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Multifrequency Pulsed EPR and the Characterization of Molecular Dynamics

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Abstract

In fluid solution motion-dependent processes dominate electron spin lattice relaxation for nitroxides and semiquinones at frequencies between 250 MHz and 34 GHz. For triarylmethyl radicals motion-dependent processes dominate spin lattice relaxation at frequencies below about 3 GHz. The frequency dependence of relaxation provides invaluable information about dynamic processes occurring with characteristic times on the order of the electron Zeeman frequency. Relaxation mechanisms, methods of measuring spin-lattice relaxation, and motional processes for nitroxide, semiquinone, and triarylmethyl radicals are discussed.

Keywords

intramolecular motions; inversion recovery; molecular tumbling; saturation recovery; solvent motions

Introduction

Extensive studies of nitroxide lineshapes have demonstrated the dependence of electron spin-spin relaxation (T_2) on molecular tumbling (Budil, Earle, & Freed, 1993; Earle, Budil, & Freed, 1993; Freed, 1976). In fluid solution motion-dependent processes also dominate electron spin-lattice relaxation (T_1), but have been less extensively studied than for T_2 . Molecular motions impact T_1 via processes that depend solely on the tumbling correlation time, τ_R , or on the product $\omega\tau$, where ω is the Zeeman frequency and τ is the characteristic time for the process. To distinguish between these types of processes it is very helpful to measure T_1 as a function of Zeeman frequency and of tumbling correlation time. Processes that have been characterized by studying the frequency dependence of T_1 include molecular tumbling, intramolecular dynamics, and solvent motions (Biller et al., 2013; Elajaili, Biller, Eaton, & Eaton, 2014; Hyde et al., 2004; Owenius, Eaton, & Eaton, 2005; Owenius, Terry, Williams, Eaton, & Eaton, 2004). In this chapter the mechanisms that contribute to electron spin relaxation of radicals in fluid solution are discussed as the basis for designing experiments to use the frequency dependence of relaxation to elucidate motions. More extensive discussion of relaxation mechanisms can be found in (Eaton & Eaton, 2000). Practical issues related to pulse methods for measuring T_1 as a function of Zeeman frequency are then discussed, followed by examples of applications to nitroxides,

triarylmethyl radicals (trityls), and semiquinones. With the emerging availability of commercial pulse EPR spectrometers at frequencies other than X-band, multi-frequency measurements are becoming more accessible to a wider range of user.

Contributions to Electron Spin Lattice Relaxation and Dependence of Relaxation on Motions in Fluid Solution

The local mode relaxation mechanism (Eq. 1) was initially proposed for defects in ionic lattices (Castle & Feldman, 1965).

$$\frac{1}{T_1^{\text{local}}} = C_{\text{local}} \frac{e^{\Delta_{\text{loc}}/T}}{(e^{\Delta_{\text{loc}}/T} - 1)^2} \quad (1)$$

where Δ_{loc} is the energy of the local mode in Kelvin and C_{local} is determined experimentally. The term local mode distinguishes this mechanism from interaction of the spins with long-range (phonon) modes. For a molecular species in a glass the local mode is interpreted as an intramolecular vibration that is impacted by interaction with the matrix. In some cases the energy of the local mode can be matched to a specific vibrational frequency of the species studied (Eaton & Eaton, 2000). In glassy matrices the local mode dominates relaxation for nitroxides (Sato et al., 2008; Sato et al., 2007), semiquinones (Kathirvelu, Sato, Eaton, & Eaton, 2009) and trityl radicals (Fielding, Carl, Eaton, & Eaton, 2005) below the glass transition temperature. For these samples in solvents that are highly viscous above the glass transition temperature there is no change in slope for a plot of $\log(1/T_1)$ vs. $\log(T)$ in the vicinity of the glass transition temperature. It is therefore proposed that the local mode relaxation mechanism persists in solution and dominates spin lattice relaxation when molecular tumbling is slow.

Spin rotation (Eq. 2) arises from coupling between spin angular momentum and rotational angular momentum (P. W. Atkins & Kivelson, 1966; Muus & Atkins, 1972).

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

where $i = x, y, z$, g_e is 2.0023 and τ_R is the tumbling correlation time.

Since this contribution depends inversely on τ_R , it can be distinguished from other mechanisms by changing the tumbling correlation time. Tumbling has been varied by changing viscosity either changing the composition of solvent mixtures ((Owenius et al., 2005; Owenius et al., 2004)) or by changing temperature ((Kundu, Kattinig, Mladenova, Grampp, & Das, 2015; Percival & Hyde, 1976; Rengan, Khakhar, Prabhananda, & Venkataraman, 1974; Robinson, Haas, & Mailer, 1994)).

Studies of the frequency dependence of spin-lattice relaxation for carbon-centered trityl radicals (Owenius et al., 2005), nitrogen-centered nitroxide radicals (Biller et al., 2013; Biller et al., 2012; Hyde et al., 2004), and semiquinones (Elajaili et al., 2014) in fluid solution have demonstrated the significance of additional relaxation mechanisms. These frequency-dependent processes modulate anisotropic interactions – g anisotropy (Eq. 3) (Robinson et al., 1994; Robinson, Mailer, & Reese, 1999), nitroxide nitrogen hyperfine (A_j) anisotropy (Eq. 5) (C. Mailer, Nielsen, & Robinson, 2005; Robinson et al., 1994; Robinson et al., 1999), dipolar coupling to protons in the molecule or in the solvent (Eq. 6) (Owenius et al., 2005), or involve a thermally-activated process (Eq. 7) (Biller et al., 2013).

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

where $g = g_{zz} - 0.5(g_{xx} + g_{yy})$, $\delta g = 0.5(g_{xx} - g_{yy})$, μ_B is the electron Bohr magneton and $J(\omega)$ is the Bloembergen, Purcell, Pound (BPP) spectral density function (Eq. 4).

$$J(\omega) = \frac{\tau_R}{1 + (\omega\tau_R)^2} \quad (4)$$

where τ_R is the tumbling correlation time of the radical and ω is the resonance frequency.

$$\frac{1}{T_1^A} = \frac{2}{9} I(I+1) \sum_i (A_i - \bar{A})^2 J(\omega) \quad (5)$$

where A_j is a component of the nitroxide nitrogen hyperfine coupling in angular frequency units, $\bar{A}$ is the average nitrogen hyperfine, and I is the nitrogen nuclear spin. Although derived for nitrogen nuclear hyperfine interaction, this equation is applicable for any $I = 1/2$ or $I = 1$ nuclear interaction.

$$\frac{1}{T_1^{\text{protons}}} = C_{\text{protons}} \frac{\tau_C}{1 + (\omega\tau_C)^2} \quad (6)$$

where τ_C is the correlation time for motion that modulates the electron-proton dipolar coupling, and C_{protons} is a function of the dipolar interactions with the protons.

$$\frac{1}{T_1^{\text{therm}}} = C_{\text{therm}} \left(\frac{\omega}{\omega_{\text{ref}}} \right) \frac{\tau_{\text{therm}}}{1 + (\omega\tau_{\text{therm}})^2} \quad (7)$$

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. Variable temperature studies of relaxation for nitroxide radicals also provided evidence for a thermally-activated process (Kundu et al., 2015).

When τ_c is varied, the contributions to relaxation described by equations 3, 5, 6, go through a maximum when $\omega\tau_c \sim 1$. When ω is varied these contributions have a sigmoidal dependence and an inflection point at $\omega\tau \sim 1$. The contribution from the thermal process (Eq. 7) is maximum when $\omega\tau \sim 1$. Because of these frequency dependences a process that is negligible at ca. 9 GHz may become dominant at another frequency. Variable frequency experiments are particularly valuable for characterization of these types of processes.

Measurement of Spin Lattice Relaxation, T_1

Early measurements of electron spin relaxation relied on analysis of CW power saturation curves, which depend on the $T_1 T_2$ product, and were more error prone than direct measurement by pulse techniques (Fig. 1). A comparison of values of T_1 and ‘effective’ values of T_1 obtained by these methods for organic radicals undergoing dynamic processes in irradiated solids is in (Harbridge, Eaton, & Eaton, 2003). For accurate T_1 measurements solutions should be thoroughly deoxygenated either by freeze-pump-thaw or by extensive purging with nitrogen gas. To avoid solvent evaporation when purging with nitrogen it is useful to put the sample in a gas permeable tube made of TPX (Hyde & Subczynski, 1989) or thin-wall Teflon tubing (Biller et al., 2011; Owenius et al., 2005; Swartz, Chen, Pals, Sentjurs, & Morse, 1986) and pass the nitrogen over the exterior of the tube. The extent to which T_1 is concentration dependent varies both with radical and with solvent, so it should be checked for each system.

Long-pulse saturation recovery

The ‘gold standard’ for measurement of T_1 is long pulse saturation recovery (Fig. 1a), which was initially developed by Rengan and co-workers (Rengan et al., 1974) and subsequently used extensively by Hyde and co-workers (Huisjen & Hyde, 1974; Hyde, 1979). In this method a long on-resonance saturating pump pulse is followed by low-power CW detection of the EPR signal (Percival & Hyde, 1975; Quine, Eaton, & Eaton, 1992; Rengan, Bhagat, Sastry, & Venkataraman, 1979). If the pump pulse is long enough, it saturates not only the electron spin transition that is on resonance, but also transitions that are accessible by spin diffusion. The recovery from saturation is then dominated by spin lattice relaxation, and the recovery curve should fit well to a single exponential. If the pump pulse is not long enough to saturate all accessible transitions, the time constant for the recovery curve may be decreased or the curve may fit better to the sum of two exponentials than to a single exponential. To ensure that the spin diffusion processes are saturated, the length of the pump pulse is extended until there is no further change in the time constant for the recovery (C. Mailer, Danielson, & Robinson, 1985).

If the microwave B_1 that is used during the low-power detection of the signal recovery is too high, it can contribute to equilibration of the spin state populations, and decrease the time constant for the recovery. To check for potential impact of B_1 , recovery curves are recorded

at decreasing values of B_1 , until there is no further change in the recovery time constant. Alternatively the reciprocal of the recovery time constant can be plotted as a function of B_1 and extrapolated to determine the limiting value.

An advantage of saturation recovery is that it can be applied to spin systems in which T_2 is too short to permit detection of a spin echo or one for which echo envelope modulation is deep. A disadvantage of saturation recovery is low signal-to-noise (S/N) because of the need to use low B_1 . To improve the S/N , experiments are performed with relatively high Q resonators, which have a rather narrow bandwidth. Bruker X-band CW resonators have Q in the range of 3000 to 9000, which corresponds to 3 dB signal bandwidths of 3 to 1 MHz, respectively. This is less than a 1 gauss segment of the spectrum. Because of the requirement for small B_1 the tip angle for these spins is small and only a small fraction of the spin magnetization is detected.

Inversion Recovery

Inversion recovery experiments (Fig. 1b) benefit from the fact that short high-power pulses excite a very large fraction of the spins at resonance within the relatively large bandwidth of the resonator Q and the tip angles are large so the full magnetization is detected. For example an X-band resonator with Q about 100, as is typical for pulse experiments, has a 3 dB signal bandwidth of 95 MHz. If relaxation processes, including spectral diffusion are not too fast, a 180° pulse can invert a large fraction of the spins within the resonator bandwidth, which provides a much larger initial signal than is observed by low power CW. This provides substantially improved S/N compared with CW saturation recovery. A disadvantage of inversion recovery is that any process that takes spins off resonance contributes to the inversion recovery time constant, not just spin lattice relaxation. Thus spectral diffusion processes that are faster than $1/T_1$ contribute to the recovery curve.

On most pulse spectrometers the initial inverting pulse (Fig. 1b) can be replaced with a picket fence of pulses. The effectiveness of the experiment in saturating spectral diffusion processes is strongly dependent on the pulse lengths and spacing within the picket fence. For rapidly tumbling radicals in solution inversion recovery often gives single exponential recoveries with time constant that is T_1 . However, for slower tumbling or immobilized radicals saturation recovery or picket fence inversion recovery is needed to measure T_1 (Eaton & Eaton, 2000).

Echo-detected saturation recovery

If the spectrometer has a path within the bridge that permits creation of a low-power pulse, echo-detected saturation recovery (Fig. 1c) can be performed (Harbridge et al., 2003). However the length of the saturating pulse may be limited by the duty cycle of the TWT power amplifier.

Pulsed ELDOR

For some samples it is important to characterize processes such as nuclear spin relaxation or cross-relaxation that occur on a time scale similar to T_1 , which is best done with pulsed electron-electron double resonance (ELDOR). For these experiments the pump pulse is

applied at a different frequency than the observe pulses. The contributions to the recovery curve from competing processes may have opposite signs, which makes separation of contributions more accurate than when fitting to a sum of exponential with the same signs of the coefficients as in saturation recovery or inversion recovery curves (Hyde, Froncisz, & Mottley, 1984; Rengan et al., 1979).

Analysis of recovery curves

At the University of Denver comparisons were performed between values of T_1 obtained by three different operators on locally-designed and Bruker spectrometers. The same deoxygenation protocol was used for all samples. When averages of three or more replicates were compared, the standard deviations, agreement between instrument operators, and between instruments was about 5% for inversion recovery and about 10% for saturation recovery when S/N was at least 60 (Meyer, 2014).

A key issue is the need to determine whether a recovery curve can be fit well with a single exponential. In our lab the first step is least squares fitting to models based on 1 or 2 exponentials. It is helpful to look at the residuals between calculated and observed curves because systematic deviations, especially at early times in the curves, may be better indicators of goodness of fit than consideration only of the RMS error. Good signal to noise is crucial for making a distinction between fits to 1 and 2 exponentials and we typically signal average enough to achieve at least $S/N > 100$. In some systems there also may be distributions of relaxation times, which can be characterized by modeling with the uniform penalty (UPEN) method (Borgia, Brown, & Fantazzini, 1998; Borgia, Brown, & Fantazzini, 2000). That approach searches for the distribution of exponentials that most closely models the experimental data. The UPEN method is also a useful method to distinguish between distributions and sums of two exponentials.

For example, for nitroxide radicals in fluid solution the nitrogen nuclear spin relaxation T_{1N} depends on τ and ω (Hyde et al., 1984; Popp & Hyde, 1982; Robinson et al., 1994). In the fast tumbling limit T_{1N} is equal to the electron spin T_1 . For τ_R less than about 20 ps and at X-band frequencies or below, both inversion recovery and saturation recovery curves for nitroxides fit well with a single exponential, showed a single peak in the distribution calculated with UPEN, and there was good agreement between values of T_1 obtained by inversion recovery and saturation recovery (Meyer, 2014). These observations are all consistent with the expectation of $T_{1N} \sim T_1$. However at Q-band the inversion recovery curves for nitroxides with τ_R in the range of 9 to 19 ps fit better to the sum of two exponentials and the UPEN analyses reproducibly showed two components (Fig. 2). The recovery curves for these analyses had S/N greater than 100. Even with very good S/N there is more uncertainty in relaxation times obtained by fitting to two exponentials than one exponential. The T_1 values at the two maxima in the distributions obtained with UPEN were in good agreement with the two values obtained by fitting the recovery curves to the sum of two exponentials. These observations are consistent with the expectation of $T_{1N} < T_1$ at higher resonance frequencies (Hyde et al., 1984; Popp & Hyde, 1982).

Examples of motions elucidated by measuring T_1 as a function of frequency

Motional process for nitroxides

Although the focus on the impact of fluid solution motion on nitroxide relaxation times has been mostly for T_2 and linewidths (Freed, 1976), motional processes also have dramatic impact on T_1 for nitroxides (C. Mailer et al., 2005; Robinson et al., 1994). Most of the fundamental studies of nitroxide relaxation times were performed at X-band (Fajer, Thomas, Feix, & Hyde, 1986; Percival & Hyde, 1976; Robinson et al., 1994), and these studies highlighted the importance of spin rotation and modulation of the nitrogen nuclear hyperfine interaction by molecular tumbling. Typical anisotropic g values for nitroxides are about $g_x \sim 2.0092$, $g_y \sim 2.0063$, and $g_z \sim 2.0023$ (Berliner, 1976; Biller et al., 2013; Griffith, Cornell, & McConnell, 1965). These values differ substantially from the free electron g value and as a result spin rotation (Eq. 2) is an important tumbling-dependent contribution to relaxation, as was recognized early in the studies of spin relaxation (P. W. Atkins, 1972; Percival & Hyde, 1976). Since this contribution increases as τ_R decreases, it tends to dominate in the fast tumbling regime. The substantial anisotropy of the nitroxide nitrogen hyperfine interactions means that modulation of that interaction by molecular tumbling (Eq. 5) makes major contributions in the intermediate tumbling regime (C. Mailer et al., 2005; Robinson et al., 1994).

In the past decade the availability of pulse spectrometers operating at frequencies other than X-band has increased the opportunity to measure relaxation times as a function of frequency. Water:glycerol mixtures have been used to examine the tumbling dependence of T_1 for piperidinyl nitroxides including tempol- d_{17} at 9.2, 3.1, and 1.9 GHz (Owenius et al., 2004). For $\tau_R < 2$ ns tumbling dependent processes dominated the relaxation, but at longer correlation times the relaxation was dominated by a tumbling-independent frequency-dependent process. A contribution from a thermally-activated process (Eq. 7) was proposed with $E_a = 1100$ K (9 kJ/mol) and $\tau_{\text{therm}} = 1.1 \times 10^{-10}$ s.

Results from a study of the frequency dependence of $1/T_1$ between 250 MHz and 34 GHz are shown in Fig. 3 for PDT in solvents that result in τ_R between 4 and 50 ps (Biller et al., 2013). For $\tau_R < \sim 20$ ps and resonance frequencies > 9 GHz, $1/T_2$ is $\sim 1/T_1$ as expected in the fast tumbling regime. At 34 GHz, $1/T_2 - 1/T_1$ is substantial (Fig. 3) due to increased contributions to T_2 from incomplete motional averaging of g-anisotropy. At $\tau_R = 4$ ps spin rotation dominates $1/T_1$ and relaxation is independent of frequency (Figure 3A). The agreement between experiment and calculation at $\tau_R = 4$ ps is not as good as at longer τ_R . For the small PDT molecule in low-viscosity toluene, Heisenberg exchange due to nitroxide-nitroxide collisions, which is not included in the simulation, may contribute more to relaxation than for other samples in that study. As τ_R increases the frequency-independent contribution from spin rotation (Eq. 2) decreases, the contributions from modulation of A and g-anisotropy (Eq. 3–5) increase, and the relaxation at frequencies less than 34 GHz changes from domination by spin rotation to domination by modulation of A anisotropy (Fig. 3). The frequency at which the contribution from modulation of A changes from frequency independent to frequency dependent is a strong function of τ_R (Eq. 3–5). The longer the value of τ_R , the lower the frequency at which the change in frequency dependence occurs. Thus the agreement between the observed and calculated frequency dependence of $1/T_1$ is a validation of the model and of the values of τ_R . For $\tau_R = 9$ ps, in either water (Fig. 3B) or light mineral oil (Fig. 3C), $1/T_1$ is smaller at 250 MHz than at 1–2 GHz. This frequency dependence is not predicted by spin rotation (Eq. 2) or modulation of A or g-anisotropy (Eq. 3–5). Modeling of the relaxation rates therefore included a contribution from a thermally-activated process, Eq. (7). The effect of the thermally-activated process is maximum when $\omega = 1/\tau_{\text{therm}}$, which was observed to occur at about 1.6 GHz (Fig. 3), and corresponds to $\tau_{\text{therm}} = 1.0 \times 10^{-10}$ s. This is the same value of τ_{therm} that was used to model the frequency dependence of $1/T_1$ for tempol with $\tau_R > \sim 3 \times 10^{-9}$ s (300 ps) in viscous water:glycerol mixtures (Owenius et al., 2004). The value of C_{therm} was similar in water (Fig. 3B) or light mineral oil (Fig. 3C), which indicates that the process does not require a protic solvent. C_{therm} is smaller in 44% glycerol (Fig. 3D), which demonstrates a dependence on viscosity (Biller et al., 2013). For comparison, the contributions to $1/T_1$ from

the thermally-activated process for tempol with $\tau_R > \sim 3 \times 10^{-9}$ s in viscous water:glycerol mixtures (Owenius et al., 2004) is shown as gold squares in Fig. 3E, and is negligibly small relative to the contributions from modulation of A and g anisotropy at $\tau_R = 50$ ps.

The characteristic time for the thermally-activated process of about 1×10^{-10} s at 295 K, is longer than the τ_R of $(10 \text{ to } 50) \times 10^{-12}$ s (Fig. 3) (Biller et al., 2013). Thus the thermally-activated process modulates an interaction that is not averaged by τ_R , which is inconsistent with modulation of anisotropic dipolar couplings. The values of C_{therm} decrease with increasing solvent viscosity, in the order PDT in water (Fig. 3B) > PDT in 44% glycerol (Fig. 3D) > tempol in highly viscous solvents (Owenius et al., 2004). Values of C_{therm} are similar for 5- and 6-membered rings, but are substantially smaller for nitroxides with more rigid rings and larger for ^{14}N than for ^{15}N (Biller et al., 2013). These observations are consistent with assignment of the thermally-activated process as modulation of the nitrogen isotropic A.

The contribution from a thermally-activated process to spin lattice relaxation of nitroxides was confirmed by variable temperature saturation recovery studies at X-band (Kundu et al., 2015). The E_a (Eq. 7) for this process for tempol and tempol in ionic liquids, sec-butyl benzene, and di-isononyl phthalate ranged from 800 to 1200 K, which is similar to the value of 1100 K for PDT in water:glycerol mixtures (Owenius et al., 2004).

One possible mechanism for modulation of g or A is conformational changes of the geometry at the nitroxide nitrogen. Substantial temperature dependence of nitroxide g and A-values in fluid solution has been observed and attributed to fast exchange between conformations (Siri, Guadel-Siri, & Tordo, 2002). The large differences in rates of interconversion of ring conformations for 5- and 6-member ring nitroxides (Rockenbauer, Korecz, & Hideg, 1993) and estimates of lifetimes substantially longer than 10^{-10} s at 298 K (Barbon, Brustolon, Maniero, Romanelli, & Brunel, 1999) indicate that interconversion of ring conformations is too slow to be assigned as the thermally-activated process for nitroxide relaxation. Recent molecular dynamics calculations have shown that the intramolecular out-of-plane motion of the N-O moiety is a 'soft' mode (Pavone, Biczysko, Rega, & Barone, 2010; Savitsky, Plato, & Mobius, 2010). For both 5- and 6-member ring nitroxides the angle

between the N-O group and the plane of the molecule can vary by $\pm 10^\circ$ within a low-energy range of about 0.5 kcal/mole (Savitsky et al., 2010). This angular variation is sufficient to cause substantial changes in the isotropic g and A values. Modulation of either or both of these parameters is an effective spin-lattice relaxation process. Modulation of isotropic A and g would be consistent with the observed nitrogen isotope effect on C_{therm} (Biller et al., 2013). The dependence of C_{therm} on viscosity could indicate that a smaller range of orientations is encompassed by these motions when solvent viscosity is higher. The value of τ_{therm} also is approximately what is predicted for methyl rotation at room temperature based on ENDOR studies of tempone (Barbon et al., 1999). In these sterically hindered molecules methyl rotation may couple to the motion of the NO bond and be involved in the bond angle variation that dominates the thermally-activated process.

Tumbling of trityl radicals

The absence of a large nuclear hyperfine coupling means that the dependence of linewidths on tumbling correlation time that has been so useful in determining tumbling correlation times for nitroxides (Freed, 1976) does not have an analogy for trityl radicals. This absence also means that modulation of nuclear hyperfine coupling (Eq. 5) is not a contributor to trityl T_1 . Trityl radicals have g values near 2.0027 and g anisotropy is small (Fielding et al., 2005), so spin rotation and modulation of g anisotropy also are relatively ineffective relaxation mechanisms. Thus relaxation times for trityls are longer than for nitroxides or semiquinones for the same tumbling correlation times and temperatures (Owenius et al., 2005).

The peak-to-peak linewidths in the fluid solution spectra of trityls such as trityl-CH₃, OX63, or OX31 are less than 200 mG (Fielding et al., 2005) because there are few nuclear spins in proximity to the unpaired electron and hence inhomogeneous broadening of the lines is small. For these narrow linewidths the signals are fully excited by the pulses in the inversion recovery sequence and spectral diffusion is not expected to contribute to the recovery curves. Values of T_1 obtained by saturation recovery and inversion recovery at X-band were in good agreement, which confirmed that spectral diffusion processes make negligible contributions to the inversion recovery curves (Owenius et al., 2005). At X-band the temperature dependence of $1/T_1$ led to the proposal that the relaxation was dominated by a local mode (Fielding et al., 2005). The electron spin relaxation rates for these four trityl radicals in

water at room temperature increase as frequency is decreased, and the magnitudes of the changes are molecule-dependent (Fig. 4) (Owenius et al., 2005).

To determine the extent to which molecular tumbling contributes to the frequency and structural dependence of T_1 , the solution viscosity was varied by changing the ratio of glycerol to water (Owenius et al., 2005). At 250 MHz the relaxation rates are strongly dependent on viscosity, which was not observed at X-band (Fig. 5), consistent with an additional contribution to relaxation at lower frequency. The enhanced relaxation at 250 MHz is attributed to modulation of electron-proton dipolar coupling (Eq. 6). Deuteration of the solvent was used to distinguish relaxation due to solvent protons from relaxation due to intramolecular electron-proton interactions. When τ_C is varied, the maximum relaxation rate occurs when $\omega\tau_C \sim 1$ (Eq. 6). At 250 MHz this maximum occurs when $\tau_C \sim 0.63$ ns. For the proton-containing trityls the percent glycerol for the maximum relaxation rate decreases in the order trityl-CH₃ (~20%) > OX63 (~10%) > OX31 (~5%), which parallels the increase in molar masses trityl-CH₃ (975) < OX63 (1335) < OX31 (1695). The smaller the molecule, the higher the viscosity that is needed to achieve the same tumbling correlation time. τ_C was therefore assigned as the tumbling correlation time for the trityl. The viscosity and frequency dependence of T_1 was modeled based on dipolar interaction with a defined numbers of protons at specified distances from the unpaired electron. For trityl-CD₃, which contains no protons, modulation of dipolar interaction with solvent protons dominates T_1 at 250 MHz, and τ_C is assigned as the correlation time for motion of the solvent molecules. For trityl-CD₃ the maximum relaxation rate was at about 50% glycerol (Owenius et al., 2005).

Hydrogen bonding interactions with semiquinones

For 25DTBSQ in C₂H₅OH or CH₃OH (Fig. 6), the spin lattice relaxation rates were about a factor of two faster at lower frequencies than at 9 GHz (Elajaili et al., 2014). The solid lines in Fig. 6 were calculated as the sum of contributions from two frequency-independent processes, (spin rotation (Eq. 2) and a local mode (Eq. 1)), and two frequency-dependent processes (modulation of dipolar interaction with solvent nuclei (Eq. 6) and a smaller contribution from modulation of g -anisotropy (Eq. 3). Spin-lattice relaxation at X-band is dominated by frequency-independent processes and is unchanged by solvent deuteration. At 295 K the viscosity of C₂H₅OH (1.08 cP) is about twice that of CH₃OH (0.545 cP) so τ_R decreases by about a factor of 2 when the solvent is changed from C₂H₅OH to CH₃OH and the relaxation rates at X-band increase (Fig. 6B). Comparison of relaxation rates for 25DTBSQ in C₂H₅OH and CH₃OH at X-band permitted separation of the tumbling-dependent spin-rotation contribution (Eq. 2) from the tumbling-independent local mode contribution (Eq. 1). The modulation of g anisotropy by molecular tumbling (Eq. 3) makes a relatively small contribution to $1/T_1$ and is significant for the semiquinones only at Q-band.

In both $\text{C}_2\text{H}_5\text{OH}$ and CH_3OH the semiquinone relaxation rates are strongly frequency dependent (Fig. 6) which suggests a major role for processes described by Eq. 6 (Elajaili et al., 2014). The dominant frequency-dependent contribution to $1/T_1$ was decreased by deuteration of the solvent (Fig. 6). The replacement of solvent OH by OD decreased the contribution from the frequency-dependent contribution by about a factor of two. Complete solvent deuteration eliminated this contribution, which demonstrated domination of the frequency dependent contribution by interaction with solvent nuclei. The contributions calculated using Eq. (6) are strongly dependent on the value of τ_{solvent} . The simulations required τ_{solvent} about twice as long as τ_R . The observation of $\tau_{\text{solvent}} > \tau_R$ indicates that the dynamic process that modulates the interaction between the unpaired electron and the solvent nuclei is not molecular tumbling. The OH proton is only one of 4 protons in CH_3OH and one of 6 protons in $\text{C}_2\text{H}_5\text{OH}$, so the factor of two reduction in the frequency-dependent contribution to relaxation (Eq. 6) when only the OH proton is replaced by a deuteron is substantially larger than predicted if all of the protons contributed equally. Water and alcohols are strongly hydrogen bonded to semiquinones (Ackermann, Barbarin, Germain, & Fabre, 1975; Flores, Isaacson, Calvo, Feher, & Lubitz, 2003; Paddock, Flores, Isaacson, Shepherd, & Okamura, 2010). The observations that (i) the contribution from Eq. (6) decreased by about a factor of 2 when only the OH was replaced by OD (Fig. 6), (ii) the frequency dependent contribution to relaxation was eliminated by solvent perdeuteration, and (iii) values of τ_{solvent} that differ from τ_R , are consistent with assignment of fluctuations in hydrogen bonding as the process that drives τ_{solvent} . It was concluded that hydrogen bonding to the semiquinone oxygens is the most probable source of the frequency-dependent contribution to relaxation (Elajaili et al., 2014).

Conclusions

The frequency dependence of electron spin relaxation provides invaluable information concerning dynamic processes that occur with characteristic time constants on the order of the Zeeman frequency.

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References

- Ackermann P, Barbarin F, Germain JP, Fabre C. Resonance Paramagnetique Electronique. Influence de la solvation sur la relaxation electronique anisotrope de radicaux stable neutres ou charges. Proceedings of the Colloquium Spectroscopicum Internationale. 1975; 2:440–445.
- Atkins, PW. Spin-Rotation Interaction. In: Muus, LT., Atkins, PW., editors. Electron Spin Relaxation in Liquids. New York: Plenum Press; 1972. p. 279–312.
- Atkins PW, Kivelson D. ESR Linewidths in Solution. II Analysis of Spin-Rotational Relaxation Data. J Chem Phys. 1966; 44:169–174.
- Barbon A, Brustolon M, Maniero AL, Romanelli M, Brunel LC. Dynamics and spin relaxation of tempone in a host crystal. An ENDOR, high field EPR and electron spin echo study. Phys Chem Chem Phys. 1999; 1:4015–4023.
- Berliner, LJ. Spin Labeling: Theory and Applications. New York: Academic Press; 1976. Principal Values of the g and Hyperfine Tensors for Several Nitroxides Reported to Date; p. 564–565.
- Biller JR, Elajaili H, Meyer V, Rosen GM, Eaton SS, Eaton GR. Electron Spin Lattice Relaxation Mechanisms of Rapidly-Tumbling Nitroxide Radicals. J Magn Reson. 2013; 236:47–56. [PubMed: 24056272]
- Biller JR, Meyer V, Elajaili H, Rosen GM, Kao JPY, Eaton SS, et al. Relaxation Times and Line Widths of Isotopically-Substituted Nitroxides in Aqueous Solution at X-band. J Magn Reson. 2011; 212:370–377. [PubMed: 21843961]
- Biller JR, Meyer VM, Elajaili H, Rosen GM, Eaton SS, Eaton GR. Frequency Dependence of Electron Spin Relaxation Times in Aqueous Solution for a Nitronyl Nitroxide Radical and Per-deuterated-Tempone between 250 MHz and 34 GHz. J Magn Reson. 2012; 225:52–57. [PubMed: 23123770]
- Borgia GC, Brown RJS, Fantazzini P. Uniform-penalty Inversion of Multiexponential Decay Data. J Magn Res. 1998; 132:65–77.
- 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.
- Budil DE, Earle KA, Freed JH. Full Determination of the Rotational Diffusion Tensor by Electron Paramagnetic Resonance at 250 GHz. J Phys Chem. 1993; 97:1294–1303.
- Castle JG Jr, Feldman DW. Temperature dependence of paramagnetic relaxation at point defects in vitreous silica. J Appl Phys. 1965; 36:124–128.
- Earle KA, Budil DE, Freed JH. 250-GHz EPR of Nitroxides in the Slow-Motional Regime: Models of Rotational Diffusion. J Phys Chem. 1993; 97:13289–13297.
- Eaton SS, Eaton GR. Relaxation times of organic radicals and transition metal ions. Biol Magn Reson. 2000; 19:29–154.
- Elajaili HB, Biller JR, Eaton SS, Eaton GR. Frequency Dependence of Electron Spin-lattice Relaxation for Semiquinones in Alcohol Solutions. J Magn Reson. 2014; 247:81–87. [PubMed: 25261741]
- Fajer P, Thomas DD, Feix JB, Hyde JS. Measurement of rotational molecular motion by time-resolved saturation transfer electron paramagnetic resonance. Biophys J. 1986; 50(6):1195–1202. [PubMed: 3026503]
- Fielding AJ, Carl PJ, Eaton GR, Eaton SS. Multifrequency EPR of Four Triarylmethyl Radicals. Appl Magn Reson. 2005; 28:239–249.
- Flores M, Isaacson RA, Calvo R, Feher G, Lubitz W. Probing Hydrogen Bonding to Quinone Anions Radicals by ¹H and ²H ENDOR Spectroscopy at 35 GHz. Chem Phys. 2003; 294:401–413.
- 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.
- Griffith OH, Cornell DW, McConnell HM. Nitrogen hyperfine tensor and g tensor of nitroxide radicals. J Chem Phys. 1965; 43:2909–2910.
- Harbridge JR, Eaton SS, Eaton GR. Electron Spin-Lattice Relaxation Processes of Radicals in Irradiated Crystalline Organic Compounds. J Phys Chem A. 2003; 107(5):598–610.
- Huisjen J, Hyde JS. Saturation recovery measurement of electron spin-lattice relaxation times. J Chem Phys. 1974; 60:1682–1683.

- Hyde, JS. Saturation Recovery Methodology. In: Kevan, L., Schwartz, RN., editors. Time Domain Electron Spin Resonance. New York: John Wiley; 1979. p. 1-30.
- Hyde JS, Froncisz W, Mottley C. Pulsed ELDOR measurement of nitrogen T1 in spin labels. *Chem Phys Lett.* 1984; 110(6):621–625.
- Hyde JS, Subczynski WK. Spin-label oximetry. *Biol Magn Reson.* 1989; 8(Spin Labeling):399–425.
- Hyde JS, Yin JJ, Subczynski WK, Camenisch TG, Ratke JJ, Froncisz W. Spin-labeled EPR T₁ Values Using Saturation Recovery from 2 to 35 GHz. *J Phys Chem B.* 2004; 108:9524–9529.
- Kathirvelu V, Sato H, Eaton SS, Eaton GR. Electron Spin Relaxation Rates for Semiquinones between 25 and 295 K in Glass-Forming Solvents. *J Magn Reson.* 2009; 198:111–120. [PubMed: 19223213]
- Kundu K, Kattnig DR, Mladenova BY, Grampp G, Das R. Electron Spin-Lattice Relaxation Mechanisms of Nitroxyl Radicals in Ionic Liquids and Conventional Organic Liquids: Temperature Dependence of a Thermally Activated Process. *J Phys Chem B.* 2015; 119:4501–4511. [PubMed: 25775000]
- Mailer C, Danielson JDS, Robinson BH. Computer-controlled pulsed electron-paramagnetic-resonance spectrometer. *Rev Sci Instrum.* 1985; 56:1917–1925.
- 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]
- Meyer, V. Applications of EPR with an Emphasis on Tau Fibril Structure. University of Denver; Denver: 2014.
- Muus, LT., Atkins, PW., editors. Electron Spin Relaxation in Liquids. Plenum Press; 1972.
- Owenius R, Eaton GR, Eaton SS. Frequency (250 MHz to 9.2 GHz) and Viscosity Dependence of Electron Spin Relaxation of Triarylmethyl Radicals at Room Temperature. *J Magn Reson.* 2005; 172:168–175. [PubMed: 15589420]
- 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.
- Paddock ML, Flores M, Isaacson R, Shepherd JN, Okamura MY. EPR and ENDOR Investigation of Rhodosemiquinone in Bacterial Reaction Centers Formed by B-Branch Electron Transfer. *Appl Magn Reson.* 2010; 37:39–48. [PubMed: 20157643]
- Pavone M, Biczysko M, Rega N, Barone V. Magnetic Properties of Nitroxide Spin Probes: Reliable Account of Molecular Motions and Nonspecific Solvent Effects by Time-Dependent and Time-Independent Approaches. *J Phys Chem B.* 2010; 114:11509–11514. [PubMed: 20715877]
- Percival PW, Hyde JS. Pulsed EPR spectrometer II. *Rev Sci Instrum.* 1975; 46:1522–1529.
- 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.
- 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 US.* 1982; 79:2559–2563.
- Quine RW, Eaton SS, Eaton GR. Saturation recovery electron paramagnetic resonance spectrometer. *Rev Sci Instrum.* 1992; 63(10):4251–4262.
- Rengan SK, Bhagat VR, Sastry VSS, Venkataraman B. Magnetic Field-Pulsed ELDOR Spectroscopy. *J Magn Reson.* 1979; 33:227–240.
- Rengan SK, Khakhar MP, Prabhananda BS, Venkataraman B. Study of molecular motions in liquids by electron spin-lattice relaxation measurements, I. Semiquinone ions in hydrogen bonding solvents. *Pramana.* 1974; 3:95–121.
- Robinson BH, Haas DA, Mailer C. Molecular dynamics in liquids: spin-lattice relaxation of nitroxide spin labels. *Science.* 1994; 263:490–493. [PubMed: 8290958]
- 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]
- Rockenbauer A, Korecz L, Hideg K. Ring pseudorotation of pyrroline N-oxyl radicals: an analysis of carbon-13 hyperfine structure of EPR spectra. *JCS Perkin Trans 2.* 1993; 2:2149–2156.

- 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]
- Sato H, Kathirvelu V, Fielding AJ, Bottle SE, Blinco JP, Micallef AS, et al. Impact of molecular size on electron spin relaxation rates of nitroxyl radicals in glassy solvents between 100 and 300 K. *Mol Phys.* 2007; 105:2137–2151.
- Savitsky A, Plato M, Mobius K. Temperature Dependence of Nitroxide Spin-Label Interaction Parameters: a High-Field EPR Study of Intramolecular Motional Contributions. *Appl Magn Reson.* 2010; 37:415–434.
- Siri D, Guadel-Siri A, Tordo P. Conformational Analysis of five-membered rings by molecular mechanics: applications to nitroxides. *J Mol Struct (Theochem).* 2002; 582:171–185.
- Swartz HM, Chen K, Pals M, Sentjurs M, Morse PD. Hypoxia-sensitive NMR contrast agents. *Magn Reson Med.* 1986; 3:169–174. [PubMed: 3959885]

Figure 1.

Pulse sequences for measurement of T_1 . a) In a saturation recovery experiment a long pulse or picket fence of pulses is used to saturate the spin transition. The return of the intensity of the observed transition to equilibrium is monitored with low power CW. The length of the initial pulse or picket fence of pulses is extended until there is no further change in T_1 . b) In an inversion recovery experiment spins are inverted with an initial π pulse, and the intensity of the spin transition is monitored with a 2-pulse spin echo as the time between the end of the saturating pulse and the start of the 2-pulse sequence is incremented. c) In an echo-detected saturation recovery experiment a long low-power pulse is used to saturate the spin transition and a two-pulse spin echo sequence is used to detect the return to equilibrium of the magnetization.

Figure 2.

Analysis of Q-band inversion recovery curves for the low-field lines and high-field lines of three ^{15}N -nitroxides in aqueous solution at 20°C with $\tau_R = 19$, 13, and 9 ps, respectively. The plots show the distribution of $1/T_1$ calculated with the uniform penalty (UPEN) method. The x axis is $\log(1/T_1)$ with T_1 in μs , so the value 0.0 corresponds to $T_1 = 1 \mu\text{s}$. Each trace is a single relaxation measurement. In each plot three or four traces are superimposed to indicate the agreement between replicate measurements. Data are from the dissertation of V. Meyer, University of Denver, 2014.

Figure 3.

Frequency Dependence of $1/T_1$ (■) and $1/T_2$ (♦) for PDT in solvents that result in τ_R between 4 and 50 ps at 293 K. (A) toluene, (B) water, (C) light mineral oil, (D) 44% glycerol in water, and (E) 69% glycerol in water. Spin lattice relaxation is modeled as the sum (—) of contributions from spin rotation (Eq. 2, —), the modulation of g- and A-anisotropy (Eq. 3 – 5, — ■ —), and a thermally-activated process (Eq. 7, — · —). In (E) the gold squares and fit line — · — are the contribution from the thermally-activated process observed previously for $\tau > \sim 3 \times 10^{-9}$ s (Owenius et al., 2004). Reproduced with permission from (Biller et al., 2013).

Figure 4. Frequency dependence of $1/T_1$ for trityl-CD₃ (●), trityl-CH₃ (■), OX63 (▲), and OX31 (◆) in water at 293 K. The fit lines are based on contributions from a local mode (Eq. 1) plus modulation of inter- and/or intramolecular electron-proton interaction (Eq. 6).

Figure 5.

Dependence of $1/T_1$ on solvent composition (and therefore viscosity) for trityl-CD₃ (○, ●), trityl-CH₃ (□, ■), OX63 (△, ▲), and OX31 (◇, ◆) at X-band (open symbols) and 250 MHz (closed symbols). To emphasize trends solid lines connect the points. Reproduced with permission from (Owenius et al., 2005).

Figure 6.

(A) Frequency dependence of $1/T_1$ ($\diamond$) for 25DTBSQ in ethanol at 293 K. The relaxation is modeled as the sum (—, short dash) of contributions from spin rotation (SR) (—, solid) Eq. (2), a local mode (local) (—, long dash) Eq. (1), modulation of g-anisotropy (g) (—, dash dot) Eq. (3) and modulation of dipolar interactions with solvent nuclei (solvent) (—, medium dash) Eq. (6). (B) Frequency dependence of $1/T_1$ in $\text{C}_2\text{H}_5\text{OH}$ ($\diamond$), in CH_3OH ($\times$), in $\text{C}_2\text{H}_5\text{OD}$ ($\circ$) and in $\text{C}_2\text{D}_5\text{OD}$ ($\triangleright$). The dashed lines through the data are the sums of contributions to

the spin lattice relaxation, calculated with Eq. 1 – 3 and 7. Reproduced with permission from (Elajaili et al., 2014)

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