34 GHz and indicates that spin rotation is the dominant contribution to $1/T_1$ at this frequency. The increase in relaxation rates for **1** with decreasing frequency is due to frequency dependent processes. In the limit where $(\omega\tau_R)^2 \ll 1$ the denominator of the equation for $J(\omega)$ (Eq. 3) is approximately 1, and the spectral density becomes equal to τ with no frequency dependence. For $\tau_R = 9$ ps, $\omega\tau_R$ is 1.9 at 34 GHz and 0.5 at 9.5 GHz, but decreases to less than 1 at frequencies below 9.5 GHz. This explains why $1/T_1^A$ and $1/T_1^g$ (Eq. (2) and (4)) increase as frequency is decreased from 34 to 9 GHz and then plateau at lower frequencies (Fig. 3). The combined contributions to $1/T_1$ for **1** from spin rotation and modulation of g and A anisotropy explain the frequency dependence between 34 and 1.5 GHz, but cannot account for the slower relaxation (longer T_1) at 250 MHz than at 1.5 GHz (Fig. 3). In the calculations of spin rotation and modulation of g and A anisotropy there were no adjustable parameters and the good agreement with the experimental data confirms both the model and the value of τ_R . Variation of τ_R by more than about 10% resulted in significantly poorer agreement with the experimental data. The fit line in Fig. 3 includes a small contribution from a thermally-activated process described by Eq. (5) and proposed by analogy with studies of tempol in water:glycerol mixtures [5]. The fit shown in Fig. 3 was obtained by adjusting only C_{therm} to match the experimental data (Fig. 3). The resulting value of $C_{therm} = 7 \times 10^{16} s^{-2}$ is within a factor of 3 of the value of $C_{therm} = 2.8 \times 10^{16} s^{-2}$ found for slowly tumbling tempol in water:glycerol mixtures [5]. The combined contributions from spin rotation, modulation of g and A anisotropy, and the thermally activated process are in excellent agreement with the experimental frequency dependence of $1/T_1$ (Fig. 3). Further experiments with a wider range of nitroxides are needed to assign the thermally activated process to a physical motion, but the maximum contribution at about 1.5 GHz supports the plausibility of the proposal that the same process dominates at long τ_R at a range of frequencies and for short τ_R at lower frequencies. As τ_R approaches 10^{-9} s, the contributions from spin rotation and modulation of g and A anisotropy are predicted to become negligible and all that remains is the contribution from the thermally-activated process.

Analysis of the frequency dependence of $1/T_1$ for nitronyl 2

Because of the equivalent couplings to two nitrogen nuclei, τ_R for nitronyl **2** cannot be determined by the Kivelson formulae [42] that were used for **1**. Analysis of the frequency dependence of $1/T_1$ for **1** demonstrated that spin rotation is the dominant contribution at 34 GHz, so the assumption was made that this is also the case for **2**. Using the literature g values [11] and Eq. (1), τ for **2** was varied to give $1/T_1$ less than the experimental value at 34 GHz. The shortest value of τ_R that is consistent with the experimental data is 25 ps. Values of τ longer than 25 ps would require a contribution from an additional process at 34 GHz, beyond those required to interpret the data for **1**. To fit $\tau = 25$ ps to the Stokes-Einstein equation ($\tau = c_{slip} V \eta / kT$, V = molecular volume calculated from the molar mass assuming a density of 0.9 g/mL which is typical of organic molecules, η = viscosity, k = Boltzmann's

constant) requires $c_{\text{slip}} = 0.20$. This c_{slip} is comparable to the value of 0.21 for 3-carboxy-2,2,5,5-tetramethyl-1-pyrrolidinyloxy [22], which also has a carboxy group that can hydrogen-bond to water, so $\tau = 25$ ps is a plausible value for **2**. The contribution from spin rotation to $1/T_1$ for **2** is shown as a solid line in Fig. 4. The slower relaxation for **2** than for **1** at 34 GHz (Table 1) is due primarily to longer τ_R for **2** than for **1**. If τ_R for **2** were similar to that for **1**, the contribution to $1/T_1$ for **2** due to spin rotation would be about 1.5 times larger than for **1** because of the larger g anisotropy (Table 3) and $1/T_{1e}$ would be about $0.7 \mu\text{s}$ with little frequency dependence. In water at 22°C , the slower tumbling of **2** than of **1** offsets the difference in g values and results in slower relaxation for **2** than for **1** at 34 GHz (Table 1 and 2). Based on this analysis a value of $\tau_R = 25$ ps was used in analyzing other contributions to $1/T_1$ for **2**.

Eq. (4), the contribution to relaxation from modulation of g anisotropy, is valid for **2** as well as for **1**. The model for the contribution to $1/T_1$ from modulation of A anisotropy (Eq. 2) was derived for a nitroxide with anisotropic hyperfine coupling to a single nitrogen nucleus, so it is not directly transferable to the nitronyl which has hyperfine couplings to two equivalent nitrogens. Molecular orbital calculations and modeling of the motional dynamics for *p*-(methylthio)phenyl nitronylnitroxide in toluene [44] found that the principal axes of the nitrogen hyperfine were not collinear and that tumbling was anisotropic, which complicates the interpretation of the contribution to $1/T_1$ for **2** from modulation of A anisotropy. However, it is reasonable to assume that modulation of couplings to the two nitrogens can be approximated by an expression of the form shown in Eq. (6).

$$\frac{1}{T_1^{A'}} = C_A J(\omega) \quad (6)$$

where C_A is determined by the nitrogen hyperfine couplings of **2**. For $\tau_R = 25$ ps $\omega\tau_R$ is 1.5 at 9.5 GHz and 0.24 at 1.5 GHz so the contribution to $1/T_1$ for **2** becomes frequency independent at lower frequency than for **1** (Fig. 4), which explains why the increase in $1/T_1$ for **2** between 34 and 2 GHz is substantially greater than for **1**. Since the frequency dependence of $1/T_1$ is strongly dependent on τ_R , the fit to the experimental data confirms the value of τ_R . The contribution to $1/T_1$ for **2** from modulation of g and A -anisotropy was modeled as the sum of contributions from Eq. (4) using literature values of g [11], $\tau = 25$ ps, and Eq. (6) with C_A treated as an adjustable parameter. The contribution from Eq. (6) that is shown in Fig. 4 was obtained with $C_A = 3 \times 10^{16} \text{ s}^{-2}$. If the parameters for **1** had been used to calculate C_A , the value would be $8.2 \times 10^{16} \text{ s}^{-2}$. The substantially smaller value of C_A for **2** than for **1** seems plausible because of the dependence on the square of the anisotropy of the hyperfine interaction and the much smaller hyperfine splittings for **2** than for **1** (Table 3).

For **2**, as for **1**, $1/T_1$ at 250 MHz is smaller than at 1.5 GHz, which requires an additional frequency-dependent contribution to relaxation. Because of the similarities in structures, it is proposed that the thermally-activated process that contributes to the relaxation of **1**, also contributes to the relaxation of **2**. The contribution shown in Fig. 4 for the thermally-activated process was calculated assuming the same parameters E_a and τ_c^0 as for **1**. To fit the data, decreasing C_{therm} requires a compensating change in C_A , within a range of about 7%. The combination of contributions from spin rotation, modulation of g and A anisotropy and the thermally-activated process is in excellent agreement with the experimental data (Fig. 4).

As shown in Eq. (1) – (4) spin lattice relaxation rates depend on both τ_R and resonance frequency. In ref. [18] the relaxation rates for nitronyl nitroxide **3** which is analogous to **2** but without the carboxyl group, were studied in toluene as a function of temperature. Based on analysis of the temperature dependence of $1/T_2 - 1/T_1$, the hydrodynamic radius of **3** was

found to be 1.5 times larger than for tempone [18]. Since τ_R is proportional to a^3 , the difference in radii predicts a ratio of τ_R values for **3** to that for tempone of ~ 3 , which is in good agreement with the ratio of τ_R values for **2** and **1** = 2.8. Based on these differences in τ_R our modeling indicates that the longer values of T_1 and T_2 for **3** than for tempone in toluene at 300 K [18] are due predominantly to the slower tumbling of **3** than of tempone, analogous to what was observed in our studies in water. We predict that if both a nitroxyl and a nitronyl had τ_R of 5 to 10 ps, the relaxation rates at X-band would be faster for the nitronyl than for tempone by up to 20%, due to the dominant effect of spin rotation. For longer τ_R in the range of 25 to 50 ps for both radicals, the X-band spin lattice relaxation rates for the nitronyl would be slower than for the nitroxyl by up to 30%. At Q-band, the nitronyl relaxation rates at the same τ_R , between 5 and 50 ps, are predicted to be faster than for tempone provided that only the mechanisms described by Eq (1) – (4) apply.

Conclusion

Spin lattice relaxation rates ($1/T_1$) for both PDT and nitronyl **2** between 250 MHz and 34 GHz are due predominantly to spin rotation and modulation of g and A anisotropy. The frequency dependence is dominated by the modulation of g and A anisotropy, and rates are approximately constant between about 250 MHz and 3 GHz. For both radicals, the smaller values of $1/T_1$ at 250 MHz than at 1.5 GHz are attributed to a contribution from thermally-activated process that has its maximum contribution at about 1.5 GHz. The decreasing ratio of $1/T_1$ for **2** to that for **1** with decreasing frequency is due to different ratios of contributions to relaxation at different frequencies. The slower spin lattice relaxation rate for **2** than for **1** at 34 GHz (Table 1) is due primarily to spin rotation and longer τ_R for **2** than for **1**. At the same τ_R relaxation rates at 9 GHz and higher would be faster for **2** than for **1** because of larger g anisotropy. The relaxation rates for **1** and **2** from 250 MHz to 3 GHz are more similar than at higher frequencies because modulation of A anisotropy is increasingly important at these frequencies, and $(\omega\tau_R)^2 < 1$, so the spectral density function is proportional to τ_R at lower frequencies. The longer τ_R for **2** than for **1** partially compensates for the smaller value of C_A for **2** than for **1** and the resulting values of $1/T_1$ differ by only a factor of 1.5 between 250 MHz and 3 GHz.

Acknowledgments

Partial support of this work by NIH grant P41 EB002034 to GMR and GRE, H. J. Halpern, PI, is gratefully acknowledged. We thank Prof. M. Brustolon for comments on this paper.

References

1. Berliner, L.J., editor. Spin Labeling II. New York: Academic Press; 1979.
2. Columbus L, Hubbell WL. A New Spin on Protein Dynamics. Trends in Biochem. Sciences. 2002; 27:288–295.
3. Eaton SS, Eaton GR. Relaxation times of organic radicals and transition metal ions. Biol. Magn. Reson. 2000; 19:29–154.
4. Robinson BH, Mailer C, Reese AW. Linewidth analysis of spin labels in liquids. I. Theory and data analysis. J. Magn. Reson. 1999; 138:199–209. [PubMed: 10341123]
5. Owenius R, Terry GE, Williams MJ, Eaton SS, Eaton GR. Frequency Dependence of Electron Spin Relaxation of Nitroxyl Radicals in Fluid Solution. J. Phys. Chem. B. 2004; 108:9475–9481.
6. Mailer C, Nielsen RD, Robinson BH. Explanation of Spin-Lattice Relaxation Rates of Spin Labels Obtained with Multifrequency Saturation Recovery EPR. J. Phys. Chem. A. 2005; 109:4049–4061. [PubMed: 16833727]
7. Hyde JS, Yin J-J, Subczynski WK, Camenisch TG, Ratke JJ, Froncisz W. Spin-labeled EPR T_1 Values Using Saturation Recovery from 2 to 35 GHz. J. Phys. Chem. B. 2004; 108:9524–9529.

8. Froncisz W, Camenisch TG, Ratke JJ, Anderson JR, Subczynski WK, Strangeway RA, Sidabras JW, Hyde JS. Saturation recovery EPR and ELDOR at W-band for spin labels. *J. Magn. Reson.* 2008; 193:297–304. [PubMed: 18547848]
9. Kao JPY, Barth ED, Burks SR, Smithback P, Mailer C, Ahn K-H, Halpern HJ, Rosen GM. Very-Low-Frequency Electron Paramagnetic Resonance (EPR) Imaging of Nitroxide-Loaded Cells. *Magn. Reson. Med.* 2007; 58:850–855. [PubMed: 17899588]
10. Subramanian S, Krishna MC. Time-Domain Radiofrequency EPR Imaging. *Biol. Magn. Reson.* 2005; 23:321–383.
11. Volodarsky, LB. Synthesis and Properties. Boca Raton: CRC Press; 1988. Imidazoline Nitroxides. Vol. I.
12. Rosen, GM.; Britigan, BE.; Halpern, HJ.; Pou, S. Free Radicals: Biology and Detection by Spin Trapping. Oxford: Oxford University Press; 1999. p. 225-226.
13. Armstrong BD, Han S. A new model for Overhauser enhanced nuclear magnetic resonance using nitroxide radicals. *J. Chem. Phys.* 2007; 127:104508/1–104508/10. [PubMed: 17867762]
14. Sezer D, Gafurov M, Prondolini MJ, Denysenkov VP, Prisner TF. Dynamic Nuclear Polarization of water by a nitroxide radical: rigorous treatment of the electron spin saturation and comparison with experiments at 9.3 Tesla. *Phys. Chem. Chem. Phys.* 2009; 11:6638–6653. [PubMed: 19639138]
15. Turke MT, Bennati M. Saturation factor of nitroxide radicals in liquid DNP by pulsed ELDOR experiments. *Phys. Chem. Chem. Phys.* 2011; 13:3630–3633. [PubMed: 21264371]
16. Sanvito S. Molecular spintronics. *Chem. Soc. Rev.* 2011; 40:3336–3355. [PubMed: 21552606]
17. Ratera I, Veciana J. Playing with organic radicals as building blocks for functional molecular materials. *Chem. Soc. Rev.* 2012; 41:303–349. [PubMed: 21850355]
18. Collauto A, Barbon A, Brustolon M. First determination of the spin relaxation properties of a nitronyl nitroxide in solution by electron spin echo at X-band: A comparison with Tempone. *J. Magn. Reson.* 2012; 223:180–186. [PubMed: 22975247]
19. Atkins, PW. Spin-Rotation Interaction. In: Muus, LT.; Atkins, PW., editors. *Electron Spin Relaxation in Liquids*. New York: Plenum Press; 1972. p. 279-312.
20. Robinson BH, Reese AW, Gibbons E, Mailer C. A unified description of the spin-spin and spin-lattice relaxation rates applied to nitroxide spin labels in viscous liquids. *J. Phys. Chem. B.* 1999; 103:5881–5894.
21. Robinson BH, Haas DA, Mailer C. Molecular dynamics in liquids: spin-lattice relaxation of nitroxide spin labels. *Science.* 1994; 263:490–493. [PubMed: 8290958]
22. Biller JR, Meyer V, Elajaili H, Rosen GM, Kao JPY, Eaton SS, Eaton GR. Relaxation Times and Line Widths of Isotopically-Substituted Nitroxides in Aqueous Solution at X-band. *J. Magn. Reson.* 2011; 212:370–377. [PubMed: 21843961]
23. Bloembergen N, Purcell EM, Pound RV. Relaxation Effects in Nuclear Resonance Absorption. *Phys. Rev.* 1948; 73:679–712.
24. Eaton SS, Harbridge J, Rinard GA, Eaton GR, Weber RT. Frequency dependence of electron spin relaxation for three $S = 1/2$ species doped into diamagnetic solid hosts. *Appl. Magn. Reson.* 2001; 20:151–157.
25. Freed, JH. Theory of slow tumbling ESR spectra of nitroxides. In: Berliner, LJ., editor. *Spin Labeling: Theory and Applications*. New York: Academic Press; 1976. p. 53-132.
26. Hwang JS, Mason RP, Hwang L-P, Freed JH. Electron spin resonance studies of anisotropic rotational reorientation and slow tumbling in liquid and frozen media. III. Perdeuterated 2,2,6,6-tetramethyl-4-piperidone-N-oxide and an analysis of fluctuating torques. *J. Phys. Chem.* 1975; 79:489–511.
27. Percival PW, Hyde JS. Saturation-recovery measurements of the spin-lattice relaxation times of some nitroxides in solution. *J. Magn. Reson.* 1976; 23:249–257.
28. Ullman EF, Osiecki JH, Boocock DGB, Darcy R. Studies of stable free radicals. X. Nitronyl nitroxide monoradicals and biradicals as possible small molecule spin labels. *J. Am. Chem. Soc.* 1972; 94:7049–7059.

29. Akaike T, Yoshida M, Miyamoto Y, Sato K, Kohno M, Sasamoto K, Miyazaki K, Ueda S, Maeda H. Antagonistic action of imidazolineoxyl N-oxides against endothelium-derived relaxation factor/ $\bullet$ NO through a radical reaction. *Biochemistry*. 1993; 32:827–832. [PubMed: 8422387]
30. Quine RW, Eaton GR, Eaton SS. Pulsed EPR spectrometer. *Rev. Sci. Instrum.* 1987; 58:1709–1723.
31. Quine RW, Rinard GA, Ghim BT, Eaton SS, Eaton GR. A 1-2 GHz pulsed and continuous wave electron paramagnetic resonance spectrometer. *Rev. Sci. Instrum.* 1996; 67:2514–2527.
32. Quine RW, Rinard GA, Eaton SS, Eaton GR. A pulsed and continuous wave 250 MHz electron paramagnetic resonance spectrometer. *Magn. Reson. Engineer.* 2002; 15:59–91.
33. Rinard GA, Quine RW, Ghim BT, Eaton SS, Eaton GR. Dispersion and superheterodyne EPR using a bimodal resonator. *J. Magn. Reson. A*. 1996; 122:58–63.
34. Rinard GA, Quine RW, Eaton GR. An L-Band crossed-loop (bimodal) EPR resonator. *J. Magn. Reson.* 2000; 144:85–88. [PubMed: 10783276]
35. Rinard GA, Quine RW, Eaton GR, Eaton SS. 250 MHz crossed loop resonator for pulsed electron paramagnetic resonance. *Magn. Reson. Engineer.* 2002; 15:37–46.
36. Quine RW, Eaton SS, Eaton GR. Saturation recovery electron paramagnetic resonance spectrometer. *Rev. Sci. Instrum.* 1992; 63:4251–4262.
37. Popp CA, Hyde JS. Electron-electron double resonance and saturation-recovery studies of nitroxide electron and nuclear spin-lattice relaxation times and Heisenberg exchanges rates: lateral diffusion in dimyristoyl phosphatidylcholine. *Proc. Nat. Acad. Sci. U.S.* 1982; 79:2559–2563.
38. Yin YJ, Hyde JS. Use of high observing power in electron spin resonance saturation-recovery experiments in spin-labeled membranes. *J. Chem. Phys.* 1989; 91:6029–6035.
39. Borgia GC, Brown RJS, Fantazzini P. Uniform-penalty Inversion of Multiexponential Decay Data II. Data Spacing, T2 Data, Systematic Errors, and Diagnostics. *J. Magn. Res.* 2000; 147:273–285.
40. Borgia GC, Brown RJS, Fantazzini P. Uniform-penalty Inversion of Multiexponential Decay Data. *J. Magn. Res.* 1998; 132:65–77.
41. Sato H, Bottle SE, Blinco JP, Micallef AS, Eaton GR, Eaton SS. Electron spin-lattice relaxation of nitroxyl radicals in temperature ranges that span glassy solutions to low-viscosity liquids. *J. Magn. Reson.* 2008; 191:66–77. [PubMed: 18166493]
42. Kivelson D. Theory of EPR [electron paramagnetic resonance]. line widths of free radicals J. *Chem. Phys.* 1960; 33:1094–1106.
43. Chasteen ND, Hanna MW. Electron Paramagnetic Resonance Line Widths of Vanadyl (IV) ahydroxycarboxylates. *J. Phys. Chem.* 1972; 76:3951–3958.
44. Barone V, Brustolon M, Cimino P, Polimeno A, Zerbetto M, Zoleo A. Development and Validation of an Integrated Computational Approach for the Modeling of CW-ESR Spectra of Free Radicals in Solution: p-(methylthio)phenyl Nitronyl nitroxide in Toluene as a Case Study. *J. Amer. Chem. Soc.* 2006; 128:15865–15873. [PubMed: 17147399]

Research Highlights

- Tumbling correlation times in H₂O are 9 ps for **1** and 25 ps for **2**.
- For **1** and **2** in H₂O $T_1 \sim T_2$ at resonance frequencies below 3 GHz.
- The frequency dependence of $1/T_1$ was modeled with spin rotation, modulation of anisotropy, and a thermally activated process.

1

2

Figure 1.
Structures of Nitroxide **1** (PDT) and Nitronyl Nitroxide **2**.

Figure 2. Frequency dependence of $1/T_1$ ($\square$) and $1/T_2$ ($\circ$) for (a) 0.5 mM **1**, $m_I = 0$ and (b) 0.5 mM **2**, $M_I = 0$ in aqueous solution at 22°C from 250 MHz to 34 GHz. .

Figure 3.

The frequency dependence of $1/T_1$ for **1** (□) was modeled (—) as the sum of contributions from spin rotation (—) (Eq. 1), modulation of g and A anisotropies (—·—) (Eq. 2 – 4), and a thermally-activated process (—·—) (Eq. 5). Parameters are discussed in the text.

Figure 4.

The frequency dependence of $1/T_1$ for **2** (□) was modeled (— — —) as the sum of contributions from spin rotation (—) (Eq. 1), modulation of g and A anisotropies (— · —) (Eq. 3, 4, 6), and a thermally-activated process (— · · —) (Eq. 5). Parameters are discussed in the text.

\$watermark-text

\$watermark-text

\$watermark-text

Table 1

Frequency Dependence of T₁ (μs) in deoxygenated H₂O at 22° C.^a

Frequency (GHz) \mI	PDT (1)			Nitronyl (2)		
	+1	0	-1	+1	0	-1
0.25	0.58	0.60	0.56	0.60	0.90	0.60
1.5	0.48	0.48	0.46	0.54	0.76	0.50
3.0	0.51	0.50	0.46	0.64	0.87	0.60
9.5	0.61	0.58	0.52	1.04	1.24	0.84
34.5	1.0	1.1	1.0	2.1	2.1	2.1

^aStandard deviations for replicate measurements were about 3%.

Table 2

Frequency Dependence of T_2 (μs) in deoxygenated H_2O at 22°C .^a

Frequency (GHz) \ m_I	PDT (1)			Nitronyl (2)		
	+1	0	-1	+1	0	-1
0.25	0.55	0.58	0.55	0.56	0.69	0.44
1.5	0.39	0.41	0.37	0.45	0.60	0.39
3.0	0.42	0.43	0.38	0.46	0.62	0.40
9.5	0.49	0.47	0.38	0.61	0.71	0.44
34.5	0.52	0.42	0.31	0.46	0.38	0.25

^aStandard deviations for replicate measurements were about 3%.

\$watermark-text

\$watermark-text

\$watermark-text

Table 3

g- and A- values.

	g_{xx}	g_{yy}	g_{zz}	A_{xx} (G)	A_{yy} (G)	A_{zz} (G)
1^1	2.0092	2.0061	2.0022	5.5	6.3	35.6
2^2	2.0110	2.0065	2.0021	3.6	3.6	18.6

1 From [26]
 2 From [11]